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Unravelling Microbial Communities in the Rhizosphere Soil of Selected Dipterocarpaceae species through Amplicon Sequencing

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ABSTRACT

The Dipterocarpaceae family, comprising nearly 700 species, is a dominant component of Southeast Asian tropical rainforest. In Malaysia, dipterocarps are widely distributed and serve as one of the essential tree families in the forest ecosystems. Their rhizosphere represents a unique microhabitat where complex interactions between roots, soils, and microbial communities occur. However, there are limited datasets on microbial diversity of Malaysian dipterocarp rhizosphere soil. This study aimed to unravel the microbial diversity of selected dipterocarp species (*Dipterocarpus oblongifolius*, *Neobalanocarpus heimii*, *Shorea sumatrana*, and *Dryobalanops aromatica*) across three environmentally different sites: the FRIM Dipterocarp Arboretum, Valencia (township), and Wetland Arboretum Centre (WAC) (nursery), using amplicon sequencing. Bacterial communities were dominated by Proteobacteria across all sites, with WAC showing significantly higher bacterial diversity (Shannon index). Eukaryotic communities were primarily fungal, dominated by Basidiomycota and Ascomycota, with WAC showing significantly higher eukaryotic diversity (Shannon index). Beta diversity analysis revealed distinct clustering patterns, with WAC harboring the most unique community structures for both bacteria and eukaryotes. This study reveals that site-specific conditions drive the microbial diversity and composition of dipterocarp rhizosphere soil.

Keywords: Microbiome; Diversity; 16S; 18S; Dipterocarp; Nanopore Sequencing

1. Introduction

Tropical rainforests represent highly biodiverse ecosystems, characterized by exceptional plant species richness (exceeding 250 species per hectare) and harboring a significant portion of global fauna (estimated at over 50%). These regions are situated in the equatorial zone and encompass roughly 6% of Earth's terrestrial surface. The prevailing climate is characterized by high levels of precipitation (ranging from 125 to 660 cm annually), constant humidity (between 77% and 88%), and

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warm temperatures (averaging 20-34°C) [1]. The Dipterocarpaceae family, well-known for its biological and economic significance, is an essential part of Southeast Asia's tropical rainforests. With 16 genera (including *Dipterocarpus*, *Hopea*, *Neobalanocarpus*, and *Shorea*) and about 700 species, the Dipterocarpaceae family is primarily found in the Malesia region [2].

The rhizosphere is the narrow area of soil directly surrounding plant roots and one of Earth's most complex ecosystems which has the most dynamic environment [3]. The microbiomes found in the rhizosphere and roots are essential for plant nutrition intake, disease resistance regulation, plant-plant competition, and nutrient redistribution. Plants and rhizosphere microbes have a close relationship, and research on the interactions between these microorganisms and the variables that affect them is beneficial to maintaining biodiversity and protecting vegetation [3]. The MinION platform from Oxford Nanopore Technologies is well-suited for amplicon sequencing for rhizosphere soil microbiome study as it produces full-length 16S rRNA and 18S rRNA reads for higher taxonomic resolution. Researchers can study the composition, organization, and spatiotemporal patterns of the bacteria and eukaryotic community development and function through amplicon sequencing [4].

2. Methodology

2.1 Rhizosphere soil sampling

Samples were retrieved from FRIM Dipterocarp Arboretum (3°13'55" N 101°37'44" E), Gamuda Valencia (3°12'42" N 101°35'11" E), and WAC (2°52'43" N 101°36'53" E). Any debris was cleaned before sampling. Fine roots were collected by tracing lateral roots 2 – 10 m from trunk, and sample were taken at 5 – 30 cm depth [5]–[7].

2.2 gDNA extraction and Amplicon amplification

Rhizosphere soil closely adhering to fine roots was isolated, pelleted by centrifugation, and used for gDNA extraction with the GF-1 Soil Sample DNA Extraction Kit (Vivantis) [4], [7], [8]. Normal PCR was conducted, targeting bacterial 16S rRNA (27F/1492R) and eukaryotic 18S rRNA (Fun18S1/FR-1) gene under optimize cycling condition (initial denaturation at 95 °C for 2 min, followed by 30 cycles (16S) or 40 cycles (18S) of denaturation at 95 °C for 20 s, annealing at 50 °C for 25 s, extension at 72 °C for 60 s, and a final extension at 72 °C for 10 min. PCR product were verified by gel electrophoresis and NanoDrop analysis.

2.3 Library preparation, Sequencing, and Bioinformatics analysis

PCR products were purified with AMPure XP beads, quantified using Qubit Fluorometer, and libraries were prepared with the Rapid Barcoding Kit V14-24 (Oxford Nanopore Technologies). Sequencing was performed on a MinION Mk1b using MinKNOW software with automated basecalling and barcode trimming. Raw fastq reads were quality-checked (FastQC and MultiQC), trimmed (Porechop and fastp) using Galaxy [9], and processed in QIIME2 (2024.5) using 90% closed reference clustering with SILVA 138 [10], [11]. Taxonomy was assigned via the q2-feature classifier [12]–[14]. Taxonomic abundance and diversity analyses (alpha and beta) were performed in MicrobiomeAnalyst [15].

3. Results and Discussion

3.1 Taxonomic Abundance

At phylum level (Figure 1), Proteobacteria dominated all samples, followed by minor portion of Firmicutes, Planctomycetota, and Acidobacteriota. WAC soils exhibited a significantly higher relative abundance of Proteobacteria compared to FRIM and Valencia ($p < 0.05$). Proteobacteria play a crucial role in nutrient cycling and pathogen suppression (Sarsaiya et al., 2025). The eukaryotic community (Figure 2) was dominated by fungi, mainly Basidiomycota and Ascomycota. Basidiomycota was significantly abundant in FRIM ($p < 0.05$), whereas Ascomycota was significantly abundance in Valencia and WAC ($p < 0.05$). Basidiomycota and Ascomycota contribute to the decomposition of animal and plant residue, cellulose, and hemicellulose, thereby providing nutrients to plant (Li et al., 2021). Sequencing data are available in the NCBI-Sequence Read Archive (SRA) with accession number: PRJNA1353651.

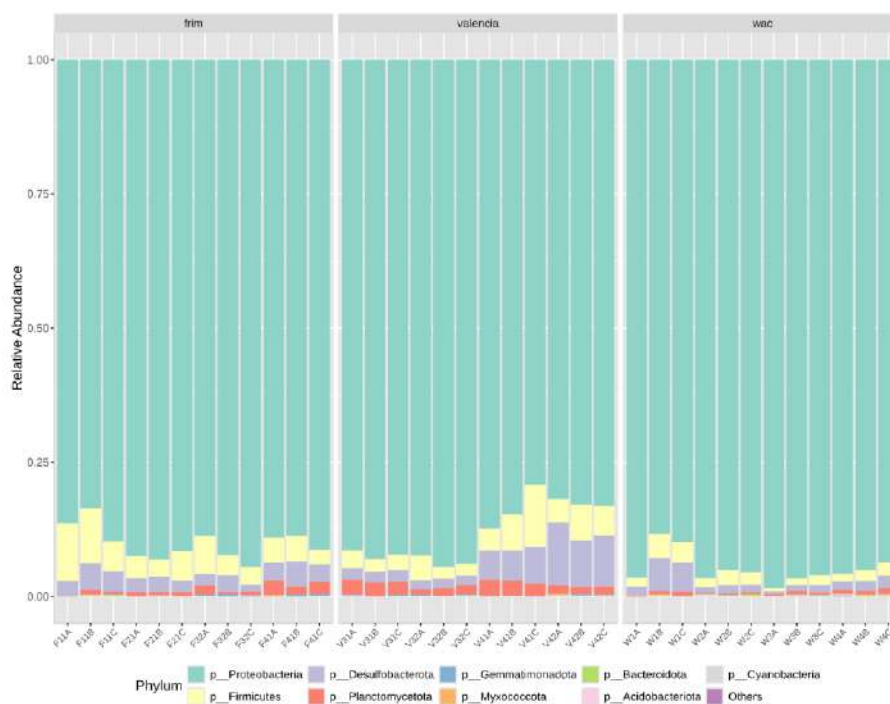


Fig. 1. Phyla abundance of bacteria from dipterocarp rhizosphere soil

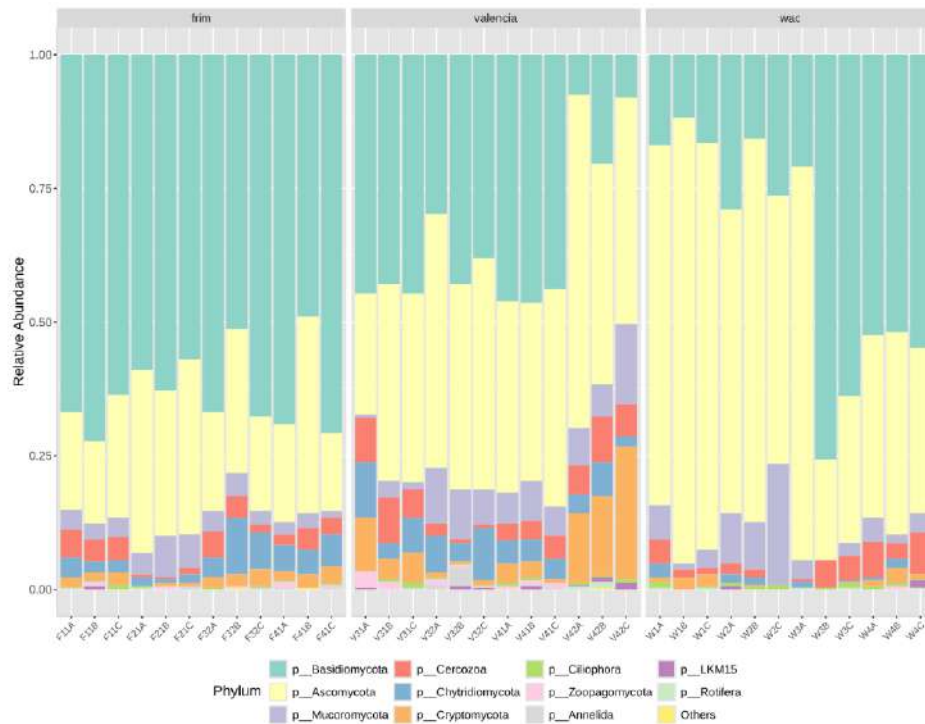


Fig. 2. Phyla abundance of eucaryotes from dipterocarp rhizosphere soil

3.2 Alpha Diversity

Shannon diversity indices showed significant differences among sites for both bacterial ($F = 3.584$, $p < 0.05$) (Figure 3, a) and eukaryotic ($F = 9.806$, $p < 0.05$) communities (Figure 3, b). In both cases, WAC showed the highest diversity and greater variability among replicates, while FRIM exhibited the lowest and most uniform diversity. Valencia showed intermediate diversity, with no significant difference compared to WAC ($p < 0.05$). This difference may be due to the fertilizer regime, land restoration and other anthropogenic activities [16].

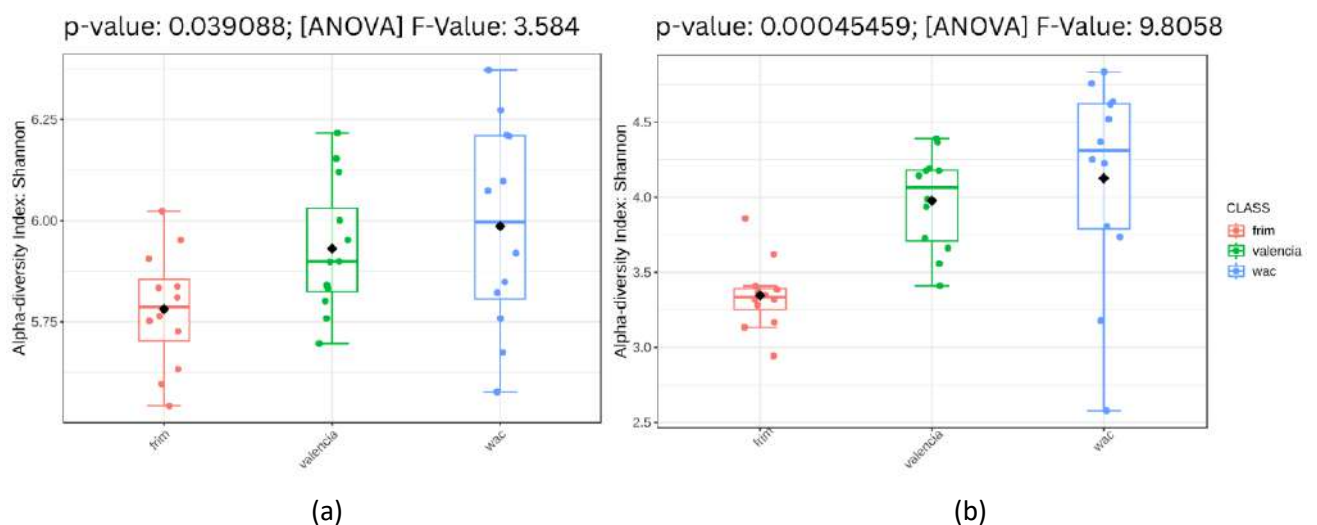


Fig. 3. Alpha diversity (Shannon index) of microbes from dipterocarp rhizosphere soil; a) Bacteria, b) Eukaryotes

3.2 Beta Diversity

Bray-Curtis PCoA and PERMANOVA analyses revealed significant differences in both bacterial and eukaryotic community structures among dipterocarp rhizosphere soils from FRIM, Valencia, and WAC ($p = 0.001$). For bacteria (Figure 4, a), the first two axes accounted for 21.7% of the variance, with WAC showing a distinct community separated from FRIM and Valencia, which overlap closely ($R^2 = 0.176$). For eukaryotes (Figure 4, b), the first two axes explained 34.2% of the variance, with clear clustering by site and WAC showing the most distinct community composition ($R^2 = 0.300$). Overall, WAC exhibited the most unique microbial community structure, while FRIM and Valencia were more similar to each other.

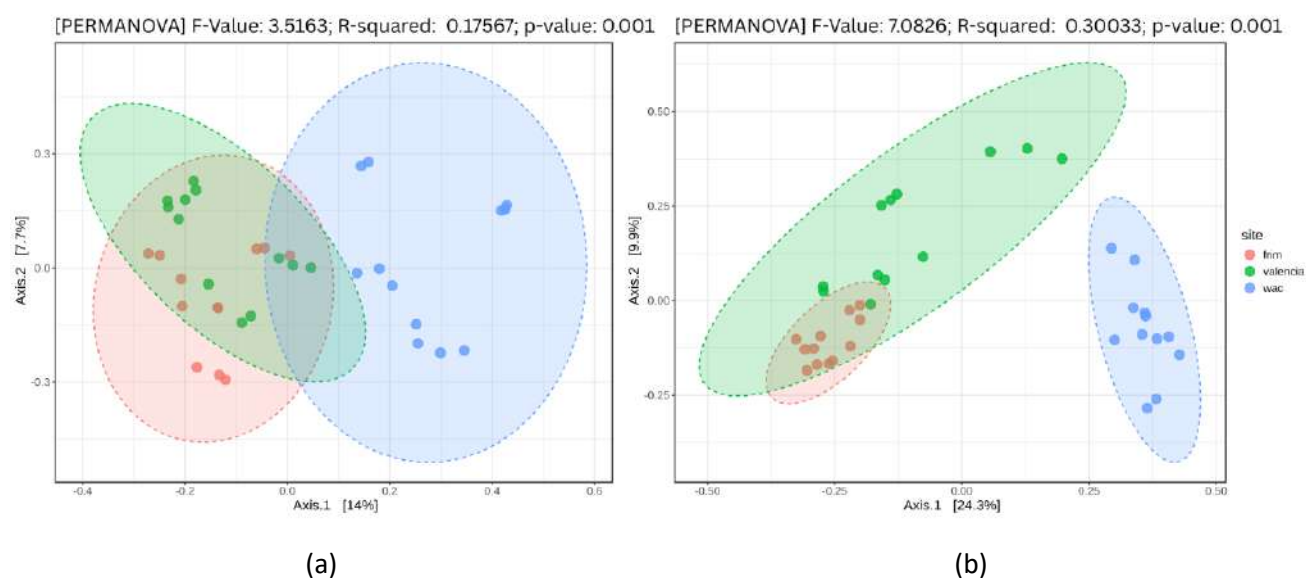


Fig. 4. Beta diversity (Bray-Curtis PCoA plot) of microbes from dipterocarp rhizosphere soil; a) Bacteria, b) Eukaryotes

4. Conclusions

Rhizosphere soil microbial communities of dipterocarp species varied significantly across different environments. Proteobacteria dominated the bacterial community, while Basidiomycota and Ascomycota were the major eukaryotic phyla, reflecting their essential roles for plant health. WAC showed highest diversity and distinct communities, whereas FRIM displayed lower diversity and greater stability. These differences suggest that site specific characteristics such as anthropogenic effects and physicochemical properties will affect the microbial community and diversity composition. The finding highlights the ecological importance of the rhizosphere soil microbiome in maintaining dipterocarp health hence preserving the tropical rainforest ecosystem.

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