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Comparison of rhizobacterial communities between secondary forest and palm oil plantations in East Kalimantan, Indonesia

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Abstract. *Yuliatin E, Hariani N, Dharma B, Patang F, Budiman. 2025. Comparison of rhizobacterial communities between secondary forest and palm oil plantations in East Kalimantan, Indonesia. Biodiversitas 26: 490-499.* The conversion of primary forests to plantations is considered a sustainable form of land management; however, its implications for soil rhizobacterial diversity remain insufficiently explored. This study compared the rhizobacterial communities associated with Palm oil Plantation (PP) and Secondary Forest (SF) in East Kalimantan using a metagenomic approach. The soil samples were collected around the Secondary Forest soil (SF) and Palm oil Plantation soil (PP) plant roots in Berambai, Samarinda. The samples were then analyzed for soil physico-chemical properties such as pH, nitrogen (N), phosphorus (P), potassium (K), organic matter, and C/N ratio. At the same time, the rhizobacterial diversity was analyzed using a metagenomic analysis through Illumina Hiseq. Soil physico-chemical assessments showed acidic conditions in both soils, with PP being more acidic (pH 4.41) than SF (pH 5.38); nutrient analysis indicated medium nitrogen levels and high organic carbon in both soils, while PP had elevated P content due to fertilization. Metagenomic analysis revealed similar rhizobacterial richness, but diversity was slightly higher in PP. Dominant phyla included Proteobacteria and Acidobacteriota, with notable orders like Rhizobiales and Acidobacteriales. The functional analysis highlighted rhizobacterial roles in organic decomposition, plant growth promotion, and nitrogen fixation, illustrating ecological adaptation to soil conditions and management practices. This study provides insights into the rhizobacterial functional diversity in distinct soil environments.

Keywords: Functional diversity, metagenomic analysis, Next Generation Sequencing, nitrogen-fixing bacteria, PGPR

Abbreviations: PGPR: Plant Growth Promoting Rhizobacteria

INTRODUCTION

Rhizobacteria have promoted soil health and plant growth for decades due to their ability to improve plant development and stress management (Santoyo et al. 2017). Their presence is around the zone surrounding the plant root, influenced by root exudates (Lucini et al. 2019). Rhizobacteria activities in this zone are crucial for uptaking nutrients and protecting against pathogens. The interaction between plants, soil, and rhizobacteria influences soil health and fertility due to root colonization, increasing nutrient availability in the rhizosphere. Consequently, the rhizosphere is highly competitive in the micro-ecosystems to compete and survive with other bacteria pathogens (Kumar and Verma 2019). Rhizobacteria release various metabolic processes in different land use systems. According to previous research, the indigenous rhizobacteria associated with plants of natural forests and palm oil plantations are beneficial for soil management in conversion land (Anggrainy et al. 2020) and improve the quality of crop production, such as soybean (Suliasih and Widawati 2020) and maize (Anggrainy et al. 2020) in East Kalimantan. They can provide nitrogen-fixing, Indole-3-Acetic Acid (IAA) production, mineralization, and solubilization of phosphorus (P), Calcium (Ca), and Calcium orthophosphates ($CaPO_4$) (Yuliatin et al. 2022), 1-Aminocyclopropane-1-Carboxylate (ACC) deaminase-producing (Anggrainy et al. 2020), and cellulose-degrading (Kusumawati et al. 2017).

Kalimantan participated in about 54.9% of the forest and the remarkable endemic flora and fauna in 23 million ha (Yuliatin et al. 2022). Berambai forest, the protected forest near a waterfall, has changed to a secondary forest and a palm oil plantation. This area is located on the outskirts of Samarinda, East Kalimantan and has a vast potential for geological diversity, biodiversity, and water catchment area (Alam et al. 2023). Moreover, this region is an essential habitat for various species of flora and fauna, including rare and endangered ones. The forest is dominated by two vegetation ecosystems: Euphorbiaceae, especially *Aluerites moluccana*, and a palm oil plantation planted over 10 years ago. This area was an undisturbed forest used for research studies on the diversity of insects (Nisita et al. 2020), bird fauna (Sason et al. 2018), amphibia (Jusmaldi et al. 2019) plants, and fish (Pratama et al. 2018; Jusmaldi et al. 2019) since 2010-2013. Nowadays,

Berambai has been experiencing forest conversion to an oil palm plantation and tourism infrastructure development.

Deforestation has emerged as a critical environmental concern in recent decades. The widespread forest was cleared for agricultural expansion, logging, and resource exploitation. One of deforestation is the forest conversion to palm oil plantations and secondary forests, which typically results in significant land changes in the soil, extensive damage to the topsoil, compaction, and erosion (Bakeri et al. 2019). The consequence is an affected soil bacteria community, a natural biomarker of soil health and quality. Soil bacteria are vital in maintaining ecosystem health and functioning, such as nutrient cycling, soil fertility, and ecosystem stability (Bakeri et al. 2019; Wiryawan et al. 2022). Land conversion can introduce pollutants and contaminants into the soil, such as heavy metals, pesticides, or fertilizers. These substances can negatively affect soil bacteria, either directly by inhibiting their growth and activity or indirectly by altering the soil environment unfavorable for microbial communities. Most bacteria are sensitive, and it is easy to change their community in the soil. The soil bacteria are a vital component of the biogeochemical cycle due to their role in decomposing carbon-based litter, breaking down organic materials, releasing carbon dioxide (CO_2) into the atmosphere through respiration, and improving nutrient uptake to enhance primary production (Bakeri et al. 2019). Hence, rhizobacterial diversity is used as a soil quality indicator.

In an omic era, the development of molecular and genomic tools can accurately assess the profile composition and diversity of bacterial communities (Bakeri et al. 2019). Next-generation sequencing is a powerful tool for genome analysis (Oulas et al. 2015). Several studies have used the structure and composition of bacterial diversity changes among community samples to analyze conversion forests' impact (Guo et al. 2021). This research will investigate the soil bacterial communities between two land uses of a secondary forest and palm oil plantation in Berambai Forest due to the lack of reports about bacterial communities. Further, the comprehensive information of this study can be helpful for soil management practices in East Kalimantan.

MATERIALS AND METHODS

Study area description and soil sampling

This study was conducted in the forest area near the waterfall tourism 135.6-140.0 m above sea level (m.a.s.l), Samarinda, East Kalimantan (Figure 1). Soil samples were collected from the rhizosphere of palm oil plantations and secondary forests using a purposive sampling method. Each soil sample was collected from 5 points around the plant roots within 20 cm soil depth in triplicate for each location. Three samples in each site were composited, mixed, and transferred in sterilized polythene bags. The soil samples were stored at 4°C for further soil physico-chemical analysis, DNA extraction, and isolation of rhizobacteria.

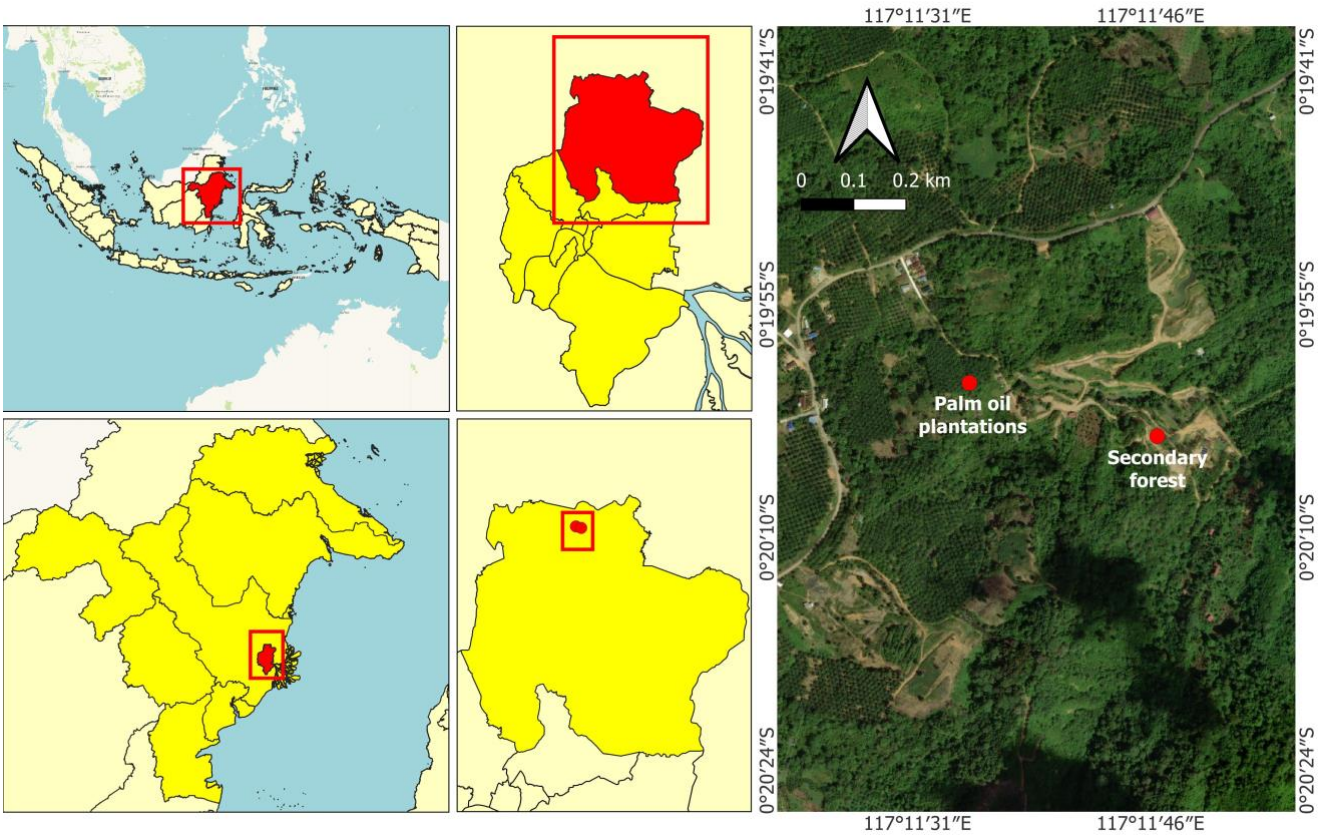

Figure 1. Sampling areas in Samarinda, East Kalimantan, Indonesia

Soil physico-chemical analysis

The soil samples were analyzed based on environmental parameters such as soil pH (1:1), Total N (Kjeldahl), organic carbon (Walkey and Black), C/N ratio, P_2O_5 (Bray-I), and K_2O availability (NH_4OAc pH 7, 1 N). The standard category of chemical soil was conducted (Eviati et al. 2023).

Rhizobacterial isolation and colony enumeration

Twenty-two grams of soil sample was dissolved in 200 mL 0.85% NaCl solution and shaken at 200 rpm for 2 h. Then, 5 mL suspension was transferred into a new glass tube, shaken, and heated at 80°C for 30 min to isolate only spore-forming rhizobacteria. One milliliter suspension was replaced into 9 mL 0.85% (10^{-1}) and repeated; hence, the serial dilution was 10^{-1}-10^{-6}. An aliquot of 0.5 mL of 10^{-3}-10^{-6} sample dilution was spread on Luria Bertani Agar (LBA) and incubated at 37°C for 18 h. The colony bacteria were enumerated using a colony counter to calculate the number of rhizobacterial colonies forming per unit (CFU/g) (Susanti et al. 2019; Kusai and Ayob 2020; Nor 2020).

Preparation and sequencing of metagenomic analysis

Total soil DNA was extracted from Palm Oil Plantation soil (PP) and Secondary Forest soil (SF) using PowerSoil DNA Isolation Kit (Mo Bio Laboratories) following the manufacturer's instructions. Rhizobacteria were assayed in the sample genome (Gel Concentration: 1%, Voltage: 100v, run time: 40 minutes), amplification products (Gel concentration: 2%, Voltage: 80v, run time: 40 minutes), and remarked using agarose gel electrophoresis (Krashevska et al. 2015; Berkelmann et al. 2020; Kaupper et al. 2020).

Data analysis procedures

Illumina Hiseq platforms (Hiseq P250) performed DNA sequencing according to the manufacturer's instructions provided by Genetika Science Laboratory (Tangerang, Indonesia). The 16S rRNA gene region (V3-V4) was used for taxonomic identification of rhizobacteria. The 16S sequence primers were 341F (5'-CCTACGGGNGGCWGCAG-3') and 785R (5'GACTACHVGGGTATCTAATCC-3').

Sequencing data processing

The raw data was obtained by sequencing; there was a certain proportion of dirty data. The data should be merged and filtered using FLASH (Fast Length Adjustment of Short Reads) V1.2.7 (Magoč and Salzberg 2011). Then, it was trimmed and filtered by QIIME (Quantitative Insights Into Microbial Ecology) V1.7.0 (Caporaso et al. 2010). The Illumina Hiseq paired-end platform sequenced the amplicon to develop 250 Raw PE (paired-end reads). It was then merged and pretreated to obtain Clean Tags (Bokulich et al. 2013). The effective tag was obtained by the Chimera sequence that was used for subsequent analysis (Haas et al. 2011). The clustering of OTU (Operational Taxonomic Units) was conducted based on effective data that represented the alpha diversity analysis (alpha diversity indices) and OTU analysis (species annotation, species distribution). OTUs were performed by clustering using Uprase (v7.0.1001) (Edgar 2013) on the Effective Tags of soil samples with 97%, afterward, identifying them based on the SSUrRNA database of SILVA138 database (http://www.arb-silva.de/). The OTU's relative abundance of Rhizobacteria (Phyla- genus level) was visualized in a bar chart using GraphPad Prism, Krona display for genus taxonomy annotation, and heatmap for species taxa richness.

RESULTS AND DISCUSSION

Soil physico-chemical and biological parameter

The soil physicochemical and biological parameters represent soil parameters compared to two rhizosphere sample types (Table 1). The pH analysis showed the acidic levels of SF and PP samples, revealing that SF exhibited a strongly acidic soil (5.38) while PP demonstrated an extremely acid (4.41). Thus, both soils were acidic, with the SF being less acidic than PP. The total nitrogen (N) content in both SF and PP soil was within the medium range at 0.22% and 0.17%, respectively, suggesting moderate nitrogen availability in each soil type. The organic carbon (C) was classified in the same range in a high range of SF and PP soil. This range showed that the organic carbon in both soil types had a relatively high effect on soil structure, water retention, and plant nutrient availability. The P_2O_5 levels illustrated a distinct between the samples: PP soil had a very high phosphorus content, attributed to fertilization practices, while SF soil contained a moderate level of P_2O_5. These indicated greater phosphorus availability in PP soil.

Similarly, both samples had high potassium (K_2O) content, suggesting that both soils had substantial potassium availability. The C/N ratio of SF and PP soil was high, indicating slower decomposer rates and reduced microbial breakdown. However, rhizobacterial activity appeared more prominent in the FR soil, potentially due to richer organic matter content, which provides a favorable environment for rhizobacterial growth.

Table 1. The soil physicochemical and biological parameters of both soil types

Parameter	Rhizosphere sample	
	SF	PP
pH	5.38 ± 0,81[a]	4.41 ± 0,55[a]
Total N (g/kg)	0.22 ± 0,01[a]	0.17 ± 0,02[a]
Organic C (%)	4.52 ± 0,67[a]	3.55 ± 0,53[a]
Available P_2O_5 (ppm)	26.45 ± 19,3[a]	144.82 ± 46.23[b]
Available K_2O (ppm)	322.60 ± 46,12[b]	211.52 ± 194,0[a]
C/N ratio	21.05 ± 2,79[a]	20.57 ± 3,7[a]
Rhizobacterial density (CFU/g)	10.30×10^{4} [b]	1.10×10^{4} [a]

Notes: Data are means±Standard Deviation (SD) from three replications, and values followed by a different letter (s) indicated a significant difference ($p<0.05$); SF: Rhizosphere sample in Secondary Forest, PP: Rhizosphere sample in Palm oil Plantation

The rhizobacterial abundance, diversity, and functional

Diversity metrics of rhizobacterial communities in SF and PP soil offered insight into species richness, evenness, and representativeness within each soil type (Table 2). Based on observed species counts, PP soil exhibited a slightly higher number of unique rhizobacterial species (2344) than SF soil (2338), suggesting similar levels of rhizobacterial richness across both samples. The Shannon and Simpson Index measured species diversity, indicating higher diversity in PP soil with values of 8.29 and 0.99, respectively, compared to SF soil. Furthermore, Good's Coverage analysis demonstrated a higher proportion of rhizobacterial community representation in PP soil, implying a more comprehensive sampling of rhizobacterial diversity. While both soils displayed high rhizobacterial diversity and representativeness, PP soil showed marginally greater diversity and evenness. It may be suggested that specific management practices in SF soil foster a highly diverse and evenly distributed rhizobacterial community structure.

The rhizobacterial phyla in both SF and PP soil had an abundant presence of Proteobacteria and Acidobacteriota as primary phyla (Figure 2). Actinobacteriota and Firmicutes exhibited slight differences in their relative abundance between SF and PP, possibly reflecting variations in soil conditions between the two ecosystems. At the class level, Alphaproteobacteria and Acidobacteriae were notably prominent in both soil types. Bacilli and Actinobacteria were also present, showing slight differences in abundance across the two environments. This similarity in class-level composition between SF and PP suggested that essential rhizobacterial functions, such as nutrient cycling and organic matter decomposition, were equally essential in both soil types, supporting ecological roles.

Meanwhile, the order-level composition highlighted Rhizobiales and Acidobacteriales as abundant across both soils. Frankiales, Chthoniobacterales, and Vicinamibacterales were in smaller quantities, underscoring their specialized roles in the soil ecosystem. At the family level, Xanthobacteraceae were highly abundant in both soils, with PP soil showing higher relative abundance. The composition of the genera level showed the *Bradyrhizobium* in each land-use soil (Figures 2 and 3). This taxonomic analysis demonstrated that while SF and PP soil display similar rhizobacterial compositions at higher taxonomic levels, significant distinctions arise at finer taxonomic levels, particularly within families and genera. These distinctions suggested that PP may selectively favor rhizobacterial genera. Conversely, the broader rhizobacterial diversity in SF likely supports more complex nutrient cycling and enhanced overall soul health within the forest ecosystem.

The rhizobacterial abundance provided a detailed profile across different soils, with each column representing a specific soil type. Individual rhizobacterial species were listed in rows, with columns categorizing their relative abundance across both soils. The color gradient represented abundance levels: dark green signified high abundance (>3000), while yellow to red shades indicated progressively lower abundance (down to a range of 1-20). This visualization allowed us to determine the dominant rhizobacterial species in various across soil types (Figure 4).

Table 2. Diversity measurement of rhizobacterial communities in secondary forest and palm oil plantation rhizosphere

Sample	Observed Species	Shannon	Simpson	Goods coverage
SF	2338	7.91	0.98	0.998
PP	2344	8.29	0.99	0.999

Notes: Observed species (the number of unique rhizobacterial species), Shannon Index (distribution of individual among species bacteria), Simpson Index (the species dominance measurement), and Good coverage (the metric estimation about representation sample in rhizobacterial diversity)

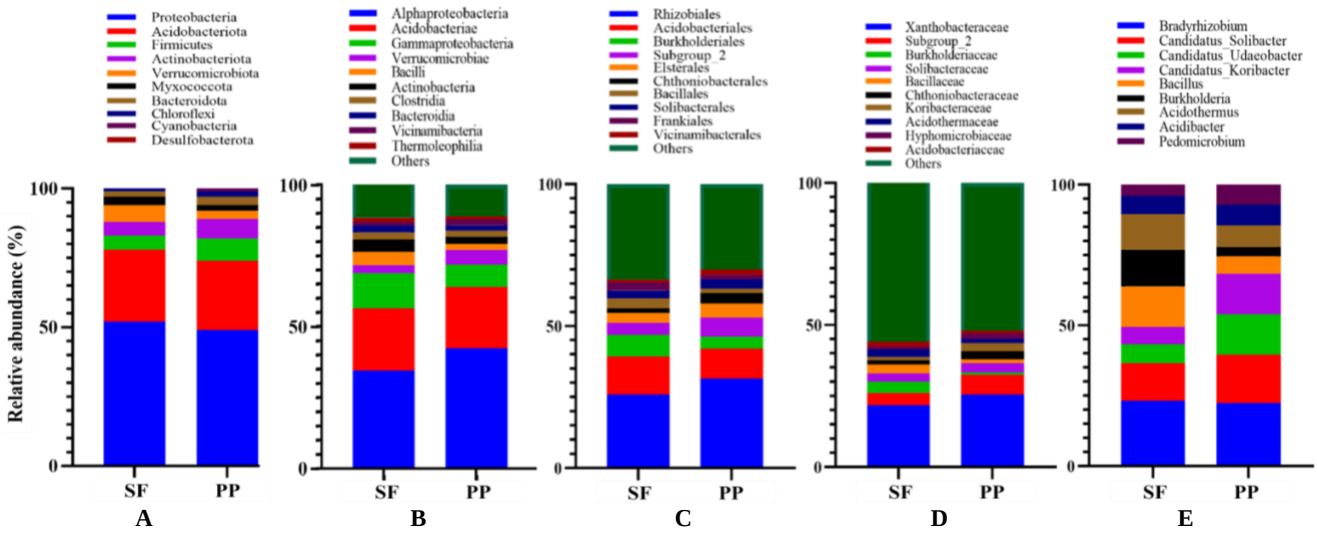

Figure 2. The top 10 taxonomic compositions of rhizobacterial communities: A. Phylum-level; B. Class-level; C. Order-level; D. Family-level; E. Genus-level

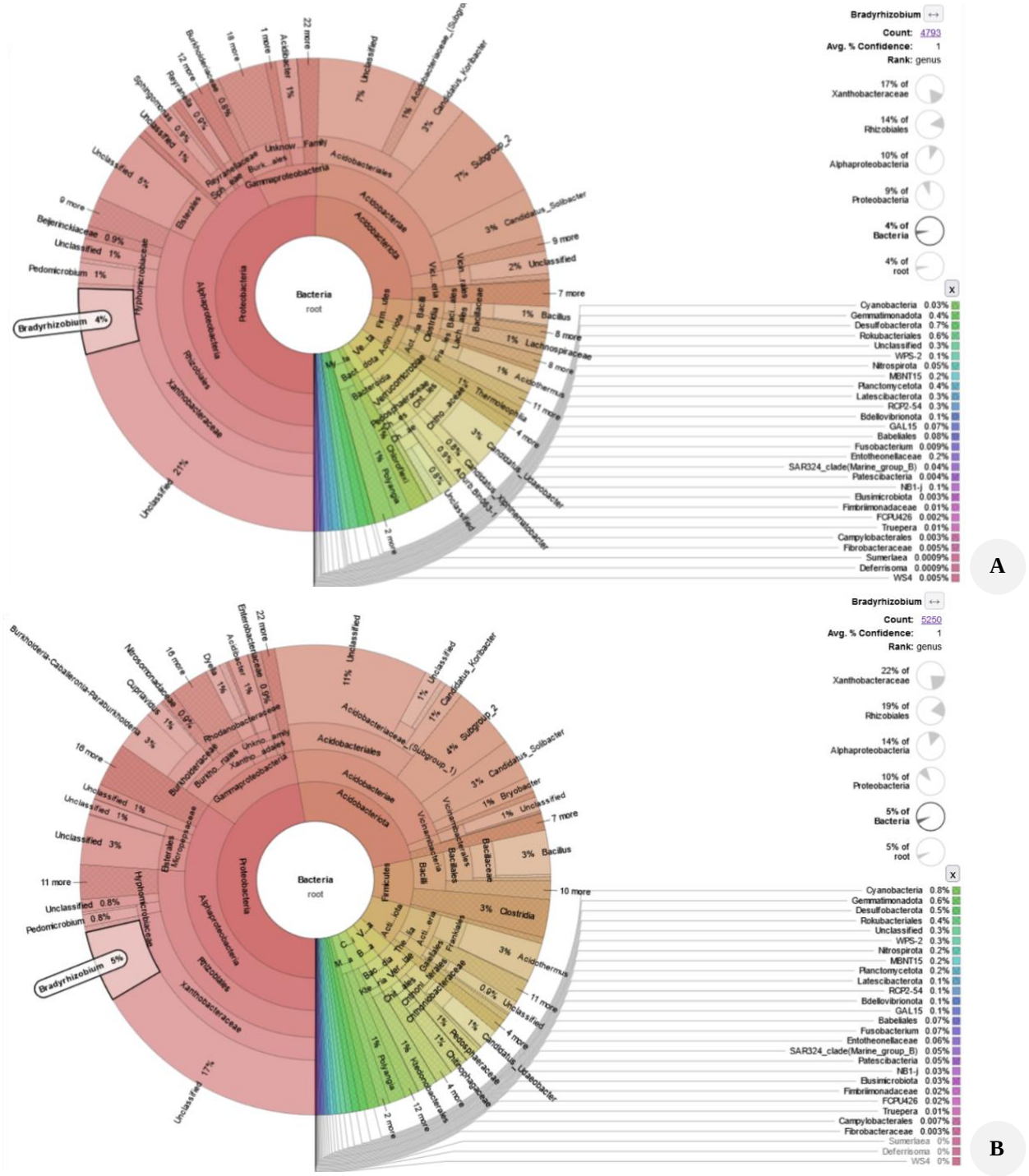

Figure 3. Taxonomic classification of rhizobacteria at the genus level: A. Secondary Forest soil (SF); B. Palm oil Plantation soil (PP) using Krona visualization

In the first column, *Bradyrhizobium elkanii* was abundant in both soil samples. Moreover, the heatmap revealed subtle differences between both soils, such as *Ruminococcus callidus*, *Comamonas testosteroni*, *Inquilinus* sp. (PP soil); *Paenibacillus cavernae*, *Bacillus wuyishanensis*, *Clostridium beijerinckii*, *Geobacter* sp., *Bacteroides massiliensis*, *Bacteroides cellulosilyticus*, *Shimazuella* sp, *Alistipes putredinis*, and *Vogesella* sp. (SF soil), which showed low abundance across all soil types. Their limited presence may reflect less common or more niche-specific functions that were not as central to soil ecosystem processes. Overall, the heatmap highlighted the diversity of rhizobacterial communities across soil types and underscored how specific species were favored by different soils, influenced by nutrient availability, organic matter, and soil management practices. These community compositions offered insight into the functional potential of each soil type.

A comparative bar plot and two labeled circles illustrated the functional rhizobacterial diversity in SF and PP soil, emphasizing their key roles in each soil type (Figure 5). The bar plot categorized rhizobacterial species based on their functional roles in the SF and PP, with distinct colors representing nine groups such as Plant Growth-Promoting Rhizobacteria (PGPR), nitrogen-fixing, nitrogen cycling, organic decomposition, chitin decomposition, mineralization, biocontrol, antibiotic production, and bioremediation. The organic decomposition rhizobacteria dominated similar species in both SF and PP, followed by PGPR and nitrogen-fixing bacteria groups. Meanwhile, the unique species bar showed that the organic decomposition rhizobacteria dominated in smaller proportions.

Figure 4. The heatmap illustrated the abundance of rhizobacterial species in soil types. The color showed the number of rhizobacterial species present: Green (>3000 species number), Yellow (21-100 species number), and Red (1-20 species number)

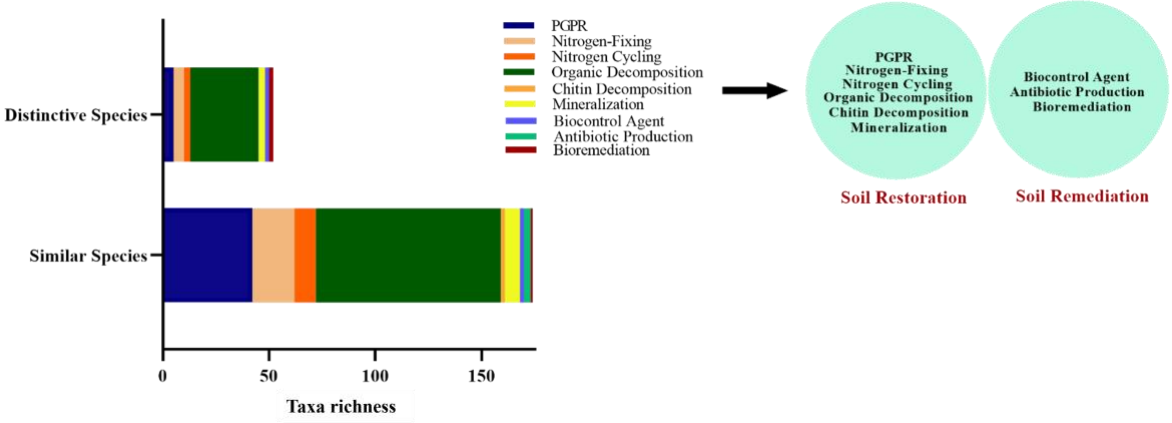

Figure 5. The functional diversity of rhizobacteria in two land-used types is represented by PGPR, nitrogen-fixing, nitrogen cycling, organic decomposition, chitin decomposition, mineralization, biocontrol agent, antibiotic production, and bioremediation

Discussion

Soil physicochemical properties

The soil physicochemical and biological analysis revealed significant differences between the SF and PP soil, primarily attributed to their distinct management practices and environmental conditions. Both soils were acidic; however, PP exhibited a higher acidity than SF due to monoculture vegetation, drainage systems, and fertilizer practices (Zhang et al. 2022). Compared to previous research, the pH of PP soil was also slightly more acidic than PP soil; however, the SF soil was higher, according to Ho et al. (2019) in Malaysia. The monoculture practices eventually produced a lack of nutrition in the litter (Meijaard et al. 2020), the roots of palm oil plantations uptake more alkali cation, the drainage system influences the cation leaching, and the fertilizer utilization (Intara et al. 2018), hence, the decomposition process releases more organic acid in the soil (Kurniawan et al. 2018). Regarding total N content, SF and PP soils were categorized within the medium range, indicating moderate nitrogen content. This moderate N aligned with findings that SF soil generally maintained stable nitrogen levels, while PP soil often experienced nitrogen depletion due to crop demand and nutrient leaching (Mahmud and Chong 2022). These findings showed that the total N was lower than the previous study, which had a range of total N around 1.2 g/kg (SF) and 1.37 g/kg (PP) (Ho et al. 2019). The Soil Organic Carbon (SOC) was higher in SF soil. It is due to contained by the litter of leaves, branches, and roots naturally decomposing with minimal human activity or other mechanical and chemical disturbances, and the forest canopy affecting the litter layers had high nitrogen with slow decomposition. Consequently, the litter was thicker, increasing the organic carbon percentage in SF soil. Meanwhile, the PP litter contained high lignin from the Palm Empty Fruit Branch (PEFB), which is challenging to decompose (Bhattacharyya et al. 2022). According to Ho et al. (2019), these results were similar to soil profiling in the secondary forest, which had a higher SOC than palm oil plantations in Malaysia's agricultural land use.

The total P content was the highest in PP soil, indicating that it was caused by fertilizer application and the mineralization process in the soil. Based on the study by Ho et al. (2019), the available P was higher in both soil than SF and PP soil in Malaysia's agricultural land use, which showed only 3.7 ppm and 19.4 ppm, respectively. The phosphorus of fertilizer increased the total P in the soil, which is not directly available for plants. The litter of palm oil plantations, such as leaves, branches, and Palm Empty Fruit Branches (PEFB), and mulch practices with palm oil residue can retransfer P into the soil. The drainage in PP soil leached only for the nitrogen, while most of the phosphorus remained in mineral or organic form in the topsoil. Moreover, the absorption of P in the PP soil was lower than that of the phosphor input of fertilizer, affecting the P accumulation in the soil (Mahmud and Chong 2022). The availability of K (potassium) was higher in the SF soil than in the PP soil, caused by the potassium input, nutrient cycle, and soil management practices. Tree roots in SF soil had deep roots that absorbed the potassium in the subsoil area and distributed it to the upper soil layers through the litter. The tree processed the potassium cycle effectively mediated by litter, thus minimizing the potassium leaching (Sardans and Peñuelas 2021). However, these results differed with SF and PP Malaysia's soil, respectively showing lower 38% and 44% than Berambai soil (Ho et al. 2019). The C/N ratio of both soils was not slightly different, and the balance of organic matter inputs and decomposition process was found in the two soil land uses. The primary factors were organic matter, external nitrogen, the decomposition process, organic matter stabilization, the source of carbon and nitrogen in the long term, soil practices, erosion, and organic matter leaching (Zhang et al. 2022). This slower decomposition may contribute to long-term organic matter stability in SF soils, while in PP's soil, a high C/N ratio may result from management practices. However, these results contradict Malaysia's SF and PP soil, which had a lower C/N ratio, about 28% and 32%, respectively, than Berambai soil (Ho et al. 2019). Soil' structure and texture may cause it to Ultisol (Fatai et al. 2017) and Oxisol (Mahmud and Chong 2022), affecting the palm oil plantation drainage and thus accelerating the organic matter process (Shamshuddin et al. 2024).

The biological parameter, the rhizobacterial density, in the SF soil revealed a higher density 10-fold than in the PP soil. It indicated the distinct habitat quality, organic matter inputs, and environmental conditions. Rhizobacteria was known for enhancing nutrient cycling and organic matter decomposition and was more widespread in SF soil due to the commendatory conditions provided by an accumulation of organic matter (Jiexiu et al. 2020) and improves soil structure, water retention, and their habitat (Bhattacharyya et al. 2022). Soil acidity plays a crucial role in influencing nutrient availability and rhizobacterial activity, with extreme acidity in PP potentially limiting essential nutrients for microbes (Wan et al. 2020). The SF soil supported a balanced nutrient cycle with moderate acidity and high microbial diversity; the intensively managed PP soils showed extreme acidity and elevated phosphorus content, posing a potential risk to long-term soil health and stability. Therefore, sustainable soil management practices are essential in PP soil settings to maintain soil fertility and ecosystem health (Rahman et al. 2021).

Rhizobacterial abundance and diversity based on metagenomic analysis

The diversity metrics of rhizobacterial communities in SF and PP soil highlighted nuanced yet significant variations in richness, evenness, and community composition. PP soil exhibited a slightly higher number of unique rhizobacterial species than SF soil. This observation aligned with findings that distributed environments, such as palm oil plantations, were due to nutrient inputs and altered soil conditions (Mahmud and Chong 2022). Diversity indices further supported this trend, with the Shannon and Simpson Index values for PP soil slightly exceeding those for SF soil, indicating more excellent species diversity and evenness in PP soil. The heightened diversity in PP soil may result from plantation practices, such as fertilization, planting methods, and irrigation, which introduce diverse nutrients

and create microhabitats favorable to various microbial communities (Xue et al. 2020).

In contrast, SF soil performed diverse rhizobacterial populations but potentially less community distribution due to minimal soil disturbance. Good's coverage analysis showed higher rhizobacterial community representation in PP soil, implying a more comprehensive sampling of its microbial diversity. This increased community coverage in PP could be linked to plantation practices that homogenized the soil environment, enhancing the detectability of rhizobacterial species. While both soils demonstrated high diversity and representativeness, PP soil displayed slightly greater diversity and evenness, likely influenced by external inputs and modified soil conditions associated with palm oil plantation management (Mahmud and Chong 2022). These findings emphasized the impact of land use and management on rhizobacterial community diversity and structure. SF soils supported a naturally rich and balanced microbial ecosystem, whereas PP soil reflected the effect of altered conditions linked to agricultural practices (Mahmud and Chong 2022). The observed human intervention in microbial dynamics highlighted the critical role of sustainable management practices in maintaining soil biodiversity and ecosystem functions within the agricultural landscape.

The rhizobacterial community composition analysis revealed similarities and differences between SF and PP soils. Proteobacteria and acidobacteria were dominant in phyla in soil land use, respectively (SF: 53%, 40%; PP: 50%, 24%). In contrast, the subsequent abundance of phyla was Verrucomicrobiota, Actinobacteriota, Firmicutes, Myxococcota, Bacteroidota, and Chloroflexi from high to low. According to a previous study, Proteobacteria also abundance in palm oil plantations and secondary forests (Yue et al. 2023) due to high of carbon and nutrients (Kusai et al. 2018) and fertilizer addition (Leff et al. 2015); hence this rhizobacterial group was known as copiotrophic organisms (Kusai et al. 2018). The Protebacteria's metabolic versatility and adaptability to diverse soil conditions reflected its typical dominance in various soil ecosystems (Mishra et al. 2023). Alphaproteobacteria and Acidobacteriae were abundant at the class level, especially in PP soil. These classes in both soils indicated the essential rhizobacterial functions contributing to overall ecosystem stability (Yue et al. 2023). In other class levels, Gammaproteobacteria had the third-highest abundance in both soils. While Verrucomicrobiae, Bacilli, Actinobacteria Clostridia, Bacteroidia, Vicinamibacteria, and Thermoleophilia were only 5-10% in both soils. These group bacteria of class level were adaptable to organic input and soil management (Kusai et al. 2018).

In the order levels, Rhizobailes was abundant at 25% and 30% in SF and PP, respectively. Others, Acidobacteriales had a proportion of about 15% (SF) and 10% (PP), while Burkholderiales, Elsterales, Chthoniobacterales, Bacillales, Solibacterales, Frankiales, Vicinamibacterales were about 1-5% in both soils. These rhizobacterial groups survived soil conditions, stimulating the specific secondary metabolites to promote nutrient cycling, biocontroling (Zheng et al. 2024), organic matter decomposition, and nitrogen fixation (Lewin et al. 2021).

At the family level, Xanthobacteraceae was most dominant in both soils, with slightly different amounts of about 5% higher in PP soil. The others, Burkholderiaceae, Solibacteraceae, Bacillaceae, Koribacteraceae, Chthoniobacteraceae, Acidothermaceae, Hypomicrobiaceae, and Acidobacteriaceace, had a proportion in both soils approximately 1-5%. Previous studies revealed Xanthobacteraceae's potential to support nitrogen fixation and soil function. While Burkholderiaceace could suppress pathogens and increase nutrient availability, Bacillaceae and Frankiaceae contributed to essential processes and enhanced soil productivity and resilience (Wang 2024).

The 10 genera at the genus level were *Bradyrhizobium*, *Candidatus_Solibacter*, *Candidatus_Udaeobacter*, *Candidatus_Koribacter*, *Bacillus*, *Burkholderia*, *Acidibacter*, *Acidothermus*, and *Pedomicrobium*. *Bradyrhizobium* was the most dominant in SF and PP soil. According to previous studies, these rhizobacteria were crucial in promoting soil nutrient cycling and inhibiting pathogens (Medici et al. 2024; Solomon et al. 2024).

Potential of rhizobacterial species based on metagenomic assessment

The analysis of rhizobacterial functions in SF and PP soils identified nine groups of rhizobacterial functional diversity. These were PGPR, nitrogen-fixing, nitrogen cycling, organic decomposition, chitin decomposition, mineralization, biocontrol, antibiotic production, and bioremediation. The similar rhizobacterial species in both soils showed their capability to decompose organic matter (rhizobacteria-degrading organic matter: 87 species) and enhance plant growth (PGPR: 45 species). The studies revealed rhizobacteria's primary role-play in breaking down organic matter and supporting nutrient cycles (Rajguru et al. 2024) and nutrient availability in limiting such as nitrogen (Wang 2024). The distinctive groups illustrated that the total species was unique in both soils, around 25% fewer than in similar species groups. However, approximately 30 rhizobacterial species assisted the litter degradation in both soils. Other species had diverse capabilities in both soils for soil ecosystem stability. This study highlighted the significant differences and commonalities in rhizobacterial communities, illustrating how land use influences rhizobacterial diversity, composition, and functionality. It also emphasized the importance of sustainable management practices to maintain rhizobacterial diversity and soil health (Wang 2024).

In conclusion, the rhizobacteria associated with secondary forest and palm oil plantation soils had different characteristics in soil physicochemical and biological properties. The metagenomic analysis performed above 3000 species in both soils. The relative abundance of rhizobacteria was slightly different at the taxonomic level: phyla (Proteobacteria), class (Alphaproteobacteria), ordo (Rhizobiales), family (Xanthobacteraceae), genus (*Bradyrhizobium*), and species (*Bradyrhizobium elkanii*) in each soil. About 114 rhizobacterial species represented their functions, such as soil restoration (litter decomposition, PGPR, nitrogen fixation, nutrient cycling, mineralization, and chitin degradation) and soil remediation (biocontrol,

antibiotic production, and bioremediation). Most dominant rhizobacterial species performed their capability in soil restoration, especially the organic matter degradation, which was abundant in secondary forest soils (24 species) and palm oil plantation soils (95 species). These findings highlighted the ecological significance of rhizobacteria among different land uses. Further, rhizobacteria in both soils can be developed to accelerate soil restoration in managing sustainable agriculture.

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