AGRI MAGAZINE
(International E-Magazine for Agricultural Articles)
Volume: 02, Issue: 12 (December, 2025)
Available online at http://www.agrimagazine.in
©Agri Magazine, ISSN: 3048-8656

Active Microbial Populations in Rice Rhizospheres: Mechanisms, Methods, and Implications for Rice Soil Management

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Flooded rice paddies' rhizosphere is a complex microbial hotspot with alternate redox conditions, strong root exudation, and steep chemical gradients. Because DNA alone might misrepresent the organisms driving biogeochemical activities, it is important to distinguish the active microbial population (measured by 16S rRNA) from the whole community (measured by 16S rDNA). According to studies, the rhizosphere effect is frequently more noticeable at the activity level than the membership level because the rice root imposes a significant selection pressure through radial oxygen loss and exudates. Greenhouse gas emissions (CH4 and N2O) and nutrient cycling (N, Fe) are driven by active, occasionally uncommon "keystone" taxa. In order to identify the metabolically involved bacteria and inform solutions for climate mitigation and sustainable rice management, paired DNA/RNA sequencing and chemical profiling are crucial.

Introduction

The rhizosphere, a limited soil zone directly impacted by plant roots, is a microbial hotspot where plant, soil, and microbial activities all interact. In flooded rice paddies, this zone is extremely complicated because to the alternation of reduced and oxidised microsites, strong root exudation, and intensive plant microbe interactions that form steep chemical and biological gradients. Historically, soil microbial ecology depended on DNA-based surveys (16S rDNA) to determine the potential or total membership of microbial communities. However, DNA endures from both active and dormant cells, as well as extracellular and relic DNA, thus DNA alone may misrepresent the organisms that are currently metabolising and communicating. On the other hand, RNA-based methods (such as metatranscriptomics or 16S rRNA transcripts) offer a close indicator of microbial activity or capability for protein synthesis, enabling the separation of active and total populations. Because redox dynamics and root exudation significantly control microbial metabolic pathways (such as aerobic respiration in oxic microzones, denitrification, iron reduction, sulphate reduction, and methanogenesis in anoxic zones), it is crucial to distinguish between active and total bacterial assemblages in paddy soils. A focused investigation of rice paddies revealed a strong rhizosphere effect that was more noticeable in profiles based on RNA than on DNA, suggesting that roots influence microbial activity in ways that DNA cannot. Because active microorganisms drive rapid biogeochemical transformations and plant-relevant processes, this has ramifications for rice agroecosystems' nutrient cycling, greenhouse gas emissions, and disease suppression (Li *et al.*, 2019). Methodologically, parsing active vs total communities requires paired sampling (rhizosphere and bulk), immediate stabilization for nucleic acids, parallel extraction of DNA and RNA, and sequencing of both 16S rDNA and 16S rRNA (or metatranscriptomes). Recent studies highlight that rhizosphere effects on activity vary with plant growth stage, soil amendments (e.g., straw return), and landscape

scale environmental factors, meaning that the rhizosphere activity relationship is dynamic and context dependent (Zhao *et al.*, 2022)

Interaction mechanisms and evidence

Root exudation as primary selection pressure: Sugars, amino acids, organic acids (acetate, lactate, succinate, and oxalate), and secondary metabolites that supply labile carbon and signaling molecules are all released by roots. These substances encourage rapidly proliferating, copiotrophic bacteria in the rhizosphere and produce chemoattractive gradients. Research conducted in paddy rhizospheres reveals taxonomic enrichment (such as fermenters and certain α-Proteobacteria) in RNA profiles associated with certain exudates, suggesting selective activation of metabolic activity (Li *et al.*, 2019).

Nutrient hotspots and cycling shifts: Local nitrogen, phosphorus, and iron pools are changed by root absorption and exudation, which modifies microbial activities (nitrification, denitrification, N fixation, Fe and S cycling). These substrates and redox-sensitive electron acceptors elicit responses from active populations. Research reveals variations in the expression and abundance of N-cycling genes (amoA, nirK, nosZ) and that, depending on soil amendments and development stage, rhizosphere impacts either promote or repress particular N-cycling activities. Gene expression and process metrics are frequently correlated with changes in RNA levels (Zhao *et al.*, 2022).

Selection of functionally important taxa (active but not always abundant in DNA): Some taxa may be low abundance in DNA but highly active (high rRNA:rDNA ratio). These "keystone" active taxa can drive key processes (e.g., iron reduction, methanogenesis) despite low total abundance. Comparative rRNA/rDNA analyses reveal taxa whose activity surges in the rhizosphere (e.g., taxa associated with acetate utilization and Fe cycling) while DNA surveys underestimate their ecological role (Li *et al.*, 2019).

Temporal dynamics: growth stage and transient responses: During the seedling, tillering, heading, and grain-fill phases, root architecture and exudation patterns vary. Microbial responses can be episodic; certain taxa react momentarily at crucial phases (e.g., early root development). Stage-dependent patterns can occasionally be seen in rhizosphere bulk differences at the RNA level, with greater activity differences during active growth stages. At the RNA level, soil compartment explains greater variance than phenological stage, according to Li et al.'s 2019 study and previous time series research, although both are important (Li *et al.*, 2019)

Effect of agricultural management (straw return, fertilization): Amendments change the background pool of labile carbon and nutrients, potentially masking or modifying rhizosphere stimuli. Straw return can create competing C sources and alter which taxa are rhizosphere responsive. Field experiments find fewer genera uniquely affected by the rhizosphere when straw is added (i.e., the rhizosphere effect can be dampened by added organic inputs), and RNA profiles change accordingly (Zhao *et al.*, 2022)

Methodological consequences: why RNA + DNA matters: DNA alone overestimates persistence and dormant pools; RNA enriches for currently active organisms. Combining both lets researchers identify: (a) taxa present but inactive, (b) taxa active but rare, and (c) taxa both abundant and active. The divergence between active vs total bacterial communities in rice paddies is robust across studies that used paired rDNA/rRNA sequencing, making a strong case for using both measures when studying rhizosphere processes. (Li *et al.*, 2019).

Microbe–microbe interactions and predator effects: Rhizosphere selection can be amplified or dampened by interactions (competition, facilitation, bacteriophage predation, protist grazing); for instance, predation on copiotrophs may liberate resources or influence the makeup of active communities. Microbial interactions are an understudied driver of active community patterns, according to some metatranscriptomic work and larger rhizosphere literature, even if fewer research have explicitly measured this in paddies (Ling *et al.*, 2022).

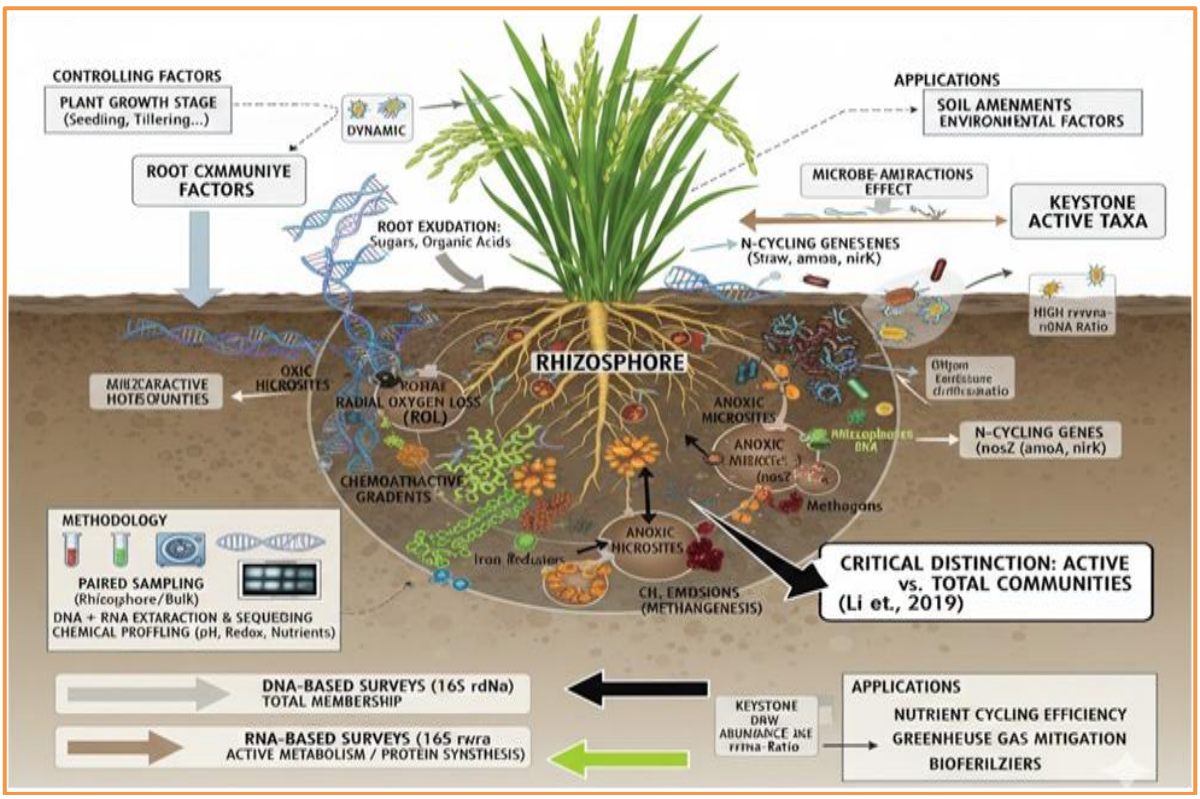

Fig. 1. Rhizosphere microbial activity in rice field

Present research scenario, applications, and gaps

In agronomy and greenhouse gas management, knowing which taxa are active in the rhizosphere can help develop strategies to: (1) encourage beneficial N-cyclers and inhibit denitrifiers that produce N2O; (2) control methanogen activity by controlling exudation and electron acceptors; and (3) create microbiome-informed amendments (biofertilizers) that target active niches. Straw return and fertilisation schedules, for instance, dramatically change the microbial taxa that actively respond to roots; modifying these procedures might direct active communities towards desired process results (Zhao *et al.*, 2022).

Conclusion

Differentiating between active and total bacterial populations in paddy rhizospheres helps us better understand which microorganisms are responsible for soil activities that are essential to rice production and environmental consequences. Roots alter who is metabolically active rather than just who is present. Plant functional selection through exudates, oxygen microgradients, and nutritional changes is highlighted by RNA-based profiling, which shows that the rhizosphere influence is often higher at the activity level than at the membership level. In terms of methodology, metabolomics, process measurements, and paired DNA/RNA techniques are becoming commonplace for reliable inference. High-resolution sampling, integrated field experiments, and multidisciplinary pipelines connecting molecular, chemical, and ecological measurements will be necessary to further these objectives.

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