Bacteria Assemble: Investigating Soil Bacterial Diversity and Interactions in a Sarawakian Oil Palm Plantation for Climate-Resilient Soil Management

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Abstract. Investigating bacterial diversity in the soil of oil palm plantations is crucial for developing effective soil management strategies to mitigate greenhouse gas emissions, thereby addressing climate change challenges. In this study, we analysed soil bacterial communities within an oil palm plantation in Sarawak, Malaysia. To capture the full spectrum of microbial diversity, samples were collected across three distinct soil depths and various managements zones within the plantation. We employed Illumina amplicon sequencing targeting the V3 region of the 16S rRNA gene, which revealed a diverse array of soil bacteria, primarily from the phyla Acidobacteriota, Actinobacteriota, and Proteobacteria. Notably, families such as RAAP2_2 and Isosphaeraceae were identified as key biomarkers predominantly inhabiting deeper soil layers (30-45 cm), while UBA7541, Streptosporangiaceae, and Sporichthyaceae were more prevalent in the surface layer (0-15 cm). Microbial correlation network analysis revealed a complex network of robust and significant interactions among 11 bacterial families, suggesting their involvement in plant material degradation and carbon cycling. Redundancy Analysis (RDA) revealed that pH, moisture content, and bulk density were significant factors that influence the microbial community structure. At the phylum level, Acidobacteriota showed a close association with pH, while Proteobacteria was associated with higher moisture content. Interestingly, we discovered that the soil was not as homogeneous as initially thought. Microenvironmental variability was evident, with specific groupings based on subplot sampled. These findings provide valuable insights into the factors shaping microbial communities in oil palm plantation soils, thus opening avenues for further exploration of the complex mechanisms driving ecosystem processes in this unique Malaysian soil environment.

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1 Introduction

In 2023, Malaysia planted 5.65 million hectares of palm oil, making it the second-larger producer in the world. Sarawak remained the largest oil palm-producing state, covering 1.62 million hectares or 28.7% of the total Malaysian oil palm planted area. Sabah came in second with 1.51 million hectares or 26.7%. Oil palm planted area in Peninsular Malaysia amounted to 2.52 million hectares, or 44.6% [1] Although oil palm growth is appropriate for humid tropical lowlands, tropical forests have been rapidly cleared in recent years to make way for the establishment of oil palm plantations, resulting in the conversion of several million hectares of forest to oil palm plantations [2]. Tropical peatland is a significant carbon reservoir, primarily composed of plant materials, accumulating peat due to varying deposition rates due to water-logged anaerobic conditions [3]. Converting peat forests to oil palm plantations can impact the environment, ecosystem, and biodiversity, including microbial diversity and activity [4].

Soil microorganisms, which comprise both prokaryotic and eukaryotic organisms constitute the majority world's biodiversity and play a crucial role in terrestrial ecosystems [5]. Understanding biodiversity trends in natural habitats can help forecast ecosystem responses to changing environments [6]. Considering oil palm plantations as an integral part of Malaysia's agricultural output, there is a significant gap in our comprehension of soil microbial communities within these plantations and their implications for biogeochemical cycling, oil palm yield, and environmental sustainability, especially in the face of climate change. To date, despite the availability of soil metagenomic studies in Malaysian oil plantations [7–10], no studies in Malaysia have thoroughly explored microbial interactions in oil palm plantation soil that are critical for managing greenhouse gas emissions and sequestration. This study aims to bridge this gap by sequencing the 16S rRNA amplicon (V3 hypervariable region) of 36 soil samples collected from diverse depths, sites, and zones within a Sarawak-based oil palm plantation, shedding light on their role in climate change mitigation and adaptation.

2 Method and Materials

2.1 Sample Collection and DNA Extraction

A total of 36 microsites samples were collected from Sabaju (SAB) oil palm plantation planted in 2007 (3° 12' 41.918" N, 113° 29' 59.709" E), located in Bintulu, Sarawak (Figure 1). The study plot was found to have an average monthly precipitation of 287 mm and categorised as Sabaju series consisting of hemic material with undecomposed wood according to the Malaysian Unified Classification of Organic Soils (MUCOS) by [11].The soil samples were collected from four different management zones and three soil depths namely 0-15 cm, 15-30 cm, and 30-45 cm within one-hectare plot [8]. For soil sampling, three subplots were randomly chosen using a peat auger (Eijkelkamp, The Netherlands). Four management zones were defined as follows: The avenue (AV) zone refers to the area between palm rows, typically covered by vegetation. The cover crop (CC) zone is where legumes (*Mucuna bracteata*) are allowed to grow freely to conserve soil moisture. The harvest path (HP) zone is the area between palm rows kept clear of weeds for easy access by workers. Finally, the weeded circle (WC) zone is the ring of soil around the oil palm trunk, kept vegetation-free with most of oil palm roots grow and fertilizers are applied. The soil samples were collected and stored in zipper bags, and kept on ice to minimise changes in microbial composition during transport. Before soil samples were processed, they were pretreated by removing the impurities and sieving (2 mm).

The pH of the supernatant from a 1: 2.5 (w/v) soil-to-distilled water was determined using a pH meter (Sartorius Stedim, Goettingen, Germany). Soil pH and moisture content

were measured according to the methodology described by [12]. Soil bulk density samples were collected, dried, and heated in a muffle furnace at 550 °C for 4 h. The organic matter content was determined by calculating the loss on ignition, which is calculated by dividing the weight of oven dried by the weight of ash. The CO_2 flux rate was measured using a portable CO_2 analyser system (EGM-4, PP-System, Amesbury, Massachusetts, United States) [13].

DNA was extracted from 0.5 g of soil using the NucleoSpin® Soil kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions for genomic DNA isolation. DNA purity was measured using a NanoDrop spectrophotometer, while DNA concentration was accurately quantified with a Qubit 2.0 fluorometer. Additionally, electrophoresis of 2 μL of genomic DNA (gDNA) from each sample was performed on a 1% TAE agarose gel to evaluate DNA integrity [8].

Fig. 1. Sampling site in oil palm plantation. (A) 11-year-old oil palm on peat; (B) woody materials presence on the soil surface; (C) Leguminous cover crop, *Mucuna bracteata* growing under oil palm shade.

2.2 Amplicon Sequencing

Genomic DNA (gDNA) was amplified using polymerase chain reaction (PCR) targeting the universal bacterial 16S rRNA V3 hypervariable region [14,15] with WizPure™ HS-PCR 2X Mastermix. Briefly, 1 µL of DNA (~1-10 ng) was combined with 10 µL of 2X PCR mastermix and 10 µL of a 2X forward and reverse primer mix, achieving a final primer concentration of 0.25 µM. The PCR program included an initial denaturation step at 95°C for 3 minutes, followed by 30 cycles of 95°C for 10 seconds, 50°C for 10 seconds, and 72°C for 10 seconds. The resulting PCR products were verified by agarose gel to confirm the presence of the correct band. Subsequently, PCR products were. purified using 0.8x volume of Ampure beads (Beckman Coulter Life Sciences, Indianapolis, Indiana, United States). A second PCR was performed under similar conditions to attach Illumina indices, facilitating sample multiplexing. The final PCR products were purified again with 0.8x volume of Ampure beads, pooled based on their concentrations, and quantified using a DeNovix fluorometer. The pooled amplicons were then normalized to the optimal concentration for sequencing on the Illumina iSeq 1000 platform (2 x 150 bp run configuration).

2.3 Bioinformatics Analysis

The raw paired-end reads were filtered using fastp to remove reads containing Illumina adapter and/or poly-G at the end typical of the two-color Illumina chemistry error. Subsequently, cutadapt was applied to trim the forward and reverse sequences from read 1 and read 2, respectively. The primer-trimmed reads were subsequently overlapped with fastp and denoised with dada2 to generate ASVs followed by taxonomic classification using QIIME2 sklearn trained on the GreenGenes2 database [16–18]. Identification of biomarkers associated with different depths used LEfSe analysis [19]. Microbial interaction at the family level was conducted using SparCC with p-value cutoff of 0.01 and relatively stringent correlation factor of 0.75 [20]. Both LEfSe and SparCC analyses were conducted in the MicrobiomeAnalyst web server [21]. For the Redundancy Analysis (RDA), the relative abundance of microbial taxa at the phylum level was utilised alongside five environmental

factors: moisture content, CO_2 flux, organic content, bulk density, and pH. The data for each sample, including both microbial abundances and environmental variables, were entered into PAST4 software for analysis.

3 Results and Discussion

Among the 36 samples examined, the predominant bacterial phyla were identified as Acidobacteriota, Actinobacteriota, and Proteobacteria (Figure 2), indicating a consistent microbial composition throughout various depths, sites, and management zones. These findings are in agreement with those reported by [22], who identified these three phyla in oil palm plantations and a logged forest in Jambi, Indonesia. The abundance of Acidobacteria in peat soil was supported by [7,23], who reported that Acidobacteria are widespread int the environment, constituting approximately 20% of all bacteria in soils globally. In particular, in acidic soils such as peat, Acidobacteria can make up to more than 50% of the total bacterial community [22,24,25] although they can also be found in relatively high numbers in more neutral soils [7,26–28]. Acidobacterium abundance is mostly influenced by pH, as these microorganisms thrive in pH ranges of 3.5 to 5.0 [22,24]. The prevalence of these bacteria in soils indicates that Acidobacteria are crucial to soil ecological processes. However, a notable shift in composition emerged particularly in samples from the 30-45 cm depth range, suggesting a distinct microbial community structure at this depth (Figure 2). Evidently, the family RAAP-2 exhibited higher relative abundance in samples from this depth (Figure 3). Bacteria in the RAAP-2 family belong to the Actinobacteria phylum, which are known to produce extracellular enzymes that break down macromolecules like cellulose, lignin, chitin, and starch [29]. Bacteria in this phylum also operate as plant disease biocontrol agents, displaying an antagonistic, competitive effect on phytopathogenic microbes while simultaneously benefiting their host plants by assisting in plant growth promotion (PGP) [30].

By utilizing Linear discriminant analysis effect size (LEfSe) (Figure 4), significant associations were uncovered at the family level: UBA7541, Streptosporangiaceae, and Sporichthyaceae were strongly linked with the topsoil layer (0-15 cm), while Acetobacteracea predominated in the 15-30 cm depth range, and RAAP_2 and Isosphaeraceae were prevalent in the 30-45 cm depth zone. According to [31], the host cells of Acidobacteriae UBA7541 contain genes linked to polysaccharide degradation, which facilitate central carbon metabolism processes such as glycolysis and the Kreb's cycle. Meanwhile, members of the Streptosporangiaceae falimy are likely involved in the primary decomposition of plant material in soils [32]. Sporichthyaceae, part of the Frankiales order, are less reliant on high carbon and nutrient concentrations due to their facultative aerobic metabolism [33]. In addition to their light-driven metabolism, this group is capable of nitrogen (N) fixation and utilise cyanobacterial biomass as an energy source [34]. Acetobacteracea, found in the 15-30 cm depth range is a family known to contain many species with biotechnological significance for industry. Members of this family have also been identified as N fixing bacteria capable of promoting plant growth through various mechanisms. Most members of this group, also referred to as diazotrophic, are commonly found in the soil rhizosphere of many plants, where they establish more specific associations as endophytes. Their roles in promoting plant growth typically involve enhancing plant biomass, solubilizing phosphate and other minerals, and suppressing plant pathogen [35]. Bacteria from the Isosphaeraceae family found in the 30-45 cm depth zone are chemoorganotrophic aerobes that derive energy from the oxidation of reduced organic compounds, including various sugars and polysaccharides. The range of compounds from which chemoorganotrophic organisms can generate energy, as well as their carbon sources

are extensive, making them highly versatile. However, some representatives are capable of growth in microoxic conditions [36].

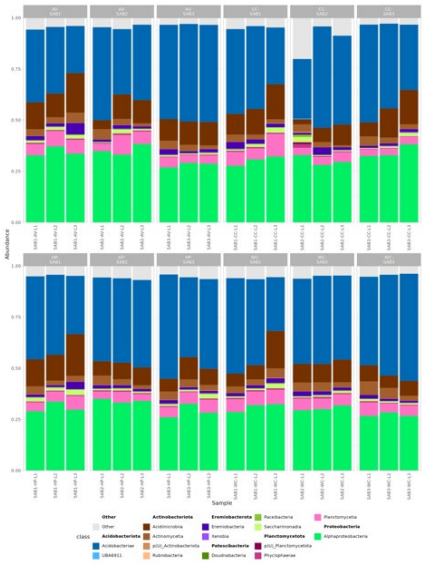

Fig. 2. Relative abundance of bacterial classes in an oil palm plantation (OPP) across different management zones and soil depths. SAB = Sabaju OPP; AV = avenue; CC = cover crop; HP = harvest path; WC = weeded circle; L1 = 0–15 cm; L2 = 15–30 cm; L3 = 30–45 cm.

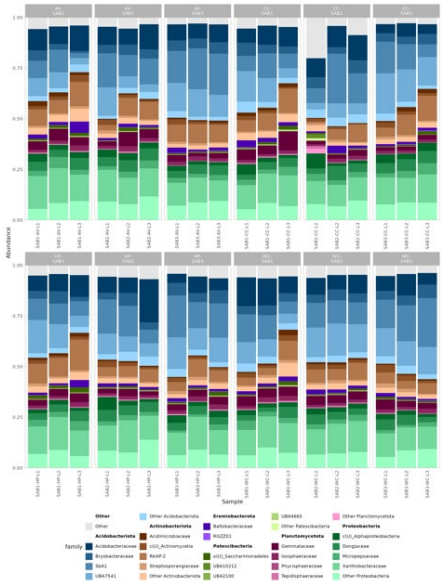

Fig. 3. Relative abundance of bacterial families in an oil palm plantation (OPP) across different management zones and soil depths. SAB = Sabaju OPP; AV = avenue; CC = cover crop; HP = harvest path; WC = weeded circle; L1 = 0–15 cm; L2 = 15–30 cm; L3 = 30–45 cm.

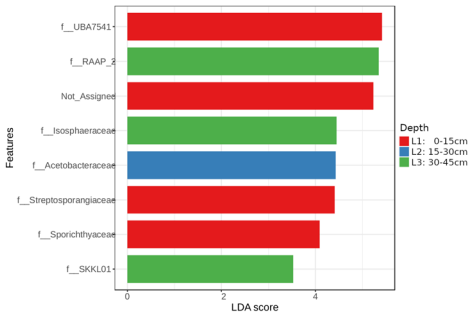

Fig. 4. Linear discriminant analysis Effect Size (LEfSe) analysis on selected families among three depth groups, 0-15cm (Red), 15-30cm (Blue), 30-45 cm (Green). Only families with Least Discriminant Analysis (LDA) values >3.5 and original p-value of < 0.01 are displayed.

Remarkably, some of these identified families also featured prominently in correlated microbial networks (Figure 5). For instance, RAAP_2 displayed strong positive correlations with families such as Baltobacteraceae, Bog 793, Dongiaceae, Gemmataceae, Micropepsaceae, and Palsa_688. Some of these families also formed tight correlations among each other, strongly suggesting the potential interdependencies in geochemical processes such as carbon (C) and N cycling among these families in the plantation soil. Most of these families are noted to be acid-tolerant consistent with the low soil pH of the area. Markedly it is, the positive inter-phylum interaction between RAAP_2 (phylum Actinobacteriota) and Baltobacteraceae (Candidatus phylum Eremiobacterota, formerly WPS-2). Members of the uncultured Baltobacteraceae are known to exhibit acid-tolerant adaptations and hydrogenotrophic activity [37]. Notably, various correlations with members of the family Gemmataceae were also observed. Additionally, Gemmataceaeare are strictly aerobic chemoorganotrophs that display several carbohydrate hydrolytic capabilities [38].

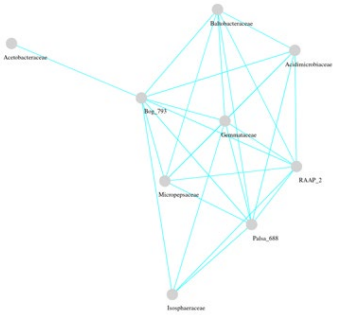

Fig. 5. SparCC network correlation plot showing abundance correlation between pairs of microbial taxa within the soil samples. Only strong correlation values of more than 0.7 with p-value of less than 0.05 are shown.

The RDA analysis showed that Acidobacteriota, one of the major phyla represented by the highest number of reads in most samples, showed higher abundance in environments with lower pH (Figure 6). This is indicated by its close proximity to the pH vector, which points in the same direction. As the name suggests, members of this phylum are well-known for their preference for acidic environments, likely playing a significant role in organic matter decomposition in such soils [39]. This affinity for low pH environments aligns with the established ecological characteristics of Acidobacteriota. In contrast, no single phylum exhibited a strong association with the bulk density (BD) vector. Bulk density, a measure of soil compaction, indicates the amount of air space within the soil. High bulk density values are typically associated with low porosity, which can lead to reduced water infiltration,

limited root penetration, and poor aeration. The absence of a close association between any particular phylum and the BD vector suggests that bulk density may not be a major factor driving the distribution of microbial phyla in the sampled soils. Nevertheless, the overall microbial composition seems to reflect some influence of bulk density, as indicated by the clustering of soil samples from the surface and 0-30 cm depths of SAB2 and SAB3 subplots near the BD vector. Neither CO_2 flux nor organic matter exhibited a strong correlation with the clustering of soil samples or the distribution of microbial phyla. A minor exception is the weak association of the phylum Planctomycetota with both vectors. This could suggest some level of involvement in processes related to CO_2 flux and organic matter turnover, although the association is not strong enough to draw definitive conclusions. The clustering of only SAB1 samples from the 30-45 cm depth away from other SAB2 and SAB3 samples from the similar depth was characterised by a notable association with Actinobacteriota. Additionally, it is important to note that the majority of the SAB1 samples were primarily positioned along the right axis. This highlights the complexity and heterogeneity of soil environments. In other words, even within seemingly homogeneous plots, microenvironments can exist, driving differential microbial colonization and activity. Actinobacteriota, known for their resilience and ability to degrade complex organic compounds, may thrive in this specific microenvironment due to factors such as unique organic matter quality, specific mineral compositions, or particular moisture regimes [40], at the SAB1 site. Proteobacteria was situated on the positive side of RDA Axis 1, near the environmental variable moisture content, suggesting members of Proteobacteria may prefer environments with higher moisture content, which could facilitate their roles in organic matter degradation and nutrient mineralization [41]. Water availability is crucial for these processes, and maintaining soil moisture may be beneficial for promoting these microbial activities, highlighting an important consideration for soil management strategies.

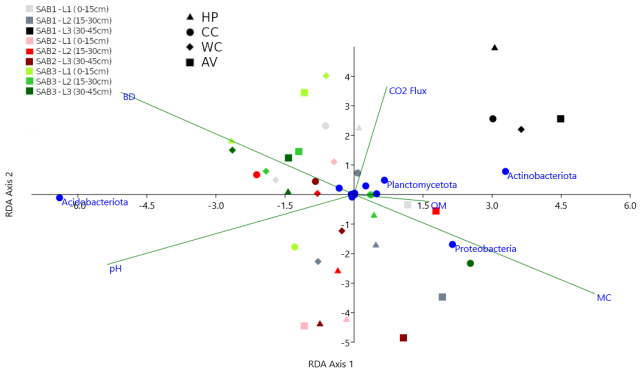

Fig. 6. Redundancy Analysis (RDA) plot depicting the relationships among soil samples, biomarkers (phylum), and environmental factors. Each point represents an individual soil sample, with different colors indicating various subplots (Black-Grey: SAB1, Green-Lightgreen: SAB2, Marron-Pink: SAB3), while shades within each color represent different soil depths (Light: Level 1, Medium: Level 2, Dark: Level 3). The shapes of the points correspond to different zones within the study area. Blue circles denote bacterial phyla that are differentially associated with the environmental variables. Green lines represent environmental factors, indicating both the direction and magnitude of their influence on the distribution of samples and associated bacterial communities. The relative positioning of samples along these vectors indicates the degree to which specific environmental variables affect the microbial composition of those samples.

4 Conclusion

The relationships between soil microbial families are essential for the effective conversion of carbon into decomposed organic matter. This process not only ensures nutrient availability

but also contributes to C sequestration, making it essential for maintaining soil productivity. Preserving this delicate microbial network is vital for enhancing sustainability and climate resilience, especially in the oil palm industry. This study highlights the varying responses of soil microbial communities to different environmental factors. pH emerged as a significant determinant for the distribution of Acidobacteriota, while factors such as bulk density and moisture content also played roles, albeit to a lesser extent. While broad environmental gradients such as pH and bulk density provide valuable context, the fine-scale heterogeneity of soils can lead to the formation of distinct microbial niches. This micro-scale variation highlights the complexity of soil environments and the need for a nuanced understanding of microbial ecology. Future research should prioritise high-resolution sampling and detailed environmental characterization to better elucidate the drivers of microbial diversity and distribution in soils. Additionally, exploring the functional attributes of microbial communities within these microenvironments via shotgun metagenomic could offer a deeper understanding of their ecological functions, particularly in nutrient cycling and organic matter decomposition.

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