RESEARCH ARTICLE Agricultural Science Digest, Volume 45 Issue 6: 964-973 (December 2025)

Comparative Analysis of Soil Fungal Profiles in Healthy and Diseased Duku (*Lansium domesticum* Corr.) Plantations at Different Altitudes in Jambi Province, Indonesia

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10.18805/ag.DF-732

ABSTRACT

Background: Soil fungal communities significantly contribute to the health and productivity of plants, with their compositions influenced by plant disease presence at various altitudes. This study compares the variations in soil fungal diversity between healthy and diseased duku (*Lancium domesticum* Corr.) plantations in Jambi, Indonesia.

Methods: This study examines the potential fungal indicators on soil health, concentrated on four sites representing various altitudes: Kumpeh (10,32 m asl), Rano (5,80 m asl), Koto Rami (623,46 m asl) and a location of the Diseased site (194,01 m asl). The fungal communities were analyzed using DNA sequencing and the NCBI ITS RefSeq database.

Result: The dominant fungal phyla across all sites were Ascomycota, most abundant in the low-altitude from Kumpeh and Basidiomycota, primarily present in the high-altitude from Koto Rami. Mucoromycota and smaller groups like Zoopagomycota were also identified. The high-altitude site at Koto Rami showed high water content and organic matter, which may have supported a unique soil microbial community. The low-altitude site at Rano exhibited a neutral pH and well-balanced phosphorus levels, both of which encourage fungal activity. In contrast, the low-altitude samples from Kumpeh displayed higher levels of calcium and magnesium, low organic matter and a sandy texture, which may have restricted the fungal community. In the Diseased site, stress was detected as indicated by reduced moisture levels and heightened microbial activity, suggesting that certain microbes might adversely affect plant health. Overall, increased moisture and good organic matter in the soil can enhance fungal growth, while a neutral pH, adequate phosphorus and suitable soil texture can facilitate fungal diversity.

Key words: Abundance, Alpha, Beta diversity, Core microbes, Kumpeh, Taxonomy.

INTRODUCTION

Fungal communities are important in the soil ecosystem because they facilitate plant growth, pathogen resistance and stabilize soil. Their interactions foster an environment conducive to ecological equilibrium. However, this equilibrium is susceptible to disruption from various environmental factors, such as altitude and plant health, which can significantly influence the composition and diversity of soil fungal populations. In Jambi, Indonesia, the emergence of diseases leading to sudden death (SD) (Fig 1) in Duku (*Lancium domesticum* Corr.) plantations poses a considerable challenge, especially in regions characterized by heterogeneous soil properties. This condition has been particularly alarming as the incidence of sudden death disease (SD) has contributed to a decline in Duku populations.

The Jambi region is known for its unique premium Duku varieties, particularly the *Kumpeh* and *Muaro Panco*. Sudden death disease, aging existing plants and inappropriate cultivation techniques have been causing a significant production decline. This phenomenon affected the economic livelihoods of local farmers and fruit industry traders. Consequently, there is an urgent need to ensure the continued conservation of the Duku plants in Jambi, especially through soil health indicators.

The characteristics of healthy soil include good structure, nutrient richness, optimal pH, organic matter content, active microbial life, adequate drainage and water retention, minimal soil erosion and absence of contaminants (Lehmann *et al*., 2020; Vincze *et al*., 2024). Despite an increasing understanding of soil biodiversity, soil health assessments rely heavily on chemical indicators (Alwazzan and Ati, 2024).

Farming practices can alter soil structure, harm microbial communities, cause erosion, reduce fertility and increase greenhouse gas emissions (Singh and Kumar, 2021). Pesticide use and intensive land management can reduce overall microbial diversity, affect crucial symbiotic fungi and

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How to cite this article: Hayati, I., Rainiyati, Marwan, H., Wilia, W. and Antony, D. (2025). Comparative Analysis of Soil Fungal Profiles in Healthy and Diseased Duku (*Lansium domesticum* Corr.) Plantations at Different Altitudes in Jambi Province, Indonesia. *Agricultural Science Digest*. **45(6):** 964-973. doi: 10.18805/ag.DF-732.

Submitted: 06-03-2025 **Accepted:** 30-09-2025 **Online:** 18-10-2025

increase pathogenic fungi, which disrupt ecosystem functions (Mohapatra *et al*., 2021; Chou *et al*., 2024). Additionally, chemical disturbances like excessive nitrogen and herbicide use can inhibit important mutualistic fungi, leading to ecological imbalances (Brochado *et al*., 2023).

Microbial communities significantly influence plant metabolism by regulating nutrient uptake and overall health through interactions with plant roots, forming the plant-rhizosphere community (Dastogeer *et al*., 2022; Petrushin *et al*., 2024; Solomon *et al*., 2024). Various studies have examined soil bacterial communities, for example, as studied by Fasusi *et al*. (2023). However, the diversity of fungal, ecological and soil character roles remains underexplored. Investigating variations in fungal communities between healthy and disease-affected soils at different altitudes is essential to understanding the relationship between soil fungi and plant health. Understanding the dynamics of soil fungi concerning these environmental variables is crucial for developing effective management strategies in affected areas and is critical to assessing soil health, as this is the basis for healthy growth and thriving plant life.

This study investigated the fungal communities in soils of diseased and healthy Duku (*Lansium domesticum*) plants across different sites in Jambi Province, Indonesia. By comparing these communities, this study aimed to identify fungal patterns associated with plant health and provide baseline data to support future disease management

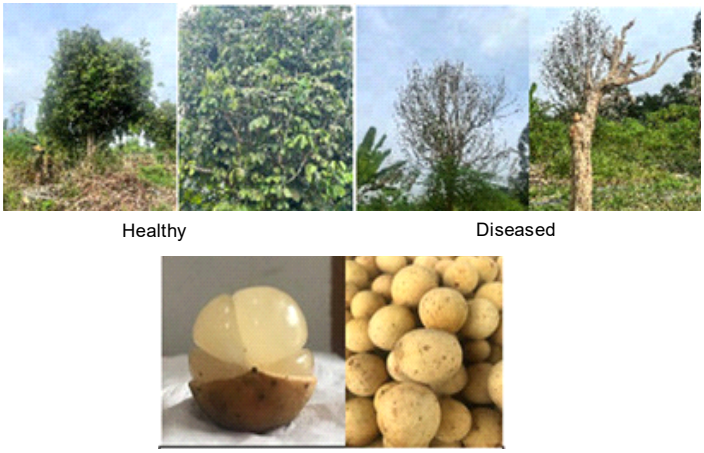


Fig 1: Performance of Duku explaining healthy and diseased plants (above) and flesh and fruit.

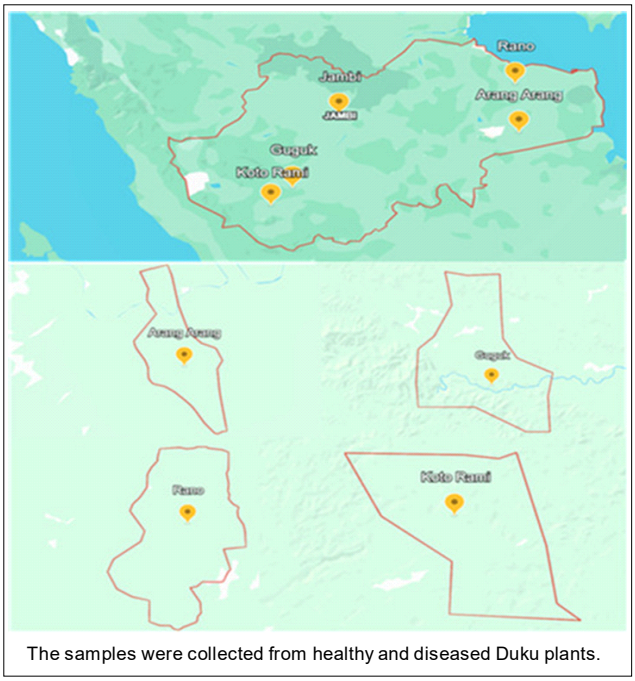


Fig 2: Location of Sampling in several villages in Jambi Province.

strategies in tropical fruit cultivation. for mitigating the impacts of disease.

MATERIALS AND METHODS

Sampling site and procedure

Soil samples were gathered from 4 locations, representing healthy and diseased Duku from various altitudes. The locations were BPP Arang-Arang (Kumpeh H), 48 366090 E 9823483 S, elevation 10,32 m above sea level, Desa Guguk Merangin (soil of Diseased plant site), 48 175230 E 9759370 S, 194,01 m asl, Desa Rano Tanjabtim (Rano H), 48 363839 E 9866389 S, 5,80 m asl and Desa Rami (Koto Rami H), 47 823898 E 9740820 S, 623,46 m asl (Fig 2). Soil samples were collected from 4 points representing the North, South, West and East directions within 30-40 cm of the base of each stem. A soil drill was used to excavate the soil to a depth of 20 cm. At each location, 5-8 plants were sampled. The soils were then combined to create a composite for each location. Physical characteristics of the soils were analyzed and are shown in Table 1. These characteristics are invaluable for assessing the health, fertility and suitability of soils that support a particular agriculture or ecology.

Exploration for fungi

To characterize the fungal community present in the soil, a metagenomic approach was employed using DNA sequencing technologies. Soil samples were processed at the Genetica Science Laboratory, where total soil DNA was extracted and quantified using both NanoDrop spectrophotometers and Qubit fluorometers to ensure purity and accurate concentration measurements. Library preparations were conducted using Kits from Oxford Nanopore Technology. Nanopore sequencing was operated by MinKNOW software version 24.02.16. Basecalling was performed using Dorado version 7.3.11 with a high-accuracy model (Wick *et al*., 2019). The quality of FASTQ files was visualized using NanoPlot and quality filtering was performed using NanoFilter (de Coster *et al*., 2018; Nygaard *et al*., 2020). Filtered reads were classified using the Centrifuge classifier (Kim *et al*., 2016). The Fungi index was built using the NCBI ITS RefSeq database. The workflow is shown in Fig 3.

Data analysis

Data collected was presented descriptively. Extensive downstream analysis and detailed visualizations were

Table 1: Physicochemical values of Duku root soils in Koto Rami, Rano, Kumpeh and Diseased site.

Parameter		Methods	Unit	Locations			
				Koto rami healthy	Rano healthy	Kumpeh healthy	Diseased
pH	H_2O	Extract 1:5	-	4.6	5.5	5.8	5.5
	KCl	(Potentiometry)	-	4.3	4.6	4.6	4.7
Water content		Gravimetry	-	52.64	16.00	19.46	6.75
C-Organic		Spectrophotometry	%	4.36	2.06	1.11	2.26
N-Total		Kjeldahl	%	0.35	0.32	0.17	0.25
P_2O_5 Potential		Spectrophotometry	mg/100 g	51.50	179.39	63.80	80.39
K_2O Potential		AAS	mg/100 g	21.26	75.35	39.68	42.28
Cation exchange capacity		Titrimetry	cmol(+)/kg	21.18	15.32	16.15	21.39
Exchangeable acidity	Al^{3+}	Titrimetry	cmol(+)/kg	<0.04	<0.04	<0.04	<0.04
	H^+		cmol(+)/kg	2.46	0.41	0.40	0.46
Three Particle-size fraction	Sand	Gravimetry	%	30	26	30	28
	Dust		%	19	27	36	28
	Clay		%	51	47	34	44
Calcium		$HClO_4$ HNO_3 - AAS	mg/Kg	292.60	868.54	1507.52	1279.77
Magnesium		$HClO_4$ HNO_3 - AAS	mg/Kg	819.29	1179.50	2654.09	1640.73
Iron		$HClO_4$ HNO_3 - AAS	mg/Kg	58520.54	54149.85	39811.39	49768.67
Zinc		$HClO_4$ HNO_3 - AAS	mg/Kg	222.38	139.40	92.36	164.07
Sulfur		$HClO_4$ HNO_3 - Spectrophotometry	%	<0.10	<0.10	<0,10	<0.10
Boron		Spectrophotometry	mg/Kg	18.08	17.84	18.26	10.39
Molybdenum		$HClO_4$ HNO_3 - AAS	mg/Kg	8.02	7.94	7.71	4.25
Al saturation		Calculations	%	0	0	0	0
Salinity		Conductometry	PPT	0.04	0.25	0.05	0.05
Soil respiration		Determination of CO_2 - Tritimetry	mg/Kg C-CO_2 /day	5.91	3.76	5.47	7.20

performed using several tools and visualizations were performed using Pavian (https://github.com/fbreitwieser/pavian) and Krona Tools (https://github.com/marbl/Krona). In addition, RStudio using R version 4.2.3 (https://www.R-project.org/) was utilized to improve the analytical capabilities and create insightful graphical representations of the data.

RESULTS AND DISCUSION

Taxonomy group

Fig 4 illustrates the main fungal groups in healthy and Diseased sites across all sites. In Koto Rami Healthy (H) soil, the dominant fungi were Ascomycota and Basidiomycota and smaller groups like Mucoromycota and Zoopagomycota.

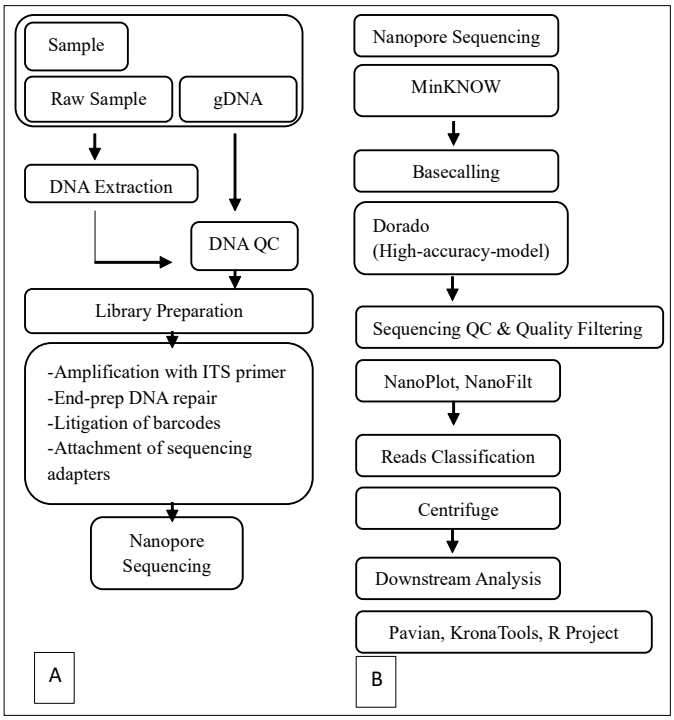


Fig 3: Experimental stages of DNA preparation and sequencing (A), Flow of metagenomic data analysis using Nanopore Technology (B).

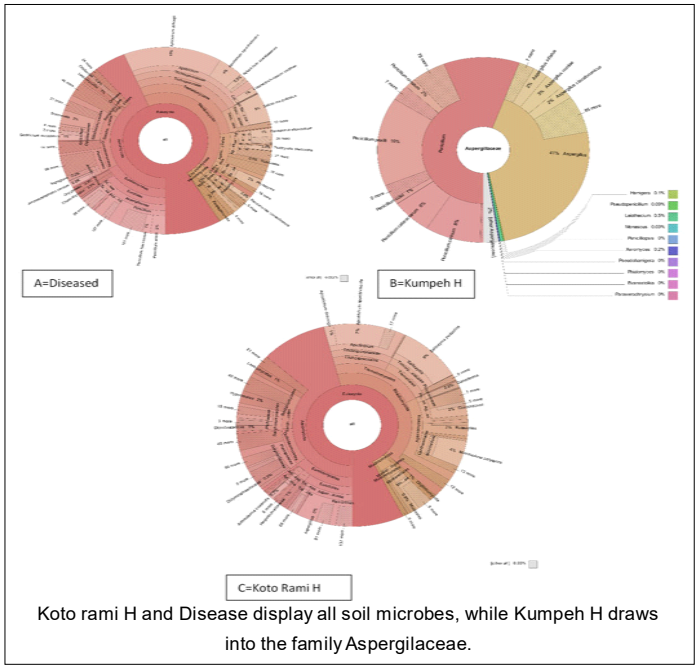


Fig 4: The Taxonomy group of Diseased site, Kumpeh H (above) and Koto Rami H (below).

The *Ascomycota phylum* includes Eurotiomycetes. When drawn into the family Aspergillaceae (Fig 4C), it was found in classes such as Tremellomycetes and notable genera like Aspergillus and Penicillium.

The quantitative distribution of Aspergillus and Penicillium is illustrated as follows (Fig 5). In Kumpeh H, out of 45.200 observed microbes, 22.400 were from the Didymosphaeriaceae family. The Trichocomaceae family had 4.110 members and the Aspergillaceae family included Aspergillus (2.620), Penicillium (3.530) and Talaromyces (4.080). In Koto Rami H, Clavariaceae led with 6.520 entities, followed by Aspergillus (3.690), Penicillium (2.510) and Ganoderma (741). Key species included *Penicillium flaviroseum* (414) and *Aspergillus fumigatus* (523). In Rano H, Aspergillus comprised 12.400 entities, while Penicillium had 31.500 entities, including *Aspergillus nomiae* (2.950) and *Penicillium citrinum* (5.900). In the Diseased site, Aspergillus counted 3.310 and Ganoderma 3.470, with various species identified.

Fungal relative abundance

Table 2 presents fungal relative abundance (%) at the phylum level under diseased site conditions and three healthy locations: Koto Rami, Kumpeh and Rano. Ascomycota and Basidiomycota were the dominant phyla across all conditions, with Ascomycota abundance ranging from 0.482 to 0.5972% and Basidiomycota from 0.3359 to 0.4849%. Kumpeh (H) had the highest Ascomycota abundance (0.5972%), while Koto Rami (H) had the highest Basidiomycota (0.4849%). Other phyla, such as Blastocladiomycota, Chytridiomycota, Cryptomycota, Olpidiomycota and Zoopagomycota, were less abundant, each below 0.05%. Blastocladiomycota was absent in Rano (H), while Chytridiomycota was highest at Kumpeh (H) (0.0122%). Mucoromycota increased at Rano (H) (0.1616%) and Zoopagomycota was most abundant at Kumpeh (H) (0.0161%). The diseased site soil, Ascomycota (0.455%) and Basidiomycota (0.4776%), displays a more balanced ratio than the Healthy sites.

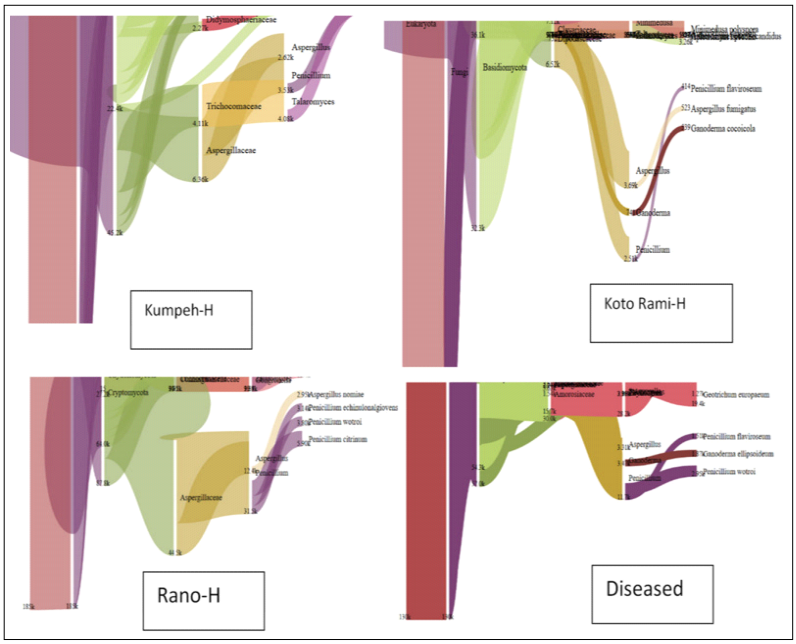


Fig 5: Quantitative distribution between the genus Aspergillus and Penicillium of all sites; Kumpeh H, Koto Rami H, Rano H and Diseased.

Table 2: The relative abundance of Phylum generated from soil samples of healthy at Rano H, Koto Rami Healthy, Kumpeh Healthy and Disease soil (%).

Phylum	Diseased	Koto rami H	Kumpeh H	Rano H
Ascomycota	0.455	0.4482	0.5972	0.4828
Basidiomycota	0.4776	0.4849	0.3359	0.3487
Blastocladiomycota	0.0005	0.0004	0.0006	0
Chytridiomycota	0.024	0.0099	0.0122	0.0051
Cryptomycota	0.0005	0.0007	0.0021	0.00021
Mucoromycota	0.0223	0.0486	0.035	0.1616
Olpidiomycota	0.001	0.0004	0.001	0.00011
Zoopagomycota	0.0198	0.007	0.0161	0.0016

Fungal Alpha diversity

The soils sampled from Diseased sites demonstrated the highest observed species richness, quantified at 3,636. This significant level of diversity is further supported by high estimates of potential richness, with Chao1 calculated at 5,370.40 and ACE at 5,361.10. The Shannon Index for these samples was recorded at 39.60, marking it the highest among all sites. Simpson's Index was measured at 5.01, indicating moderate species-level dominance within these communities. Additionally, the InvSimpson's Index, at 0.96, suggests a less equitable species distribution compared to Kumpeh_H. Despite the Diseased site showcasing the highest observed species richness, it remains evident that its potential richness is lower relative to other studied areas.

Koto Rami documented 3105 species, surpassing Rano_H (2900) but still behind Diseased site (3636) and Kumpeh_H (3297), indicating a diverse ecosystem. Chao1 and ACE estimates suggested potential species richness of around 4693,01 and 4586,20, respectively. The Shannon index was 36,79, slightly lower than Kumpeh_H (38,35), while both the Simpson's Index (5,38) and InvSimpson Index (0,97) indicated an even distribution of species. Koto Rami's Fisher's Alpha of 34,81 suggested a high potential for species richness. In contrast, Rano_H had the lowest species richness, 2900 species and potential richness estimated at 4482,63. Its Shannon Index of 35,74 and Simpson Dominance Index of 4,65 revealed an uneven distribution, dominated by certain species. Rano_H's Fisher's Alpha of 30,40 indicated limited diversity. Rano_H was the least diverse, with an imbalanced species distribution (Fig 6).

Site distinctive of fungal diversity

Each site had unique microbial communities, as shown in Fig 8. The Diseased site soil contains the highest number of exclusive fungi (805) (Fig 7 B) and has a similarity with Kumpeh H (296). Overall, 1064 microbes were identified. Kumpeh H had the highest variety with 1036 unique species, while Rano H and Koto Rami H had 741 and 980, respectively. Shared species included 409 between Rano H and Koto Rami H, 545 between Rano H and Kumpeh H and 511 between Koto Rami H and Kumpeh H, totaling 1205 common microbial phyla across all sites.

Beta diversity

Beta diversity describes the variation or differences between healthy and Diseased site of duku plantations. Fig 8 presents a principal coordinate analysis (PCoA) based on the Bray-Curtis distance, which evaluates the soil microbial community structure at Kumpeh H, Koto Rami H, Rano H

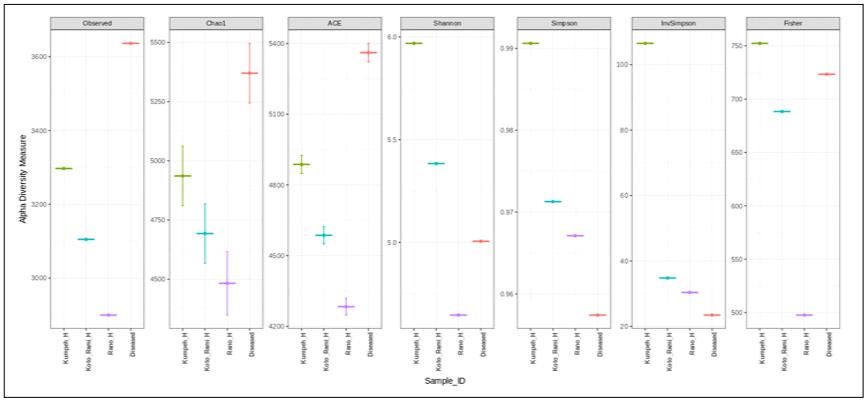


Fig 6: Comparison of alpha diversity index in fungal soil at various sites.

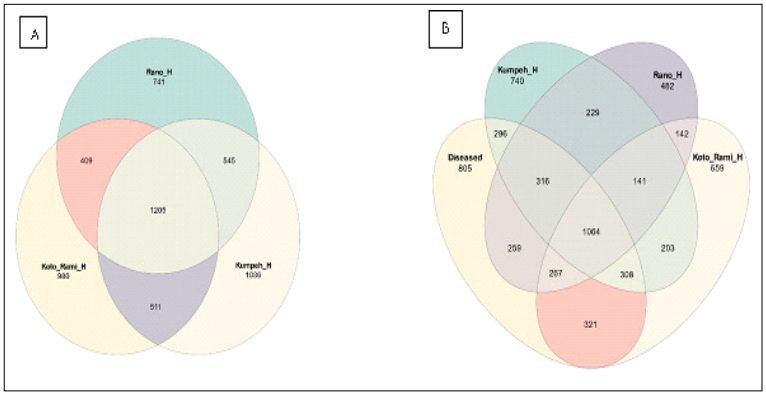


Fig 7: The distinctive soil fungal diversity across Rano_H, Koto_Rami_H and Kumpeh_H (A) and all across those plus Diseased site soil (B).

and the Diseased site. The analysis showed distinct microbial distributions among the sites, with PCoA1 (40.16%) and PCoA2 (24.54%) on the left and PCoA1 and PCoA3 (25.21%) on the right.

This study compares soil fungal profiles in healthy and Diseased duku plantations at various altitudes in Jambi. It highlights how soil properties affect fungal abundance. The results show that differences in soil characteristics-such as pH, organic matter, nutrient availability and altitude-influence fungal communities across the study sites.

Rano H, 5.80 m above sea level, showed the highest abundance of Aspergillus and Penicillium. It also has elevated Phosphorus levels and a relatively neutral pH. This finding is in line with previous studies suggesting that Phosphorus availability boosts fungal growth, especially among Ascomycota species such as Aspergillus and Penicillium (Wang *et al*., 2018). In contrast, Kumpeh H, located 10.32 m above sea level, had a moderately acidic pH and low organic matter content, leading to lower counts of Aspergillus and Penicillium, while Didymosphaeriaceae thrived instead. The thriving of Didymosphaeriaceae indicates that certain fungi can flourish in nutrient-poor soils in lowland areas, which may contribute to soil degradation and increased susceptibility to diseases (Treseder and Lennon, 2015).

At an elevation of 623.46 m above sea level, Koto Rami H exhibited distinct soil characteristics, including the highest levels of organic matter and total Nitrogen. Despite low nutrient levels of phosphorus and Kalium, Koto Rami H supported a diverse fungal community, with higher counts of Aspergillus and Penicillium compared to Rano H. Typically, as altitude increases, temperatures drop, which slows the decomposition of organic matter. This slowdown leads to a greater buildup of organic materials and, in turn, fosters a richer diversity of fungi (Hartmann *et al*., 2015; Zahra *et al*., 2021). Additionally, Koto Rami H showed the highest concentrations of Iron and Zinc, which can enhance the enzymatic activities of fungi. However, excessive iron can negatively affect fungal communities (Luo *et al*., 2024).

The presence of Aspergillus and Ganoderma at the Diseased site hints at a fungal-driven disease progression, as elevated microbial respiration and CEC are frequently linked to microbial competition and shifts in fungal community structure in degraded soils (Zhou *et al*., 2023). The disease site's intermediate altitude, due to its specific soil conditions, may have created an environment that favors fungal communities associated with plant disease.

At the Diseased site, high microbial activity but lower diversity was observed. It reflects stress conditions that can favor pathogenic fungi over beneficial mutualists (Gupta *et al*., 2022). This finding resonates with studies indicating that Diseased plant soil often hosts more complex fungal networks than healthy soil (Jia *et al*., 2022). The elevated CEC in Diseased soil implies improved nutrient cycling; however, this does not necessarily translate to enhanced plant health, as high microbial respiration may signify nutrient competition or stress (Meimaroglou and Mouzakis, 2019; Lalkhumliana *et al*., 2024).

Across all sites, Ascomycota and Basidiomycota were the dominant fungal groups, though variations were observed depending on environmental conditions (Feng *et al*., 2024). The ratio of Ascomycota to Basidiomycota can serve as a soil health indicator, as a dominance of Ascomycota in organic-rich soils may limit the presence of beneficial fungal groups such as Basidiomycota and arbuscular mycorrhizal fungi (Manici *et al*., 2024; Martínez-García *et al*., 2018). The lower Nitrogen content in Kumpeh H could help explain its reduced fungal abundance (Di Lonardo *et al*., 2020). Higher Phosphorus levels in Rano H might support the growth of Mucoromycota, which is important for hyphal development (Bhalla *et al*., 2022). Furthermore, the greater moisture levels in Koto Rami H and Kumpeh H may be linked to increasing Zoopagomycota, which thrive in damp conditions (Chen *et al*., 2023).

Fungi play vital roles in soil health as decomposers, mutualists and pathogens (Li *et al*., 2019; Yiallouris *et al*., 2024). The prevalence of Penicillium and Aspergillus across various sites contributes to organic matter decomposition, Phosphorus solubilization and microbial antagonism (Díaz-Urbano *et al*., 2023). While Basidiomycota

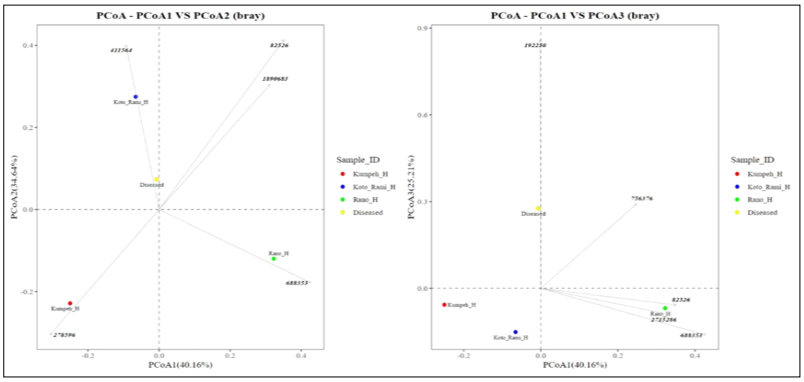


Fig 8: Beta diversity metrics showing differences in soil microbial communities across Rano H, Koto Rami H, Kumpeh H and Disease site soil, using Principle Coordinate Analysis (PCoA) plot, based on Bray-Curtis dissimilarity.

populations can stabilize microbial interactions, disruptions such as land use changes and agricultural practices may diminish their abundance, ultimately impacting soil health (Egidi *et al*., 2019; Xiang *et al*., 2024). Moreover, fungal activity significantly influences soil structure by producing microbial exudates that improve soil aggregation (Arias *et al*., 2023; Sudheer *et al*., 2024).

The metagenomic analysis conducted in this study successfully identified a diverse fungal community across all sampling sites, including healthy and diseased duku plantations. Dominant genera such as *Aspergillus*, *Penicillium*, *Ganoderma* and members of the *Didymosp-haeriaceae* and *Clavariaceae* families were detected in varying abundance. However, it is notable that previously reported pathogens associated with duku diseases, such as *Phytophthora palmivora* (Handoko, 2014) and *Ceratocystis* sp (Firmanto, 2023), were not detected in the current dataset.

Several factors may account for this discrepancy. First, metagenomic sequencing is highly dependent on the DNA extraction method and sequencing depth. It is possible that the DNA of *Phytophthora* or *Ceratocystis* was present at very low concentrations, falling below the detection limit of the sequencing pipeline (Sharpton, 2014). Additionally, the reference database used for taxonomic classification (ITS RefSeq) may have limitations in resolving certain oomycete taxa like *Phytophthora*, which often require other markers such as *cox1* or *cox2* for accurate identification (Robideau *et al*., 2011).

Second, the symptoms observed in the diseased site may not be caused solely by fungal or oomycete pathogens, but could be the result of complex biotic interactions, including latent infections, bacterial co-infections, or abiotic stressors that mimic pathogen-induced symptoms (Scholthof, 2007). Without pathogen isolation and pathogenicity testing (fulfilling Koch's postulates), it remains speculative to attribute symptoms to specific taxa based solely on sequencing data.

Finally, spatial and temporal variation in pathogen presence could have influenced the results. Pathogens like *P. palmivora* may not be uniformly distributed in soil or plant tissues and may only be detectable during specific stages of infection (Hardham and Blackman, 2018). The findings highlight the need to combine metagenomic approaches with traditional plant pathology methods like culturing, microscopy and pathogenicity tests for better disease diagnostics.

CONCLUSION

This study found that fungal communities differed between healthy and diseased duku plantations. Healthy soils, such as those from Koto Rami, Kumpeh and Rano, showed a more even distribution of fungi, with common genera like *Aspergillus* and *Penicillium*. These communities were supported by better soil conditions, such as higher organic matter or phosphorus levels. In contrast, the diseased site had the highest fungal richness but showed dominance by certain taxa, such as *Ganoderma*, suggesting microbial imbalance. This imbalance may reflect stress conditions in the soil, which could suppress beneficial fungi and promote opportunistic or harmful species. Known pathogens like *Phytophthora palmivora* and *Ceratocystis* were not detected, possibly due to low DNA abundance or database limitations. These results highlight that fungal community structure can reflect soil health status. Maintaining balanced and diverse fungal communities is important for sustaining healthy duku plantations. Soil management practices that support microbial diversity are key to preventing disease outbreaks.

ACKNOWLEDGEMENT

The present study was supported by the Ministry of Higher Education, Science and Technology of the Republic of Indonesia, as part of the Fundamental Research 2024 program, Contract number: 1612/UN2.11/PT.01.05/SPK/2024.

Disclaimers

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Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal Care and Handling Techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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