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Exploration of Microbial Diversity of Anoxic Soil Under Submerged Low-Land Paddy Cultivation by Culture-Independent Methods for Its Consequent Ramifications on Environment, Soil Health, and Sustainability

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ABSTRACT

Soil microbes, the primary components of soil biodiversity, provide crucial ecosystem services. Anthropogenic activities, such as agriculture, bring about innumerable biotic and abiotic changes in the soil ecosystem that can have short and long-term impacts on the environment and soil sustainability. Lowland paddy cultivation is a typical example of anthropogenic disturbance to soil, where the paddy field fluctuates between waterlogged conditions and extreme dry spells, which have some immediate effects, such as greenhouse gas emissions, and a probable long-term impact on soil, altering soil oxidoreduction states and influencing microbe-driven nutrient and carbon cycling. Although rice is a dominant crop in Northeast India, microbial responses to paddy cultivation remain poorly documented. In this study, we compared the soil microbiomes of paddy fields and adjacent non-paddy land in the Bhagawatipara region of Kamrup district of Assam, India, using 16S rRNA amplicon sequencing. Paddy soils exhibited clear shifts in microbial abundance, with an increased representation of methanogens, nitrogen-transforming archaea, diazotrophs, and phosphate-solubilizing bacteria. Distinct changes in alpha and beta diversity further indicated strong hydrological and cultivation-driven impacts on microbial community dynamics. This study provides the first comprehensive microbial dataset for paddy soils in the Kamrup district (Assam) and highlights key microbial signatures associated with methane cycling and soil nutrient processes.

1. INTRODUCTION

Various anthropogenic activities, including agriculture, contribute to the environmental crisis, posing a serious threat to the sustainability and existence of life on this planet. Agricultural practices such as dependence on fertilizers, pesticides, and mechanized plowing contribute to environmental degradation and loss of soil sustainability. Lowland paddy cultivation poses a higher environmental threat as the practice of flooding paddy fields creates anaerobic conditions under which certain bacteria and archaea produce methane gas, which is more potent than carbon dioxide in greenhouse effect. However, rice, being the staple diet of more than 50% of the global population, is indispensable for food security. The microbes present in the soil play a major role in this emission, and at the same time, these microbes are essential for soil sustainability as they maintain biogeochemical cycles, mitigate stress, and even oxidize some greenhouse gases. Although the effect of agricultural activities on microbial diversity is invisible, it can have serious consequences for long-term sustainability. Thus, the characterization of microbial communities can play a very important role in designing mitigation strategies. Although paddy is the major crop of Assam and other northeastern states of India, there is limited literature available on metagenomic studies of paddy field microbiomes. Earlier studies were mostly culture-dependent and revealed only a minor fraction of the total microbiome.

Microbes, including archaea, bacteria, and fungi, play a pivotal role in overall soil health and offer innumerable ecosystem services. These microbial communities display significant diversity in both structure (community composition) and function (collective physiology of all species) (Escalas et al. 2019). This diversity varies across different locations and changes over time. Soil microorganisms that significantly impact biogeochemical cycles, are influenced by varied fertilizer practices, and are closely associated with crop growth, nutrient cycling, and sustainable soil productivity (Yang et al. 2022, Börjesson et al. 2011). Exploring bacterial diversity and biogeographic patterns can offer insights into the fundamental ecological processes and mechanisms that support bacterial diversity and ecosystem function, along with the scope of application in diverse fields such as agriculture, the environment, and many innovative areas. Microbes can be used for soil stabilization instead of chemical and physical methods (Shah et al. 2024). Various agricultural management practices, including crop type, tillage, fertilizer application, and irrigation patterns, affect soil microbial activity and community structure (Camacho-Sanchez et al. 2023, Gupta et al. 2022). However, the majority of microbial ecosystem research relies on a few dominant species due to their easy detectability. In microbial communities, a few abundant species are prevalent alongside a multitude of rare species, which contributes to high diversity (Hanson et al. 2012). Therefore, a thorough investigation of the complexity and diversity of soil microorganisms is vital for understanding the role of different microbial communities in soil ecosystems and the environment as a whole. Understanding the link between microbial diversity and the environment is crucial for developing predictive models of microbial diversity-driven ecosystem processes and functions (Wan et al. 2023). The identification of uncultured microorganisms requires rigorous scientific examination. Among the techniques developed to elucidate the physiology and genetics of non-cultured organisms, metagenomics is a transformative centerpiece in microbiology. Metagenomics has notably propelled novel research avenues by facilitating unparalleled analyses of genome heterogeneity and evolution in environmental settings. Additionally, it grants access to a substantially broader spectrum of microbial diversity than traditional cultivation methods (Handelsman 2004).

In the present age, facing extreme environmental crises, climate change, food security concerns, and various environment-related complexities, the analysis of soil microbiomes associated with agriculture has become imperative for understanding the role of microbiomes in environmental degradation and restoration of soil health towards developing climate change resilient, sustainable agriculture. In the case of paddy cultivation, the anaerobic environment of the flooded paddy field soil becomes suitable for methanogens, a type of microorganism that produces the greenhouse gas methane (CH_4) as a metabolic byproduct (Xuan et al. 2025). The effect of prolonged flooding and fertilization practiced in paddy cultivation changes the abiotic factors of the soil ecosystem, which are closely tied to the structure and function of microbial communities. Unlike higher organisms, disturbance diversity relationships in microbial ecology are contradictory and difficult to generalize. Although paddy field soil in some cases displays higher diversity than non-paddy field soil, the increase in microbial diversity of paddy fields does not always correlate with positive sustainability impacts, as higher diversity can sometimes correlate with trade-offs in ecosystem services, such as soil organic carbon (SOC) retention or nitrogen (N) retention.

Although India is one of the major producers of paddy, research on paddy field soil microbiomes is not proportionate. A study on the effect of agricultural practices on the soil microbiome showed that fertilizer application did not have a significant effect on the microbiome, indicating the resilience of soil microflora to short-term disturbances (Kumar et al. 2025). Studies on the rhizospheric microbiome of basmati rice have shown a unique assemblage of bacteria, such as Actinobacteria, *Bacillus subtilis*, *Burkholderia, Enterobacter, Klebsiella, Lactobacillus, Micrococcus, Pseudomonas*, and *Sinomonas* (Raina et al. 2025). In the paddyfield microbiome, the prevalence and diversity of beneficial microbes, such as nitrogen fixers, nitrifiers, and phosphate mineralizers, were lower, whereas cellulose and hemicellulose decomposing microbes and phosphorus solubilizers were abundant (Prasannakumar et al. 2021). Proteobacteria, Chloroflexi, Acidobacteria, Actinobacteria, and Bacteroidetes are the most abundant bacterial phyla in rice field soil (Singh et al. 2024).

The northeastern region of India, situated within the Eastern Himalayan biodiversity hotspot, serves as a gateway to diverse flora and fauna. The diverse physiography of the area offers a distinct and varied environment for the evolution of microorganisms. Studies on microbes isolated from these diverse areas can provide opportunities to explore genetic diversity and contribute to understanding ecological processes on Earth. Numerous studies have investigated the microbial biodiversity of soil across various ecosystems in northeastern India. Nevertheless, the implementation of high -throughput sequencing methods has the potential to enhance and contribute to in-depth analysis and documentation of microbial diversity of the samples

There is significantly less research based on metagenomic approaches to study the paddy field microbiome of northeast

India. Very few reports on the paddy field microbiome of this region indicate a major research gap, as the region has unique rice cultivation practices and diverse agroecosystems. Microbiome studies have mostly been conducted on local fermented foods and culture-dependent analysis of plant growth-promoting and other beneficial microbes of diverse crops. Sediments of the Brahmaputra River, India, showed increased abundance of Pseudomonadota at the phylum level, *Bradyrhizobium*, *Pseudomonas* and *Variovorax* at the genus level and T. *denitrificans* as the most abundant bacterial species (Sharma et al. 2024). An increase in the abundance of Proteobacteria and Firmicutes was observed in paddy field soil supplemented with vermicompost, increasing paddy grain yield (Deka et al. 2025). In culture-dependent studies, the bacterium *Alcaligenes faecalis* isolated from the rice rhizosphere of the upper Brahmaputra Valley region of Assam was found to be effective as a biocontrol agent against rice root-knot nematodes (Jena et al. 2023). Consortia of indigenous PGPR isolates of *Bacillus cereus, Pseudomonas fluorescens,* and *Azospirillum oryzae* improved the productivity and soil health of paddy under jhum cultivation in Nagaland (Giri et al. 2023).

This study analyzed the composition of microbial communities found in paddy field and non-paddy field soil samples using a metagenomic approach. Metagenomic analysis has provided comprehensive insights into the previously uncharted microbial realm, and the data generated from global research endeavors have the potential to enhance our comprehension of global microbiome patterns (Chakraborty et al. 2023). While some microbial communities are essential for preserving soil fertility, others are responsible for harmful gaseous emissions, denitrification, and may also be pathogenic. Therefore, this study aimed to identify the specific types of microorganisms present in both paddy field and non-paddy field soil samples. This analysis would provide insights into how different environments shape microbial diversity. Assessing the environmental factors influencing microbial diversity requires the evaluation of various environmental parameters, such as pH, temperature, moisture content, and nutrient availability, to understand their influence on shaping microbial communities in both paddy and non-paddy soils.

This study was conducted in the Bhagwatipara paddy field situated in Rangamati, Kamrup District of Assam, India. Metagenomic analysis was employed to examine the microbiome of paddy soil in Bhagwatipara. A comparative analysis was conducted using non-paddy soil from the same location as the reference point. This study aimed to comprehend the impact of paddy cultivation on indigenous microflora and gain insights into the general effects of ecological disturbance on microbial community diversity.

2. MATERIALS AND METHODS

2.1. Soil Sampling

To study the microbial diversity of paddy field and non-paddy field soil samples (control/reference), soil samples were obtained from the Bhagawatipara area (26°22’60” N and 91°58’0” E, site elevation: ~32 m above sea level) (Fig. 1). Soil was collected from three adjacent paddy

Fig. 1: The study area is located in Bhagawati Para in Kamrup district, situated in Assam state.

fields in Bhagwatipara using sterilized trowels, and each of the collected soil samples was a composite sample of three random subsamples from each paddy field. Soil samples from adjacent non-agricultural land with natural, undisturbed vegetation were used as a reference or control. A V-shaped hole was dug in the soil, and the soil samples were collected from a depth of about 10cm in sterilized sample bottles, properly labeled and brought to the laboratory at a low temperature with ice packs.

2.2. Soil Physical Parameters

The study of soil moisture content (%), pH, and temperature (°C) was conducted on the samples obtained from three different locations of paddy and non-paddy sites of the Bhagawatipara area.

2.3. DNA Isolation

DNA was isolated from the soil samples using a DNeasy PowerSoil kit (Qiagen, United States). The DNA present in the soil samples was isolated, and the concentration was measured using a Qubit Fluorimeter (V.3.0) (Thermo Fisher Scientific, Malaysia).

2.4. Illumina Library Preparation:

The region V3-V4 of 16S rRNA was amplified using specific V3 primer CCTACGGGNBGCASCAG (forward) and V4 primer GACTACNVGGGTATCTAATCC (reverse). The resulting amplification product was verified on agarose gel (2%), which was purified to eliminate non-specific amplifications. A 5 nanogram aliquot of the amplified product was used for library preparation using the NEB Next Ultra DNA library preparation kit. (Illumina, California, United States). The quantification and its quality were estimated using an Agilent 2200 Tape Station. After ligation with Illumina adaptors, the amplicons were gel-purified and amplified using conventional Illumina methods. Subsequently, the prepared library was sequenced on an Illumina HiSeq 2500 platform at AgriGenome Labs, Kochi, India.

2.5. Analysis of 16S rRNA Dataset Using Bioinformatics

In this study, raw sequences were quality-filtered to remove reads containing ambiguous bases, homopolymer runs >6 bp, primer mismatches, or read lengths <100 bp. High-quality reads were processed using QIIME v1.9.1. OTUs were clustered at 97% sequence similarity using the UCLUST algorithm (Edgar 2010), and taxonomy was assigned using the SILVA reference database based on 97% identity to the reference sequences. Chimeric sequences were filtered during the workflow to ensure an accurate taxonomic classification. Taxonomic summaries from phylum to genus levels were generated directly from the final OTU table without rarefaction, and taxa contributing less than 0.005% of the total abundance were excluded from downstream analysis. A BIOM file was created to support the functional and statistical analyses. Alpha-diversity indices (Shannon, Chao1, and Observed Species) and beta-diversity metrics were computed using QIIME to assess within-sample and between-sample microbial variation. Principal Coordinate Analysis (PCoA) was performed using weighted and unweighted UniFrac distance matrices. Functional predictions were derived using PICRUSt, and KEGG pathway profiles were generated for comparative functional assessments (Kanehisa & Goto 2000).

Statistical analyses were performed in Python and R. Group differences in taxonomic abundance were assessed using one-way ANOVA, followed by Linear Discriminant Analysis (LDA) to identify the taxa that most strongly differentiated the paddy and non-paddy soils. PERMANOVA (Adonis function, vegan package) was used to evaluate differences in beta diversity among sample groups. Non-parametric comparisons of taxon abundance were performed using the Kruskal–Wallis test, where applicable. All analyses were performed in R v3.6.2 using the vegan package to compute richness, evenness, and community diversity.

3. RESULTS

Table 1 presents the physicochemical properties of the soil samples from both paddy and non-paddy fields. The moisture content of soil samples from the paddy field was relatively higher (average 27.32% ± 1.4) than that of non-paddy soil (average 9± 0.5%).

3.1. Quality Filtering and Read Retention

Six environmental samples were processed for quality assessment following read joining. The input read count per sample ranged from 30,302 to 42,429 sequences. After applying stringent quality filters, including the removal of reads that were too short post-trimming,

Table 1: Physical parameters of samples collected from both paddy and non-paddy fields of Bhagawatipara.

Samples	Tempertaure	pH	Moisture
1 Bhagwatipara paddy	32.3	7.2	24.49
2 Bhagwatipara paddy	30.1	6.85	28.87
3 Bhagwatipara paddy	31.1	7.1	28.60
4 Bhagwatipara non-paddy	29.6	6.9	10
5 Bhagwatipara non-paddy	27.8	7.1	8
6 Bhagwatipara non-paddy	28.6	6.8	9

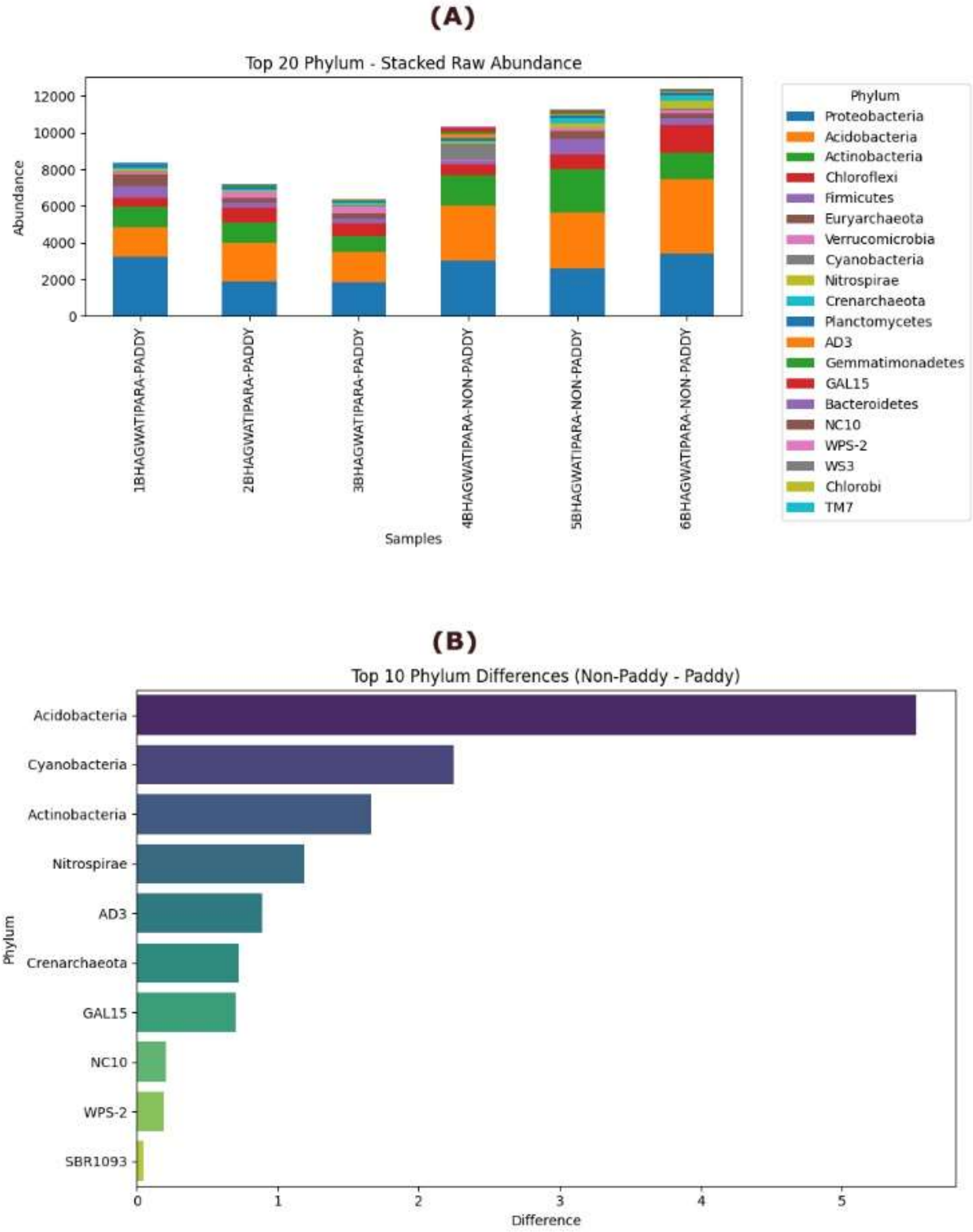

Fig. 2: (A) Stacked bar plot showing top 20 microbes at the phylum level, (B) Top 10 Phylum-Level Differences Between Non-Paddy and Paddy Soils.

exceeded the limit of ambiguous bases (N), or failed barcode criteria, each sample retained a substantial number of high-quality reads suitable for downstream microbiome analysis. The number of high-quality sequences retained per sample was 18,483 for 1BHAGWATIPARA-PADDY, 18,889 for 2BHAGWATIPARA-PADDY, 16,373 for 3BHAGWATIPARA-PADDY, 18,318 for 4BHAGWATIPARA-NON-PADDY, 23,379 for 5BHAGWATIPARA-NON-PADDY, and 23,262 for 6BHAGWATIPARA-NON-PADDY groups. The median read lengths after filtering remained consistent across samples (440–441 bp), indicating a high-quality sequencing performance. The 16S rRNA gene sequencing data are available at the Sequence Read Archive (SRA), NCBI (http://www.ncbi.nlm.nih.gov/sra), SRR21547403, SRR21547404, and SRR21547406.

Operational Taxonomic Units (OTUs) picking and taxonomy classification were performed using pre-processed consensus V3-V4 sequences. Pre-processed reads from all samples were pooled and clustered into OTUs based on their

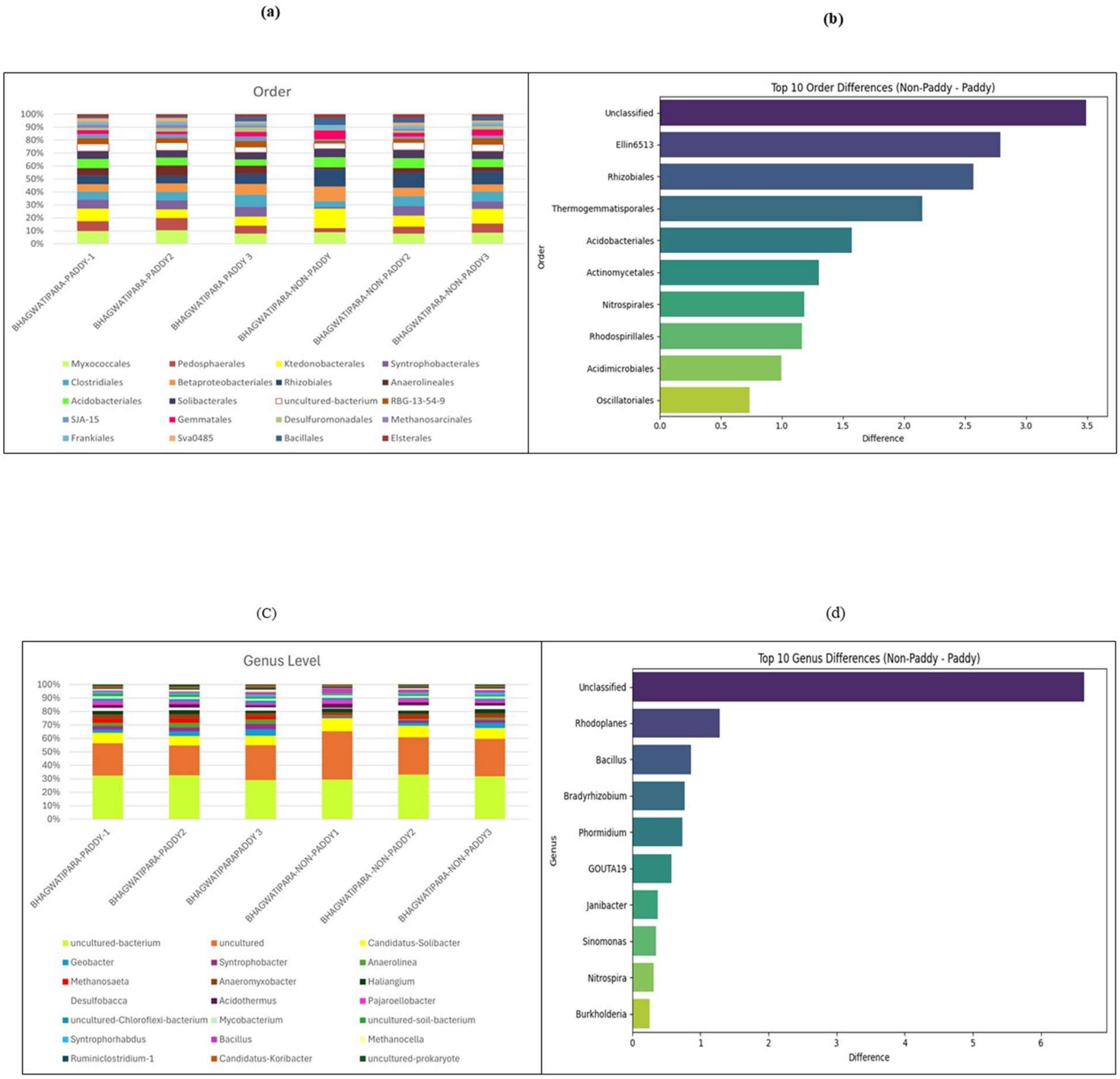

Fig. 3: (a) Stacked bar plot showing top 20 microbes at the Order level, (b) Top 10 Order-Level Differences Between Non-Paddy and Paddy Soils, (C) Stacked bar plot showing top 20 microbes at the Genus- level, (d) Top 10 Genus-Level Differences Between Non-Paddy and Paddy Soils.

sequence similarity using the Uclust program (similarity cut-off = 0.97) available in the QIIME software.

3.2. Relative Abundance of Microbes

Analysis of the microbial community structure revealed clear ecological distinctions between paddy and non-paddy soils (Fig. 2). In paddy soils, a higher relative abundance of *Chloroflexi*, known for degrading complex hydrocarbons and pollutants, was observed, which may be linked to organic matter deposition from crop residues and the recurrent use of fertilizers and pesticides. An increased representation of *Euryarchaeota*, a phylum associated with methanogenesis under anaerobic conditions, further reflects the waterlogged, low-oxygen environment that is typical of paddy fields. In contrast, the comparative difference analysis (non-Paddy vs. Paddy) highlighted that several functionally important phyla were markedly more abundant in non-Paddy soils. *Acidobacteria* exhibited the highest positive difference, indicating a strong preference for well-aerated, stable soil environments. Their reduced abundance in paddy fields suggests suppression under anaerobic conditions. *Cyanobacteria*, responsible for nitrogen fixation and primary production, and *Actinobacteria*, key decomposers and antibiotic producers, were also substantially enriched in non-paddy soils, implying diminished nutrient cycling and lower decomposition activity in paddy fields compared to non-paddy fields. Additional phyla, such as *Nitrospirae*, *AD3, Crenarchaeota, GAL15, NC10,* and *WPS-2,* also showed higher abundance in non-paddy soils, reinforcing that non-paddy environments support a richer and more functionally diverse aerobic microbial community. Overall, these shifts reflect the strong impact of paddy field flooding and management practices on the microbial ecological balance and nutrient cycling potential.

Higher relative abundances of *Myxococcales, Pedosphaerales, Syntrophobacterales, Clostridiales, Anaerolineales* and *Methanosarcinales* were observed in the paddy field samples than in the non-paddy field soil samples, whereas *Ktedonobacterales, Betaproteobacteriales, Rhizobiales, Acidobacteriales, Solibacterales, Gemmatales, Frankiales, and Bacillales* were more abundant in the non-paddy soil than in the paddy field soil (Fig. 3).

At the genus level, *Geobacter, Syntrophobacter, Anaerolinea, Methanosaeta, Desulfobacca, Acidothermus,* and *Methanocella* have higher abundance in paddy field soil, but *Candidatus-Solibacter, Anaeromyxobacter, Haliangium, Bacillus,* and *Paenibacillus* had higher abundance in non-paddy soil. *Methanobrevibacter, Listeria, Clostridium, Methanobrevibacter, Syntrophus,* and *Geobacter* had significantly different relative abundances across the samples ($p < 0.05$). *Methanobrevibacter* and *Listeria* have been observed only in paddy fields, whereas *Clostridium, Syntrophus,* and *Geobacter* have very low abundance in non-paddy soil. *Methanocella* are ubiquitous in paddy soils and are key methane producers

Genus-and species-level patterns further reinforced the phylum-level differences observed between paddy and non-paddy soils. In alignment with the higher abundance of *Acidobacteria* in non-paddy fields, several Acidobacteria-associated species, including *Unclassified Acidobacteria*, *Candidatus Solibacter celatum*, and *Solibacter gordonii*, showed big positive differences, indicating their substantially greater enrichment in non-paddy soils. These species are typically involved in soil carbon turnover and are sensitive to agricultural disturbances, supporting the observation that non-paddy soils maintain a more stable and diverse heterotrophic community than paddy soils. Similarly, species such as *Nitrospira leptosphaera* and *Nitrospira flexus*, belonging to the Nitrospirae phylum, contribute to nitrification processes and suggest more efficient nitrogen cycling under non-flooded conditions.

In contrast, paddy fields showed lower representation of many Actinobacteria-associated species, consistent with our earlier finding of reduced Actinobacteria abundance at the phylum level. Species such as *Actinobacteria radiopugnans*, *A. tuberum*, *A. fastidiosa*, and *A. muraliis* all showed negative differences in the comparison, indicating a reduced presence in the flooded environment. This decline may contribute to the slower degradation of complex organic matter and an ecological shift that favors anaerobic and methanogenic taxa. This trend also aligns with the higher abundance of Euryarchaeota (methanogens) observed in paddy soils,

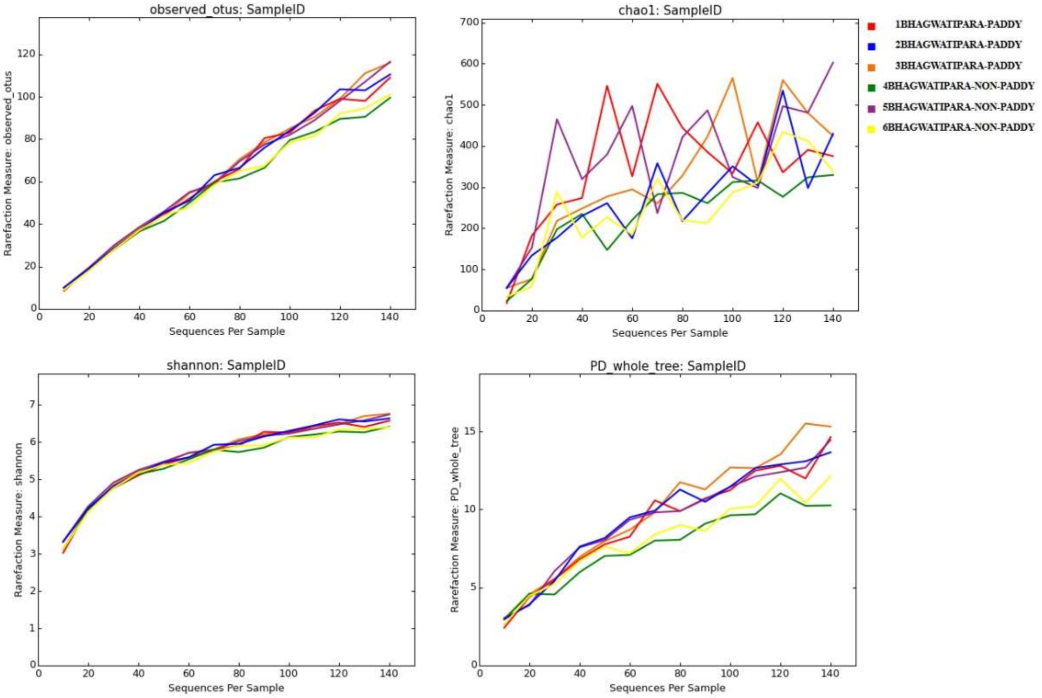

Fig. 4: Rarefaction curves showing alpha diversity estimates across paddy and non-paddy soil samples.

suggesting enhanced methane-producing activity under waterlogged and oxygen-limited conditions.

The stacked species-level abundance plot further confirmed that non-paddy soils contained a broader distribution of key bacterial species, whereas paddy soils were dominated by a narrower set of taxa with lower species-level diversity. Altogether, these species-level observations complement the phylum-level findings and highlight how land use type and soil flooding conditions distinctly shape microbial community structure, ecological functions, and nutrient cycling potential in the studied habitats.

3.3. Alpha Diversity and Beta Diversity Analysis

Rarefaction analysis was performed to evaluate the sequencing depth and compare the alpha diversity patterns across paddy and non-paddy soil samples (Fig. 4). The observed OTUs rarefaction curves showed a consistent increase with sequencing depth for all samples, indicating adequate sampling coverage and comparable sequencing effort across groups. Non-paddy soils (Samples 4–6) generally exhibited slightly higher richness values at higher sequence depths compared to paddy soils (Samples 1–3), suggesting a more diverse microbial community under non-flooded conditions.

The Chao1 richness estimator revealed greater variability among samples, with several non-paddy samples showing higher estimated richness than the paddy samples. This pattern reflects the presence of additional rare taxa in non-paddy soils, further supporting the increased richness of aerobic environments. The Shannon diversity index, which accounts for both richness and evenness, showed smooth and gradually rising curves for all samples, with non-paddy samples displaying marginally higher diversity, indicating a more even community composition than that of paddy soils.

Similarly, the Phylogenetic Diversity (PD whole tree) rarefaction curves demonstrated a higher phylogenetic breadth in non-paddy soils, implying the presence of more evolutionarily distinct microbial lineages under non-flooded conditions.

Beta diversity analysis using Bray-Curtis dissimilarity clearly showed a separation between the microbial communities of paddy and non-paddy soil samples. The principal coordinate analysis (PCoA) plot demonstrated that the samples clustered according to their land type, indicating distinct community compositions between the two environments (Fig. 5). PC1 explained 41.4% of the total variation, whereas PC2 explained 22.9%, together accounting for a substantial proportion of the community differences. Non-paddy samples formed a more dispersed cluster, suggesting higher heterogeneity in this group. In contrast, paddy samples clustered more tightly, reflecting a more uniform microbial community structure. This separation supports the findings observed in alpha diversity, phylum-level shifts, and species-level differences, collectively indicating that land use practices strongly influence soil microbial composition.

3.4. Differential Abundance Statistical Analysis

Differential abundance testing using one-way ANOVA and the non-parametric Kruskal–Wallis test revealed several taxa that were significantly different between the paddy and non-paddy soil microbiomes. ANOVA

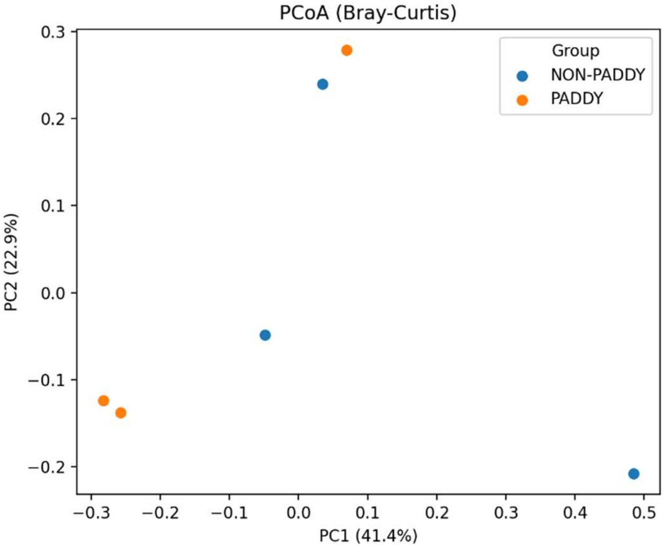

Fig. 5: Principal Coordinates Analysis (PCoA) based on Bray-Curtis dissimilarity.

identified strong separation driven by members of *Lentisphaerae* (Victivallaceae), Betaproteobacteria (*Ramlibacter*), methanogenic archaea including Candidatus Methanoregula and Methanobrevibacter, and multiple anaerobic lineages of Chloroflexi (*Anaerolineae*), alongside enriched Firmicutes taxa such as *Listeria*, *Clostridium*, *Pelosinus*, and representatives of Ruminococcaceae. Several taxa associated with anaerobic respiration and redox cycling, including Geobacter, Syntrophus, and Nitrospirae, showed significant differences. The Kruskal–Walli's test supported these patterns, consistently highlighting significant shifts in methanogenic archaea (*Methanobrevibacter, Methanosarcina, Methanomassiliicoccus*), Chloroflexi Anaerolineae, and Firmicutes lineages, along with key soil groups such as Acidobacteria, Actinobacteria, and multiple Proteobacteria families (Comamonadaceae, Moraxellaceae, Enterobacteriaceae). The recurrence of these taxa across both statistical tests confirms robust microbial distinctions between the two soil types, driven largely by the increased abundance of anaerobic and methanogenic groups in paddy soils and the enrichment of aerobic Acidobacteria, Actinobacteria, and several Proteobacteria in non-paddy soils. These findings align with the taxonomic and ordination-based patterns observed at the community level, reinforcing the strong ecological divergence between paddy and non-paddy soil microbial communities.

3.5. Differential Abundance Analysis Using LDA (LEfSe)

LEfSe analysis was performed to identify the taxa that significantly discriminated between the paddy and non-paddy soil microbiomes, as shown in Fig. 6. Using the non-paddy group as a reference, several bacterial and archaeal lineages exhibited strong differential enrichment patterns. Taxa belonging to the phylum GAL15 showed the highest positive LDA coefficients, indicating strong enrichment in non-paddy soils. Members of Verrucomicrobia (particularly *Candidatus Udaeobacter*) and Chloroflexi (including *Ktedonobacter* lineages) were also strongly associated with non-paddy environments, suggesting their ecological preference for relatively dry and less waterlogged soil conditions. Conversely, certain Acidobacteria subgroups (subgroups 1 and 2) were enriched in paddy soils, reflecting their adaptability to the acidic, submerged, and nutrient-variable conditions typical of flooded rice fields. Enrichment of Thaumarchaeota, specifically ammonia-oxidizing archaea (*Candidatus Nitrosotalea*), in paddy soils indicates enhanced nitrification activity under flooded conditions.

Overall, the LDA results complemented the phylum- and species-level observations by highlighting the distinct microbial signatures shaped by contrasting soil environments. These discriminant taxa provide deeper insights into the functional and ecological differences between paddy and non-paddy soil ecosystems.

3.6. Functional Prediction of Microbial Communities (PICRUSt Analysis)

PICRUSt-based metagenome prediction revealed clear functional distinctions between paddy and non-paddy soil microbiomes across multiple metabolic and stress response pathways (Fig. 7). The functional shifts broadly reflected the contrasting environmental conditions of waterlogged, anaerobic paddy soils versus relatively oxygenated and nutrient-variable non-paddy soils.

Pathways associated with stress tolerance and cellular repair were strongly enriched in paddy soils. ABC transporters and oxidative phosphorylation were markedly higher in several paddy samples, indicating enhanced nutrient uptake and energy generation under fluctuating redox conditions in paddy fields. Likewise, DNA repair and recombination proteins were consistently elevated in paddy soils, suggesting increased genomic maintenance, potentially driven by oxidative stress, electron acceptor limitations, and microbial competition. In contrast, non-paddy samples showed comparatively higher contributions to inorganic ion transport and sulfur metabolism, reflecting their more aerated environment, where sulfur oxidation and ion exchange processes remain active.

The functional genes associated with nitrogen transformation exhibited significant differences between

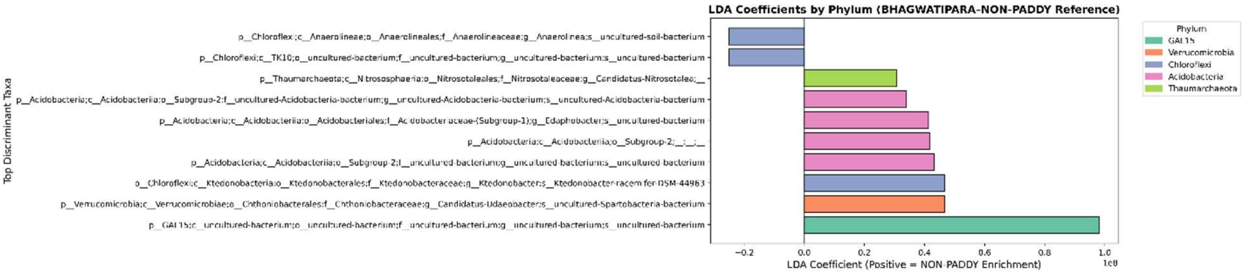

Fig. 6: LEfSe LDA scores identifying discriminant taxa between paddy and non-paddy soils.

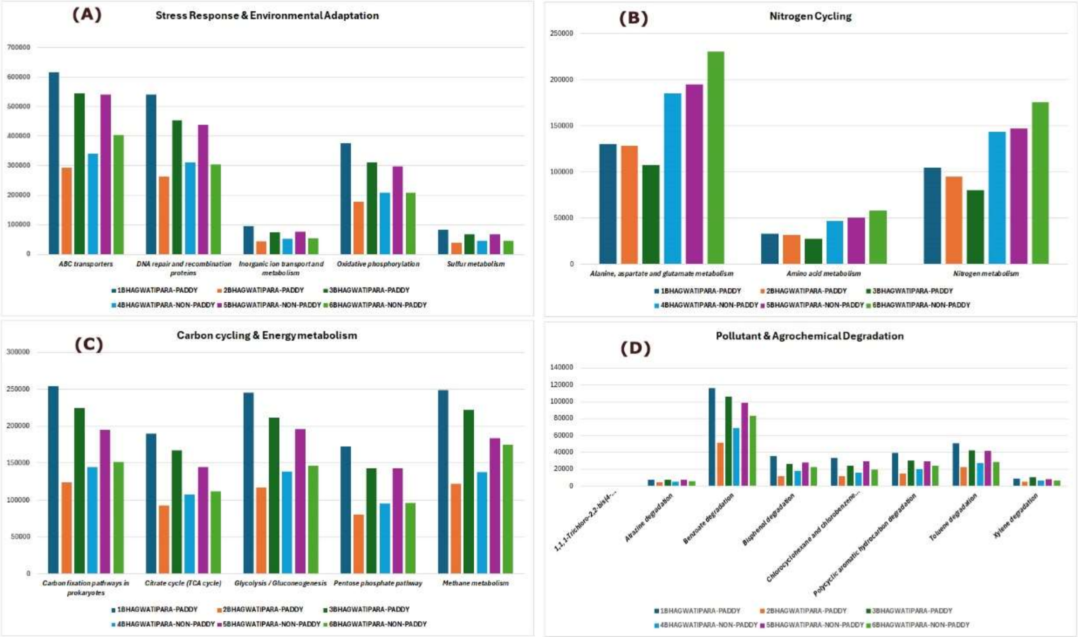

Fig. 7: PICRUSt-based functional predictions of microbial communities in paddy and non-paddy soils.

the two soil types. Alanine, aspartate, and glutamate metabolism, which is central to nitrogen assimilation, showed notable enrichment in non-paddy soils, aligning with higher microbial activity associated with organic nitrogen turnover. Conversely, paddy soils displayed elevated nitrogen metabolism gene counts in some samples, likely driven by the presence of methanogens and denitrifiers thriving under reduced flooded conditions. Amino acid metabolism was also more pronounced in non-paddy soils, indicating diverse heterotrophic nitrogen utilization in oxygenated environments.

Carbon transformation pathways revealed a strong ecological fingerprint in each habitat. Non-paddy soils constantly exhibited higher activity in carbon fixation pathways, the TCA cycle, glycolysis/glyconeogenesis, and the pentose phosphate pathway, reflecting active aerobic heterotrophy and carbon turnover. In contrast, methane metabolism was substantially higher in paddy soils, supporting the dominance of anaerobic methanogenic archaea under flooded and low-oxygen conditions. The significant contribution of these pathways in paddy samples mirrors the taxonomic enrichment of methanogenic groups observed in earlier analyses. PICRUSt predictions also highlighted the functional capacities related to xenobiotic degradation. Non-paddy soils demonstrated enriched pathways for benzene, chlorocyclohexane, chlorobenzene, and polycyclic aromatic hydrocarbon (PAH) degradation functions typically associated with diverse aerobic Proteobacteria and Actinobacteria. Paddy soils showed notable contributions to specific degradation pathways, such as alkane and benzoate degradation, suggesting adaptive responses to agricultural inputs and anaerobic breakdown processes. Although several pathways were shared between the two environments, their magnitudes differed, indicating distinct pollutant-processing roles shaped by oxygen availability and microbial community composition.

4. DISCUSSION

A comparative analysis of soil microbiomes from paddy and non-paddy fields at Bhagawatipara revealed strong ecological and functional divergence driven by differences in hydrology, redox chemistry, and land-use practices. At the phylum level, paddy soils were characterized by the enrichment of Chloroflexi, Euryarchaeota, and other anaerobic or facultative anaerobic groups, consistent with earlier reports that waterlogged and reduced environments select for microorganisms capable of fermentation, syntrophy and methanogenesis (Lee et al. 2015, Jiang et al. 2021). The elevated abundance of methanogenic archaea, particularly Euryarchaeota, aligns with the anaerobic conditions typical of flooded rice systems and supports previous observations that paddy soils are major methane-emitting ecosystems (Sugano et al. 2005). In contrast, non-paddy soils exhibited markedly higher levels of Acidobacteria, Cyanobacteria, Actinobacteria, and Nitrospirae, which are commonly associated with aerobic, nutrient-rich, and stable soil environments. Acidobacteria showed the strongest positive enrichment in non-paddy soils, reinforcing their preference for well-aerated, mature soils with stable moisture and organic matter turnover (Fierer et al. 2007, Griffiths et al. 2011). Several Acidobacteria-associated species, including *Candidatus Solibacter celatum* and *Solibacter gordonii*, were dominant in non-paddy samples, highlighting their role in carbon turnover and sensitivity to disturbance or prolonged flooding.

At the species level, non-paddy soils supported a broader and more evenly distributed microbial community, with enriched populations of *Nitrospira* species involved in nitrification processes and aerobic nitrogen cycling than paddy soils. This pattern aligns with the functional characteristics of upland soils, where oxygen availability favors autotrophic nitrifiers and other aerobic chemoorganotrophs (Prosser & Nicol 2012). Meanwhile, the reduced abundance of Actinobacteria-associated species and several Proteobacteria in paddy soils suggests diminished decomposition and nutrient mineralization under flooded, oxygen-limited conditions, as Actinobacteria are well-known decomposers and producers of extracellular enzymes (Hayat et al. 2010). The enrichment of Chloroflexi, particularly members of *Anaerolineae*, in paddy soils indicates active syntrophic interactions with methanogens, where complex hydrocarbons and long-chain fatty acids are degraded anaerobically, producing substrates that fuel methane production (Liang et al. 2015, Xia et al. 2016). This microbial consortium is characteristic of flooded rice ecosystems. The increased abundance of methanogenic taxa, such as *Candidatus Methanoregula*, *Methanobrevibacter*, and *Methanosarcina,* further strengthens the evidence of enhanced methanogenic activity in paddy soils. These findings support previous studies demonstrating that methanogenic archaea respond strongly to waterlogging, redox shifts, and organic matter deposition in rice fields (Sugano et al. 2005).

Conversely, non-paddy soils displayed higher abundance of bacterial groups such as *Ktedonobacterales, Rhizobiales, Solibacterales, Frankiales, Bacillales*, and other aerobes involved in nutrient solubilization, nitrogen fixation, and carbon decomposition (Beneduzi et al. 2008, Hayat et al. 2010). Their reduced presence in paddy fields suggests that flooding suppresses important soil fertility functions mediated by these taxa, such as oxidative decomposition of recalcitrant organic compounds and aerobic nitrogen transformations.

The diversity analyses further supported the strong environmental filtering imposed by land use type. Alpha diversity indices, for example, Chao1, Shannon diversity, and PD whole tree, were consistently higher in non-paddy soils, indicating a richer and more phylogenetically diverse community structure. These findings contrast with those of earlier studies suggesting higher OTUs in paddy soils (Hou et al. 2018), but the present dataset clearly shows that oxygen availability and environmental stability in non-paddy soils promote broader microbial diversity. The tighter clustering of paddy samples in the Bray-Curtis PCoA plot, compared with the greater dispersion among non-paddy samples, further reflects the uniformity of microbial communities formed under consistently flooded conditions.

Differential abundance analyses using ANOVA, Kruskal-Wallis, and LEfSe consistently identified anaerobic Chloroflexi (*Anaerolineae*), methanogenic archaea (*Methanobrevibacter, Methanosarcina, Methanomassiliicoccus*), and Firmicutes (*Clostridium, Pelosinus, Listeria*) as key discriminant taxa enriched in paddy soils. These groups contribute to anaerobic respiration, fermentation, syntrophic metabolism, and methane production, which are central to flooded rice ecosystems (Xia et al. 2016). In contrast, Acidobacteria, Actinobacteria, Proteobacteria (*Comamonadaceae* and *Moraxellaceae*), and several Nitrospirae lineages were significantly enriched in non-paddy soils, reflecting their ecological preference for oxygenated, nutrient-variable, and stable terrains.

The PICRUSt-based functional predictions revealed that environmental differences between the two fields also translated into distinct functional potentials. Paddy soils showed high contributions to ABC transporters, oxidative phosphorylation, DNA repair, and methane metabolism, consistent with microbial adaptation to redox fluctuations, oxidative stress, and anaerobic carbon transformation. Non-paddy soils exhibited enriched pathways for carbon fixation, the TCA cycle, glycolysis/gluconeogenesis, nitrogen assimilation (alanine/aspartate/glutamate metabolism), and xenobiotic degradation, reflecting their more active aerobic metabolism and pollutant-processing capacities driven by Proteobacteria and Actinobacteria (Spain et al. 2009). Collectively, taxonomic, diversity, and functional analyses converge to demonstrate that paddy soil microbiomes are dominated by anaerobic, methanogenic, and syntrophic communities, whereas non-paddy soils host diverse aerobic decomposers, nitrifiers, and nutrient-cycling bacteria. These findings underscore the importance of land use type, moisture regime, and flooding-driven redox conditions as major ecological filters that shape soil microbial structure and function. Understanding these shifts is essential for developing sustainable agricultural practices, mitigating methane emissions, and maintaining soil fertility under diverse cultivation systems.

5. CONCLUSIONS

This study revealed a distinct restructuring of soil microbial communities between paddy and non-paddy ecosystems, primarily driven by waterlogging, redox fluctuations, and land-use practices. Non-paddy soils supported microbial communities characteristic of aerobic and oligotrophic environments, marked by the enrichment of Acidobacteria, Actinobacteria, and several Proteobacteria groups. The strong representation of Acidobacteria underscores their importance as indicators of soil health, organic matter turnover, and carbon cycling efficiency in well-drained soils. In contrast, paddy soils exhibited a pronounced shift toward anaerobic, methanogenic, and syntrophic microbial groups, reflecting the prolonged flooding and low-oxygen conditions typical of rice cultivation. The presence of Thaumarchaeota and other nitrogen-transforming archaea indicates modified nitrogen cycling pathways under reduced conditions, whereas elevated methanogenic lineages highlight the potential for enhanced methane emissions from waterlogged soils.

Diversity analyses further supported these trends in the study. Although both environments contained taxonomically rich communities, their functional profiles differed substantially. Paddy soils showed enrichment in pathways associated with anaerobic metabolism, methane production, stress response systems, and nutrient limitation, whereas non-paddy soils exhibited a higher representation of pathways involved in aerobic heterotrophy, carbon fixation, and degradation of complex organic pollutants. Together, these results illustrate how hydrological management and cultivation practices significantly influence the microbial community structure and functional potential. Such shifts have direct implications for soil fertility, greenhouse-gas emissions, and long-term ecosystem sustainability. Therefore, understanding these microbial signatures is essential for predicting how land-use changes affect nutrient cycling and environmental health. Expanding these findings through larger, multi-location studies will enable the development of predictive models and targeted mitigation strategies, particularly those aimed at reducing methane production in rice agriculture while maintaining its soil productivity.

Data availability: Raw data generated in the study have been submitted to NCBI. The accessions for this submission are: SRR21547403, SRR21547404, and SRR21547406.

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