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ARTICLE

BEYOND THE CONTROVERSY: SOIL MICROBIOME DYNAMICS IN OIL PALM VS. NATURAL PEAT ECOSYSTEMS

[*Di Balik Kontroversi: Dinamika Mikroba Tanah di Kebun Kelapa Sawit dan Ekosistem Lahan Gambut*]

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ABSRACT

The increasing global demand for palm oil has driven the expansion of oil palm cultivation into peatland areas, raising concerns over environmental sustainability. This study investigates the impact of different land uses on soil microbial diversity and physicochemical properties by comparing three ecosystems: peat soil planted with oil palm (GS), peat soil with natural fern vegetation (GNS), and mineral soil cultivated with oil palm (MS). Physicochemical parameters including organic carbon, cellulose, and lignin content were analyzed, followed by 16S rRNA gene sequencing (V3-V4 regions) using the Illumina MiSeq platform. Sequencing data were processed using the QIIME2 pipeline and classified with the SILVA v.138 database. Results show that while MS had the highest microbial diversity, GS and GNS also supported diverse bacterial communities. Organic carbon, cellulose, and lignin were more abundant in GNS and GS than in MS, reflecting the influence of vegetation on soil composition. Dominant bacterial phyla across all sites included Proteobacteria, Actinobacteria, Firmicutes, and Acidobacteria. These findings suggest that oil palm cultivation on peatlands, when properly managed, does not necessarily reduce microbial diversity. Environmental factors such as vegetation cover, soil type, and water table levels play key roles in shaping microbial communities in tropical soils.

Keywords: metagenomics, microbial diversity, NGS, peatland

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ABSTRAK

Permintaan global minyak sawit yang terus meningkat telah mendorong perluasan budidaya kelapa sawit ke wilayah lahan gambut, yang menimbulkan kekhawatiran terkait keberlanjutan lingkungan. Studi ini mengevaluasi dampak penggunaan lahan yang berbeda terhadap keanekaragaman mikroba tanah dan sifat fisikokimia tanah dengan membandingkan tiga ekosistem: tanah gambut yang ditanami kelapa sawit (GS), tanah gambut dengan vegetasi alami paku-pakuan (GNS), dan tanah mineral yang juga ditanami kelapa sawit (MS). Parameter fisikokimia termasuk karbon organik, selulosa, dan lignin telah dianalisis, dilanjutkan dengan sekuensing gen 16S rRNA (region V3-V4) menggunakan platform Illumina MiSeq. Data sekuensing dianalisis menggunakan pipeline QIIME2 dan diklasifikasikan dengan basis data SILVA v.138. Hasil menunjukkan bahwa meskipun tanah MS memiliki keanekaragaman mikroba tertinggi, tanah GS dan GNS juga mendukung komunitas mikroba yang beragam. Kandungan karbon organik, selulosa, dan lignin lebih tinggi pada tanah GNS dan GS dibandingkan dengan MS, mencerminkan pengaruh vegetasi terhadap komposisi tanah. Filum bakteri dominan di semua lokasi meliputi Proteobacteria, Actinobacteria, Firmicutes, dan Acidobacteria. Temuan ini menunjukkan bahwa budidaya kelapa sawit di lahan gambut, jika dikelola dengan baik, tidak selalu menurunkan keanekaragaman mikroba tanah. Faktor lingkungan seperti tutupan vegetasi, jenis tanah, dan tinggi muka air tanah memainkan peran penting dalam membentuk komunitas mikroba di tanah tropis.

Kata kunci*: keanekaragaman mikroba, lahan gambut, metagenomik, NGS*

INTRODUCTION

Oil palm (*Elaeis guineensis*) cultivation offers rural communities an effective pathway out of poverty (McCarthy *et al*., 2012). A key advantage of this crop is its exceptionally high oil yield per unit of cultivated land. Its fruits produce two valuable products: palm kernel oil derived from the seeds and crude palm oil extracted from the fresh fruit (Chiriacò *et al*., 2022). *E. guineensis* contains a variety of fatty acids, carotenoids, vitamin E, and phenolic compounds, which have a range of pharmacological qualities that address society's greatest health problems (Sulaiman *et al*., 2022). Benefit of oil palm can be obtained not only from its fruit, but also from other organs. For example, the leaves extract of oil palm (OPLE) has been proven to contain high antioxidants, antimicrobial activity and skin brightening as well as skin protectant against UV irradiation (Yusof *et al*., 2016). The stem part of oil palm can be harnessed as a soil conditioner in the replanting process, with high moisture content of 22.99%, C-organic of 10.72%, and C/N ratio of 13.48% (Alfarisi *et al*., 2022). Moreover, waste materials from palm oil production, including shells, empty palm fruit bunches, fibers, leaves, midribs, and Palm Oil Mill Effluent (POME), have a number of potential uses as bioenergy sources, including biogas, biodiesel, bio-briquettes, electricity generators, and activated charcoal. In addition to being utilized as bioenergy, waste palm oil can be added to concrete, fed to animals, fertilized, and used as an antioxidant (Noerrizki *et al*., 2019). Because of the multifunctional benefits that humans have gained from oil palm, the demand is significantly increasing over the year.

Despite all the benefits we can harness from oil palm, the negative drawbacks of oil palm plantation are continuously being discussed worldwide. In Southeast Asia, oil palm is linked to the burning and draining of peatlands. Decreases in biodiversity, increase in greenhouse gas emissions, and air pollution are only a few of the documented adverse environmental effects of such expansion. Because of its potential and effects on society and the environment, palm oil vegetation is divisive. The most direct environmental effects of oil palm production are habitat loss, decreased woody biomass, and peatland drainage that arise during site preparation. When such conversion occurs, biodiversity and water quality are usually decreased, greenhouse gas emissions are increased, and smoke and haze are produced when fire is employed (Dislich *et al*., 2017). A study in 2019 by Wijayanti *et al*. (2022) proved that transformation of natural forest into oil palm plantation area directly impacted the bacterial community, even though the abundance of phyla and genera in oil palm plantation is higher than the natural forest. In addition, the micro and macro nutrients availability also directly affected the soil composition of microbia, so that the forest soil has higher microbia diversity and evenness compared to tilled land. Moreover, the lack of diversity of soil bacteria in oil palm plantation is associated with soil alkaline characteristics compare to the natural forest (Tripathi *et al.*, 2012).

In converted land, the soil moisture also shifting, indicated by the decrease in the soil quality index (SQI) (Ramadhan *et al.*, 2022). Total soil organic carbon (TC), total nitrogen (TN), and pH are examples of soil chemical characteristics commonly used as the parameter to convey the data. Furthermore, the variety of soil bacteria is influenced, either directly or indirectly, by the pH of the soil (Yan *et al.*, 2015) The pH of the soil had a significant impact on the variety of bacteria, for instance, being lower in acidic samples and higher in neutral samples. An ordination analysis revealed that soil pH also has a significant role in determining the structure of the bacterial population. Certain dominant phyla showed varying relative abundances over the pH gradient, and the enrichment of certain phylotypes indicated that they were adapted to low pH conditions (Wu *et al.*, 2017) Together, biotic and abiotic forces were responsible for the shift in microbial abundance. Microbial abundance was negatively impacted by physical factors (pH and soil water content) as well as the microbial co-occurrence network; microbial abundance was positively impacted by nutritional variables (total nitrogen and total phosphorus) (Zhao *et al.*, 2022).

To investigate the microbial diversity in peat land planted with oil palm compared to another type of soil, here we performed amplicon sequencing analysis of three soil sources: peatland planted with oil palm (GS), peat soil with ferns (GNS), and mineral soil planted with oil palm (MS). According to Mocali and Benedetti (2010), metagenomics is a potent method for exploring the molecular ecology of soil, evaluating the diversity of intricate microbial communities, and gaining access to a number of new species, genes, or unique compounds that are important for biotechnology and agricultural applications.

MATERIALS AND METHODS

Sampling location

The soil samples were collected from the oil palm peatland area in PT Kimia Tirta Utama (KTU), Indonesia in April 2019. The area specifically located in 0°46'14"-0°39'13" NL and 101°40'36"-101°47'45" EL. Three soil types were collected: peatland planted with oil palm (GS), peat soil with ferns vegetation (GNS), and mineral soil planted with oil palm (MS). Each sample was collected from three different point area in a location (equivalent to three biological replicates), and the soil were retrieved± 50 cm from the rhizosphere of the plant. The peat soil has been planted with oil palm for 15 years, equipped with an irrigation system to maintain the water table stable between 50-60 cm under the surface. While mineral soil has been planted with oil palm for 17 years. All of the peat location is considered as sapric peatland, characterized by rotten organic material with low pH of ±3.37-3.41. All soil samples were stored at 4 °C in an icebox to preserve microbiome DNA stability until further laboratory processing.

Soil physicochemical analysis

Cellulose content was determined by weighing one gram sample and refluxed for 2 hours with 150 mL H_2O in 100 °C. Solution was then filtered using a paper filter and dried. Re-reflux the residue using 0.5M of H_2SO_4 in 100 °C resulting in hemicellulose. Next, the samples were dried 72% (v/v) H_2SO_4 in room temperature for 4 hours, diluted with 0.5 M of H_2SO_4, and refluxed in 100 °C for 2 hours. In this step, cellulose was calculated. The samples from cellulose determination were dried one more time in 575 °C, until ashes were formed and the weight is constant.

$$Cellulose\ (\%) = \frac{c-d}{a}\ x\ 100\% \tag{1}$$

$$Lignin\ (\%) = \frac{d-e}{a}\ x\ 100\% \tag{2}$$

c: oven dry weight after reflux with 0.5 M of H_2SO_4; d: oven dry weight after 72% H_2SO_4
a: oven dry weight biomass sample lignocellulose

The C-organic content was calculated by collecting 0.5 grams sample and added with $K_2Cr_2O_7$ 2N and shaked. Subsequently, a 7.5 mL of H_2SO_4 were added and incubated overnight. The value of C-organic was determined using a spectrophotometer at 561 nm.

DNA extraction and sequencing

Fifty grams of each soil sample was collected by drill tool and stored in 4 °C. DNA extraction was performed using Soil DNA Isolation Plus Kit (Norgen Biotek, Canada). Amplification is performed targeting the V3-V4 region of 16S rRNA employing specific primers: Bakt_341F 5’-CCTACGGGNGGCWGCAG-3’ (forward) and Bakt_805R 5’-GACTACHVGGGTATCTAATCC-3’(reverse). Next, the sequencing process was performed utilizing the MiSeq Illumina platform (Macrogen, Inc).

Bacterial community analysis

The data were analyzed following the QIIME2 v.2019.10 pipeline (Bolyen *et al*., 2019). The sequence quality data was assessed using DADA2 (Callahan *et al*., 2016) integrated into QIIME2, with threshold Q10. Taxonomy classification is performed using the classifier database SILVA v.138 (Quast el al., 2013). The diversity analysis involving alpha (ACE Chao1, Margalef, Shannon, and Simpson index) and beta diversity (Bray-curtis, Weighted Unifrac and Unweighted Unifrac index) was performed. To visualize the data, QIIME webserver were employed by submitting the data collected.

RESULTS

Soil Physicochemical Properties

The physicochemical characteristics of three soil samples: peatland cultivated with oil palm (GS), peat soil with fern vegetation (GNS), and mineral soil cultivated with oil palm (MS), are presented in Figure 1. The result shows that the contents of organic carbon, cellulose, and lignin are significantly higher in GS and GNS compared to the MS sample. GS soil contains 52.83% organic carbon, 36.78% cellulose, and 21.22% lignin. Similarly, GNS exhibits comparable values, with organic carbon at 54.67%, cellulose at 38.25%, and lignin at 21.67%. In contrast, MS soil contains lower levels of these components, with only 4.6% organic carbon, 16.59% cellulose, and 10.82% lignin.

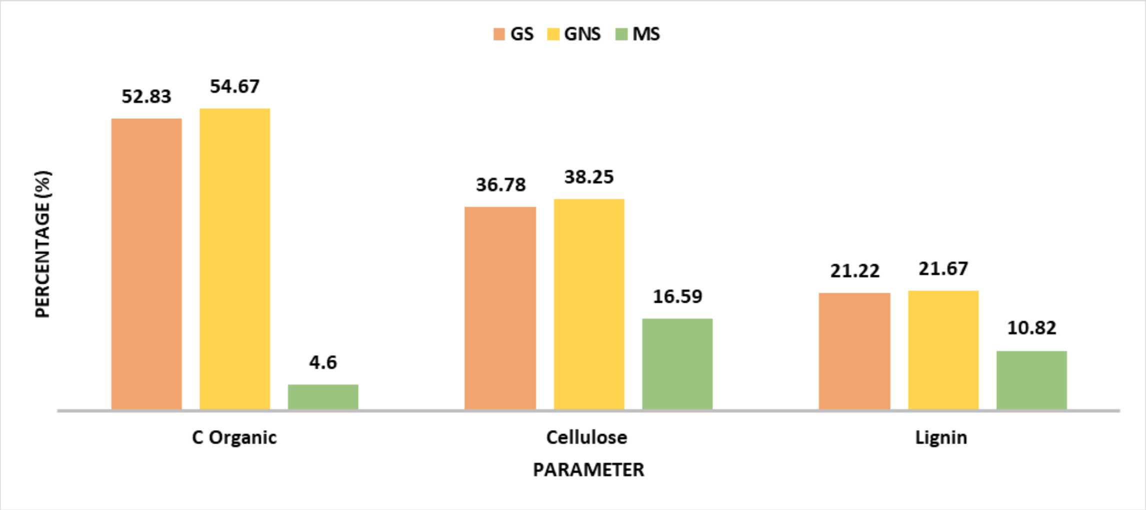

Figure 1. Physicochemical properties of peatland planted with oil palm (GS), peat soil with ferns vegetation (GNS), and mineral soil planted with oil palm (MS) (*Sifat fisikokimia lahan gambut yang ditanami kelapa sawit (GS), tanah gambut yang ditanami tumbuhan paku-pakuan (GNS), dan tanah mineral yang ditanami kelapa sawit (MS)*).

Sequencing output overview

Metagenomic 16S sequencing of the three soil samples yielded a total of 367,400 reads, identifying 1,029 bacterial taxa with a cumulative abundance of 42,267. The mean sequencing depth per sample was 14,089, while the average frequency per taxonomic feature was 41.07 (Supplementary data).

Statistical analysis of alpha diversity

To assess microbial diversity, alpha diversity indices such as ACE, Chao1, Shannon, and Simpson were calculated. The mineral soil (MS) exhibited the highest richness and diversity, as reflected by Chao1 (464) and Shannon (8.3) indices. GS and GNS soils displayed slightly lower values, indicating marginal differences in microbial diversity (Table 1).

Table 1. Alpha diversity indices data in three soil samples (*Data indeks keanekaragaman alfa dalam tiga sampel tanah*).

Sample (*Sampel*)	**Indexing** (*Indeks*)		
	Ace	**Chao1**	**Shannon**
GS	333.3	333	8.0
GNS	237.3	237	7.4
MS	464	464	8.3

A rarefaction curve based on the Shannon diversity index (Figure 3) for three soil samples: peat soil cultivated with oil palm (GS), mineral soil with oil palm (MS), and peat soil with fern vegetation (GNS). The x-axis represents the sequencing depth, while the y-axis indicates the Shannon index, which measures microbial diversity considering both richness and evenness.

All three curves demonstrate a rapid increase in microbial diversity as sequencing depth increases, followed by a plateau phase. Among the samples, MS soil exhibits the highest Shannon diversity index, followed closely by GS, while GNS consistently shows the lowest microbial diversity across the sequencing depth. The close proximity of GS and MS curves indicates similar diversity levels, while the separation of GNS shows fewer or less evenly distributed microbial taxa.

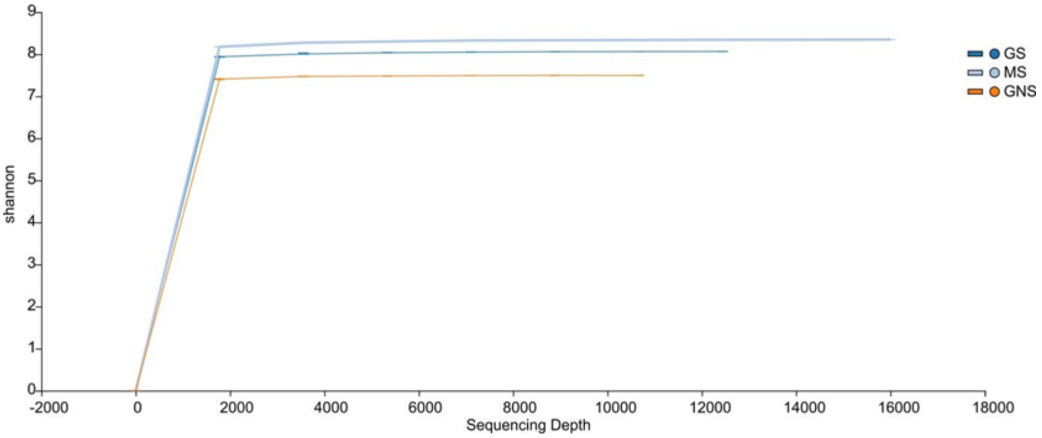

Figure 2. Shannon index of GS, MS and GNS sample. Peat soil with oil palm vegetation (GS), mineral soil planted with oil palm (MS), and fern vegetation soil (GNS). (*Kurva rarefaksi GS, GNS, dan MS. Tanah gambut dengan vegetasi kelapa sawit (GS), tanah mineral yang ditanami kelapa sawit (MS), dan tanah vegetasi pakis (GNS)*).

The rarefaction curve based on observed ASVs (Amplicon Sequence Variants) illustrates species richness across three soil types: GS (peat soil with oil palm), MS (mineral soil with oil palm), and GNS (peat soil with fern vegetation). As sequencing depth increases, MS consistently exhibits the highest number of observed ASVs, followed by GS, with GNS showing the lowest richness. The curves plateau, indicating that sequencing depth was sufficient to capture the majority of species. These results suggest that mineral soil (MS) supports the highest microbial richness, while fern-covered peat soil (GNS) harbors the least diverse microbial community among the samples.

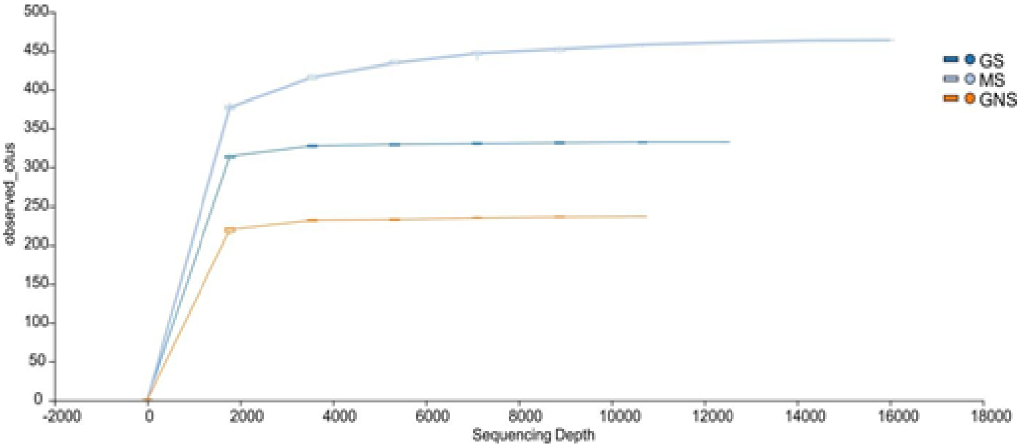

Figure 3. Rarefaction curve of GS, GNS and MS. Peat soil with oil palm vegetation (GS), mineral soil planted with oil palm (MS), and fern vegetation soil (GNS). (*Kurva rarefaksi GS, GNS, dan MS. Tanah gambut dengan vegetasi kelapa sawit (GS), tanah mineral yang ditanami kelapa sawit (MS), dan tanah vegetasi pakis (GNS)*).

Taxonomic composition of microbial

A total of 366 bacterial species were identified across the samples. MS soil had the highest number of taxa (225), followed by GS (168), and GNS (73). At the order level, dominant bacterial groups in MS included Acidobacteriales (9.28%), Subgroup 2 (6.92%), Ktedonobacteriales (6.39%), Solibacterales (6.27%), and Rhizobiales (5%). In GS soil, the predominant orders were Elsterales (9.3%), Frankiales (6.02%), Rhizobiales (6%), Solirubrobacteriales (5.07%), and Corynebacteriales (3.01%). GNS soil was primarily dominated by Acidobacteriales (14.9%), Acidobacteriae (9.4%), and Elsterales (9.15%).

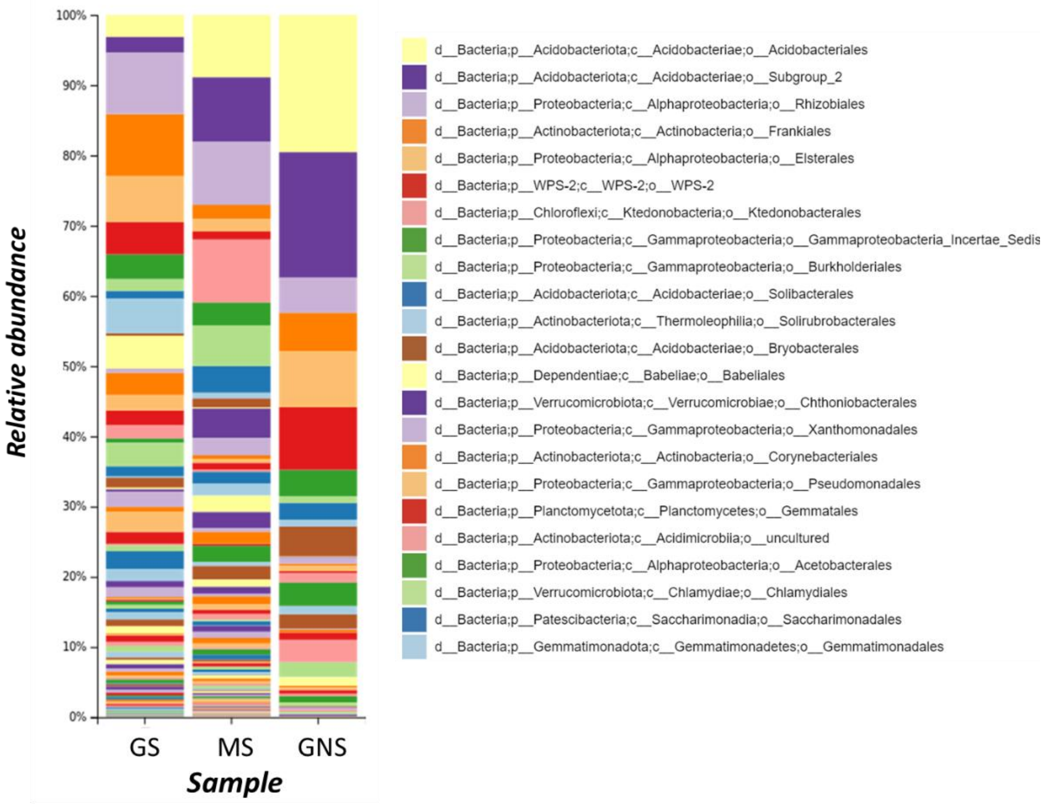

Figure 4. Relative abundance of ordo level in the soil samples. Peat soil in oil palm area (GS), fern vegetation soil (GNS), and mineral soil planted with oil palm (MS) (*Kelimpahan relatif tingkat ordo dalam sampel tanah. tanah gambut di area kelapa sawit (GS), tanah vegetasi paku-pakuan (GNS), dan tanah mineral yang ditanami kelapa sawit (MS)*).

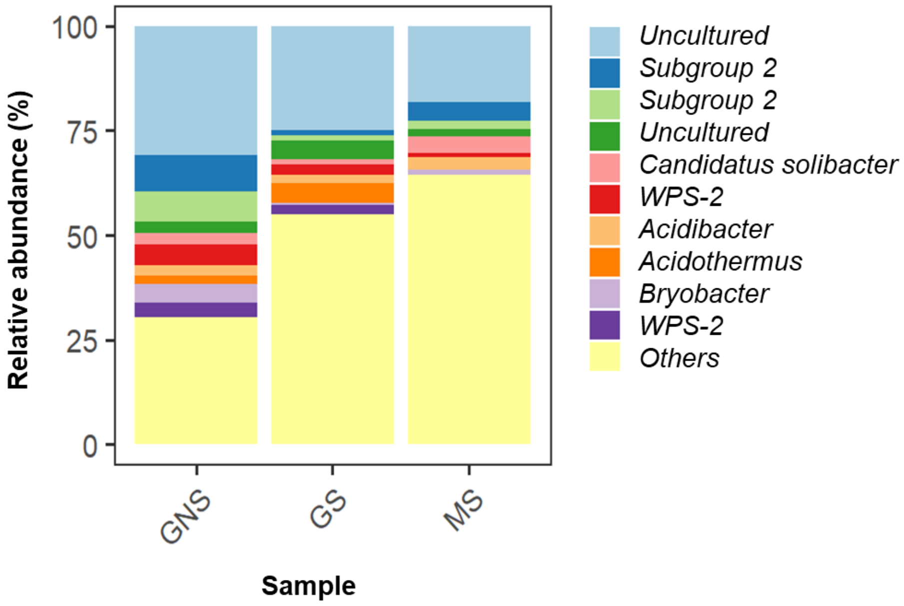

Figure 5. Relative abundance of top 10 genus level in the soil samples. Peat soil in oil palm area (GS), fern vegetation soil (GNS), and mineral soil planted with oil palm (MS) (*Kelimpahan relatif 10 terbaik tingkat genus dalam sampel tanah. tanah gambut di area kelapa sawit (GS), tanah vegetasi paku-pakuan (GNS), dan tanah mineral yang ditanami kelapa sawit (MS)*).

The bacterial community composition showed that the genus uncultured dominated all sites (Figure 5), accounting for 54.98% in GS, 51.39% in MS, and 44.39% in GNS. In contrast, Subgroup 2 were markedly enriched in MS (12.67% and 5.20%, respectively) and GNS (12.41% and 10.55%) but remained minor in GS (2.78% and 2.74%). Candidatus Solibacter was a prominent genus in MS

(11.48%) but much lower in GS (2.87%) and GNS (3.70%), suggesting a microhabitat-specific association with MS soils.

Similarly, Acidibacter showed increased abundance in MS (8.24%) relative to GS (4.68%) and GNS (3.60%). Genera uniquely enriched in GNS included Bryobacter (6.60%) and WPS-2 (7.25%), both showing more than five-fold increases compared to GS and MS. Additionally, WPS-2 was present in GS (5.24%) and GNS (4.91%) but entirely absent from MS.

Conversely, Acidothermus was exclusively found in GS (10.58%) and absent in MS and GNS. These findings highlight distinct genus-level signatures, with several taxa uniquely enriched in MS or GNS, indicating niche-specific bacterial assemblages.

DISCUSSION

The highest concentration of organic carbon was found in GNS soil, followed closely by GS, whereas MS showed the lowest value. This pattern may be attributed to vegetation cover; GNS and GS are enriched with ferns and oil palm, respectively, which contribute to higher organic matter accumulation. In contrast, the lack of substantial vegetation in MS reduces its capacity to retain carbon. Previous research supports the vital role of vegetation and land use in determining soil organic carbon (SOC) stocks at regional levels (Billings *et al*., 2021; Esteban & Jobbágy, 2000). Vegetation influences SOC through organic input, root biomass, and microbial interactions. However, dense understory layers such as mosses or ferns may hinder SOC decomposition and inhibit forest regeneration (Begerow *et al*., 2010).

A similar trend was observed in the cellulose and lignin content, with the highest percentages in GNS and GS, and the lowest in MS. This observation aligns with earlier studies that reported a preferential breakdown of polysaccharides in the upper peat layers, while recalcitrant compounds such as lignin tend to accumulate in deeper horizons (Tfaily *et al*., 2013; Treat *et al*., 2014; Kuder & Kruge, 1998).

Although MS soil demonstrated the highest microbial diversity based on alpha diversity indices, GS and GNS also supported diverse bacterial communities. The reduced diversity in peat soils may be due to limitations in root penetration, leading to microbial concentration in upper layers. In mineral soil, the broader root distribution of oil palm facilitates extensive interactions with soil microbes. This finding is consistent with previous studies suggesting that soil mineral composition can shape microbial community structures (Carson *et al*., 2009).

Interestingly, microbial diversity in peat soils cultivated with oil palm (GS) was comparable to that in undisturbed peat (GNS), suggesting that well-managed plantations may not necessarily reduce microbial richness. In fact, our findings show that microbial abundance in GS slightly exceeded that of GNS. Dominant phyla observed across all samples: Proteobacteria, Actinobacteria, Firmicutes, and Acidobacteria, are consistent with findings from both culture-dependent and independent studies (Ayob & Kusai, 2021; Kraigher *et al*., 2006; Danilova *et al*., 2016).

Moreover, the site's irrigation management could have contributed to maintaining microbial activity. Earlier studies noted that groundwater levels and peat depth significantly affect microbial composition and abundance (Ayob & Kusai, 2021). Vertical stratification of bacterial phyla, particularly Proteobacteria and Firmicutes, has been detected in oil palm plantations, with richness often highest at 0–30 cm depths (Wibowo *et al*., 2022). Our findings highlight the critical ecological role of sustaining diverse bacterial populations in peatland ecosystems, which are essential for nutrient cycling, organic matter decomposition, and overall soil health. Maintaining this microbial diversity supports ecosystem resilience, particularly under land-use changes such as oil palm cultivation. The results further indicate that, with appropriate management practices—such as minimal soil disturbance, reduced chemical inputs, and the adoption of sustainable farming techniques—oil palm plantations can coexist with a robust and functional soil microbiome. This underscores the need for integrating biodiversity conservation into agricultural strategies to ensure both productivity and long-term environmental sustainability.

CONCLUSION

This study shows that soil type and vegetation strongly affect physicochemical properties and microbial diversity. Peat soils under ferns (GNS) and oil palm (GS) contained more organic carbon, cellulose, and lignin, while mineral soil (MS) supported the highest microbial diversity, likely due to better root-soil interactions. Microbial richness in GS was comparable to GNS, indicating that well-managed oil palm cultivation can sustain biodiversity. Dominant taxa remained consistent across sites. These findings underscore the importance of responsible agricultural practices in maintaining soil health and microbial diversity in peat ecosystems.

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AUTHOR CONTRIBUTIONS

HW, HBP, S, DT: collecting research data, drafting the article, final revision of the manuscript; HW, HM, SA, DT: Creating research concept, final revision of manuscript; GWP, HW, BS, SS: Create research concepts, draft articles, revise manuscripts.

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