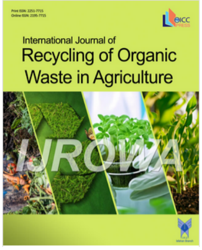

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Assessing microbial biodiversity in oil palm seedlings using oil palm waste compost mixed media

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Abstract:

Purpose: The oil palm nursery plays a pivotal role in ensuring optimal replanting materials for sustained productivity. However, conventional practices that rely exclusively on inorganic fertilizers can degrade soil over time, highlighting the need to reassess planting materials. This study examines the impact of incorporating compost into soil media on oil palm seedling development in primary nurseries.
Method: The study compared microbial biodiversity in soil media with compost amendments by employing DNA sequencing to assess microbial communities. The analysis focused on bacterial species richness and the prevalence of key phyla, including *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, and others.
Results: The compost-amended media (T2) demonstrated significantly higher microbial biodiversity, with 60,769 sequences compared to 45,741 sequences in media containing only soil and inorganic fertilizer (T1). This compost-enriched media notably increased bacterial species richness, particularly benefiting nutrient-cycling bacteria such as *Proteobacteria* and *Actinobacteria*. While *Acidobacteria* showed sensitivity to compost, other phyla like *Firmicutes*, *Planctomycetes*, and *Chloroflexi* remained stable under the nutrient dynamics of elevated organic content. Streptomyces, known for its metabolic versatility, dominated across all media compositions. The co-occurrence of *Bacteroidetes* and *Acidobacteria* in compost-treated soils suggested pH stability that supports plant growth and ecosystem resilience.
Conclusion: Integrating compost into nursery media significantly enhances microbial biodiversity and dynamics, particularly favoring beneficial nutrient-cycling bacteria. This suggests that compost amendments can serve as a viable pathway for improving oil palm nursery practices, promoting sustainable soil management, and ensuring long-term productivity.

Keywords: Compost; Biofertilizer; Beneficial microbes; Microbial diversity; Oil palm nursery

1. Introduction

The oil palm (*Elaeis guineensis*) is a cornerstone of tropical agricultural economies, particularly in Malaysia, which commands 44% of global palm oil exports, including by-products (Hassan et al., 2024). This industry spans 4.49 million hectares, yielding 17.73 million tons of palm oil, underscoring its vital economic significance. Meeting this demand necessitates optimal planting practices, including meticulous care within nurseries, which serve as the developmental hub for plantlets for up to 12 months prior to field transplantation. Ensuring high-quality plantlets demands intensive care encompassing nutrient provision, irrigation, light management, and pest and disease control (Salamat et al., 2019).

In oil palm seedling cultivation, current practices for fertilization and planting mediums are designed to optimize seedling health and productivity. Fertilization involves the use of balanced fertilizers with a specific ratio of nitrogen (N), phosphorus (P), and potassium (K), such as the common 12-6-27 composition (Purnomo et al., 2022). Micronutrients like calcium (Ca), magnesium (Mg), and trace elements are also included to prevent deficiencies. Fertilizer application begins in the month of planting and is repeated every 15 days, typically using slow-release granules and occasionally foliar sprays to address any nutrient imbalances. For planting mediums, a mix of peat, perlite, and compost is commonly used to ensure good drainage, aeration, and nutrient content, with a pH range of $4.7-5.1$ being ideal (Rosenani et al., 2016). Seedlings are often grown in poly bags or containers filled with this medium, designed to prevent waterlogging and facilitate effective drainage (Salamat et al., 2019). Recent trends in the industry include a shift towards organic and sustainable materials to manage nutrient application more sustainably (Supriatna et al., 2023). These practices collectively aim to provide a strong foundation for the healthy growth of oil palm seedlings and the success of future plantations.

Critical to plant development within nurseries is the choice of planting medium, a factor acknowledged to profoundly influence oil palm growth (Radin et al., 2018). Traditionally, the industry has relied on topsoil supplemented by inorganic fertilizers due to topsoil scarcity. However, this practice has contributed to soil acidification and diminished fertility (Khatun et al., 2017; Ding et al., 2023). The presence of plant growth-promoting rhizobacteria (PGPR) such as *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacteria*, *Rhizobium*, *Erwinia*, and *Flavobacterium* is enhanced when inorganic fertilizers containing nitrogen, phosphorus, and potassium (NPK) are applied (Compant et al., 2019). These bacteria act as biofertilizers by improving nutrient bioavailability to plantlets, thereby increasing plant growth and production via nitrogen fixation and potassium solubilization. However, optimal NPK fertilizer proportions are imperative, as excessive application can disrupt the delicate balance of microbe-plant interactions, leading to media degradation (Ajeng et al., 2020). Additionally, the conventional practice of layering second soil contributes to nursery media deterioration due to its low organic matter content.

In response to these challenges, integrating compost into soil pre-mixtures has emerged as a promising strategy for enhancing microbial diversity and soil health within oil palm nurseries. Compost, derived from organic matter through controlled decomposition, represents a valuable source of nutrients and microbial inoculants, fostering a dynamic ecosystem conducive to plant growth and development. Anaerobic sludge from palm oil mills and other waste products, such as empty fruit bunches (EFB), offer a sustainable alternative (Baharuddin et al., 2009; Baharuddin et al., 2010). These materials are ideal for composting into soil amendments due to their high organic matter content and essential nutrients like NPK. This practice has shown to enhance soil fertility significantly, crucial for fostering optimal growth conditions for oil palm seedlings (Salamat et al., 2019). Recycling nutrients in this way has shown promise as an approach to increase soil fertility, water retention, microbial activity, cation exchange capacity (CEC), nitrogen and phosphorus content, and overall soil health (Han et al., 2016). Studies have indicated that nurseries employing mixed media comprising topsoil and EFB compost yield comparable results to those utilizing topsoil with conventional fertilizer applications (Xiong et al., 2015). The utilization of EFB in nurseries has demonstrated enhanced soil chemical properties and vegetative growth (Ovie et al., 2015). Additionally, the method and quantity of fertilizer application significantly impact root and shoot morphology, with microbial assistance playing a crucial role in bolstering oil palm seedling vigor and development (Ashraf et al., 2017).

Soil nutrients and microbiota are essential for plant health and productivity, where their roles in the early growth of oil palm seedlings (*Elaeis guineensis*) under nitrogen, phosphorus, and potassium (NPK) fertilizers has recently reported (Ding et al., 2023). Findings show that NPK fertilizers enhanced the growth of copiotrophic bacteria such as *Pseudomonadota* and *Bacteroidota* in normal soil. In contrast, soil sterilization reduced microbial diversity and altered microbiota composition, adversely affecting seedling growth. Many beneficial microbes were depleted in sterilized soils, emphasizing the importance of assessing soil microbiomes for optimizing fertilizer recommendations and improving plant health.

This evidence that harnessing the symbiotic relationship between plants and compost-contained microbes holds promise for enhancing nutrient acquisition and fostering microbial activity in oil palm nurseries, thus optimizing plant growth. This was further confirmed by Kirkman et al. (2022), showcasing the microbial community found in the rhizosphere of oil palm seedlings was evidenced to be vital for nutrient cycling, disease control, and overall soil fertility. Therefore, it is critical to comprehend the effects of compost application on the composition and diversity of microorganisms in the nursery environment to gain the most from the cultivation of oil palm. This research focuses on soil composition and fertilizer treatments to understand how compost-enriched mixed media biofertilizers affect microbial diversity and community structure in oil palm nurseries. Furthermore, by assessing the compatibility of compost-enriched biofertilizers with standard nursery practices, we aim to offer practical recommendations for sustainable and environmentally conscious oil palm cultivation. By filling this specific gap, this study seeks to provide a foundation for improving current nursery practices, contributing to more resilient and productive oil palm plantations, and addressing global concerns related to sustainable agricultural practices and environmental conservation.

2. Materials and methods

Location and materials

The temperature varied from 24 to 33 °C throughout the investigation, which was conducted at Ladang 10, Universiti Putra Malaysia (coordinate: 2.991778925465053, 101.714618615345). Seri Hulu Langat Palm Oil Mill provided the pressed-shredded EFB, while FELDA Trolak Palm Oil Mill supplied the POME anaerobic sludge. The Dura x Psifera oil palm seeds were purchased from FELDA Agricultural Services Sdn. Bhd.

Compost production

Thorough mixing of pressed-shredded Empty Fruit Bunches (EFB) with thickened Palm Oil Mill Effluent (POME) anaerobic sludge, at a 1:1 v/v ratio, initiated the composting process (Baharuddin et al., 2009; Zainudin et al., 2013; Dzulkurnain et al., 2017). This pre-mixture underwent an extended period of composting, spanning up to 40 days, and was periodically monitored to guarantee its full maturation. The composting procedures were meticulously executed following established protocols, ensuring optimal conditions for microbial activity and compost development.

Temperature was monitored daily, ensuring it remained within the optimal range of 55 – 65 °C for effective microbial activity. Moisture content was maintained between 50 – 60%, measured twice weekly, and adjusted by adding water or dry materials as necessary. The pH levels were checked weekly, kept within the ideal range of 6.5 – 8.0, and adjusted using lime or sulfur if deviations occurred. Aeration was facilitated through turning the compost pile every 3 – 4 days to ensure sufficient oxygen supply for aerobic microbes, thus preventing anaerobic conditions that could hinder the composting process.

Media formulation and preparation

The media formulation utilized a 1:1 weight ratio of soil to compost, with 1 kg each. The experiment comprised several treatments, as detailed in Table 1. The table outlines the composition of different treatment mixtures used in the experiment. The C15 mixture consists solely of compost, while S100 is made up of 100% soil before any treatment is applied. S50 is a pre-treatment mixture containing an equal 50:50 ratio of soil to compost. Treatment 1 (T1) involves 100% soil combined with 100% inorganic fertilizer. In Treatment 2 (T2), a 50:50 mixture of soil and compost is used with 100% of the standard amount of inorganic fertilizer. Treatment 3 (T3) maintains the same soil-to-compost ratio as T2 but reduces the inorganic fertilizer to 75%. Treatment 4 (T4) further decreases the inorganic fertilizer to 50%, while Treatment 5 (T5) reduces it even more to 25%, all while maintaining the same 50:50 soil-to-compost ratio. The oil palm seedlings were fertilized in the main nursery of FGV Agri Services Sdn. Bhd. using the standard methods, which involved applying an inorganic fertilizer called NPK Yellow (15:15:6). Each week, beginning in week 1, the application rates were 10 g per plant, increasing to 15 g per plant in week 2, 20 g per plant in weeks 3 to 5, and 30 g per plant in weeks 6 to 8 (Salamat et al., 2019).

Understanding the impact of various compost-inorganic fertilizer pre-mixtures on microbial diversity in oil palm nurseries is critical for optimizing agricultural sustainability and enhancing crop productivity (Keni et al., 2023). Microorganisms in soil are pivotal for nutrient cycling, organic matter decomposition, and soil structure maintenance, directly influencing soil health and fertility crucial for supporting robust oil palm seedling growth. By investigating the effects of these pre-mixtures (T1 to T5) on microbial community dynamics, it may refine nutrient management strategies to improve nutrient availability for oil palms while minimizing reliance on synthetic fertilizers.

Table 1 shows the different amounts of inorganic fertilizer added to five different polybag media, which were all prepared from a combination of 100% Serdang series topsoil and compost (in a volume ratio of 50% topsoil: 50% compost). After the compost had matured, a four-replicated randomized complete block design (RCBD) was used for the experiment. Polybags were spaced 3 ft. × 3 ft. apart in a triangular pattern, as suggested by Nemergut et al. (2010). In polybags measuring 38 cm × 45 cm, one oil palm seed was planted, ensuring equal volume of media per polybag. The seedlings received irrigation twice daily via a drip system and underwent manual weeding. During each sampling event, four replications were employed to generate composite samples consisting of ten seedlings per replication. Soil samples for microbial community analyses (0 to 5 cm) were gathered using an ethanol-cleaned trowel and sieved through a 4 mm screen. Equal-weight subsamples from each of the four replicate plots were blended thoroughly to create a single composite sample for each trial entry at the

Table 1. Oil palm nursery media treatment and soil composition with various compost-inorganic fertilizer composition pre-mixtures.

Treatment	Composition of pre-mixture
C15	Compost only
S100	100% soil (before treatment)
S50	50% soil : 50% compost (before treatment)
T1	100% soil + 100% inorganic fertilizer
T2	50% soil : 50% compost + 100% inorganic fertilizer
T3	50% soil : 50% compost + 75% inorganic fertilizer
T4	50% soil : 50% compost + 50% inorganic fertilizer
T5	50% soil : 50% compost + 25% inorganic fertilizer

sampling time. These composite samples were then stored at −20 °C after collection (Baek et al., 2020).

DNA extraction

Following the instructions provided by Mo Bio Laboratories (Carlsbad, CA, USA), the POWERSOILTM SterivexTM DNA Isolation Kit was used to carry out the procedure (Zainudin et al., 2017). The protocol began with the collection of 40 soil samples, each weighing 10 g. The sample in the bead tube was mixed with 500 µL of lysozyme (100 mg/mL) and incubated at 37 °C for 30 min to facilitate hydrolysis. DNA purification was performed using the phenol and ethanol precipitation method. Specifically, 100 µL of extracted DNA was mixed with 100 µL of phenol:chloroform alcohol solution in a 25:24:1 ratio in a 1.5 mL centrifuge tube. The mixture was vortexed and centrifuged at 10,000 × g for 5 min. The upper phase was transferred to a new 1.5 mL centrifuge tube, and 100 µL of chloroform alcohol solution (24:1 ratio) was added and centrifuged at 10,000 × g for 5 min. The upper phase was again transferred to a new 1.5 mL centrifuge tube, where 10 µL of 3 M sodium acetate and 80 µL of 2-propanol were added. The solution was gently mixed and centrifuged at 10,000 × g for 30 min. The supernatant was removed, and 1 mL of cold 70% ethanol was added to the tube. The solution was centrifuged again at 10,000 × g for 30 min. The supernatant was decanted, and the precipitated DNA was air-dried for 10 min. Finally, the dried DNA was dissolved in 100 µL of Tris-EDTA buffer.

The purity and integrity of the purified DNA were evaluated through the utilization of a Nanodrop® ND-1000 Spectrophotometer (Thermo Scientific Inc., DE, USA), measuring absorbance at 260 nm and 280 nm to determine the A260/A280 ratio, which is indicative of DNA purity. Additionally, the absorbance at 230 nm was checked to assess the presence of potential contaminants. The mean A260/A280 ratio was 1.85 ± 0.05, with a range of 1.75 to 1.95, and the mean A260/A230 ratio was 2.1 ± 0.1, with a range of 1.9 to 2.3, indicating high purity. Then, until further investigation, the genomic DNA that had been isolated was kept at −20 °C.

High-throughput 16S rRNA sequencing

The desired region from the collected DNA was amplified using the primers that target the V4-V5 region: forward primer 515F (5′-GTGCCAGCMGCCGCGG-3′) and reverse primer 907R (5′-CCGTCAATTCMTTTRAGTTT-3′) (Su et al., 2024). For PCR reaction, 50 ng of cDNA template was used with a final volume of 25 µL, included 10 µM of both forward and reverse primers, 10x Taq buffer, and Taq polymerase (Bio Labs), 2 mM dNTPs, and 25 mM $MgSO_4$ (Toyobo). The PCR amplification process involved 35 cycles with the following parameters: an initial denaturation step at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 50 °C for 60 s, and extension at 72 °C for 90 s. A final extension step was performed at 72 °C for 10 min to ensure complete elongation of the DNA strands. Each cycle was conducted in a Thermo Fisher Scientific PCR machine with a temperature accuracy of ±0.2 °C.

After amplification, the PCR products were purified using the Macherey-Nagel purification system (Düren, Germany). The purification process involved a recovery rate of approximately 90% of the amplicons. Post-purification, the amplicons were prepared for sequencing using the Nextera XT DNA Library Preparation Kit, adhering to the Illumina protocol for library construction. The prepared libraries were quantified using the Qubit dsDNA HS Assay Kit (Life Technologies, Oregon, USA), with an average concentration of 1.8 ± 0.3 ng/µL across samples, which is within the optimal range for sequencing.

Processing high-throughput data

The purified PCR products were sequenced using an Illumina MiSeq system (San Diego, CA). Post-sequencing, the output was processed using Quantitative Insights into Microbial Ecology 2 (QIIME2) (Caporaso et al., 2010). Sequences were filtered and sorted according to sample-specific tags, and sequences shorter than 300 base pairs were excluded from further analysis (Zainudin et al., 2017). Taxonomic classification of the sequences was performed using the Ribosomal Database Project (RDP) classifier in conjunction with the Greengenes database v13.8 (DeSantis et al., 2006). The RDP classifier assigned taxonomic labels to 85% of the reads with a confidence threshold of 80%, providing a detailed taxonomic profile for each sample.

For alpha diversity analysis, the De novo OTU picking pipeline in QIIME2 was used to cluster high-quality reads into operational taxonomic units (OTUs) at a 97% sequence similarity threshold. An average of 450 OTUs per sample was identified. Shannon diversity indices were calculated for each sample, with values ranging from 2.5 to 4.5, indicating varying levels of microbial richness and evenness across samples. Rarefaction curves were generated, reaching asymptotes at approximately 90% of the maximum read depth, suggesting sufficient sampling depth.

Beta diversity was assessed using the Bray-Curtis dissimilarity metric to compare microbial community composition across samples. The Bray-Curtis dissimilarity indices ranged from 0.2 to 0.6, reflecting varying degrees of dissimilarity between sample pairs. Principal Coordinate Analysis (PCoA) was performed using the dissimilarity matrix, with the first two principal coordinates explaining 60% of the variance in microbial community composition. PCoA plots illustrated clear clustering of samples based on their treatment groups, indicating significant differences in microbial communities (Jones et al., 2009; Wang et al., 2012).

3. Results and discussion

Diversity and richness of the bacterial communities across all treatments

The integration of compost into soil formulations for oil palm nursery management offers multiple benefits, notably a decreased dependency on synthetic fertilizers and a favorable impact on microbial activity. Compost, abundant in organic matter and key nutrients like nitrogen, phosphorus, and potassium, enriches the soil when mixed, providing a sustainable and slow-release nutrient source (Farid et al., 2019). Recycling nutrients such as nitrogen, phosphorus, and potassium (NPK) through composting anaerobic sludge and EFB waste from palm oil mills offers a sustainable alternative, potentially enhancing soil fertility and improving planting conditions (Farid et al., 2019). This enrichment diminishes the need for synthetic fertilizers, promoting a more environmentally friendly approach.

Moreover, compost enhances soil structure, improving water retention, aeration, and drainage (Norrrahim et al., 2022). These enhancements facilitate superior root development and nutrient absorption by oil palm seedlings while minimizing nutrient leaching, thereby optimizing the efficiency of inorganic fertilizers, as evidenced in our prior research (Salamat et al., 2019). By utilizing compost in nursery management, dependency on synthetic fertilizers decreases, nurturing beneficial soil microbes.

The microbial richness in compost can function as a biofertilizer, enhancing plant growth and productivity through processes like nitrogen fixation and potassium solubilization. (Omara et al., 2022). The application of compost has been shown to cause major changes in the architecture of microbial communities, introduce new microorganisms that survive over time, and modify the networks and key taxa of soil microorganisms (Heisey et al., 2022). On the other hand, the use of synthetic fertilizers did not have a significant effect on the overall composition of microorganisms in the soil. However, it decreased the complexity of the microbial network and caused changes in the dominant taxa. This emphasizes the important role of organic matter fertilization in shaping soil microbiomes and supporting soil health via the use of organic fertilizers.

To assess the impact of compost-enriched mixed media on soil biodiversity within oil palm nurseries at a small scale, diverse diversity indexes were employed. Data was subsampled at random from 0 to 80,000 readings to investigate these effects using rarefaction curves. Fig. 1 shows flattened curves at low sequencing levels, which means that the samples were adequately represented for microbial activities in the media and that the sequencing effort was enough to capture most of the diversity (Aguirre-Von-Wobeser et al., 2018). Based on our research, the two treatments with the greatest levels of microbial biodiversity were T1 (100% soil with 100% inorganic fertilizer) and T2 (50% soil: 50% compost with 100% inorganic fertilizer). The greater richness observed in mixed soil with compost compared to oil palm media with 100% soil at all rarefaction sampling depths indicates the enriching effect of mixed media on soil bacterial species richness.

These findings were consistent with the number of sequences, which quantitatively measures the amount of genetic material sampled from the microbial community, which was analyzed using MiSeq gene amplicon data across all samples. The sequence counts varied across treatments, with C15 containing 5,812 sequences, T2 with 60,769 sequences, T1 with 45,471 sequences, T3 with 12,672 sequences, T4 with 11,374 sequences, T5 with 9,882 sequences, S100 with 6,249 sequences, and S50 with 8,509 sequences. Samples T1 and T2 have notably higher numbers of sequences compared to the others, suggesting potentially richer or more diverse bacterial communities in these treatments. Conversely, samples C15 and S100 have lower numbers of sequences, indicating potentially less diverse bacterial communities. These libraries effectively reflected the majority of 16S rRNA sequences in each sample, as validated by the Fastq quality check tool in the QIIME2 process. The quality values ranged from 90 to 99%.

Dynamic of bacterial community across all treatments

Relative abundance of the dominant bacterial phyla

The identification of nine phyla from all samples is depicted in Fig. 2. Among these, *Proteobacteria*, *Actinobacteria*, *Chloroflexi*, *Acidobacteria*, *Firmicutes*, TM7, *Planctomycetes*, *Verrucomicrobia*, and *Bacteroidetes* exhibited dominance in the community composition of both composts. Between 80 and 90% of all readings belonged to only five phyla. *Actinobacteria*, *Proteobacteria*, *Chloroflexi*, *Acidobacteria*, and *Firmicutes* were the most numerous phyla among these communities.

These phyla are prevalent in compost-rich environments because of their essential functions in breaking down lignocellulose, cycling nutrients, and producing antimicrobial substances. Research has shown that *Actinobacteria* have a crucial function in microbial enzymatic deconstruction of recalcitrant compounds like lignin and cellulose, hosting a wide range of enzymes that may degrade lignocellulose

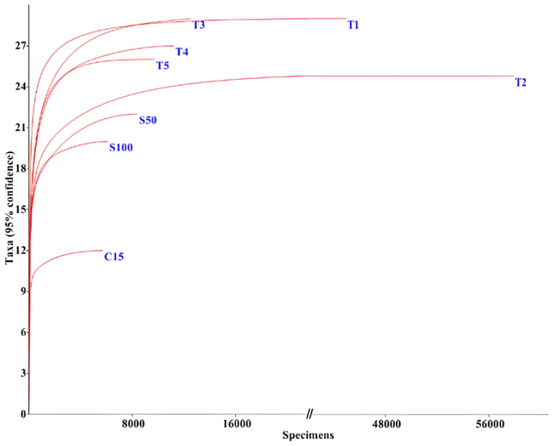

Figure 1. Rarefaction curves of microbiomes present in all treatment media.

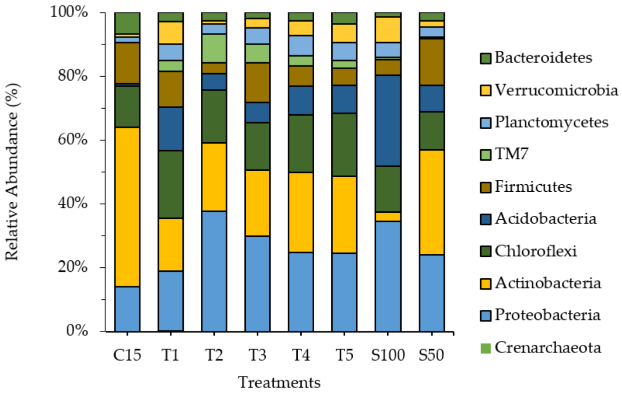

Figure 2. Bar plot of the relative abundance of dominant phyla across all treatments.

(Wang et al., 2016). Moreover, *Proteobacteria*, *Firmicutes*, and *Chloroflexi* are abundant in compost ecosystems, playing a significant role in the decomposition of organic waste and the recycling of nutrients. A study by Tumuhairwe and Tenywa (2018) evidenced that nutrient cycling relies on *proteobacteria* to fix atmospheric nitrogen for plants by degrading organic materials. *Firmicutes* also were shown to break down cellulose and hemicellulose, contributing to nutrient cycling and producing antimicrobial compounds like bacteriocins, which may affect compost microbial community dynamics and nutrient availability (Wang et al., 2022). The presence of *Chloroflexi* and *Acidobacteria* suggests its participation in the composting process, possibly participating in the decomposition of complex organic compounds and are involved in the cycling of carbon and nitrogen transformations in various environments (Wang et al., 2016). The abundance of these bacterial phyla in compost medium emphasizes their importance in facilitating the composting process, converting nutrients, and contributing to the general functioning of the microbial community in compost ecosystems.

All these dominant phyla are notably prevalent in T1 media, characterized by the highest soil and compost composition with 100% inorganic fertilizer application. This suggests that in nutrient-rich environments, microbial species experience minimal competition, allowing dominant phyla to flourish to a similar extent. Interestingly, despite a consistent supply of inorganic fertilizer, reduction in soil and compost compositions by 50% in T2 media caused changes in microbial community dynamics, resulting in alterations in the richness of dominant phyla. *Actinobacteria*, *Proteobacteria*, and *Chloroflexi* persisted as dominant phyla, whereas TM7 exhibited a marginal increase, accompanied by a reduction in the population sizes of other phyla. This indicates the resilience of these dominant phyla in nutrient-deficient environments, suggesting their capacity to adapt to the scarcity of nutrients, especially since less inorganic fertilizers are being used (Zheng et al., 2020).

The discrepancy observed in phyla' abundance across media with varying fertilizer concentrations from T2 to T5 media, despite constant in soil and compost composition, highlights the intricate interplay of nutrient dynamics, microbial competition, and soil properties. Lower fertilizer concentrations, even in compost-rich media, may have limited crucial nutrient availability for dominant phyla, while heightened microbial competition, exacerbated by lower fertilizer levels, could have restrained their proliferation. There are noticeable trends as the numbers of *Proteobacteria*, *Firmicutes*, and TM7 in the medium drop when the quantity of inorganic fertilizer is reduced. However, *Bacteroidetes*, *Verrucomicrobia*, and *Chloroflexi* exhibit increases, while *Planctomycetes* and *Acidobacteria* persist under conditions of fertilizer deprivation.

The observation that microbial biodiversity measurements were higher in treatments where soil was mixed, particularly in T1 and T2, emphasizing the importance of incorporating organic input and promoting microbial diversity for enhancing soil health and fertility. Mixed media applications not only provided additional organic matter but also fostered the establishment of diverse microbial communities critical for sustaining soil productivity and ecosystem resilience (Martínez-García et al., 2018).

Proteobacteria are a diverse group of bacteria known for their metabolic versatility and adaptability to various environments (Zhou et al., 2020). It is observed that *Proteobacteria* were highly abundant in the C15 treatment where compost was utilized, as well as in as well as in pre-treatment samples (S100 and S50). The prevalence of *Proteobacteria* in compost-rich environments suggests their proficiency in utilizing the wide array of organic compounds present, thereby contributing to their dominance and proliferation.

Actinobacteria are known for their role in decomposing organic matter and their ability to produce bioactive compounds (Fang et al., 2023). In the study, they are prevalent in C15 and S50, indicating a preference for environments with organic inputs like compost, suggesting roles in organic matter decomposition and nutrient cycling. *Acidobacteria* were notably abundant in media lacking compost, such as S100 and T1. This observation suggests a potential sensitivity of *Acidobacteria* to the presence of compost, possibly due to competition with other microbial groups favored by the compost-rich environment or due to specific nutritional requirements not met in compost-amended media. *Firmicutes* were prominent in C15, T1, T3, and S50, showing adaptability to organic-rich environments and potential resilience to high inorganic fertilizer application. Additionally, other phyla such as TM7, *Planctomycetes*, *Verrucomicrobia*, and *Bacteroidetes* were observed, each likely playing specific roles in soil ecosystems such as nutrient cycling or organic matter degradation.

Discussion on the bacterial community shift with each treatment

The intricate dynamics of microbial communities within oil palm nurseries were explored through detailed analysis, as depicted in Fig. 3 (A-H). This investigation provided useful insights into the impact of diverse soil treatment methods on soil health and ecosystem functioning by investigating the distribution and dominance of several bacterial phyla across different treatments.

The dominance of *Acidobacteria* in T1 media could be correlated with their preference for acidic conditions and oligotrophic nature. Media enriched with organic fertilizer typically have lower pH levels, especially nitrogen (N) because it forms ammonium ions in soil that increases soil acidity. Contrarily, a neutral or slightly alkaline environment is characteristic of soil-rich media, which usually have higher pH values caused by the breakdown of organic materials. Hence, the acidic pH in T1 may be conducive, whereas T2 media, which *Acidobacteria* prefer for their acidic conditions, does not appear to be as favorable (Jog et al., 2016). Notwithstanding, compost-rich T2 media are often nutrient-rich due to the abundance of organic matter, providing ample resources for other microbial competitors. In compost-rich conditions, *Acidobacteria*, which are oligotrophic, may have less dominance than in soils with lower pH levels and less organic matter content because other microbial species provide better competition (Castro et al., 2013). In

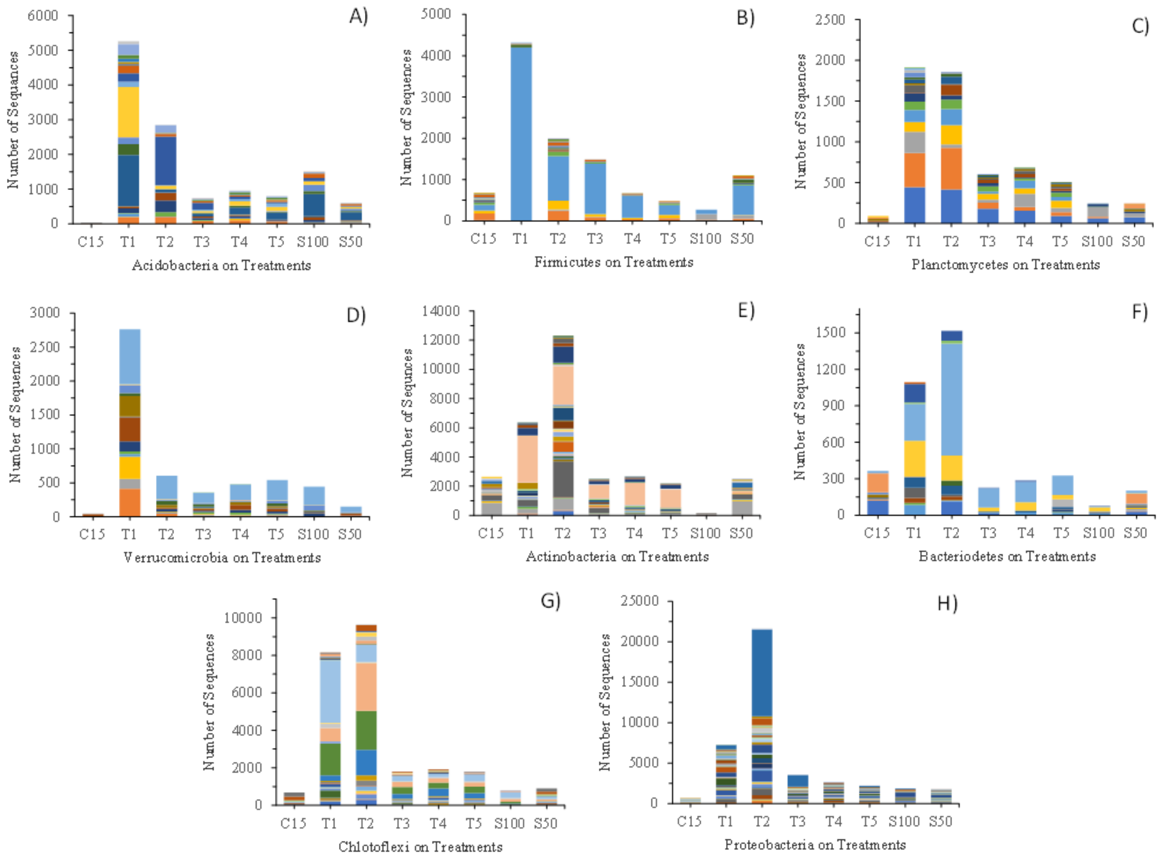

Figure 3. Distribution of phyla sequence abundance across each treatment.

T1 media, characterized by the highest compost composition, *Acidobacteria*'s pivotal role in efficiently decomposing complex plant-derived polysaccharides like cellulose and lignin may contribute to its dominance (Adileksana et al., 2020). Much like *Verrucomicrobia*, these microbes thrive in nutrient-rich environments with elevated concentrations of NPK, even though they are often found in soils with poor fertility (Navarrete et al., 2015). In T1 media supplemented with inorganic fertilizer, while nutrients are also abundant, may favour the growth and dominance of *Verrucomicrobia* due to their competitive advantage in utilizing certain nutrients over other microbial taxa.

Acidobacteria and *Verrucomicrobia* were also predominantly found in media with elevated soil composition, specifically in T1 and S100 media. Soil serves as a rich reservoir of nutrients essential for microbial growth, and its elevated presence in the media results in heightened nutrient availability (Miransari, 2013). This advantage promotes bacterial proliferation, leading to an overall increase in their abundance. Additionally, in environments characterized by abundant nutrient availability, microbial species engage in heightened competition for resources. In environments with limited nutrient availability, bacteria may encounter formidable competition from other microbial groups, such as fungi or archaea, which possess adaptations that enable them to thrive under nutrient-poor conditions (Kim et al., 2021). These competitive interactions shape the microbial community, favoring *Acidobacteria* and *Verrucomicrobia* proliferation while inhibiting others.

The dominance of *Actinobacteria* and *Bacteroidetes* in S50 media, characterized by lower soil composition, can be attributed to their adaptability to nutrient-poor environments. This is due to the fact that both species are capable of displaying copiotrophic and oligotrophic traits in response to the environmental stresses they encounter. Copiotrophs, on the one hand, thrive in environments with an abundance of nutrients, while oligotrophs specialize in nutrient-poor environments in order to avoid competition (Morrissey et al., 2016; Stone et al., 2023; Su et al., 2024). These bacterial phyla are known to thrive in conditions with limited nutrient availability, such as those found in media with reduced soil content. Their abundance noticeably rose in T2 media, despite a 50% decrease in soil compared to T1 media. This increase in nutrient availability, likely facilitated by compost and inorganic fertilizer in T2 rather than S50 media, facilitated their proliferation. Additionally, *Actinobacteria* and *Bacteroidetes* flourish in T2 soil due to their involvement in nutrient turnover and nitrogen cycling.

The initial dominance of *Firmicutes* in S50 media, indicative of its metabolic versatility to thrive under nutrient-poor conditions. *Firmicutes* are known to possess metabolic pathways enabling them to efficiently utilize limited resources, making them well-suited for environments with reduced soil composition (Aguirre-Von-Wobeser et al., 2018). Regardless, the increased soil composition and added nutrients from compost and inorganic fertilizer in T1 media, which showed an increase in *Firmicutes* abundance. This highlights its ecological plasticity in capitalizing on the additional nutrients, thereby outcompeting other microbial taxa and reinforcing its dominance. *Planctomycetes* and *Chloroflexi* show stability across soil and compost composition variations (between T1 and T2, S100 and S50 treatments), but exhibit a more pronounced response to shifts in nutrient availability (between T2 to T5 treatment). The phylum *Chloroflexi* is known for its participation in the biogeochemical chlorine cycle, thereby indicating its role in transforming chlorine compounds within the media (Krzmarzick et al., 2012b).

Proteobacteria's strong response to shifts in compost availability in T2 media indicates their ability to adapt quickly to increased nutrient influx. This is signified by their roles in biogeochemical cycles like carbon, nitrogen, and sulfur cycling (Song et al., 2023). In nutrient-deficient environments like T2 media, their contributions are vital for sustaining microbial communities and facilitating nutrient cycling. However, in media with nutrient supplementation, they may be surpassed by other phyla better suited to utilize the specific nutrients provided. This shift in microbial dominance could serve as an indicator of ecosystem health within compost-based media.

This research is consistent with previously documented results on the most prevalent groups of organisms after compost is applied. Xu et al. (2023) found that in saline soils treated with compost made from sheep dung and maize straw, *Proteobacteria*, *Firmicutes*, and *Actinobacteria* were the most prevalent phyla. The application of compost led to a notable rise in the abundance of *Actinobacteria* and *Chloroflexi* by 7.6% and 6.6%, respectively. In contrast, the prevalence of *Firmicutes* reduced from 25.12 to 18.77%, indicating that compost could change the makeup of microbial communities. Moreover, composts made from a combination of municipal biowaste and wood chips have been shown to have comparable bacterial compositions (Partanen et al., 2010). The composts also exhibited a high occurrence of bacteria belonging to the phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. The consistent findings across several research highlights the influence of compost application on microbial communities, highlighting changes towards certain phyla that have important functions in nutrient cycling, breakdown of organic waste, and general soil health. The findings of this study corroborate existing literature, demonstrating that compost application can significantly influence microbial community dynamics by promoting certain bacterial phyla while reducing others, despite different agricultural settings. This shift in microbial composition is critical for enhancing soil fertility and resilience, which are vital for sustainable agricultural practices.

Fig. 4 prominently displays the results of a heatmap analysis that examined the bacterial community change at the genera level. Given *Actinobacteria*'s prominence as the predominant phylum in this study, notably in compost-rich media treatments, a thorough exploration of the intricacies within this dominant group becomes essential. The predominant community in nutrient-deprived media (T3, T4, and T5 samples) consisted mainly of genera closely related to *Streptomyces*, *Nocardioides*, and *Amycolatopsis*. On the contrary, nutrient-rich media (T1) showed dominance by *Streptomyces*, *Mycobacterium*, and *Nocardia*. However, there was a shift in *Actinobacteria* genera in T2 media due to a 50% reduction in soil and compost. *Rathayibacter* emerged as dominant alongside *Streptomyces* in this altered environment. This suggests that *Streptomyces* tends to dominate in nearly all treated media, except C15 with fully compost, showcasing its versatility in nitrogen-fixing abilities crucial for nutrient cycling. This positions them as promising candidates for natural fertilizers (Wang et al., 2012; Krzmarzick et al., 2012a). Additionally, it has a long history of use and demonstration in enhancing plant growth, mineral availability, and nutrient absorption. Biofortification techniques may also take use of its metal mobilization capabilities to improve seed mineral nutrients including silica (Si), iron (Fe), and zinc (Zn) (Jog et al., 2016; Gopalakrishnan et al., 2020).

Figure 4. Distribution of *Actinobacteria* genera by treatment in a heatmap layout.

Correlations between pH and dominant phyla

As shown in Fig. 5, there was a clear relationship between the number of bacteria and soil pH. *Acidobacteria*, in particular, exhibited a negative relationship. The bacterial community composition was shown to be greatly affected by soil pH, with the most diverse communities found in soils around neutral pH (Fierer et al., 2012). This is because in neutral to alkaline soils with higher pH levels, microbial activity and enzyme function are generally more favourable for carbon mineralization (Li et al., 2007). A compost-rich environment fosters the proliferation of carbon mineralization due to the accelerated decomposition of organic matter facilitated by the heightened microbial activity in these soils.

To put it into perspective, the relationship between *Acidobacteria* and *Bacteroidetes* populations and carbon min-

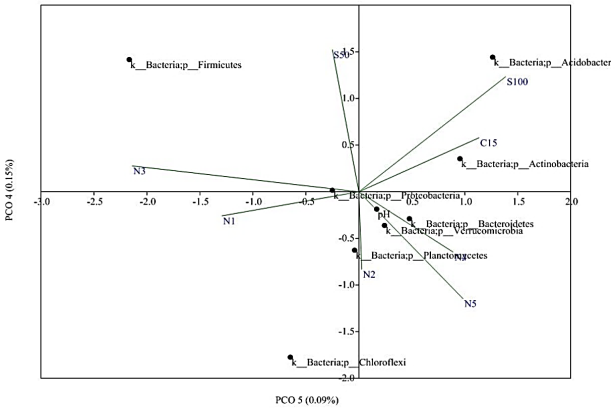

Figure 5. PCoA plot of the Bray-Curtis dissimilarity between dominant phyla to pH.

eralization is often inverse due to their differing pH preference, as evident in Fig. 5. *Acidobacteria* are typically oligotrophic, flourishing in environments characterized by limited nutrient availability, including such as soils low in organic carbon, and exhibiting resilience to pH variations (Castro et al., 2013). In such environments, where carbon mineralization rates may be lower due to reduced microbial activity, *Acidobacteria* populations tend to increase. Conversely, *Bacteroidetes* are often copiotrophic, favoring nutrient-rich environments with higher pH levels (Haindl et al., 2021; Song et al., 2023). Regardless of pH-dependent, the coexistence of *Bacteroidetes* and *Acidobacteria* in compost-amended soils serves as an indicator of good soil health.

Potential limitations and prospects of compost-based mixed media

Based on the results that showed compost mixed media improving soil microbial communities, the potential of this method in oil palm nurseries seems to be encouraging. Anticipate a wider acceptance as the advantages for soil health and plant development gain more recognition. Nevertheless, there is still potential for additional improvement in the technique by acknowledging and resolving key shortcomings.
Fluctuations in compost quality can lead to inconsistent seedling development, and the potential introduction of diseases and pests poses a threat to plant health (Azim et al., 2018). Environmental factors, such as the sensitivity of compost to moisture and temperature, can impact its effectiveness (Biswas et al., 2018). Economic considerations, including the costs of production and transportation, can also be significant (Pergola et al., 2018). Furthermore, the presence of competing microorganisms in the compost may inhibit the growth of beneficial bacteria, and the use of compost-based media can disrupt native soil microbial communities (Fuchs, 2010). Depending only on one kind of compost may limit the applicability of results, since compost materials differ significantly in terms of nutritional composition, microbial variety, and physical properties. Therefore, the findings drawn from this research may not accurately represent the many circumstances found in real-life situations (Palaniveloo et al., 2020). These limitations highlight the necessity for improved compost formulations and management strategies to maximize the benefits while minimizing potential drawbacks in the context of oil palm nurseries.

4. Conclusion

The study's analysis showed that the mixed media incorporating compost (T2) had the highest microbial biodiversity, with 60,769 sequences, compared to 45,741 sequences in the media without compost (T1) and 6,249 sequences in the soil-only media (S100). This indicates a significant enrichment in soil bacterial species richness in the compost-enhanced treatment. The predominant phyla, *Actinobacteria*, *Proteobacteria*, *Chloroflexi*, *Acidobacteria*, and *Firmicutes*, collectively constituted 80 to 90% of all sequences. *Proteobacteria* and *Actinobacteria* thrived prominently in compost-amended environments, implicating their pivotal roles in decomposition and nutrient cycling dynamics. *Streptomyces*, a versatile *Actinobacteria* genus, exhibited dominance across varied media compositions, highlighting its potential for enhancing natural fertilization and nutrient cycling processes. *Acidobacteria* displayed sensitivity to soil pH levels, while *Bacteroidetes* showed affinity for nutrient-rich conditions. While compost integration significantly enhanced microbial activity in oil palm nurseries, it was found not to obviate the requirement for synthetic fertilizers. These findings highlight the study's contributions to advancing knowledge in agricultural microbiology and sustainable nursery management, emphasizing the potential of compost-based strategies to augment soil biodiversity and fertility while synergistically supporting conventional nutrient management practices.

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results and approved the final version of the manuscript.

Availability of data and materials
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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