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Article

Impact of Long-Term Paddy Monoculture on Soil Microbial Diversity and Soil Macronutrients

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***Abstract*— This study investigated the long-term impacts of paddy monoculture on soil health in Tanjung Karang, Malaysia, through integrated physicochemical and metagenomic analyses, with comparisons to a pristine Raja Musa Forest Reserve (RMFR) reference site. Our findings demonstrate that prolonged monoculture significantly alters soil physicochemical properties of nitrogen, phosphorus, and potassium, thereby creating a distinct edaphic environment. Metagenomic analyses revealed a substantial increase in microbial diversity and richness in monocultured paddy soils compared to RMFR, with specific taxonomic groups becoming dominant. Crucially, functional annotation highlighted significant shifts in the abundance of genes associated with nitrogen, phosphorus, and potassium metabolism, underscoring the adaptive responses of the soil microbiome to continuous cultivation pressures and their influence on critical nutrient cycling processes.**

***Keywords*— Paddy monoculture; Soil health; Metagenomics; Microbial diversity; Nutrient cycling; Tanjung Karang**

I. INTRODUCTION

Rice (Oryza sativa L.) stands as a fundamental staple crop globally, sustaining over half of the world's population, with a particularly critical role in Asian food security [1]. In Malaysia, paddy cultivation is a cornerstone of the agricultural sector, significantly contributing to national food self-sufficiency [2]. Regions designated as "Rice Bowls," such as Tanjung Karang in Selangor, have been pivotal in the nation's rice production for decades, characterized by continuous and intensive paddy monoculture practices. Notably, the extensively cultivated paddy soils of Tanjung Karang were historically part of a larger ecological complex, specifically, once encompassing portions of the Raja Musa Forest Reserve (RMFR) [3]. This historical connection is particularly salient as RMFR now serves as a crucial pristine reference soil in this study, providing a valuable baseline for comparative analysis against the long-term monocultured paddy areas. While this agricultural system offers benefits in terms of simplified farm management and enhanced operational efficiency, its prolonged application has raised substantial concerns regarding long-term soil health and the sustainability of these vital agricultural ecosystems.

Soil health is intrinsically linked to sustainable agricultural productivity, underpinned by the complex interplay between its physicochemical properties and the resident microbial communities [4]. Intensive monoculture, involving the repetitive cultivation of a single crop species on the same land, frequently leads to a decline in soil organic matter, nutrient imbalances, and alterations in soil structure [5], [6]. The persistent reliance on chemical fertilizers and pesticides, common in such intensive systems, can further exacerbate soil degradation and detrimentally impact the diversity and functional capacity of soil microbial communities [7]. Consequently, a comprehensive understanding of these cumulative impacts on the soil environment is paramount for developing effective and sustainable management strategies.

Soil microbes are indispensable drivers of numerous ecological processes, including nutrient cycling, soil aggregation, and plant health maintenance [8]. They are central to the biogeochemical transformations of essential macronutrients such as carbon, nitrogen, phosphorus, and potassium, which are vital for crop growth and yield [9]. Any perturbation in the diversity, composition, or metabolic activities of these microbial communities can directly influence soil fertility and agricultural productivity [10]. Traditional microbiological methods often fall short in capturing the full spectrum of microbial diversity and function due to the unculturable nature of most soil microorganisms [11]. In contrast, metagenomic sequencing offers a powerful, culture-independent approach to comprehensively characterize the entire genomic content of microbial communities within an environmental sample. This enables a deeper exploration of both microbial species composition and their functional potential, providing invaluable insights into how these communities adapt and respond to anthropogenic pressures like long-term monoculture [12].

Building upon existing knowledge and addressing the specific context of Malaysian paddy fields, monoculture is hypothesized to be linked to shifts in soil diversity and functional capacity [13]. This study aims to elucidate the multifaceted effects of prolonged paddy monoculture on soil health in Tanjung Karang. Specifically, our objectives are threefold: (1) to assess the total nitrogen (N), available phosphorus (P), and available potassium (K); (2) to characterize the microbial species composition and evaluate metagenome assembly statistics following metagenomic DNA isolation and sequencing; and (3) to investigate the impact of monoculture on key genes involved in nitrogen, phosphorus, and potassium metabolism within the soil microbial community. The findings from this research are anticipated to provide critical insights into the ecological consequences of intensive paddy monoculture on soil health in Malaysia's prime rice-producing areas, thereby informing the formulation of more sustainable soil management practices to ensure long-term agricultural productivity and ecosystem resilience.

II. MATERIALS AND METHODS

A. Sampling Sites

This study investigated the impact of long-term paddy monoculture on soil health by integrating macronutrient analyses with metagenomics sequencing. Soil samples were collected from three cultivation sites in Tanjung Karang, Selangor (TK1: 3°27'15"N, 101°12'10"E; TK2: 3°26'48"N, 101°11'57"E; TK3: 3°26'59"N, 101°12'08"E). At each site, five subsampling points were taken from the surrounding area. The pH of the soil samples was recorded in the range of 6.4–6.8. Yield records indicated that TK1 produced more than 10 tons of rice per 1.2 acres, TK2 produced around 5 tons, while TK3 yielded below 2 tons, reflecting different productivity levels across the sites. The Tanjung Karang area has been subjected to over five decades of continuous rice farming. As a natural reference, data from the pristine Raja Musa Forest Reserve (RMFR; 3°25'N, 101°26'E) peat swamp forest were included. Soil sampling in Tanjung Karang was conducted in December 2023, targeting the topsoil layer (0–15 cm) using a grid-based approach to ensure representative coverage of the cultivation area. The samples were then stored at −80°C to prevent DNA degradation [14] - [16].

B. Key Macronutrients Analysis

Following collection, soil samples were processed for a series of physicochemical analyses. Air-dried samples were sieved through a 2 mm mesh before determination of various parameters. Key macronutrients, including total nitrogen (N), available phosphorus (P), and available potassium (K), were quantified using standard methods, specifically the Kjeldahl method for total N and the Olsen method with sodium bicarbonate extraction for available P, followed by colorimetric analysis. [17].

C. DNA Extraction, Quality Control, and Library Preparation

Metagenomics DNA was extracted from 0.25–0.30 g of soil using the DNeasy PowerSoil Kit (Qiagen) following the manufacturer's instructions. DNA concentration was determined using a Qubit™ 4 Fluorometer (Invitrogen) with the Qubit™ dsDNA HS Assay Kit, and samples were purified with magnetic beads to remove potential inhibitors. DNA was then fragmented to an average size of 300–350 bp using a Covaris ultrasonicator, and fragment size distribution was verified with an Agilent 2100 Bioanalyzer. Library preparation was performed using the VAHTS Universal Pro DNA Library Prep Kit for Illumina (Vazyme). Fragmented DNA underwent end-repair, phosphorylation, and 3′ adenylation before ligation with index adapter sequences. Adapter-ligated DNA was size-selected with magnetic beads and amplified by eight cycles of PCR using P5 and indexed P7 primers. The resulting libraries were purified, validated on the Agilent 2100 Bioanalyzer, and quantified with the Equalbit 1x dsDNA HS Assay Kit. Qualified libraries were sequenced on the Illumina NovaSeq 6000 platform.

D. Bioinformatics workflow

Raw metagenomics reads were subjected to quality control and preprocessing using SolexaQA++, where DynamicTrim removed low-quality bases ($Q \geqslant 20$) and LengthSort retained sequences longer than 50 bp. Contaminant sequences such as PhiX were filtered out using Bowtie2, followed by Python scripts to ensure correct pairing and shuffling of reads. High-quality reads were assembled into contigs using MetaSPAdes, which applied a multi-k-mer strategy to optimize assembly of complex microbial communities. The assembled sequences were then aligned against the NCBI non-redundant (NR) protein database using DIAMOND BLASTx with frameshift-

aware alignment and an e-value threshold of 1e-5 (specified by the --evalue parameter) to improve alignment accuracy. Annotation and binning were performed in MEGAN6 using the interval-union Lowest Common Ancestor (LCA) algorithm. This enabled both taxonomic profiling and functional annotation, referencing KEGG, SEED, eggNOG, and GTDB databases. Finally, data visualization was carried out with MEGAN6, providing hierarchical taxonomic trees and interactive functional profiles [14], [18].

E. Statistical Analysis

Statistical analyses were applied to microbial diversity, alpha diversity metrics (Shannon index) and beta diversity (Bray-Curtis dissimilarity) were computed, with alpha and beta diversity specifically calculated using KrakenTools. Appropriate statistical tests were employed to determine differences between sample groups and to identify correlations between soil microbial community each sites [19], [20].

III. Results and Discussions

A. Key Macronutrients in Tanjung Karang Paddy Fields and Raja Muda Musa Forest Reserve (RMFR) Soils.

Our analyses revealed significant alterations in the key macronutrients properties of soils subjected to long-term paddy monoculture in Tanjung Karang compared to the reference RMFR soil as stated in Figure 1. The concentrations of key macronutrients varied considerably. The evaluation of total nitrogen, phosphorus, and potassium across the paddy monoculture sites and the undisturbed Raja Musa Forest Reserve (RMFR) revealed substantial variability linked to productivity levels and land use history. The total nitrogen content in the Raja Muda Musa Forest Reserve (RMFR) was notably lower (0.28%) compared to the cultivated paddy field sites in Tanjung Karang, where values ranged from 0.56% to 0.67%. In contrast, the nitrogen content in RMFR soils is derived primarily from natural organic matter decomposition, which occurs at a slower rate in undisturbed forest ecosystems [22].

Phosphorus levels showed significant variation across the sites. TK3 exhibited an exceptionally high phosphorus content (5.54%), substantially exceeding levels observed at RMFR (1.06%) and the other two Tanjung Karang sites, TK1 (0.23%) and TK2 (0.60%). The elevated phosphorus concentration in TK3, despite its low paddy yield, may result from localized over-application of phosphate fertilizers or from variations in soil properties that influence phosphorus retention. [21]. In forest soils like those in RMFR, phosphorus is typically bound in organic forms and becomes available through mineralization, resulting in moderate levels [23].

Potassium content was relatively high across all sites, ranging from 3.66% in RMFR to 4.41% in TK3. The elevated potassium levels in both natural and cultivated soils may be influenced by the parent material and mineral weathering processes, especially in Malaysian soils known to be rich in K-bearing minerals [24]. The slightly higher values in the cultivated fields may also reflect the regular input of potassium fertilizers and residues from previous cropping cycles [21].

The variability of macronutrient concentrations across sites appears to correspond with observed differences in rice yield. TK1, which recorded the highest yield (>10 tons per 1.2 acres), also exhibited balanced macronutrient composition with relatively high total nitrogen (0.62%) and moderate phosphorus and potassium levels, supporting optimal crop growth [25]. In contrast, TK2, with a moderate yield (5 tons), showed slightly higher nitrogen (0.67%) but lower phosphorus availability, suggesting possible nutrient imbalance [26]. TK3, which produced the lowest yield (<2 tons), displayed an abnormally high phosphorus concentration (5.54%) but lower nitrogen, indicating nutrient saturation or fixation that may limit plant uptake. Excess phosphorus can reduce micronutrient availability and disrupt root-associated microbial activity, potentially constraining productivity [27]. This disparity is likely due to the routine use of nitrogen, phosphorus, and potassium based NPK fertilizers in the agricultural sites as recommended by Jabatan Pertanian Malaysia, which increases soil nitrogen, availability. Fertilization in rice fields is typically applied four times during the growth cycle (15–90 days after sowing), with compound fertilizers and urea supplied at rates of 50–140 kg/ha depending on the stage [21].

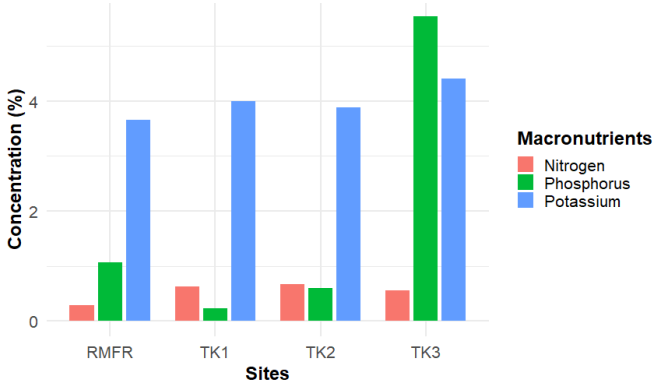

Figure 1. Comparative Analysis of Key Macronutrients in Tanjung Karang Paddy Fields and Raja Muda Musa Forest Reserve (RMFR) Soils.

B. DNA Extraction and purification

Shotgun metagenomic sequencing using the Illumina NovaSeq 6000 platform began with the extraction of total DNA from three paddy soil samples collected from long-term monoculture plots in Tanjung Karang. This step ensured the comprehensive capture of microbial DNA, enabling unbiased taxonomic and functional analysis of the soil microbiome. High-quality DNA is essential for reliable downstream sequencing and bioinformatics.

As shown in Table 1, DNA concentrations ranged from 58.22 to 70.59 ng/µL across the samples. TK1 had the highest concentration at 70.59 ng/µL, with a total yield of 1764.75 ng, followed by TK3 at 63.33 ng/µL with 1773.24 ng. TK2 showed the lowest concentration at 58.22 ng/µL with a total yield of 1455.50 ng. The purity of DNA, assessed using a NanoDrop spectrophotometer, showed A260/A280 ratios between 1.8 and 2.0. Integrity was further confirmed by agarose gel electrophoresis, which showed clear high-molecular-weight bands without significant degradation. Overall, the DNA obtained from all samples was of sufficient quality and quantity for downstream metagenomics sequencing [28].

TABLE 1. The details of DNA extraction and purification quality for three paddy soil metagenomics samples

Sample ID	A260/A280 ratio	Qubit	
		Concentration (ng/µl)	Amount (ng)
TK1	1.8	70.59	1764.75
TK2	2.0	58.22	1455.50
TK3	1.9	63.33	1773.24

C. Metagenome Assembly Statistics

The whole-genome shotgun sequencing generated substantial raw reads for all samples. After quality trimming with SolexaQA++ and removal of PhiX contaminants using Bowtie2, high-quality clean reads were obtained for de novo assembly with SPAdes [13]. Assembly statistics for all samples have N50 values of 377–410 bp and N90 values of 286–288 bp, with total contigs ranging from 2.0 to 2.8 million and maximum contigs lengths between 47 kb and 103 kb. The L50 values ranged from 794,000 to 855,000 contigs, while the L90 values were between 1.79 and 2.07 million contigs, confirming that the assemblies were highly fragmented, as expected for complex soil metagenomes. The relatively low N50 and high L90 values reflect the fragmented nature of soil metagenome assemblies, which is expected due to the immense microbial diversity and uneven species abundance in such environments. Importantly, the consistency of these metrics across sites indicates reliable sequencing, while the presence of large contigs (up to 100 kb) supports functional annotation [29].

Sequencing was performed on soils from the Raja Muda Musa Forest Reserve (RMFR) and three Tanjung Karang sites (TK1, TK2, TK3) as shown in Figure 2 with a read length of 150 bp. RMFR produced 16,559,928 raw reads, of which 15,496,792 were retained after assembly. Among the cultivated sites, TK2 yielded the highest number of raw reads (77,639,274), followed by TK3 (69,143,644) and TK1 (62,476,396), with assembled reads of 75,088,646, 66,425,838, and 59,696,808, respectively. All samples were processed and analysed using identical workflows to minimize potential bias and minimal read loss post-assembly indicates consistent sequencing depth and efficient assembly across samples. These results confirm the generation of high-quality metagenomics datasets suitable for taxonomic and functional profiling of soil microbiomes [30].

D. Dominant Bacterial Phyla in Long-Term Paddy Monoculture and Pristine Soils RMFR

Analysis of top 10 microbial species composition (Figure 3) using MEGAN6 revealed distinct differences between the long-term paddy monoculture soils of Tanjung Karang and the pristine RMFR soil. Metagenomics analysis revealed substantial variation in the abundance of dominant bacterial phyla across the Raja Muda Musa Forest Reserve (RMFR) and the three cultivated paddy field sites in Tanjung Karang (TK1, TK2, and TK3). The number of hits, representing sequence alignments to specific taxa, was used as a proxy for relative abundance at the phylum level.

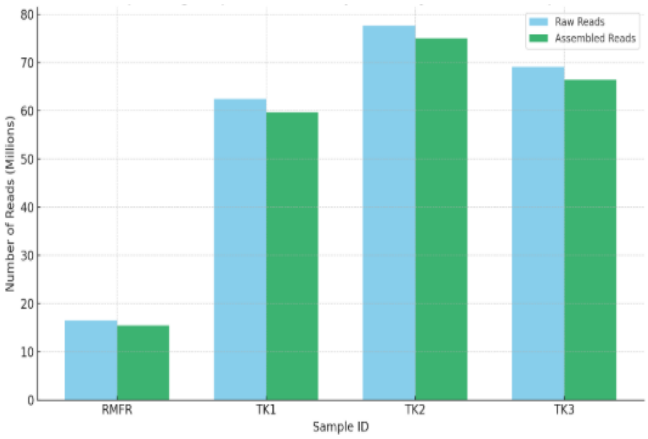

Figure 2. Metagenome Assembly Statistics

Proteobacteria were the most dominant phylum across all sites, with higher relative abundance in the cultivated soils (TK2 > TK1 > TK3) than in RMFR, reflecting the influence of fertilizer inputs and root exudates on nitrogen-cycling microorganisms [27]. Acidobacteria ranked second in dominance, particularly in TK3 and TK2, and are associated with carbon degradation and soil acidification processes linked to long-term cultivation [32]. Actinobacteria were moderately represented across sites, with higher abundance in TK2 and TK3, indicating their role in organic matter decomposition and nutrient turnover [33].

Chloroflexi were notably enriched in paddy soils (TK1 and TK2) but scarce in RMFR, consistent with their function in organic matter degradation and photosynthetic metabolism [34]. Verrucomicrobia and Nitrospirae, though less dominant, were more prevalent in paddy soils, with Nitrospirae enrichment in TK3 suggesting enhanced nitrification potential [35], [36]. Minor phyla such as Planctomycetes, Gemmatimonadetes, Firmicutes, and Candidatus Rokubacteria were also more represented in cultivated sites, reflecting broader microbial functional diversity under agricultural management [37].

The shift in dominant bacterial phyla between the forest (RMFR) and cultivated paddy soils (TK1, TK2, TK3) reflects substantial ecological reorganization in response to long-term agricultural management. In RMFR, Proteobacteria and Acidobacteria were the major phyla, characteristic of undisturbed soils with balanced nutrient turnover and organic matter decomposition. In contrast, cultivated sites, particularly TK1 and TK2, showed marked enrichment of Proteobacteria and Chloroflexi, groups commonly associated with active nutrient cycling and adaptation to higher nutrient availability resulting from fertilizer input. The dominance of Proteobacteria indicates enhanced nitrogen and carbon cycling potential, while the proliferation of Chloroflexi suggests intensified degradation of complex organic compounds under flooded and anaerobic paddy conditions. The high representation of Acidobacteria in TK3 indicates adaptation to reduced nutrient availability and lower productivity, consistent with their preference for oligotrophic conditions. The increased abundance of Nitrospirae in TK3 further implies intensified nitrification activity, potentially driven by nitrogen fertilizer application.

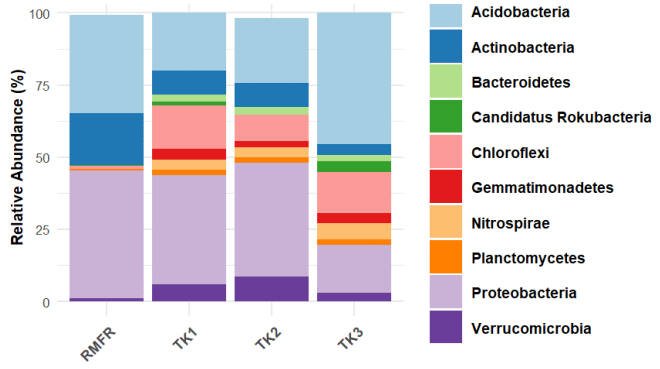

Figure 3. Relative abundance of Top10 Dominant Bacterial Phyla in Paddy and Pristine Soils RMFR.

E. Statistical Analysis

Alpha diversity analysis (TABLE 2) was conducted to evaluate microbial richness and evenness across the four soil sites: RMFR, TK1, TK2, and TK3. Diversity metrics, including the number of observed species (richness) and the Shannon index (accounting for richness and evenness), were calculated using MEGAN6 based on taxonomic classifications. TK2 exhibited the highest alpha diversity, with 990 observed species and a Shannon index of 2.19, followed by TK1 (970 species, 2.15). RMFR recorded 657 species with a Shannon index of 1.337, indicating lower microbial richness and evenness compared to the cultivated sites. TK3, despite being an agricultural site, showed lower richness (644 species) and a Shannon index of 1.563, slightly higher than RMFR but substantially lower than TK1 and TK2. These findings suggest that long-term paddy cultivation, particularly under flooded conditions with increased nutrient and organic inputs, promotes a more diverse soil microbial community [38].

TABLE 2. Alpha diversity indices of soil microbial communities across all sampling sites

Sites	Observed (sp.)	Shannon
RMFR	657	1.337
TK1	970	2.15
TK2	990	2.19
TK3	644	1.563

Beta diversity analysis (TABLE 3) based on Bray–Curtis dissimilarity was performed using KrakenTools to assess compositional differences in microbial communities between the Raja Muda Musa Forest Reserve (RMFR) and the three paddy field sites (TK1, TK2, and TK3) in Tanjung Karang. The Bray–Curtis index quantifies community dissimilarity on a scale from 0 (identical) to 1 (completely different). The highest dissimilarity was observed between RMFR and TK2 (0.798), followed by TK1 (0.772) and TK3 (0.691). These results indicate that intensive cultivation practices at TK1 and TK2 have caused greater divergence in microbial community composition compared to the undisturbed RMFR soil, while TK3 maintained a community structure more similar to RMFR. likely due to their shared origin from the same parent soil type [3]. The higher Bray–Curtis dissimilarity values support the finding that Proteobacteria enhance the growth of other microbial groups by contributing to nutrient bioavailability [39].

TABLE 3. Beta diversity analysis of paddy field soils in comparison to the Raja Musa Forest Reserve (RMFR), based on Bray-Curtis dissimilarity metrics.

Sample	Bray-Curtis
TK1	0.772
TK2	0.798
TK3	0.691

F. Nitrogen, Phosphorus, and Potassium Metabolism in Microbial Communities

Functional annotation of the metagenomes using KEGG revealed significant differences in the abundance of genes associated with nitrogen, phosphorus, and potassium metabolism between the monoculture paddy soils and the RMFR reference soil, as well as across the paddy yield gradients (Figure 4).

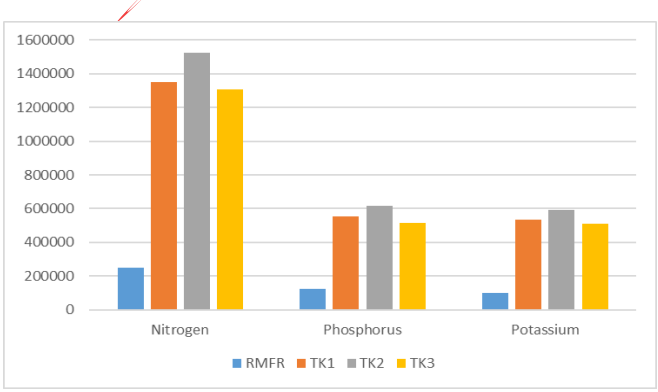

Figure 4. Nitrogen, Phosphorus, and Potassium Metabolism in paddy soil and RMFR

For nitrogen metabolism, genes involved in nitrogen fixation (*nifH*), nitrification (*amoA*, *nxrA*), and denitrification (*nirK*, *nirS*, *norB*, *nosZ*) showed varying abundances. For instance, genes related to denitrification were highly abundant in paddy soils compared to RMFR, reflecting the prevalence of anaerobic conditions that favour denitrifying bacteria [40].

In terms of phosphorus metabolism, genes associated with phosphate solubilisation (*gcd*, *pqqC*) and organic phosphate mineralization (*phoD*) were identified. The abundance of these genes was relatively higher in paddy soils compared to forest soils, suggesting that microbial communities employ multiple strategies to enhance phosphorus availability. This reflects adaptation to phosphorus limitation or fixation, which typically occur under flooded and fertilized paddy environments [41].

For potassium metabolism, genes associated with potassium solubilisation and transport (*trkA*, *kup*, *kdpA*) were identified. The abundance of these genes was relatively consistent across all paddy soil samples, compare to forest soils, suggesting a microbial capacity to maintain potassium availability. This functional trait is critical in agricultural soils

like paddy fields, where continuous cropping deplete available potassium despite fertilization. The presence of these genes indicates microbial mobilizing potassium from insoluble mineral forms, thereby supporting plant potassium nutrition under varying soil conditions [42].

The enrichment of nitrogen metabolism genes supports the low nitrogen levels observed (Figure 1), indicating that fertilizer-derived nitrogen is actively utilized and transformed by the microbial community. In contrast, the relatively low abundance of phosphorus and potassium metabolism genes corresponds with the high concentrations of these elements in the soil (Figure 1), suggesting limited microbial turnover and potential nutrient accumulation. Overall, the functional analysis highlighted that long-term paddy monoculture not only alters the microbial community structure but also profoundly impacts the functional potential related to critical nutrient cycling processes. The observed shifts in gene abundances for nitrogen, phosphorus, and potassium metabolism reflect the adaptive responses of the soil microbiome to the specific environmental pressures and nutrient dynamics imposed by continuous paddy cultivation.

IV. CONCLUSIONS

This study provides comprehensive insights into the long-term impacts of paddy monoculture on soil health in Tanjung Karang, Malaysia, by integrating macronutrients and metagenomic analyses, with comparisons to the pristine Raja Musa Forest Reserve (RMFR). Our findings unequivocally demonstrate that prolonged paddy monoculture significantly alters the availability of key macronutrients like nitrogen, phosphorus, and potassium. These changes create a unique edaphic environment that profoundly influences the resident microbial communities.

Metagenomic analyses revealed substantial divergence in microbial species composition between the monocultured paddy soils and the pristine RMFR, indicating an increase in overall microbial diversity and richness in the intensively farmed areas. Specific taxonomic groups adapted to the unique conditions of paddy fields were observed to be dominant. Crucially, the functional annotation of metagenomes highlighted significant shifts in the abundance of genes associated with nitrogen, phosphorus, and potassium metabolism. These molecular insights underscore the adaptive responses of the soil microbiome to the pressures of continuous cultivation and associated agricultural practices, influencing the critical nutrient cycling processes essential for plant growth.

To mitigate nutrient imbalance and preserve soil microbial integrity, sustainable management practices such as crop rotation, periodic fallowing, incorporation of organic amendments, and the use of biofertilizers are recommended. These practices can enhance nutrient recycling, restore microbial balance, and maintain long-term soil productivity. From a policy perspective, the results highlight the need for site-specific nutrient management guidelines and monitoring programs to prevent over-fertilization and soil degradation in Malaysia's rice-growing regions. Strengthening farmer education through integrated soil health management policies could ensure sustainable intensification while preserving ecosystem resilience.

CONFLICT OF INTEREST

I hereby declare that the disclosed information is correct and that there are no conflicts of interest with the other authors.

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