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APPLICATION OF OLD OIL PALM TRUNKS AFFECTS THE GROWTH PERFORMANCE OF OIL PALM SEEDLINGS

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ABSTRACT

*Oil palm plantations generate substantial biomass waste, primarily old oil palm trunks (OPT), during replanting. As part of a sustainable plantation management program, old OPT are returned to the plantation and released nutrients into the soil for new oil palm seedlings. However, whether this method improves soil nutrient levels is unclear. In this study, we investigated the effects of OPT on oil palm seedling growth and soil microbial communities. The plant height, chlorophyll content, leaf area, and biomass weight were low in seedlings grown in soil containing OPT [44.3 cm, 44, 278.9 cm^2, 19.6 g (dry shoot), and 14.9 g (dry root)]. Similar results were obtained for seedlings grown in soil containing cellulose or soil containing OPT and fertiliser. Leaf nitrogen, phosphorus, and potassium contents were similar in seedlings grown in soil amended with OPT and control seedlings. However, the calcium content was significantly lower in seedlings grown in soil containing OPT (0.424 ± 0.004%) than in control seedlings (0.496 ± 0.006%). Metagenomic analysis of soils showed that three lignocellulose-degrading fungal genera (*Chaetomium, Mortierella, *and* Staphylotrichum*) were abundant in soil containing OPT. Thus, the return of OPT promotes the growth of lignocellulose-degrading microorganisms and decreases fertiliser nutrient availability.*

Keywords: *Chaetomium*, lignocellulose-degrading microorganisms, oil palm trunk, plant growth performance, soil metagenomics.

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INTRODUCTION

Palm oil is regarded as one of Malaysia's primary sector industries and is a major agricultural product exported globally. Oil palm productivity decreases every 20 to 25 years, and new oil palm seedlings are replanted (Corley & Tinker, 2015). The old oil palm trees are felled to clear the land, and are left in the plantation to decompose. Some plantations use the chip and windrow method (*e.g.*, Guan Soon Plantation, Alor Pongsu, Perak, Malaysia), whereas some use the pulverisation technique, such as Kuala Lumpur Kepong Berhad (Kuala Lumpur Kepong Berhad, 2018), whereby entire old oil palm trunks (OPT) are pulverised into smaller pieces that are then spread on the cleared land (Pulingam *et al.*, 2022). OPT left using the windrow method takes at least 2 years to decompose completely, whereas those that are pulverised and spread onto soil degrade within approximately 1 year, which helps to shorten the fallow period. The decomposing old OPT is considered to serve as mulch (Sung, 2016) while simultaneously replenishing soil nutrients as they break down (Pulingam *et al.*, 2022).

However, during the decomposition period, various environmental issues may arise. For example, OPT eventually become a breeding site for pests, such as *Oryctes rhinoceros* (Manjeri *et al.*, 2014), and the fungus *Ganoderma boninense*, which causes basal stem rot disease (Gorea *et al.*, 2019).

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Additionally, the high carbon-to-nitrogen (C:N) ratio (Lin *et al.*, 2019) lengthens the decomposition period (Akratos *et al.*, 2017) and deprives the soil of nitrogen, which is needed for plant growth (Lin *et al.*, 2019). Usually, a C:N ratio between 25 and 35 is optimal for agricultural applications; however, OPT has a high C:N ratio of 155 (Loh *et al.*, 2013) and it takes a long time for this ratio to decrease. Therefore, the substantial OPT biomass waste generated during replanting and left in oil palm plantations to decompose may negatively affect plant growth. The application of OPT results in decreased growth performance compared with empty fruit bunches (Mohammed *et al.*, 2014). It also alters the soil microflora and decreases the availability of nutrients, such as nitrogen, calcium and magnesium, for plant uptake (Uke *et al.*, 2021). Nitrogen promotes leaf growth. The leaf is one of the most important plant organs because it is a nitrogen storage site that synthesises amino acids. Calcium is an important component of cell walls and membranes, thereby contributing to the structural integrity of cells. This helps to maintain the physical barriers that protect plants from pathogens, leading to enhanced immunity. Magnesium is a component of chlorophyll, which is essential for photosynthesis. A magnesium deficiency may cause chlorosis.

The soil type and quality of organic matter affect the diversity and abundance of soil microflora (Li *et al.*, 2018; Savy *et al.*, 2020). The soil and rhizosphere microorganisms are bio-indicators of soil quality because they are sensitive to the smallest changes in abiotic conditions such as environmental stress and soil perturbation. In addition, land fertility and environmental biodiversity are affected by the cultivation of oil palm (Ashton-Butt *et al.*, 2018; McGuire *et al.*, 2014).

To understand how OPT breakdown affects soil health and microbial diversity within a plantation, it is important to determine the effect of applying OPT fibre directly to the soil, mimicking the pulverisation technique, on oil palm seedling growth and the soil microbial community. In this study, we explored the effects of OPT fibre and the contrasting effects of fertiliser applications on the growth of oil palm seedlings. We experimented with various soil amendments: Unamended soil (T1), soil + OPT fibre (T2), soil + cellulose (T3), soil + fertiliser (T4), soil + OPT fibre + fertiliser (T5), and soil + cellulose + fertiliser (T6). Soil metagenomics analyses were conducted to explore the diversity and abundance of soil microflora. Thus, this study provides valuable insights into the sustainability and productivity of oil palm plantations by determining the effects of OPT biomass waste on the growth of oil palm seedlings and the soil microbial community.

MATERIALS AND METHODS

Polybag Assays

To establish the different treatments, dried OPT fibre, cellulose powder as cellulosic material, and fertiliser was added to the soil that was collected from an MPOB-certified oil palm nursery in Alor Pongsu, Perak, Malaysia. Fresh OPT fibre (>20 years old) was air-dried under the sun until the moisture content reached <10% (w/w) to prevent any microbial growth (Lai *et al.*, 2014). Fifty-five four-month-old oil palm seedlings (*Elaeis guineensis*) were used for the polybag assay, which was conducted in an open space at an oil palm seedling distributor in Alor Pongsu (5°04′37.8″N, 100°35′52.4″E). Twin Arrow Fertiliser (TAF) (15:15:6:4/N:P:K:Mg+TE), a commercial fertiliser from TAF Sdn. Bhd., Selangor, Malaysia and cellulose fibre powder (Arbocel®, Rettenmaier, Germany) were used. The polybag assay was further modified according to our previous study (Uke *et al.*, 2021). The four-month-old seedlings were transplanted into polybags (33×35 cm) containing 10 kg soil. The six treatments were prepared as shown in *Table 1*. In total, 55 polybags of oil palm seedlings were prepared, with 10 replicates for Treatments 1, 2, 4, and 5; seven replicates for Treatment 3; and eight replicates for Treatment 6. All polybags containing seedlings were arranged in rows with spacing of 0.75×0.75 ×0.75 m following the "Code of Good Nursery Practice for Oil Palm Nurseries" published by the Malaysian Palm Oil Board (MPOB) (MPOB, 2015). A standardised average of at least seven replicates of oil palm seedlings per treatment was used to validate the results. The treatments were applied using a factorial and randomised design. The amount of OPT fibre added to each polybag (200 g) was determined using Equation (1). The OPT fibre was added to the soil and mixed thoroughly. This was based on an initial dry weight of OPT of approximately 1,315 kg. However, the amount was reduced by half to ensure that the OPT fibre could fit in each polybag. Seedlings were grown under a natural photoperiod and were watered daily. Fertiliser was added to certain treatments of oil palm seedlings (Treatment 4, 5 and 6) at the dose recommended by the manufacturer at the start of the experiment.

$$\textit{Dry weight of OPT} \times \textit{Number of oil palm trees per hectare} \times \textit{Area of polybag} \quad (1)$$

Plant Measurements

The plant height, relative leaf area, relative chlorophyll content [as determined using a Soil

TABLE 1. TREATMENTS APPLIED TO OIL PALM SEEDLINGS OVER A FOUR-MONTH GROWTH PERIOD

Treatment label	Treatments	Amounts of treatments applied (g/polybag)
T1	Unamended soil (control)	No amendments
T2	Oil palm trunk (OPT) fibre	200 g
T3	Cellulose powder	200 g
T4	Fertiliser	7 g
T5	OPT fibre + Fertiliser	200 g of OPT fibre + 7 g fertiliser
T6	Cellulose + Fertiliser	200 g of cellulose + 7 g fertiliser

Plant Analysis Development (SPAD) meter], soil pH, and biomass weight were measured to determine the growth performance of oil palm seedlings in the various treatments (Uke *et al.*, 2021). The plant height was measured from the ground level up to the top shoot (Adip *et al.*, 2022). The relative leaf area was determined by multiplying the length and width of the fully extended leaves (length×width). The relative chlorophyll content was measured using a Minolta SPAD-520 Plus meter (Konica Minolta Sensing, Inc., Osaka, Japan). A soil pH and moisture tester (Model DM-15, Takemura Electric Works Ltd., Tokyo, Japan) was used to determine the soil pH. Measurements were taken at the start of the experiment and every month for four consecutive months. The biomass dry weight was determined at the end of the four-month growth period by oven-drying the shoots and roots to a constant weight at 70°C.

Leaf Nutrient Analysis

The leaf samples were analysed by Applied Agricultural Resources Sdn. Bhd., Selangor, Malaysia. The nitrogen, phosphorus, potassium, calcium, and magnesium contents of oil palm seedling leaves were determined. Kjeldahl's method was used to analyse the total nitrogen content and the acid digestion method was used to analyse the phosphorus content. The potassium content was determined using the ammonium acetate leaching method followed by atomic absorption spectrophotometry using a Perkin Elmer Analyst 100 Atomic Absorption Spectrometer (Waltham, MA, USA).

High-throughput Sequencing

DNA extraction. Soil from each treatment was used for DNA extraction. Before soil samples were collected, 1 to 2 cm of the top soil layer was removed. For each treatment, soil was collected through mixing. The samples were immediately stored at -20°C until DNA extraction was conducted. Total genomic DNA was extracted from 250 mg soil using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA concentration was determined using a NanoDrop One Microvolume UV-VIS Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). A polymerase chain reaction (PCR) targeting the internal transcribed spacer 1 (ITS) region of the fungal rRNA gene was conducted using the primers ITS1F_KYO1 (CTHGGTCATTTAGAGGAASTAA) and ITS2_KYO2 (TTYRCTRCGTTCTTCATC) as well as Tks Gflex DNA Polymerase Low DNA (TaKaRa, Kusatsu, Japan). The DNA amplification conditions were as follows: Initial denaturation at 94°C for 1 min; 35 cycles of 98°C for 10 s, 55°C for 15 s, and 68°C for 1 min; and final extension at 68°C for 5 min. The reaction products were separated on a 2% (w/v) agarose gel, stained with ethidium bromide, and purified (Ungkulpasvich *et al.*, 2021). After diluting to 25 pM, all sample libraries were combined for an automated emulsion PCR and bead enrichment and then loaded onto 510™, 520™, and 530™ chips for sequencing using an Ion Chef™ instrument (Thermo Fisher Scientific). The libraries were sequenced using the Ion GeneStudio™ S5 System with 850 flow cycles.

Operational taxonomic unit clustering and community analyses. The raw sequences generated by the Ion Chef™ instrument were subjected to further filtering and trimming to obtain sequences >150 bp and <300 bp using CLC Genomics Workbench v.23.0.4 and CLC Microbial Genomics Module v.20.1 (Qiagen, Valencia, CA, USA). The UNITE database was used to identify the operational taxonomic units (OTUs) with 97% nucleotide identity (Uke *et al.*, 2021). The software automatically identified and discarded chimeric sequences. The filtered read sets were then grouped into OTUs with 0.02 distance unit cut-offs according to the BLASTN algorithm in the National Center for Biotechnology Information GenBank database. The abundance and diversity of the fungi present in the soil were analysed.

Statistical analysis. Statistical analyses were conducted using SPSS (version 24.0) (IBM Inc., Armonk, NY, USA). Data were subjected to a one-way ANOVA, with means separated using the least significant difference test. Differences were considered significant at $P \leq 0.05$

RESULTS AND DISCUSSION

Effects of Adding OPT Fibre to Soil on Plant Growth Performance

The effects of adding OPT fibre and other substances to soil on plant growth performance (*i.e.*, plant height, relative leaf area, chlorophyll content and biomass) were determined after four months of growth (*Figure 1*). At the end of the 4th month after application (MAA), the height of oil palm seedlings was significantly lower in the OPT fibre (T2) and cellulose treatments (T3) than in the unamended (T1) and fertiliser treatments (T4). Moreover, the growth indexes of seedlings in T2 and T3 were significantly different from those of seedlings in the T1, T4, OPT + fertiliser (T5) and cellulose + fertiliser (T6) treatments. This implied that fertiliser nutrient availability decreased in the presence of OPT fibre and cellulose. Interestingly, the comparison with the T1 revealed plant growth and development were significantly inhibited in the T2 and T3 treatments. Similarly, the relative leaf area and the chlorophyll content of the oil palm seedlings were significantly lower in T2 and T3 treatments than in the other treatments and T1 (*Figure 1b* and *1c*).

The small relative leaf area, homogenous yellowing of leaves, and stunted growth observed in T2 and T3 are symptoms of chlorosis. The lower height of the oil palm seedlings in these treatments, compared with that in T1, was associated with decreases in the chlorophyll content and leaf area. The chlorophyll content (Wen *et al.*, 2019), total nitrogen content, soluble protein content in leaves, and the net photosynthetic rate are all affected by nitrogen availability (Qu *et al.*, 2022). Plants under nitrogen deficiency stress have a reduced photosynthetic capacity (Qu *et al.*, 2022), which eventually leads to poor growth and root development (Kang *et al.*, 2023). Our results also show that the seedlings in T2 and T3 had less fibrous roots than T1 (*Figure 2*). It has been reported that the morphological development of oil palm roots (length, surface area, and volume) is restricted under nitrogen-deficient conditions (De la Peña *et al.*, 2024).

The reduced plant biomass (both shoot and root) in T2 and T3 may be because of microbial nitrogen immobilisation (Uke *et al.*, 2021). The carbon content affects the amount of nitrogen immobilised by the soil microbial community. Microbial biomass and respiration are relatively low under high C:N conditions because of the limited availability of nitrogen for microorganisms, leading to delayed degradation of plant fibre. Crop residues with high C:N ratios include OPT fibre (155) (Loh *et al.*, 2013) and sugarcane bagasse (213) (Bhat *et al.*, 2015). In another study, the application of sugarcane straw affected the growth and development of sugarcane plants, although the yield and quality of sugarcane juice were unaffected (Souza *et al.*, 2020). Our results show that OPT fibre left in the soil eventually decreased the efficiency of soil microbe-mediated degradation of cellulosic biomass waste because of its high C:N ratio. Additionally, it takes approximately two years for OPT to decompose and the high C:N ratio of OPT is expected to decrease during the degradation period. This finding highlights the effects of OPT degradation in soil. Although this study was conducted using polybags, similar effects may be observed in oil palm plantations.

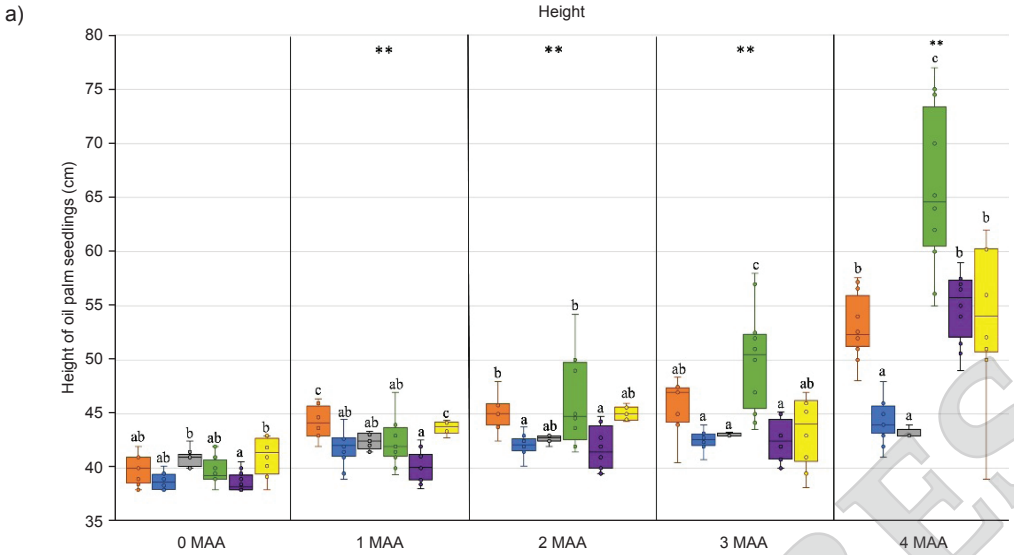
Effects of Various Treatments on Nutrient Contents in Oil Palm Seedling Leaves

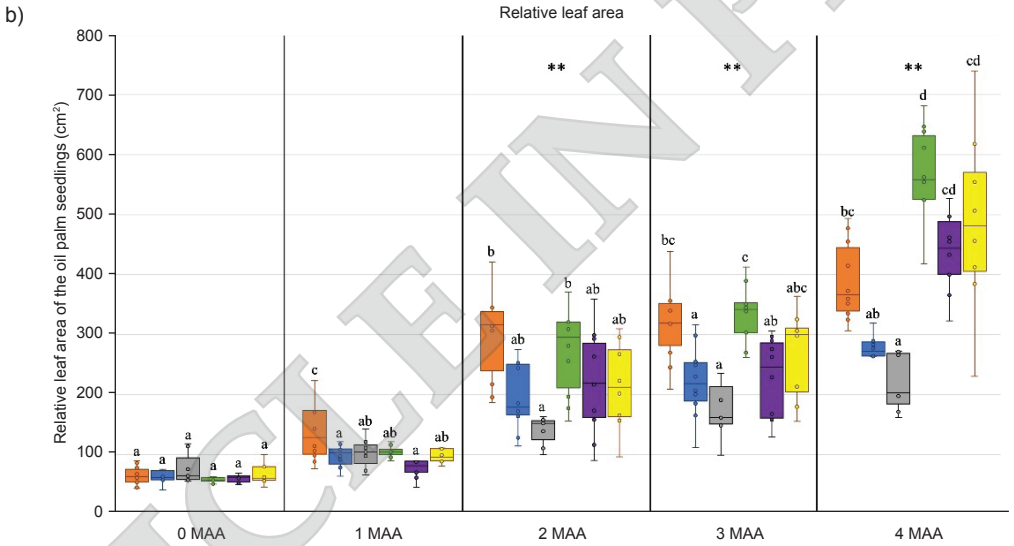
The nitrogen content in oil palm seedling leaves was higher in T2 (2.19 ± 0.01) and T5 (3.41 ± 0.008) than in T1 (1.94 ± 0.01). The calcium content in oil palm seedling leaves was higher in T1 (0.50 ± 0.006) than T2 and T5 (0.42 ± 0.004 and 0.32 ± 0.002, respectively) and the magnesium content was also higher in T1 (0.46 ± 0.004) than in T2 and T5 (0.43 ± 0.005 and 0.38 ± 0.002, respectively) (*Table 2*). These results show that the addition of fertiliser to the soil amended with OPT fibre did not increase the availability of nutrients for new oil palm seedlings.

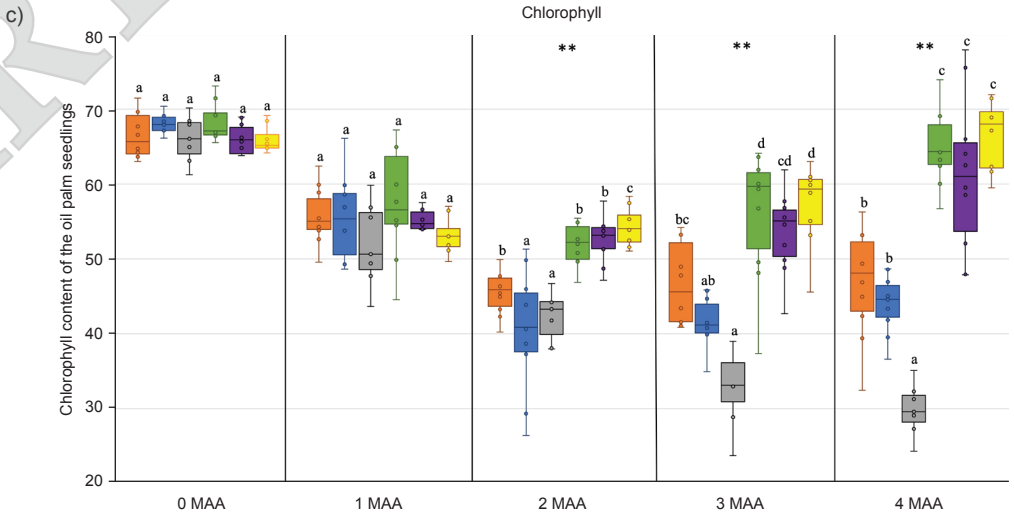
According to the results of the foliar analysis of oil palm seedlings, we speculated that the OPT fibre may have negatively affected leaf growth because of the low contents of certain nutrients, including nitrogen, magnesium, and calcium. This is supported by the overall plant growth parameters (*i.e.*, height, chlorophyll content, relative leaf area, and shoot and root biomass) that were adversely affected. Similarly, a previous study showed the addition of wheat residue to soil (applied to the soil surface or mixed with the soil) delayed winter wheat seed emergence; the resulting seedlings were shorter than normal and exhibited an abnormal geotropic response, which may reflect the phytotoxic effect of the residue (Wuest *et al.*, 2000). The application of winter wheat residue is similar to the application of OPT in oil palm plantations using the chip and windrow method and the pulverisation technique. Thus, to replenish nitrogen losses in soil, larger amounts of nitrogen fertiliser were required for the new plants to grow and achieve a satisfactory yield.

Effects of Various Treatments on the Soil Microbial Community

Plant growth is affected by soil fertility, which in turn is affected by the soil microbial population. The structure of soil microbial communities determines the quality of soil in oil palm plantations because microbes function as carbon recycling agents







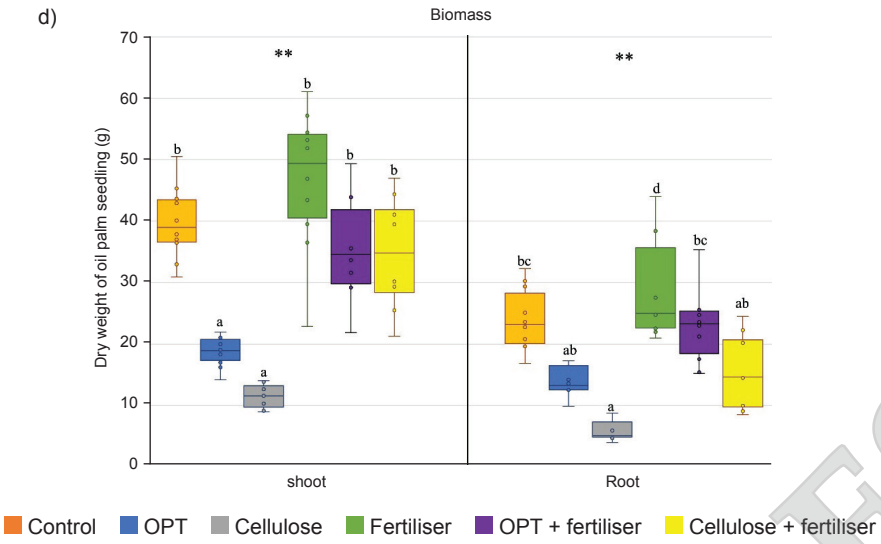


*Figure 1. Growth performance of oil palm seedlings in soil containing OPT fibre, cellulose, fertiliser, OPT fibre + fertiliser, and cellulose + fertiliser. (a) height, (b) leaf area, (c) chlorophyll content, and (d) dry weight of the oil palm seedling shoot and root. Significant differences were observed among the treatments at 4 MAA (ANOVA; F = 22.8; P<0.001 for height, ANOVA; F = 16.9; P<0.001 for relative leaf area, ANOVA; F = 41.1; P<0.001 for chlorophyll content, ANOVA; F = 16.8; P<0.001 for shoot dry weight, ANOVA; F = 8.591; P<0.001 for root dry weight). N = 10 for control, OPT fibre, fertiliser, and OPT fibre + fertiliser, N = 7 for cellulose only, and N = 8 for cellulose + fertiliser. Significant differences among treatments at specific time points are indicated with asterisks (** P<0.001).*

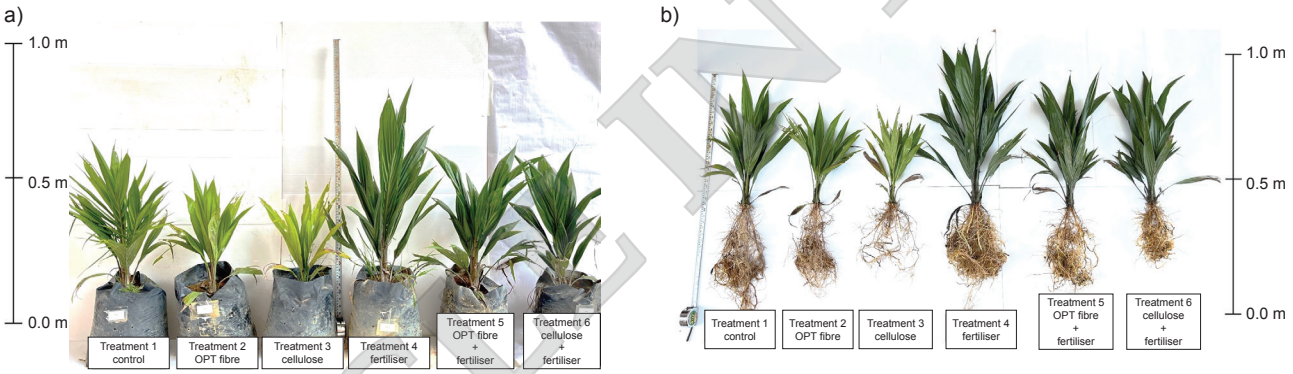


Figure 2. (a) Growth of oil palm seedlings in various treatments. (b) Roots and above-ground parts of oil palm seedlings. Treatment 1: Unamended soil (control), Treatment 2: OPT fibre (200 g), Treatment 3: Cellulose (200 g), Treatment 4: Fertiliser (7 g), Treatment 5: OPT fibre (200 g) + fertiliser (7 g), and Treatment 6: Cellulose (200 g) + fertiliser (7 g).

TABLE 2. NUTRIENT CONTENTS IN OIL PALM LEAVES

Elements	Unamended soil (T1) (%)	OPT fibre (T2) (%)	OPT fibre + fertiliser (T5) (%)
Nitrogen (N)	1.942 ± 0.010^{e}	2.188 ± 0.010^{d}	3.410 ± 0.008^{c}
Phosphorus (P)	0.205 ± 0.001^{c}	0.227 ± 0.001^{b}	0.241 ± 0.0003^{a}
Potassium (K)	1.918 ± 0.008^{e}	2.398 ± 0.030^{ab}	2.294 ± 0.010^{c}
Calcium (Ca)	0.496 ± 0.006^{a}	0.424 ± 0.004^{b}	0.316 ± 0.002^{d}
Magnesium (Mg)	0.460 ± 0.004^{d}	0.432 ± 0.005^{d}	0.382 ± 0.002^{b}

and as a source of nutrients (Situmorang *et al.*, 2016). Microbes that function as carbon recycling agents include bacteria and fungi. Bacteria are active decomposers in soil and are responsible for decomposing dead plants and animals. Fungi are the predominant microbes in the soil microbial community (in terms of biomass) and they metabolise carbon-rich substrates (Wu *et al.*, 2024). Therefore, we analysed the eukaryotic microbial community structure in all the soil treatments (T1-T6). We focused on eukaryotic microbes because analyses of the ITS1 sequence data suggested that fungal OTUs showed the widest variation among treatments. The fungal genera *Chaetomium*, *Mortierella*, and *Staphylotrichum* were most abundant in T2 and T5 (*Table 3*).

TABLE 3. RELATIVE ABUNDANCE OF MICROBIAL SEQUENCES IN DIFFERENT SOIL AMENDMENT TREATMENTS

Abundance	T1.1	T1.2	T2	T3	T4	T5	T6
Chaetomium spp.	484	1, 222	6,050	111	109	23,095	2,465
Mortierella spp.	4,756	4,224	1,445	296	2,549	1,076	91
Staphylotrichum spp.	0	2	4,497	0	0	601	0

Note: T1.1 - unamended soil at 0 MAA; T1.2 - unamended soil at 4 MAA; T2 - OPT only; T3 - cellulose only; T4 - fertiliser only; T5 - OPT + fertiliser; T6 - cellulose + fertiliser.

This observation implies the application of OPT fibre results in a unique soil microflora community composition.

Chaetomium spp. in the *Chaetomiaceae* family has been found in soils amended with compost (Zhang *et al.*, 2017). Members of this genus contain genes encoding enzymes that degrade lignocellulose biomass (Banerjee *et al.*, 2016). *Chaetomium* spp. thrive and reproduce in the presence of lignin (Dicko *et al.*, 2020), which is one of the major components of OPT. Additionally, *Mortierella* spp. accumulate lipids that are produced from sugars that are used as a carbon source (Ruan *et al.*, 2012). OPT (70% moisture) have a high sugar content, especially during its decomposition period (Hanis *et al.*, 2024; Yamada *et al.*, 2010). Therefore, it can be inferred that the high relative abundance of these genera in the soil microflora was because the decaying OPT served as their main carbon source. Despite the abundance of *Staphylotrichum* spp. in the microflora in soil amended with OPT, to date, few studies have explored their role as lignocellulose-degrading fungi. However, one of the species in this genus, *Staphylotrichum longicolleum*, can degrade chitin in wood, sugarcane bagasse, and maize leaves (Ali *et al.*, 2021).

During the decomposition of OPT, the starch in parenchyma cells is degraded and further fermented into sugars by wild endophytic fungi within the trunk (Abdul-Hamid *et al.*, 2015). The high starch and sugar contents (*i.e.,* glucose, fructose, and sucrose) of OPT (Hanis *et al.*, 2024; Yamada *et al.*, 2010) serve as the energy source for the growth and metabolism of microorganisms. Additionally, OPT has a high C:N ratio (Loh *et al.*, 2013) and contains a considerable amount of xylan and cellulose (Uke *et al.*, 2021), which influence and promote the accumulation of lignocellulose-degrading fungi. Thus, the composition of the soil microbial community may be affected by OPT degradation. All of the events that occur in the presence of OPT (De Lima Brossi *et al.*, 2016) increase the abundance and diversity of certain soil microflora; this may result in the decreased availability of nutrients for plants, which was previously reported for corn plants grown in soil amended with OPT residue (Uke *et al.*, 2021). According to soil metagenomics data, *Chaetomium* spp., *Mortierella* spp., and *Staphylotrichum* spp. substantially affect nutrient dynamics. These microorganisms were highly effective at capturing and using available soil nutrients for their growth processes, which further decreased the availability of these nutrients for plants. The ability of these microorganisms to immobilise nutrients suggests that their presence and abundance may significantly influence soil nutrient profiles and, subsequently, plant productivity. These findings were results of previous studies, but they provide specific insights into the microbial species that predominantly affect nutrient availability in the current study.

CONCLUSION

Based on our results, we conclude that leaving the old OPT fibre residue in the plantation soil negatively affects the growth of oil palm seedlings because of soil nutrient deficiencies. Our results indicate that new oil palm seedlings were directly affected and grew relatively poorly (*i.e.,* decreased height, leaf area, chlorophyll content and biomass) because of competition for nutrients with abundant lignocellulose-degrading fungi. In addition, fertiliser nutrient availability decreased in the presence of OPT. Thus, it is highly recommended that OPT be removed from oil palm plantations, even in small quantities, to decrease the amount of fertiliser required and to reduce fertiliser waste. The current practice of leaving OPT in the plantation requires a supplemental fertiliser application to compensate for the deficiency in available nutrients during the decomposition of OPT. To ensure a sustainable oil palm industry, the large amount of biomass in OPT should be used to its utmost potential for the production of value-added products (*i.e.,* bioethanol, bio-pellets, and bioplastics).

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