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The Role of Microbial Community Structure in Rice Rhizosphere Over the Growing Season

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Abstract The microbial community structure in the rice rhizosphere plays a crucial role in plant health and soil nutrient cycling throughout the growing season. This study investigates how rice plants (*Oryza sativa*) influence the microbial community in rice field soil over different growth stages. Using quantitative PCR and 16S rRNA gene pyrotag analysis, we compared the microbial communities in the rhizosphere of rice plants to those in unplanted bulk soil. Our findings indicate that the rhizosphere harbors a significantly higher abundance of 16S rRNA genes, suggesting enhanced microbial growth. The rhizosphere effect was more pronounced than temporal changes, with notable shifts in the presence of specific microbial phyla such as *Gemmatimonadetes*, *Proteobacteria*, and *Verrucomicrobia*. Functional groups like potential iron reducers and fermenters were enriched in the rhizosphere. Additionally, a *Herbaspirillum* species was consistently more abundant in the rhizosphere, particularly during the early growth stages. These results underscore the dynamic interactions between rice plants and their associated microbial communities, highlighting the importance of the rhizosphere in shaping microbial diversity and function over the growing season.

Keywords Rice rhizosphere; Microbial community structure; 16S rRNA gene analysis; Plant-microbe interactions; Soil nutrient cycling

1 Introduction

The rice rhizosphere microbial community plays a core role in agricultural ecosystems, significantly influencing plant health, nutrient cycling, and soil structure. The rhizosphere, the narrow region of soil influenced by root secretions and associated microbial activity, is a hotspot for microbial interactions that are crucial for plant growth and ecosystem functioning (Lu et al., 2006; Breidenbach et al., 2016; Zhang et al., 2021). Microorganisms in the rhizosphere contribute to nutrient availability, disease suppression, and stress tolerance, thereby enhancing crop productivity and sustainability (Philippot et al., 2013).

The dynamics of the microbial community in the rice rhizosphere are profoundly impacted by the growing season. As rice plants progress through different growth stages, the composition and functionality of the microbial community in the rhizosphere undergo significant changes (Ding et al., 2019; Li et al., 2019). These changes are driven by various factors, including root exudates, soil type, and environmental conditions (Edwards et al., 2015; Hakim et al., 2021). For instance, the abundance and diversity of microbial taxa such as *Proteobacteria*, *Firmicutes*, and methanogenic archaea fluctuate in response to plant growth stages and soil conditions (Zecchin et al., 2023). Understanding these dynamics is essential for optimizing agricultural practices and improving crop yields.

This study primarily elucidates the temporal changes in the structure of the rhizosphere microbial community during the rice growing season and identifies key microbial taxa and functional groups involved in nutrient cycling and plant health. By employing advanced molecular techniques such as quantitative PCR and 16S rRNA gene sequencing, it aims to comprehensively understand how rice plants influence microbial communities at different growth stages. The expected significance lies in informing sustainable agricultural practices by leveraging beneficial microbial interactions to enhance soil health and improve rice productivity.

2 Composition and Function of the Rice Rhizosphere Microbial Community

2.1 Key microbial groups and their ecological roles

The rice rhizosphere hosts a diverse array of microbial groups, including bacteria, fungi, and actinomycetes, each playing crucial ecological roles. Bacterial communities are particularly abundant and diverse, with key phyla such as *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, and *Bacteroidetes* being predominant (Breidenbach et al., 2016). These bacteria are involved in various processes, including nutrient cycling and organic matter decomposition. Fungi, including *Ascomycota* and *Basidiomycota*, also play significant roles in the rhizosphere, contributing to nutrient cycling and plant health (Wei et al., 2023). Actinomycetes, known for their ability to decompose complex organic materials, are another important group in the rhizosphere (Oliveira et al., 2022).

2.2 The role of microbial communities in nutrient cycling

Microbial communities in the rice rhizosphere are integral to nutrient cycling, particularly for nitrogen (N) and phosphorus (P). Bacteria such as those from the genera *Geobacter* and *Anaeromyxobacter* are involved in iron reduction, which is closely linked to nutrient availability (Breidenbach et al., 2016). Nitrogen cycling is facilitated by various microbial processes, including nitrogen fixation, nitrification, and denitrification. For instance, certain bacteria in the rhizosphere possess genes related to denitrification, enhancing nitrogen availability for rice plants (Pramanik et al., 2020). Phosphorus cycling is also mediated by microbial activity, with some microbes capable of solubilizing phosphate, making it more accessible to plants. The interactions between these microbial processes and the redox conditions in the rhizosphere further influence nutrient dynamics (Wei et al., 2019).

2.3 The potential role of microbes in rice rhizosphere health and disease resistance

Microbial communities in the rice rhizosphere contribute significantly to plant health and disease resistance. Beneficial microbes can suppress soil-borne pathogens through competitive exclusion, production of antimicrobial compounds, and induction of plant defense mechanisms (Ding et al., 2019). For example, certain strains of *Herbaspirillum* are consistently more abundant in the rhizosphere and are known to promote plant growth and health (Breidenbach et al., 2016). Additionally, the microbial diversity and network complexity in the rhizosphere can enhance resilience against environmental stresses and pathogen invasion. The presence of specific microbial groups, such as sulfate-reducing and sulfur-oxidizing bacteria, also plays a role in mitigating arsenic toxicity, thereby protecting rice plants from harmful effects (Zecchin et al., 2023).

3 Seasonal Stages and Microbial Community Changes

3.1 Criteria for dividing the growing season

The growing season of rice can be divided into several distinct stages: seedling, tillering, heading, and maturation. These stages are critical for understanding the dynamics of microbial communities in the rhizosphere. The seedling stage marks the initial growth phase, followed by the tillering stage where the plant begins to produce multiple stems. The heading stage is characterized by the emergence of the rice panicles, and finally, the maturation stage is when the grains fully develop and ripen (Schmidt and Eickhorst, 2013; Breidenbach et al., 2016; Wang et al., 2019).

3.2 Key environmental factors affecting microbial communities at each stage

Several environmental factors influence the microbial communities in the rice rhizosphere at different growth stages. Soil pH, nitrogen availability, and soil texture are significant factors that shape the microbial community structure. For instance, soil pH has been shown to be a major determinant of bacterial and archaeal community composition, while nitrogen levels influence microbial population dynamics and functional capabilities (Deng et al., 2017; Wang et al., 2019). Additionally, root exudates, which vary with plant growth stages, play a crucial role in shaping the microbial community by providing specific nutrients and signaling molecules (Li et al., 2019; Berg and Smalla, 2009).

During the seedling stage, the microbial community is influenced by initial soil conditions and the early root exudates, which are rich in simple sugars and organic acids. As the plant progresses to the tillering stage, the microbial community becomes more diverse, with an increase in aerobic bacteria such as β-*Proteobacteria*, which are involved in iron oxidation. The heading stage sees a peak in methanogenic archaea and methanotrophic bacteria, driven by the increased availability of root exudates and changes in soil redox conditions (Schmidt and Eickhorst, 2013). Finally, during the maturation stage, the microbial community stabilizes, with a notable presence of bacteria involved in nitrogen and carbon cycling (Figure 1) (Wang et al., 2019; Liu et al., 2023; Tang, 2024).

3.3 General trends in rhizosphere microbial community structure across seasonal stages

Throughout the growing season, the microbial community in the rice rhizosphere undergoes significant changes. Initially, the microbial diversity is relatively low but increases as the plant grows and root exudates become more complex. The rhizosphere effect, where the microbial community in the rhizosphere is distinct from that in the bulk soil, is evident across all stages but is most pronounced during the active growth phases (tillering and heading) (Breidenbach et al., 2016).

Proteobacteria, Chloroflexi, Bacteroidetes, and Acidobacteria are predominant phyla across all stages, but their relative abundances shift with plant development. For example, Proteobacteria are more abundant during the tillering and heading stages, while Acidobacteria increase towards the maturation stage (Liu et al., 2023). Functional groups such as iron reducers and fermenters are enriched in the rhizosphere, particularly during the early growth stages, indicating a dynamic interaction between plant roots and microbial communities.

4 Dynamic Changes in Rhizosphere Microbial Community Structure

4.1 Seasonal dynamic changes in microbial diversity and abundance

The microbial community structure in the rice rhizosphere undergoes significant changes over the growing season. Studies have shown that the abundance of 16S rRNA genes in the rice rhizosphere is generally higher compared to unplanted bulk soil, indicating a stimulation of microbial growth in the rhizosphere (Breidenbach et al., 2016). Seasonal effects are particularly evident, with shifts in bacterial community structure observed at different plant developmental stages such as tillering, panicle initiation, flowering, and maturity (Schmidt and Eickhorst, 2013). For instance, the abundance of methanogenic archaea peaks at the flowering stage, highlighting the dynamic nature of microbial populations in response to plant growth stages. Additionally, the diversity of active microorganisms in the rhizosphere remains relatively stable across the growth stages, although the total microbial diversity may show little change (Li et al., 2019).

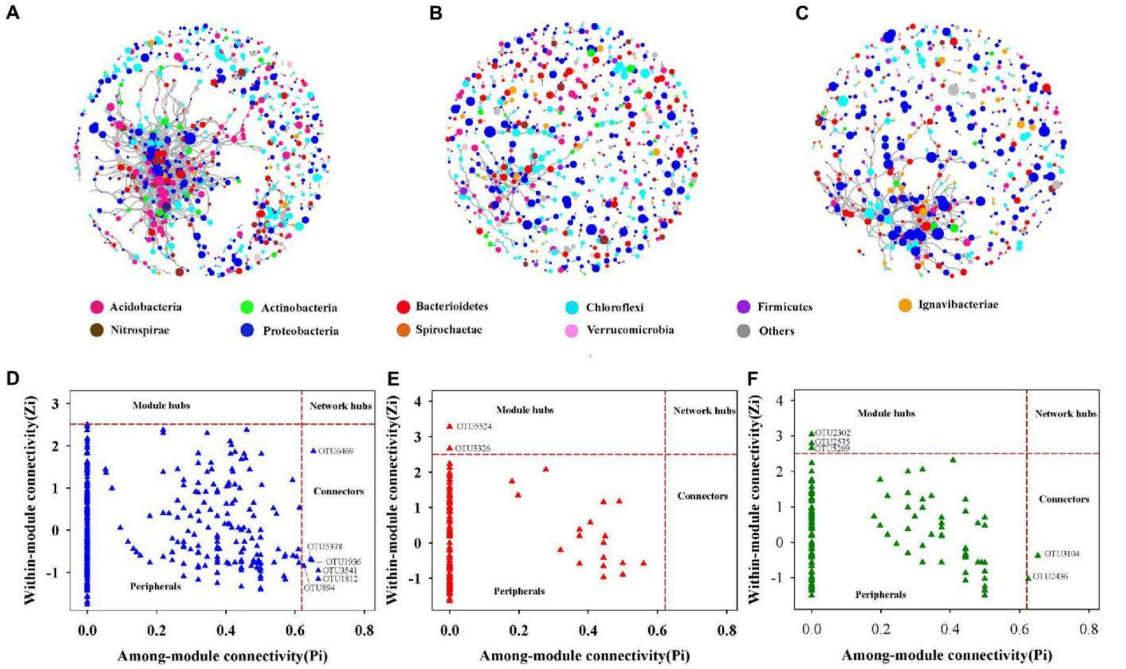

Figure 1 The co-occurrence network of soil bacterial community in bulk (A), rhizosphere (B) and rhizoplane soils (C), the size of each node is proportional to its relative abundance. Zi-Pi plots showing the distribution of soil bacterial OTUs based on their topological roles in bulk (D), rhizosphere (E) and rhizoplane soil (F) (Adopted from Liu et al., 2023)

4.2 Differential impact of each growth stage on major microbial groups

Each growth stage of the rice plant differentially impacts major microbial groups in the rhizosphere. During the early stages of plant growth, certain species such as *Herbaspirillu*m are more abundant in the rhizosphere compared to bulk soil. At the tillering stage, β-*Proteobacteria*, particularly aerobic, iron-oxidizing bacteria of the genus Sideroxydans, become predominant in the rhizosphere (Schmidt and Eickhorst, 2013). The flowering stage sees an increase in methanotrophic bacteria and methanogenic archaea, indicating a shift towards microbial groups involved in methane metabolism. Furthermore, the fungal community is notably enhanced in the rhizosphere compared to unplanted soil, with higher fungal-to-bacterial ratios observed throughout the growth stages (Hussain et al., 2018). These shifts suggest that different microbial groups are selectively enriched at various stages of plant development, influenced by root exudates and changing soil conditions (Asiloglu and Murase, 2016).

4.3 Ecological significance of dynamic changes in rhizosphere microbial community structure

The dynamic changes in the rhizosphere microbial community structure have profound ecological significance. The enrichment of specific microbial groups at different growth stages can enhance nutrient cycling and soil health, thereby supporting plant growth and productivity. For example, the presence of iron reducers and fermenters in the rhizosphere can facilitate the breakdown of organic matter and improve nutrient availability. The seasonal shifts in microbial populations, such as the increase in methanotrophic bacteria during the flowering stage, play a crucial role in mitigating methane emissions from rice paddies, contributing to greenhouse gas regulation (Schmidt and Eickhorst, 2013). Additionally, the stable yet distinct microbial communities in the rhizosphere compared to bulk soil underscore the importance of the rhizosphere effect in shaping microbial diversity and function. Overall, understanding these dynamic changes can inform strategies to optimize microbial interactions for sustainable rice cultivation and environmental management (Fu et al., 2023; Ding et al., 2019).

5 Environmental Factors Influencing Rhizosphere Microbial Communities

5.1 Variability in major environmental parameters, such as soil temperature, moisture, and pH

The structure and function of microbial communities in the rice rhizosphere are significantly influenced by various environmental parameters, including soil temperature, moisture, and pH. Soil pH, in particular, has been identified as a critical factor affecting the diversity and composition of rhizosphere bacterial communities. Studies have shown that soil pH can explain a substantial portion of the variation in microbial community structure, with different pH levels fostering distinct microbial populations (Deng et al., 2017; Han, 2024). For instance, acidic soils ($pH<5.5$) tend to support different bacterial communities compared to neutral or alkaline soils, affecting both the diversity and functional capabilities of these communities (Wan et al., 2020). Additionally, soil moisture and temperature also play crucial roles, although their specific impacts on microbial community dynamics in the rice rhizosphere are less well-documented compared to pH (Lopes et al., 2021).

5.2 Regulatory effects of different environmental factors on rhizosphere microbial composition

Environmental factors such as soil type, nutrient levels, and climatic conditions have regulatory effects on the composition of rhizosphere microbial communities. Soil type has been shown to be a primary determinant of bacterial community structure, with different soil types (e.g., Albic, Black, and Chernozem) hosting distinct microbial populations (Zhang et al., 2021). Soil nutrients, particularly nitrogen forms (NH_4-N, NO_3-N, NO_2-N, and TN), also significantly influence microbial community composition, especially for bacteria and archaea (Deng et al., 2017). Climatic factors, including temperature and precipitation, further modulate these effects by altering soil properties and nutrient availability, thereby shaping the microbial community structure in the rhizosphere (Figure 2) (Zhang et al., 2021).

5.3 Influence of seasonal environmental changes on microbial metabolic activity and community stability

Seasonal changes in environmental conditions, such as temperature and moisture, can lead to shifts in microbial metabolic activity and community stability in the rice rhizosphere. The microbial community in the rhizosphere is generally more dynamic and responsive to these changes compared to bulk soil. For example, the abundance of certain microbial taxa, such as potential iron reducers and fermenters, can fluctuate with plant growth stages and associated environmental changes. Moreover, the stability of microbial communities is influenced by the interactions between environmental factors and microbial functional groups. High organic matter content in soil, for instance, can lead to more stable and convergent microbial communities, as observed in soils with rich organic matter (Zhang et al., 2021). Seasonal variations can also impact the metabolic activity of microbes, with certain functional groups becoming more active during specific growth stages of the rice plant, thereby contributing to the overall stability and functionality of the rhizosphere microbial community (Li et al., 2019; Fu et al., 2023).

6 Case Study of the Relationship between Rhizosphere Microbes and Rice Growth

6.1 Criteria for case study selection

The case study was selected based on several criteria to ensure consistency and relevance. The geographic location chosen was Vercelli, Italy, known for its extensive rice cultivation, providing a representative environment for studying rice rhizosphere dynamics. Consistency in the growing season was maintained by conducting the study under controlled greenhouse conditions, which allowed for precise monitoring of plant growth stages and environmental factors (Breidenbach et al., 2016).

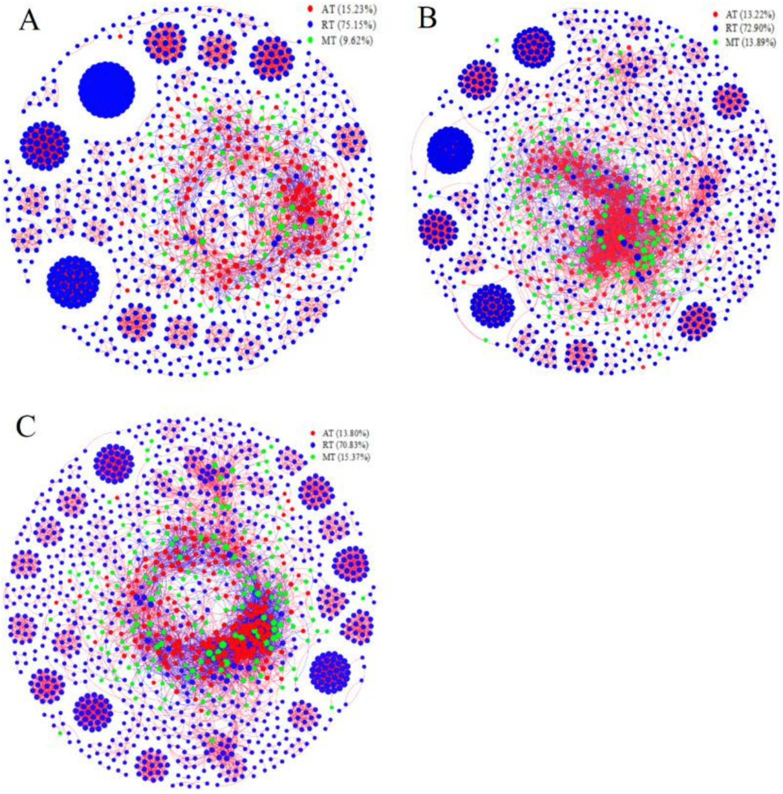

Figure 2 Co-occurrence networks of the bacterial communities in the rhizosphere of rice across three soil types based on correlation analysis. The capital letters represent different soil type, where A represent albic soil, B represent black soil, C represent chernozem soil. A connection indicates a strong (Spearman's $r > 0.8$ or $r < -0.8$) and significant ($p < 0.01$) correlation. Each node represents one genus; the size of each node is proportional to the number of connections (i.e., degree); nodes are colored according to the categories, in which red nodes represent abundant taxa, blue nodes represent rare taxa, and green nodes represent moderate taxa. Percentage inside parentheses represents the ratio of the number of genus with different categories to the number of all genus in the network. Each edge represents a strong and significant correlation between two nodes; the thickness of the edge is proportional to the value of the Spearman's correlation coefficient; and edges are colored according to the positive or negative correlation, in which red edges represent positive correlations, blue edges represent negative correlations (Adopted from Zhang et al., 2021)

6.2 The relationship between microbial community dynamics and rice yield or stress resistance

The relationship between microbial community dynamics and rice growth was analyzed by examining the microbial populations in the rhizosphere at different growth stages of the rice plant. The study utilized quantitative PCR and 16S rRNA gene pyrotag analysis to characterize the microbial community structure. It was observed that the rhizosphere had a significantly higher abundance of 16S rRNA genes compared to bulk soil, indicating a stimulation of microbial growth due to the presence of rice plants (Breidenbach et al., 2016).

The microbial community in the rhizosphere was found to be more influenced by the soil environment type (rhizosphere vs. bulk soil) than by the plant growth stage, suggesting that the rhizosphere effect plays a crucial role in shaping microbial communities. Specific microbial taxa, such as potential iron reducers (e.g., *Geobacter*, *Anaeromyxobacter*) and fermenters (e.g., *Clostridiaceae*, *Opitutaceae*), were notably enriched in the rhizosphere, which could contribute to nutrient cycling and plant growth promotion.

6.3 Key findings of the case study and their potential practical applications

Key findings from the case study include the identification of specific microbial taxa that are enriched in the rice rhizosphere and their potential roles in enhancing rice growth and stress resistance. For instance, the presence of *Herbaspirillum* species, which was consistently more abundant in the rhizosphere, suggests its potential role in promoting early-stage plant growth. Additionally, the study highlighted the importance of root exudates in shaping the microbial community, with certain taxa being enhanced in the rhizosphere due to the presence of compounds like acetate, lactate, oxalate, and succinate (Li et al., 2019).

These findings have several practical applications. Understanding the specific microbial taxa that promote rice growth can lead to the development of microbial inoculants or biofertilizers that enhance crop yield and stress resistance. Moreover, the insights into the rhizosphere effect and the role of root exudates can inform agricultural practices aimed at optimizing soil health and plant-microbe interactions for sustainable rice production (Xiao et al., 2022). The study also underscores the potential for manipulating the rhizosphere microbiome to improve phosphorus uptake and aluminum tolerance in rice, which could be particularly beneficial in nutrient-poor or acidic soils.

7 Role of Rhizosphere Microbes in Nutrient Acquisition and Disease Control in Rice

7.1 Mechanisms by which microbes promote nutrient absorption

Rhizosphere microbes play a crucial role in enhancing nutrient acquisition in rice plants through various mechanisms. One of the primary ways is phosphate solubilization, where specific microbial taxa such as Bacillus and *Pseudomonas* are known to solubilize phosphorus, making it more available for plant uptake (Sun et al., 2022; Xiao et al., 2022). Additionally, nitrogen fixation is another critical process facilitated by diazotrophic bacteria, which convert atmospheric nitrogen into a form that plants can utilize. Studies have shown that irrigated rice fields have higher counts of diazotrophs, which contribute significantly to nitrogen availability in the soil. Furthermore, the presence of iron reducers like *Geobacter* and *Anaeromyxobacter* in the rhizosphere indicates their role in iron cycling, which is essential for plant growth (Breidenbach et al., 2016).

7.2 The role of microbial communities in disease control and key beneficial species

Microbial communities in the rice rhizosphere are not only pivotal for nutrient acquisition but also play a significant role in disease control. Beneficial species such as *Bacillus* and *Pseudomonas* are known for their plant growth-promoting properties and their ability to suppress pathogenic microbes through the production of antimicrobial compounds (Xiao et al., 2022). The complexity and diversity of the microbial community, including fungi like *Aspergillus* and *Rhizopus*, contribute to a robust defense mechanism against soil-borne diseases. The presence of specific microbial taxa in aluminum-tolerant rice genotypes, for instance, has been linked to enhanced disease resistance and better overall plant health.

7.3 Research on microbial community structural changes and their mechanisms in suppressing rhizosphere diseases

Research has shown that the structure of microbial communities in the rice rhizosphere undergoes significant changes in response to various environmental factors and plant growth stages. For example, the microbial community structure is influenced more by soil type and environmental conditions than by the plant genotype itself (Zhang et al., 2021). These structural changes are crucial for the suppression of rhizosphere diseases. The enrichment of specific microbial taxa under phosphate deficiency conditions, for instance, has been shown to enhance the plant's ability to mobilize phosphorus and suppress pathogenic microbes (Sun et al., 2022). Additionally, the dynamic changes in microbial communities, such as the increased abundance of beneficial microbes like *Herbaspirillum* during early plant growth stages, play a vital role in disease suppression.

8 Future Research Directions and Applications

8.1 Future directions for in-depth microbiome research across different growing seasons

Future research should focus on the temporal dynamics of microbial communities in the rice rhizosphere across different growing seasons. Studies have shown that the microbial community structure in the rhizosphere is influenced more by the soil environment than by the plant growth stage (Breidenbach et al., 2016; Li et al., 2019). However, understanding how these communities shift over multiple growing seasons could provide deeper insights into the stability and resilience of beneficial microbial populations. Longitudinal studies employing advanced molecular techniques such as metagenomics and metatranscriptomics could elucidate the functional roles of these microbes and their interactions with rice plants over time (Marín et al., 2011; Hussain et al., 2018).

8.2 Feasibility of utilizing rhizosphere microbial regulation to enhance rice health and yield

The potential to manipulate rhizosphere microbial communities to improve rice health and yield is promising. Specific microbial taxa, such as those involved in phosphorus solubilization and plant growth promotion, have been shown to be recruited by rice genotypes tolerant to soil stresses like aluminum toxicity (Xiao et al., 2022). Additionally, the use of synthetic microbial communities (SynComs) offers a controlled approach to study and harness beneficial microbial interactions (Marín et al., 2021). By selecting and introducing beneficial microbes, it may be possible to enhance nutrient uptake, suppress pathogens, and improve overall plant health, leading to increased yields.

8.3 Potential impacts of microbial community management on sustainable rice agriculture

Managing microbial communities in the rhizosphere could have significant implications for sustainable rice agriculture. The rhizosphere effect, which shapes microbial communities through root exudates, plays a crucial role in nutrient cycling and soil health (Li et al., 2019; Fu et al., 2023). By promoting beneficial microbial populations, it is possible to reduce the need for chemical fertilizers and pesticides, thereby minimizing environmental impact. Moreover, understanding the interactions between rice plants and their associated microbiomes can help in developing strategies to mitigate methane emissions from paddy fields, contributing to climate change mitigation (Ding et al., 2019). Sustainable practices that incorporate microbial management could lead to more resilient agricultural systems capable of withstanding biotic and abiotic stresses.

9 Conclusion

The study has provided significant insights into the dynamic patterns of microbial communities in the rice rhizosphere and their implications for rice cultivation and soil health management. Throughout the growing season, the microbial community structure in the rice rhizosphere exhibited distinct dynamic patterns. The rhizosphere environment, influenced by root exudates, consistently showed higher microbial diversity and abundance compared to bulk soil. Key microbial groups such as *Proteobacteria*, *Gemmatimonadetes*, and *Verrucomicrobia* were notably enriched in the rhizosphere, indicating their critical roles in nutrient cycling and plant health. The presence of rice plants significantly altered the microbial community structure, with specific functional groups like potential iron reducers and fermenters being particularly enriched in the rhizosphere. These dynamic changes underscore the importance of the rhizosphere effect in shaping microbial communities, which remained relatively stable across different plant growth stages.

The implications of these findings for rice cultivation and soil health management are profound. The enrichment of beneficial microbial groups in the rhizosphere can enhance nutrient availability, promote plant growth, and improve stress tolerance, thereby contributing to higher crop yields and sustainable agricultural practices. Understanding the microbial dynamics in the rhizosphere can also aid in developing targeted soil management strategies that leverage beneficial microbes to suppress soil-borne pathogens and reduce the need for chemical fertilizers and pesticides.

Future applications of rhizosphere microbial research in agriculture are promising. By harnessing the beneficial interactions between rice plants and their associated microbiomes, it is possible to develop biofertilizers and biocontrol agents that enhance crop productivity and resilience to environmental stresses. Additionally, insights into the microbial community structure can inform breeding programs aimed at developing rice varieties with optimized root exudate profiles that favor beneficial microbial associations. Overall, the study highlights the critical role of rhizosphere microbial communities in sustainable rice cultivation and offers a foundation for future research to further explore and exploit these complex interactions for agricultural innovation.

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Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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