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# Macronutrient Interactions and Microbial Population in Ultisols and Spodosols Affecting the Incidence of Ganoderma Disease

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#### ABSTRACT

The spread of Ganoderma disease in oil palm plantations is affected by several factors, one of the most significant being the soil type. Different soil types influence the availability of nutrients and moisture, which can affect the growth and spread of Ganoderma. This study investigates the impact of spodosols and ultisols on soil macronutrient dynamics and their effect on microbial populations, ultimately influencing Ganoderma distribution. Using a nested sampling design, we collected data from 120 palms across 411 hectares. We analyzed the impact of soil type (sand, ultisol) on soil pH, organic carbon, macronutrients (P-total, P-Bray, Exchangeable K, Exc-Mg, Exc-Ca), and bacterial and fungal populations at 1 m, 2 m, and 3 m distances from infected and healthy palms. Data analysis employed Two-Way ANOVA. The results reveal that soil pH and organic carbon positively influenced Ganoderma incidence in spodosol soil. At the same time, macronutrients P-total, P-Bray, and K had positive interactions with the disease in both soil types. Conversely,

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cindy.fitriana@id.wilmar-intl.com (Cindy Diah Ayu Fitriana) her.wirianata@gmail.com (Herry Wirianata) astutimaria2000@gmail.com (Yohana Maria Theresia Astuti) farihawilis@gmail.com (Fariha Wilisiani) sukarman.ace@id.wilmar-intl.com (Sukarman) septa.primananda@id.wilmar-intl.com (Septa Primananda) utami.wahyunipuji@gmail.com (Wahyuni Puji Utami) \*Corresponding author Exc-Mg and Exc-Ca showed positive interactions in spodosol soil but negative interactions in ultisol soil. Nitrogen had no significant impact on spodosol soil. Regarding microbial populations, ultisol soil exhibited higher bacterial populations around infected palms (23.4% at 1 m and 12.5% at 3 m). Spodosol soil showed higher bacterial populations further away (2.3% at 1m and 41.3% at 3 m). Fungal populations were higher in ultisol soil compared to spodosol soil for infected palms (27.01 x  $10^6$  cfu/g and 26.00 x  $10^6$  cfu/g, respectively). This study highlights the complex interplay between soil type, macronutrients, microbial populations, and the spread of Ganoderma. These findings inform the development of effective disease management strategies for oil palm plantations.

Keywords: Ganoderma spp, macronutrient, microbes, oil palm, soil type

# INTRODUCTION

Ganoderma "spp" is a soil-borne pathogen in the Basidiomycota Fungal Family (Corley & Tinker, 2015). The fungus can infect plants through roots and airborne basidiospores, capable of degrading lignin, a key plant structural component (Paterson, 2007). Some Ganoderma species act as pathogens in oil palm plants, causing basal stem rot in both lower and upper stem regions. Oil palm plants affected by Ganoderma "spp" tend to be challenging to detect in the early stages of infection. The dispersal of Ganoderma "spp" spores during the initial infection occurs within the plant's tissues, and further attacks manifest as physiological changes (Chong et al., 2017). In the initial stages of Ganoderma infection, physiological changes in the palm are characterized by the formation of more than three spear leaves and withered fronds, and further attacks lead to the development of fruiting bodies on oil palm trees (Pilotti, 2005). The formation of fruiting bodies on oil palm trees has high potential as an active source of inoculum, dispersing spores with wind assistance in the morning and evening. Ganoderma's fruiting bodies (basidia) release spores, visually resembling mist. Spore dispersion originating from basidia formed in the trunk of oil palm trees has the potential to accelerate the spread of the disease in healthy palms (Hamzah et al., 2021). When the palm trunk becomes hollow, the plant reaches a high severity level and finds it difficult to maintain productivity. Ganoderma fungal attacks exhibit internal symptoms, such as a color change from white to brown in the cortex tissue, causing the cortex tissue to become brittle and the trunk to hollow out (Soetopo et al., 2022).

One of the factors influencing the spread of Ganoderma disease in the field is the variation in soil characteristics. The characteristics of different soil types can significantly affect the soil's physical, chemical, and nutritional properties, consequently influencing the growth and development of Ganoderma in oil palm plantations. Soil pore characteristics, such as size, shape, and availability, can impact spore migration within the soil (Aziz et al., 2019). Larger pores can facilitate spore movement, allowing them to travel faster and farther (Gałązka et al., 2017). Widiastuti et al. (2018) discovered a correlation between soil nutrient status and Ganoderma infestation, with potassium (K) and magnesium (Mg) exhibiting significant effects. Goh et al. (2020) emphasize the role of a more diverse soil microbial community (bacteria) in soils with high pH and calcium levels, which can suppress the incidence of the disease. Shariffah-Muzaimah et al. (2020) supported this by demonstrating the potential of Streptomyces sp. GanoSA1, a soil actinomycete, in reducing disease incidence. Rakib et al. (2017) highlight the importance of micronutrients,

especially copper (Cu) and zinc (Zn), in the spatial distribution of Ganoderma species, with lower concentrations of these nutrients in infected areas. Zhang et al.'s (2012) research indicates a positive correlation between the availability of organic matter in the soil and the Ganoderma population.

Several previous studies have identified an interaction between spodosol soil type, pH acidity, and a 25–35°C temperature range as optimum conditions for developing G. lucidum (Kapoor & Sharma, 2014). Rees et al. (2007) found that the disease is more severe in areas with high humidity, while growth is inhibited at high temperatures (>40°C). Soil texture can also impact nutrient availability and soil moisture, influencing spore growth and dispersion (Nazir et al., 2010). Soils with high sand content tend to have good drainage but may struggle to retain water and nutrients (Gerendas et al., 2013; Matichenkov et al., 2020). When nutrients are easily leached or dissolved from spodosol soil, pathogenic microorganisms may face limitations in the nutritional resources required for their growth and development (Matichenkov et al., 2020). However, there is no research on the differences in physical, chemical, and biological soil characteristics around Ganodermainfected and healthy plants in two different soil types (spodosol and ultisol). This study analyzes the differences in physical, chemical, and biological soil characteristics around plants infected with Ganoderma and healthy plants in two soil types, namely Spodosols and Ultisols). Therefore, this study is crucial for analyzing the interrelated factors influencing the vulnerability of oil palm plants to Ganoderma infection. This research is intended to provide recommendations regarding nutrient management for plants in Ganoderma-endemic areas and to offer a general overview of the developmental patterns of Ganoderma in two distinct soil types.

### **MATERIALS AND METHODS**

The research was conducted in oil palm plantations in Wilmar International Plantation Region Central Kalimantan at four locations, each with two different soil type characteristics (Figure 1). Ultisol soil sampling was carried out at MS and KSY, while Spodosol soil sampling was conducted at MS and KKP.

# Materials

The instruments utilized in this research include a thermohygrometer for measuring temperature and humidity, a lux meter for light intensity measurements, a Global Positioning System (GPS) for geographical location, a soil auger for soil sampling, and petri dishes and beaker glasses for sample preparation. A Laminar Air Flow Cabinet (LAFC), autoclave for sterilization, colony counter for bacterial enumeration, orbital shaker, and stirrer lever are employed for biochemical processes. Additional laboratory equipment includes an L-stick, forceps, and a Bunsen burner.



Figure 1. Location of research (Wilmar Plantation, 2023)

The materials comprise Nutrient Agar (NA) and Potato Dextrose Agar (PDA) for microbial growth media, King's B Medium for specific cultivation, and soil and leaf samples. Ganoderma sp. isolates are also included as primary research material.

### Methods

The flow chart of this research is presented in the following Figure 2.

# Soil Sample Selection Technique

The selection of plant samples was carried out using cluster and purposive sampling, with the details provided in Table 1.

The selection of test plant samples, palm in spodosols (PS) and palm in ultisols soils (MS), was obtained from 30 plants in different locations with similar soil characteristics and plant morphology. Diseased plants selected exhibited characteristic symptoms of Ganoderma infection (wilting fronds, spear leaves not opening, and the presence of basidiocarps). Healthy plant samples were chosen 20 meters from the infected trees at three different sampling distances (1 meter, 2 meters, and 3 meters) (Figure 3).

Soil samples taken using an auger from the same category (soil type, plant health category, and distance) were composited into 5 test samples. Each soil sample from 6 different plants with the same category was composited into 1 test sample (Figure 4).

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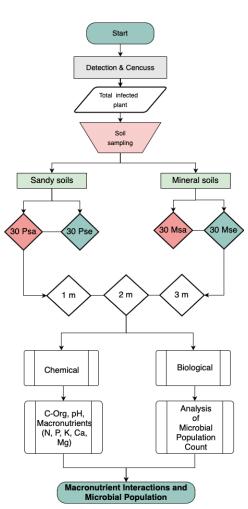
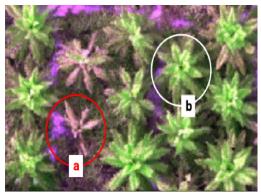


Figure 2. Flow chart macronutrient interactions and microbial population in disease plant and healthy plant

Table 1



*Figure 3.* Illustration of sampling point for: (a) infected palms (b) healthy palms (Internal documentation of Wilmar Plantation Indonesia, 2023)



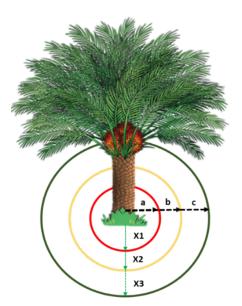
*Figure 4.* Illustration of soil sampling using auger (Internal documentation of Wilmar Plantation Indonesia, 2023)

Soil sampling scheme	for physical	chamical and	hiological analysis
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Sample Specifications	Ssa	Usa	Sse	Use
a. Sampling Location (Location)	30	30	30	30
b. Samples(Nutrient & Microbe Testing)	5	5	5	5
c. Sample Distance (1, 2 & 3) m	3	3	3	3
Total Samples	15	15	15	15

\*Ssa (Diseased palms in Spodosols), Sse (Healthy palms in Spodosols), Usa (Diseased palms in Ultisols), Use (Healthy palms in ultisols)

In this experiment, there are 90 diseased palms (Usa) and 90 healthy palms (Use) in ultisol soil, as well as 90 (Ssa) and 90 (Sse) in spodosol soil. Each soil sample was taken from different depths of 0-30, 30-60, and 60-90 cm, composited based on the sampling distance from oil palm trees, which are 1 m, 2 m, and 3 m at 4 points around the sample plant (Figure 5).



*Figure 5.* Scheme for soil sampling in ultisol and spodosol soils at different depths. (a: 1 meter, b: 2 meters, c: 3 meters, x1: soil depth 10-20 cm, x2: soil depth 20-40 cm, x3: soil depth 40-60 cm)

# Analysis of Soil Macronutrients and Micronutrients

The soil samples collected according to the specified categories in Figures 1 and 2 were analyzed for their macronutrients and micronutrients in the chemical laboratory of the Ecological Management Unit R&D Department at Wilmar Central Kalimantan. Nitrogen analysis in the soil was conducted using the Kjeldahl distillation method, P-bray using the Bray I method, total phosphorus (P-Total) with spectrophotometry using molybdate fanadat reagent, Exc-K, Exc-Ca, and Exc-Mg were analyzed using percolation techniques and detected using tomic Absorption Spectroscopy AAS type AAcle900F.

# Analysis of Microbial Population Count

Microbial Population Analysis on Soil Samples from the Root Zone of Healthy and Diseased Plants was conducted following a modified method based on Chaudhary et al. (2019). The composited soil samples were filtered using a 2-mesh sieve, and then 3 grams of soil were randomly sampled according to the treatment. The 3-gram soil samples were mixed with 300 ml of NB and PDB media in Erlenmeyer flasks and incubated in a shaker at room temperature for four days using Flask shaker orbital incubator Unimax: HEIDOLP. A 10 ml sample from the solution was taken, and serial dilution was performed using a 0.85% NaCl saline solution with incremental dilution from  $10^{-1}$  to  $10^{-6}$ . At each dilution stage, 100 µL was taken and spread-plated on KB and PDA media, followed by incubation at room temperature for four days after inoculation. Different colonies on each petri dish were counted using the following formula. Observations in this test only counted the number of bacterial and fungal populations. The fungi/bacteria analyzed resulted from spread plate

activities in dilutions from 10<sup>-2</sup> to 10<sup>-7</sup>, and the number of single colonies formed on PDA/ KB/NA media in each petri dish was counted. The colony count range for each dilution was 25–250 colonies; if the count was below or above this range, calculations were not performed on the petri dish. Microbial population calculation analysis was based on the formula (Bellali et al., 2019):

$$Cf u/m l(g) = \frac{number \ of \ counted \ cells}{Concentration \ x \ Dillution \ factor}$$
[1]

#### **Data Analysis**

The data analysis used in this research includes the Two-Way ANOVA test, the Mann-Whitney Test, and the Friedman Test. The data analysis from the treatments of this study was performed with the Duncan Multiple Range Test (DMRT) at a significant level of 5%.

#### **RESULTS AND DISCUSSION**

#### Analysis of pH and Organic Carbon on the Development of Ganoderma

The soil pH and organic carbon analysis results indicate that all soil samples fall into the acidic category (Table 2). This analysis aims to understand the development of soil microorganisms in two different host plants with varying plant health categories.

#### Table 2

Soil Type	Plants Condition	Sig	pH-KCl
Ultisols	Diseased (Usa)	0.00	3.680ª
	Healthy (Use)	0.01	3.210 <sup>ab</sup>
Spodosols	Diseased (Ssa)	0.41	4.218 <sup>b</sup>
	Healthy (Sse)	0.00	3.783°

pH analysis results using the KCl method in spodosol and ultisol soil types for diseased and healthy plants

Note: Different letters indicate a significant difference

In general, the pH values around healthy plants in ultisol or spodosol soils tend to be more acidic compared to the soil pH around plants infected with Ganoderma. This condition aligns with the typical characteristics of acidic tropical soils, and plants like palm trees can grow well. The higher pH in soils around infected plants may be due to the specific activities of microbes or pathogens. Some microbes or pathogens can produce acids or reduce the availability of certain nutrients for plants, which generally lowers the soil pH, making it more acidic. However, there are specific conditions where microbial activity can lead to more alkaline soil pH if substances that increase pH are produced. It can occur if the decomposition processes in the soil are reduced; thus, the soil pH tends to remain higher or more alkaline (Supriyanto et al., 2020). The analysis results indicate that the highest organic carbon (C-organic) content, 2.50%, is found in soil samples from the roots infected with Ganoderma (diseased soil). In ultisol soil types, the results differ, with higher C-organic values in the soil area that is not infected with Ganoderma (healthy plants). The lowest C-organic value is observed in the healthy spodosol soil treatment, with a value of 0.95% (Figure 6).

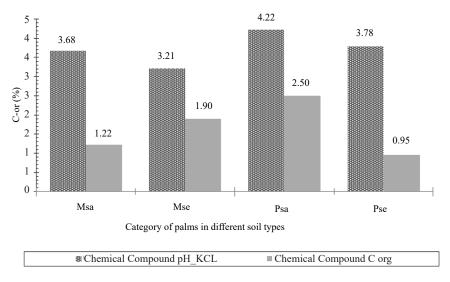


Figure 6. Comparison of pH and C organic values in spodosol soil (Usa & Use) and ultisol soil (Ssa & Sse)

The sandy soil selected as the sampling site belongs to the Spodosols order. Spodosols are characterized by a spodic layer containing a high organic matter content, typically resulting from the accumulation of organic material in the surface layer (Hartati et al., 2021; Santoso et al., 2013). On the other hand, Ultisols tend to form from parent materials that are more acidic and less leached, leading to higher levels of organic matter and lower cation exchange capacity (Fujii et al., 2009). Microorganisms in the soil produce enzymes to decompose organic matter and generate nutrients available for plants and other microbes. The activity of these enzymes is highly influenced by soil pH. Some enzymes operate most efficiently at low pH, while others function optimally at higher pH levels (Reardon et al., 2022).

# The Role of Soil Macronutrients in the Development of Ganoderma

The soil macronutrient contents analyzed in this study include total phosphorus (P total), P-Bray, total nitrogen (N-total), Exc-K, Exc-Mg, and Exc-Ca, as presented in Figure 7.

Analysis of P-Total and P-Bray in Soil with Ganoderma-Infected Plants in Spodosol (Ssa) and Ultisol (Usa) Soils, and Ganoderma-Uninfected Plants in Spodosol (Sse) and Ultisol (Use) Soils. The P-total values were higher in Ssa and Usa than in Sse and Use.

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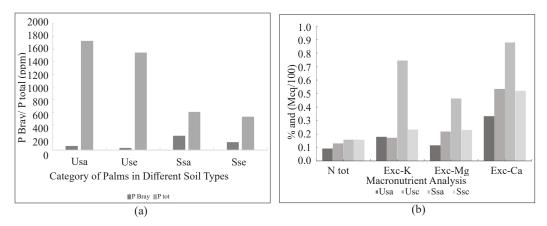


Figure 7. Soil macronutrient analysis in ultisol and spodosol soils, a) Total phosphorus (P-Total) and Bray phosphorus (P-Bray), b) Total nitrogen (N-total), Exc-K, Exc-Mg, Exc-Ca

The P-total value around Ganoderma-infected plants in Usa was 64.8% higher than in Ssa. The variance in P-total around infected plants in ultisol soil was 10.8% higher than healthy plants. Meanwhile, in infected plants in spodosol soil, it was 13.3% higher than in healthy plants. The P-Bray value in Ssa was 71.6% higher than in Usa. The variance in P-Bray around infected plants in spodosol soil was 52.6% higher than healthy plants, whereas in ultisol soil, it was 45.7%. The analysis results show that macronutrient values in spodosol soil are higher than in ultisol soil. Three macronutrient elements in ultisol soil (N-tot, Exc-Mg, and Exc-Ca) were higher in Use than in Usa. Meanwhile, the average nutrient content of Exc-K was higher by 0.1 ppm in Usa and Ssa compared to Use and Sse. Exc-K, Exc-Mg, and Ca values were higher in Ssa and Usa, while there was no difference in nutrient content values for N-total in the soil around sick or healthy plants.

Sandy soil has better drainage than other ultisol soils due to its coarse texture, reducing the risk of waterlogging that can lead to oxidation and phosphorus loss in easily soluble forms (Brown et al., 2017; Byrne et al., 2022). The total phosphorus content in sandy soil potentially tends to be lower than in other ultisol soils; however, phosphorus in sandy soil is more easily accessed by plants and is more available for root absorption (Mayakaduwa et al., 2023). Some plants facing stress or specific pathogen infections can increase the secretion of organic acids or enzymes that alter nutrient availability around the root zone (Castro-moretti et al., 2020). This response can affect the total phosphorus and P-Bray levels in the soil. Phosphorus is one of the essential nutrients Ganoderma requires for growth, cell division, and reproduction (Li et al., 2016). The availability of phosphorus in the soil can also influence the interaction between Ganoderma and its host plants (Hou, 2009). The total nitrogen (N-total) in sandy soil is usually lower than in ultisol soil.

The high N-total content in spodosol soil is presumed to be due to the spodic layer in spodosols, which has a high organic matter content. The difference in N-total between Cindy Diah Ayu Fitriana, Herry Wirianata, Yohana Maria Theresia Astuti, Fariha Wilisiani, Sukarman, Septa Primananda and Wahyuni Puji Utami

spodosol and ultisol soils is typically influenced by several factors, including organic matter content, the speed of organic matter decomposition, nitrogen leaching rate, and microorganism activity in the soil (Burgos et al., 2006). Higher N-total levels allow for additional nutrient presence in spodosol soil areas for pathogen development (Tong et al., 2020). Soilborne pathogens can positively respond to increased nitrogen availability in the soil (Shen et al., 2022). High magnesium levels can disrupt calcium absorption, leading to an increased incidence of diseases in plants (Huber & Jones, 2013). Elevated Mg levels in sick oil palm plants in sandy soil can negatively impact calcium absorption by plants. Impaired calcium absorption makes plants susceptible to pathogen attacks and diseases (Gupta et al., 2022).

# Role of Soil Biology in the Spread of Ganoderma Enumeration of Bacterial Populations in the Rhizosphere of Oil Palm Trees

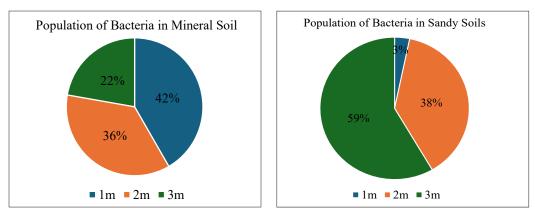
The highest bacterial population enumeration was found in the ultisol soil around healthy plants (Use), reaching 294.05 x  $10^8$  cfu/g of soil (Table 3).

 Table 3

 Comparison of bacterial populations in the roots of infected and healthy plants (cfu/g)

Code		Distance (Meter)			Bacteria
		1 2		3	(cfu/g)
Usa	Ultisol soils around infected palm	20.60 x 10 <sup>8</sup>	26.37 x 10 <sup>8</sup>	48.39 x 10 <sup>8</sup>	95.36 x 10 <sup>8</sup>
Use	Ultisol soils around non-infected palms	161.74 x 10 <sup>8</sup>	131.97 x 10 <sup>8</sup>	0.34 x 10 <sup>8</sup>	294.05 x 10 <sup>8</sup>
Ssa	Spodosol soils around infected palms	8.98 x 10 <sup>8</sup>	126.87 x 10 <sup>8</sup>	97.56 x 10 <sup>8</sup>	233.41 x 10 <sup>8</sup>
Sse	Spodosol soils around non-infected palms	2.05 x 10 <sup>8</sup>	4.02 x 10 <sup>8</sup>	2.41 x 10 <sup>8</sup>	8.48 x 10 <sup>8</sup>

In ultisol soil, the highest bacterial population is found at 1 meter (23.4% of the total population), and the lowest is at 3 meters (12.5% of the total population) (Figure 8). Meanwhile, in spodosol soil, the lowest bacterial population is at 1 meter (2.3% of the total population), and the highest is at 3 meters from the plant (4.3%). Thus, it can be concluded that the bacterial population from the exploration results in ultisol soil at distances of 1, 2, and 3 meters is inversely proportional to spodosol soil.



*Figure 8.* Comparison of bacterial population percentages in spodosol soil (Ssa & Sse) and ultisol soil (Usa & Use) around healthy (Se) and Ganoderma-infected (Sa) plants

The results are presented in Table 4 based on the analysis data of fungal populations in each soil sample. The highest fungal population is around diseased plants in spodosol and ultisol soils. The fungal population is quite high in ultisol soil around diseased plants, reaching 27,012 cfu/g. Meanwhile, in spodosol soil, the fungal population around diseased plants reaches 26,009 cfu/g.

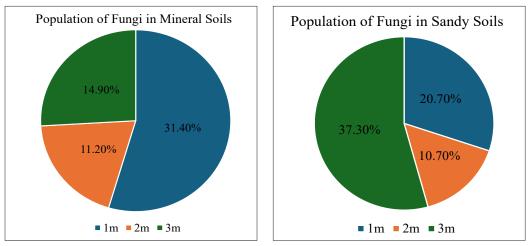
#### Distance (Meter) Code Fungi (cfu/g) 1 2 3 Ultisol soils around 16.30 x 10<sup>6</sup> 67.9 x 10<sup>5</sup> 3.92 x 10<sup>6</sup> Usa 27.01 x 10<sup>6</sup> infected Palm Ultisol soils around 2.78 x 10<sup>6</sup> 0.06 x 10<sup>5</sup> 0.61x 10<sup>6</sup> 3.40 x 10<sup>6</sup> Use non-infected palms Spodosol soils around 15.54 x 10<sup>6</sup> 0.97 x 10<sup>5</sup> 10.37x 10<sup>6</sup> Ssa 26.00 x 10<sup>6</sup> infected palms Spodosol soils around 3.12 x 10<sup>6</sup> 95.29 x 10<sup>5</sup> 6.44 x 10<sup>6</sup> Sse 19.08 x 10<sup>6</sup> non-infected palms.

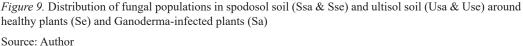
Fungal population in spodosol soil (Ssa & Sse) and ultisol soil (Usa & Use) around healthy plants (Se) and Ganoderma-infected plants (Sa)

Table 4

Considering the proportion of fungal populations at each sampling distance, differences in the distribution patterns of fungi in the two analyzed soil types were observed (Figure 9). In ultisol soil, the highest proportion of fungal population is found at 1 meter from the planting point (31.4%), while the lowest is at 2 meters. It contrasts with spodosol soil, where the highest fungal population is 3 meters from the planting point, while the lowest population is at the same distance as in ultisol soil, which is 2 meters.

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The higher bacterial population in ultisol soil near the roots of healthy plants at 2-3 meters may be attributed to several factors related to plant root activity and environmental conditions around the roots (Cheng et al., 2022; Jin et al., 2022; Liu et al., 2022). The fungal population in diseased oil palm plants infected with Ganoderma tends to be higher than in healthy plants due to the influence of Ganoderma on soil structure, which can inhibit the growth and activity of microbes in the rhizosphere (Anandyawati et al., 2017).

# CONCLUSION

This study demonstrates the significant influence of soil type on macronutrients, microbial communities, and the distribution of Ganoderma in oil palm plantations. In spodosol soil, pH, organic carbon, P-total, and P-Bray positively impacted Ganoderma occurrence, while exchangeable Mg and Ca showed mixed interactions. Notably, bacterial populations around infected palms were higher at 2–3m in ultisol soil than in spodosol soil. These findings highlight the complexity of soil-disease interactions and emphasize the need for soil-specific management strategies. Optimizing nutrient management based on soil type can enhance plant resistance to Ganoderma. Further research should explore the microbiome differences between healthy and infected areas to understand the broader ecological impact of this disease. By elucidating the intricate relationships between soil properties, microbial dynamics, and the spread of Ganoderma, it can develop more effective and sustainable disease management strategies for diverse oil palm plantation environments.

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