ASIAN JOURNAL OF AGRICULTURE
Volume 10, Number 1, June 2026 E-ISSN: 2580-4537
Pages: 9 https://doi.org/10.13057/asianjagric/g100105

Rhizosphere microbial functional traits associated with basal stem rot suppression in oil palm (*Elaeis guineensis*)

EVAN PURNAMA RAMDAN[1,♥], GIYANTO[2], ARIEF HARTONO[3], SRI HENDRASTUTI HIDAYAT[2], WIDODO[2,♥♥]
[1]Department of Agrotechnology, Faculty of Industrial Technology, Universitas Gunadarma. Jl. Margonda Raya 100, Depok 16424, West Java, Indonesia. Tel.: +62-21-78881112 ext. 234, ♥email: evan_ramdan@staff.gunadarma.ac.id
[2]Department of Plant Protection, Faculty of Agriculture, Institut Pertanian Bogor. Jl. Kamper, Babakan, Dramaga, Bogor 16680, West Java, Indonesia. Tel.: +62-251-8629364, ♥♥email: widodo@apps.ipb.ac.id
[3]Departement of Soil Science and Land Resource, Faculty of Agriculture, Institut Pertanian Bogor. JI. Meranti Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia

Manuscript received: 9 October 2025. Revision accepted: 18 January 2026.

Abstract. *Ramdan EP, Giyanto, Hartono A, Hidayat SH, Widodo. 2026. Rhizosphere microbial functional traits associated with basal stem rot suppression in oil palm (*Elaeis guineensis*). Asian J Agric 10 (1): g100105. https://doi.org/10.13057/asianjagric/g100105.* This study aimed to investigate the abundance, diversity, and functional characteristics of rhizospheric microbial communities associated with different levels of Basal Stem Rot (BSR) disease incidence in oil palm plantations, with the understanding that the observed relationships represent associations rather than causal effects. Rhizospheric microbial communities play an essential role in soil health and disease suppression in oil palm plantations. Fifteen composite soil samples were collected from three field blocks with low, moderate, and high BSR incidence in the Rejosari Unit, PT Perkebunan Nusantara VII, Lampung, Indonesia. Microbial populations were quantified using standard plate counts, while diversity indices were assessed using the Shannon-Wiener, evenness, and dominance indices based on morphospecies counts. The results showed that total microbial, bacterial, and fungal populations were significantly higher in soils with low disease incidence (4.99×10^7, 4.94×10^7, and 5.18×10^5 CFU g^{-1}, respectively) compared to moderate and high categories. Soils with low BSR incidence also exhibited greater microbial and bacterial diversity (H′=1.07 and 0.74) and lower dominance, indicating a more balanced community structure. Non-pathogenic, antagonistic, and Volatile Organic Compound (VOC)-producing microbes predominated in low-incidence soils, contributing to natural disease suppression. LASSO regression identified VOC-producing and antagonistic microbes as predictors associated with BSR incidence, whereas correlation analysis revealed a significant negative association only for VOC-producing microbes (r=-0.60, p=0.02). Nitrogen-fixing microbes were positively associated with disease severity (r=0.63, p=0.01). Although causality was not tested, the results suggest that reduced BSR incidence was more closely associated with specific functional microbial groups, particularly VOC-producing microbes, than with overall microbial abundance or diversity, emphasizing that soil management strategies that support beneficial functional microbial groups may contribute to enhanced soil resilience.

Keywords: Functional microbes, *Ganoderma boninense*, Shannon-Wiener index, suppressive soil

INTRODUCTION

Basal Stem Rot (BSR), caused by *Ganoderma boninense*, remains the most destructive disease threatening the productivity and longevity of oil palm (*Elaeis guineensis*). Biological control using beneficial microorganisms has long been regarded as a sustainable alternative to chemical fungicides. Numerous studies have demonstrated the potential of rhizosphere-associated microbes to suppress *G. boninense* infection under controlled conditions. However, despite encouraging laboratory results, the consistent suppression of BSR under field conditions has been difficult to achieve. One major limitation arises from ecological competition between beneficial microbes and *G. boninense*, as biological agents often fail to establish and persist in the same niche within the soil environment (Niu et al. 2020; Pirttila et al. 2021).

Previous studies reported that *G. boninense* possesses exceptional adaptability, enabling it to survive at considerable depths in the soil profile. Mustapha et al. (2011) observed that the pathogen could inhabit soil up to 3 m below the surface, whereas the vertical distribution of functional microbes is typically limited to 0-120 cm (Wafa 2017; Sun et al. 2021; He et al. 2023). This spatial separation restricts direct microbial-pathogen interactions in deeper soil layers, reducing the efficacy of many biocontrol agents. Nevertheless, functional microbes remain essential in sustainable disease management due to their roles in pathogen inhibition, soil fertility, and plant nutrition, while offering environmentally safe alternatives to chemical fungicides (Chen et al. 2023).

The composition and activity of rhizosphere microbial communities are also important indicators of soil health. Microbial abundance and diversity are closely linked to soil chemical properties, particularly Soil Organic Carbon (SOC). Ramdan et al. (2023) reported that *G. boninense*-infected oil palm soils typically exhibit lower fertility levels, with SOC contents below 1%. Such infertile conditions reduce the proliferation of beneficial microbes, further aggravating disease severity. Nutrient elements such as potassium (K), magnesium (Mg), manganese (Mn), and Base Saturation (BS) have also been correlated with

BSR incidence (Ramdan et al. 2024), highlighting the ecological linkage between nutrient status and disease suppression.

Studies on suppressive soils-soils that naturally exhibit low disease incidence-have highlighted the role of soil microbial communities in regulating pathogen activity. Wahyudi et al. (2017) reported significantly higher fungal abundance in BSR-suppressive soils compared to conducive soils, suggesting a potential antagonistic role of certain microbial taxa. Recent advances in sequencing and microbiome analyses have enabled comprehensive characterization of both bacterial and fungal communities, including their potential functional roles. However, relatively few studies have integrated culture-based assessments of specific functional microbial groups with disease incidence under field conditions, particularly in oil palm soils affected by basal stem rot. Addressing these research gaps requires an integrated assessment of both microbial abundance and diversity across oil palm fields exhibiting different levels of BSR incidence. Identifying microbial taxa that contribute to natural disease suppression-through antagonism, nutrient competition, or induction of host resistance-could enhance the design of targeted and resilient biological control strategies. Moreover, linking microbial community profiles with soil chemical properties will advance the understanding of disease ecology in the oil palm rhizosphere.

Therefore, this study aims to characterize the abundance, diversity, and functional composition of rhizosphere microbial communities in oil palm plantations exhibiting low, medium, and high incidences of BSR. The findings are expected to identify microbial indicators of natural disease suppression and provide a scientific foundation for developing more effective and ecologically sustainable management strategies for *G. boninense*. This study hypothesized that oil palm soils exhibiting low basal stem rot incidence harbor higher abundance and functional activity of beneficial rhizosphere microbes-particularly antagonistic and Volatile Organic Compound (VOC)-producing groups-compared with soils showing moderate to high disease incidence.

MATERIALS AND METHODS

Time and place

The research was conducted at the Plant Mycology Laboratory and the Soil Science and Land Resources Laboratory, Faculty of Agriculture, Institut Pertanian Bogor, Bogor, Indonesia, from March 2022 to November 2022.

Soil sample source

Soil sampling was conducted in June 2021 at oil palm (*E. guineensis*) plantations managed by PT Perkebunan Nusantara VII, Rejosari Unit, Lampung, Indonesia. Disease incidence in each block was determined through a systematic census of oil palm trees using the 5-2 transect method (Siregar et al. 2020), in which all palms encountered along the transects were visually assessed for Basal Stem Rot (BSR) symptoms. BSR assessment was based on characteristic visual indicators, including the presence of three or more unopened spear leaves, leaf chlorosis, skirt-like drooping fronds, basal stem decay, and the occurrence of basidiocarps. Based on this assessment, the study area comprised three field blocks representing low (0-10%), moderate (10-50%), and high (>50%) levels of BSR incidence (Ramdan et al. 2024).

Each block contained five plots of 50 m², resulting in a total of 15 sampling plots. Within each plot, five subplots (10 m² each) were selected-four located at the edges and one at the center. Within each subplot, three representative oil palm trees arranged in a triangular planting pattern were selected for disease assessment, resulting in a total of 15 palms assessed per 50 m² plot. Rhizosphere soils were collected from the root zones (0-30 cm depth) of three palms per subplot and composited into a single representative sample per plot. In total, 15 composite soil samples were obtained. Samples were stored in sterile plastic bags, transported on ice, and processed within 24 hours.

In this study, rhizosphere soil was operationally defined as soil collected from the vicinity of the roots after removal of visible coarse root fragments, without specific separation of soil tightly adhering to the root surface. The composite soil sample was considered the experimental unit for all statistical analyses. A total of fifteen independent composite samples were analyzed, representing three BSR incidence categories (low, moderate, and high), with five biological replicates per category (n=5). Each composite sample was derived from multiple subsamples within a plot and treated as a single biological replicate, thereby avoiding pseudo-replication. Replicate measurements within each assay were considered technical replicates and were averaged prior to statistical analysis.

Soil physicochemical characterization

The basic physicochemical characteristics of the soils used in this study were obtained from our previous research (Ramdan et al. 2024), which analyzed the same experimental plots and sampling sites. The parameters included soil pH, C-organic content, texture class, and exchangeable cations (K, Mg, and Na). The measurements followed standard soil analysis procedures. These parameters describe the general environmental conditions that may influence rhizosphere microbial communities and disease development. The results (Table 1) show relatively homogeneous soil characteristics among the three disease incidence categories, indicating that the observed differences in microbial abundance and activity were mainly associated with disease status rather than major variations in soil properties.

Source of *G. boninense* isolates

The *G. boninense* isolate used in this study was obtained from the culture collection of the Indonesian Oil Palm Research Institute (IOPRI), originally isolated from basidiocarps of symptomatic oil palm trees collected from the Rejosari plantation, Lampung, and maintained on Potato Dextrose Agar (PDA) medium at 27±2°C.

Table 1. Physico-chemical properties of soils from different disease incidence categories in oil palm plantations (Ramdan et al. 2024)

Parameter	Unit	Low Incidence	Moderate Incidence	High Incidence
pH (H_2O)	-	6.2	6.1	6.2
N	-	-	-	-
P Olsen	ppm	44.2	42.9	50.3
P Hcl	ppm	499.1	478.6	87.4
C-organic	%	0.7	0.7	0.8
K	cmol(+)/kg	1.6	1.4	1.2
Ca	cmol(+)/kg	4.3	3.6	4.6
Mg	cmol(+)/kg	2.5	2.2	2.2
Na	cmol(+)/kg	0.7	0.6	0.6
BS	%	71.6	57.5	58.8
Texture	-	Sandy clay loam	Sandy clay loam	Sandy clay loam

Microbial isolation

Bacterial and fungal isolates were obtained following the methods of Schaad et al. (2001) and Zain et al. (2014), respectively. Ten grams of each soil sample were suspended in 95 mL of sterile 0.85% NaCl solution and shaken at 120 rpm for 30 min. Serial dilutions (10^{-1}-10^{-4}) were prepared, and 0.1 mL aliquots were spread on 10% (w/v) Potato Dextrose Agar (PDA) and Martin Agar (MA) for fungal isolation, and on 10% (w/v) Tryptic Soy Agar (TSA) for bacterial isolation. All plates were incubated at 28±2°C for 3-5 days. Colonies differing in morphology were purified and maintained for further testing.

Enumeration of microbial morphospecies abundance

The abundance of microbial morphospecies was determined using the standard plate count method (Ramdan et al. 2020), calculated according to the formula:

$$N = \frac{\sum c}{[(1 \times n_1) + (0{,}1 \times n_2)]d}$$

Where, N indicates the total number of microorganisms, ∑c indicates the total number of colonies, n_1 indicates the total number of colonies in the first Petri dish, and n_2 indicates the number of colonies in the second Petri dish. Colony-Forming Units (CFU) were expressed per gram of dry soil (CFU g^{-1} soil).

Morphospecies were distinguished based on observable colony morphology, including differences in color, size, shape, margin, elevation, surface texture, and the presence or absence of sporulation

Assessment of microb diversity, evenness, and dominance indices

Microbial diversity indices were determined for soil samples from different BSR incidence levels to describe community structure. Microbial diversity (H′), evenness (E), and dominance (C) indices were calculated using standard ecological formulas (Shannon 1948; Simpson 1949; Magurran 2004), where n_i represents the abundance of each morphospecies, *N* denotes the total microbial count, and *S* represents the total number of species.

$$H' = -\sum \left(\frac{n_i}{N}\right) \times \ln\left(\frac{n_i}{N}\right)$$

$$E = \frac{H'}{LnS}$$

$$C = \sum \left(\frac{n_i}{N}\right)^2$$

Pathogenicity testing

Hemolysis test

The hemolytic activity of bacterial isolates was assessed following Gilligan (2013). Each isolate was streaked on blood agar medium containing 5% (v/v) fresh goat blood and incubated for 24-48 h at 28±2°C. Colonies showing clear (β-hemolysis) or greenish (α-hemolysis) zones were considered potentially pathogenic, while those showing no color change (γ-hemolysis) were deemed non-pathogenic.

Hypersensitivity test

Bacterial isolates negative for hemolysis were further tested for hypersensitivity reactions on tobacco (*Nicotiana tabacum*) leaves following Schaad et al. (2001). Bacterial suspensions grown in Tryptic Soy Broth (TSB) for 24-48 h were injected into the abaxial side of the leaves and incubated under ambient conditions for 48 h. Isolates causing necrosis were considered pathogenic and excluded from further analyses.

Fungal pathogenicity test

Fungal pathogenicity was assessed using a modified method of Levic et al. (2015) on cucumber (*Cucumis sativus*) seeds. Ten surface-sterilized seeds were placed 20 mm from a 7-day-old fungal culture on Water Agar (WA) medium and incubated for 7 days at 28°C. Fungal pathogenicity was evaluated based on the percentage of seed necrosis or mortality observed after incubation. Controls were maintained without fungal inoculum. Each treatment was conducted in triplicate. A seven-day incubation period was considered sufficient for detecting early pathogenic effects in this screening assay, following the modified protocol of Levic et al. (2015). These pathogenicity assays were used solely for preliminary screening of general pathogenic traits and were not intended to directly evaluate soil suppressiveness or identify the causal agent of BSR

Functional characterization of microbes

Antibiosis assay

Antagonistic activity was evaluated using the dual-culture technique modified from Karunasinghe et al. (2020). A 5 mm agar plug of *G. boninense* and the test fungus were placed 5 cm apart on PDA plates. For bacterial isolates, a 2 cm line streak was made opposite the *G. boninense* colony. Plates were incubated for 7 days, and inhibition (%) was calculated as:

$$\text{Inhibition rate (\%)} = \frac{R_2 - R_1}{R_2} \times 100\%$$

Where, R_1 indicates colony radius of *G. boninense* in control, and R_2 indicates colony radius in co-culture.

Volatile Organic Compound (VOC) assay

VOC-producing microbes were operationally defined as isolates that inhibited the growth of the target organism through volatile-mediated effects without physical contact

in a sealed dual-plate assay. VOC production was functionally verified based on reproducible growth inhibition relative to blank controls under standardized assay conditions. VOC-mediated inhibition was tested following Danaei et al. (2014). For VOC assays, paired Petri dishes were sealed face-to-face using parafilm to prevent gas exchange with the external environment while allowing volatile diffusion between plates. Plates were positioned in base-to-base contact and incubated under identical conditions and incubated under identical conditions for 7 days at room temperature. Control treatments consisted of *G. boninense* cultures paired with uninoculated medium, allowing inhibition effects to be attributed specifically to microbial volatile emissions. Inhibition (%) was calculated as:

$$\text{Inhibition rate (\%)} = \frac{D_2 - D_1}{D_2} \times 100\%$$

Where, D_1 indicates radial growth of *G. boninense* in the control, and D_2 indicates growth in the treatment. Each assay was conducted in triplicate.

Phosphate solubilization test

Phosphate-solubilizing microbes were identified using 10% Pikovskaya medium (PVK) following serial dilution plating. Plates were incubated for 2-5 days at 28°C. The presence of a clear halo zone surrounding the colony indicated phosphate solubilization activity.

Nitrogen fixation test

Nitrogen-fixing microbes were isolated using Nitrogen-Free Medium (NFM). Ten grams of soil were suspended in 90 mL sterile 0.85% NaCl solution, shaken for 30 min, and serially diluted (10^{-1}-10^{-5}). One milliliter from each dilution was plated and mixed with molten NFM at 45°C. Plates were incubated for 5 days at 28°C, and colony morphology was recorded.

Statistical analysis

Data were analyzed using one-way Analysis of Variance (ANOVA) to compare microbial abundance, diversity, and functional traits across disease incidence categories (low, medium, high). Where significant differences were found, Tukey's HSD test ($p<0.05$) was used for post hoc comparisons. Additionally, the Pearson correlation test was performed to determine the relationship between rhizosphere microbial populations and Basal Stem Rot (BSR) disease incidence and severity. The strength of associations was interpreted using commonly applied correlation coefficient thresholds.

To evaluate which rhizosphere microbial variables significantly influenced disease development, LASSO regression (Least Absolute Shrinkage and Selection Operator) was conducted to minimize multicollinearity and overfitting among the 17 predictor variables. Seventeen predictor variables representing rhizosphere microbial abundance, diversity indices, and functional microbial groups were included in the regression analysis, namely: total microbial population, total bacterial population, total fungal population, Shannon diversity index (H′), evenness index (E), dominance index (C), antagonistic bacteria, antagonistic fungi, total antagonistic microbes, VOC-producing bacteria, VOC-producing fungi, total VOC-producing microbes, nitrogen-fixing bacteria, nitrogen-fixing fungi, total nitrogen-fixing microbes, phosphate-solubilizing bacteria, and phosphate-solubilizing fungi. The optimal penalty parameter (λ=4.59) was determined through 10-fold cross-validation using the minimum Mean Squared Error (MSE) criterion. LASSO regression was applied to identify key predictors associated with BSR incidence and severity. Predictor selection was based on non-zero regression coefficients, and the resulting models were presented as mathematical equations. Statistical analyses were performed using R Studio, and results were expressed as mean ± Standard Deviation (SD).

RESULTS AND DISCUSSION

Microbial abundance at different levels of disease incidence

Microbial abundance differed significantly among disease incidence categories ($p<0.05$). Total microbial, bacterial, and fungal populations were significantly higher in soils with low disease incidence compared to soils with moderate and high disease incidence (Table 2). No significant differences were observed between the moderate- and high-incidence categories for any of the microbial groups. Overall, microbial abundance declined markedly once disease incidence increased from low to moderate levels.

Diversity, evenness, and dominance of microbes at different levels of disease incidence

Microbial and bacterial diversity and evenness were generally higher in soils with low disease incidence, while dominance increased with disease severity (Table 3). Conversely, fungal diversity did not follow a monotonic trend across disease incidence categories; the highest Shannon diversity index was observed in the moderate-incidence category (H′=1.75). Lower dominance (C) values in low-incidence soils indicated a more balanced microbial community structure.

Table 2. Microbial population in soil with different levels of disease incidence

Microbial group (unit)	Low incidence	Moderate incidence	High incidence	p-value
Total Microbes ($\times10^7$ CFU g^{-1} soil)	4.99±0.38^a	2.60±0.27^b	2.35±0.22^b	0.032
Bacteria ($\times10^7$ CFU g^{-1} soil)	4.94±0.36^a	2.58±0.25^b	2.34±0.19^b	0.027
Fungi ($\times10^5$ CFU g^{-1} soil)	5.18±0.47^a	1.50±0.13^b	1.64±0.11^b	0.041

Note: Numbers followed by the same letter within the same row are not significantly different according to Tukey's test at the 5% level

Pathogenic microbes and biological agents at different levels of disease incidence

Hypersensitivity and pathogenesis tests indicated that non-pathogenic microbes predominated in soils with low disease incidence, accounting for approximately 2.5-, 2-, and 3-fold higher proportions of total, bacterial, and fungal populations, respectively, compared to pathogenic counterparts (Figure 1). The proportion of non-pathogenic microbes decreased with increasing disease incidence, with non-pathogenic to pathogenic ratios of approximately 4:3 and 1:1 in moderate and high categories. Functional microbial groups exhibited a similar trend, as antagonistic and Volatile Organic Compound (VOC)-producing microbes were most abundant in low-incidence soils and decreased progressively with increasing disease incidence (Figure 2). In the moderate-incidence category, antagonistic and VOC-producing microbes accounted for 66.67% and 16.67% of isolates, respectively, whereas VOC-producing microbes were not detected in high-incidence soils. Total microbes represent the aggregated culturable microbial population, whereas bacterial and fungal counts were quantified separately; therefore, these categories are not intended to sum directly.

Inhibition assays further revealed significant differences ($p<0.05$) among disease incidence levels (Table 4). Both antagonistic and VOC-producing microbes exhibited the strongest inhibition in soils with low Basal Stem Rot (BSR) incidence, with inhibition capacity declining progressively in moderate and high categories. Antagonistic microbes consistently showed slightly higher inhibition potential than VOC-producing microbes, suggesting that direct antibiosis played a more prominent role in pathogen suppression than volatile-mediated inhibition. Overall, inhibition capacity decreased in parallel with increasing disease incidence, reflecting reduced functional microbial activity under severe BSR conditions.

Abundance of functional microbes at different levels of disease incidence

The abundance of phosphate-solubilizing microbes was consistently higher than that of nitrogen-fixing microbes across all disease incidence categories (Table 5). Although no significant differences were detected among disease categories ($p>0.05$), a slight increasing trend was observed with higher disease incidence.

Table 3. Diversity index (H'), evenness (E), and dominance (C) of microbes, bacteria, and fungi at different levels of disease incidence

Microbial group	Disease incidence	Diversity (H')	Evenness (E)	Dominance (C)
Total microbes	Low	1.07	0.31	0.36
	Moderate	0.93	0.30	0.44
	High	0.88	0.32	0.47
Bacteria	Low	0.74	0.24	0.50
	Moderate	0.60	0.21	0.73
	High	0.64	0.25	0.73
Fungi	Low	1.46	0.39	0.25
	Moderate	1.75	0.57	0.24
	High	1.27	0.63	0.31

Note: Higher H′ and E indicate greater microbial diversity and evenness, while higher C indicates stronger dominance within the community

Table 4. Inhibition activity of antagonistic and VOC-producing microbes against *G boninense* at different levels of disease incidence

Microbial group	Low	Moderate	High	p-value
Antagonistic microbes	51.5±8.5^{a}	38.4±6.5^{b}	26.5±5.8^{c}	<0.001
VOC-producing microbes	45.1±7.3^{a}	31.4±5.2^{b}	18.8±4.5^{c}	0.002

Note: Numbers by the same letter within each microbial group are not significantly different according to Tukey's test ($p<0.05$)

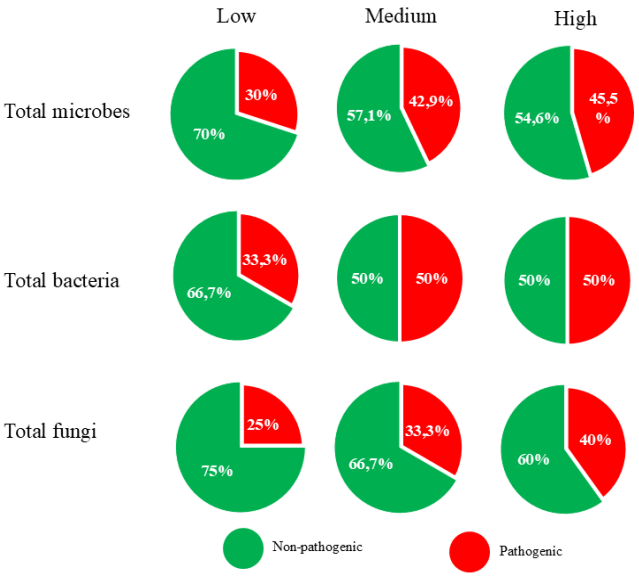

Figure 1. Percentage of pathogenic and non-pathogenic microbes across different levels of disease incidence

Table 5. Functional microbial populations (phosphate-solubilizing and nitrogen-fixing microbes) at different levels of disease incidence

Functional microbial group (Unit)	Low incidence	Moderate incidence	High incidence	p-value
Phosphate-solubilizing ($\times 10^5$ CFU g^{-1} soil)	1.21±0.12[a]	1.69±0.14[a]	1.80±0.16[a]	0.412
Nitrogen Fixer ($\times 10^3$ CFU g^{-1} soil)	5.48±0.48[a]	5.64±0.57[a]	7.46±0.61[a]	0.087

Note: Numbers followed by the same letter within a row are not significantly different according to Tukey's test at the 5% level

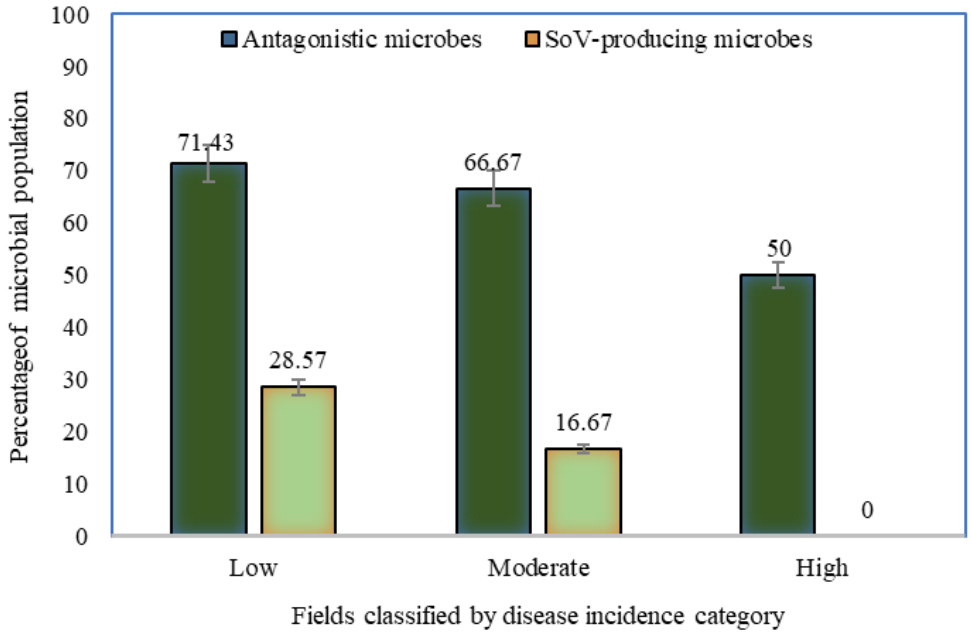

Figure 2. Percentage of antagonistic microbes and VOC-producing microbes detected under in vitro assay conditions across different levels of basal stem rot disease incidence. VOC-producing microbes were defined based on growth inhibition in a sealed dual-plate assay without physical contact

Relationship and regression analysis of rhizosphere microbes associated with basal stem rot disease

Correlation analysis revealed distinct relationships between rhizospheric microbial attributes and BSR disease parameters (Table 6). Disease incidence and severity were positively correlated with the abundance of nitrogen-fixing microbes (r=0.47, p=0.08; r=0.63, p=0.01), indicating that higher N-fixer populations tended to occur in soils with more severe BSR symptoms. In contrast, VOC-producing microbes exhibited negative correlations with disease incidence and severity (r=-0.60, p=0.02; r=-0.44, p=0.10), suggesting that their presence may be associated with reduced pathogen activity. Other microbial parameters, including total microbial counts, diversity, and evenness indices, showed weak and non-significant relationships with disease variables (p>0.05). These results indicate that specific functional groups, rather than overall microbial abundance or diversity, are more closely associated with variations in BSR incidence and severity (Table 6). Regression analysis indicated associations between specific-fungtional microbial groups and BSR incidence and severity. LASSO regression identified antagonistic, VOC-producing, and as a predictor explaining 63.3% of the variance in disease incidence (R^2=0.633) and 68.9% in disease severity. Regression analysis indicated that BSR incidence and severity were negatively associated with the abundance of antagonistic and VOC-producing microbes, and positively associated with nitrogen-fixing microbes (Equations 1-2).

Equation 1: Y (Disease incidence) = 43.89 − 1.47(Antagonistic microbes) − 6.49(VOC-producing microbes) (R^2 = 0.63)

Equation 2: Y (Disease severity) = 32.66 − 4.33(VOC-producing microbes) − 1.37(Phosphate-solubilizing microbes) (R^2 = 0.69)

Discussion

Functional microbial groups influencing Basal Stem Rot (BSR) dynamics could be distinguished based on the level of statistical support. VOC-producing and nitrogen-fixing microbes were supported by both correlation analysis and LASSO regression, indicating consistent associations with BSR incidence and severity. In contrast, antagonistic and phosphate-solubilizing microbes were primarily identified through LASSO selection, suggesting their potential contribution to BSR dynamics despite the absence of significant pairwise correlations. These findings underscore the importance of considering both statistical association and multivariate predictor selection when interpreting the role of rhizosphere microbiota in disease suppression.

The mechanism by which antagonistic and VOC-producing microbes suppress plant pathogens primarily involves antibiosis and enzymatic lysis, which can damage pathogen cell walls and membranes, resulting in hyphal thickening, melanization, shrinkage, curling, or collapse (Widiantini et al. 2020; Istifadah et al. 2022; Zhao et al. 2022). VOC-producing microbes are also known to release volatile metabolites that inhibit *Ganoderma* growth through interference with mycelial respiration and signaling. Such antagonistic interactions may disrupt *Ganoderma* infection both directly-by inhibiting pathogen growth-and indirectly-by altering the chemical and microbial equilibrium of the rhizosphere.

Beyond the role of specific functional microbial groups, overall rhizospheric microbial abundance was associated with field-level differences in BSR incidence rather than acting as a strong continuous determinant of disease suppression. Fields categorized by low disease incidence were characterized by higher overall microbial abundance and fungal diversity, suggesting a more competitive and resilient rhizosphere environment. A dense microbial population may suppress pathogens through competitive exclusion for root exudate nutrients and space. Moreover, beneficial soil microbes produce siderophores that chelate Fe^{3+} ions, thereby reducing its availability for pathogen metabolism and virulence (Ning et al. 2023). Similar mechanisms have been widely reported in disease-suppressive soils, where enriched and functionally active rhizosphere microbiomes are associated with reduced establishment or activity of soil-borne pathogens (Schlatter et al. 2017; Spooren et al. 2024). These observations suggest that high microbial abundance may function as a field-level buffering factor against pathogen proliferation, rather than a strong continuous predictor of disease suppression.

Microbial diversity further contributes to the ecological stability of the soil environment and serves as a key indicator of soil health (Che and Jin 2024). In the present study, lower diversity, evenness, and dominance indices in soils with higher disease incidence suggest that BSR-affected rhizospheres were ecologically stressed and less resilient (Odum 1971; Di et al. 2025). Reduced microbial diversity is often associated with impaired ecosystem functioning, including slower organic matter decomposition, weaker nutrient cycling, and diminished pathogen suppression (Iqbal et al. 2023). Although fungal diversity was moderate to high, bacterial dominance was pronounced in severely infected fields, indicating a community imbalance that may favor opportunistic pathogens. This trend aligns with the findings of Rahman and Othman (2020), who reported that microbial community disruption is closely linked to increased BSR severity in oil palm plantations.

The compositional shift between pathogenic and non-pathogenic microbes across disease incidence levels provides further insight into disease ecology. Non-pathogenic and antagonistic microbes were predominant in soils with low disease incidence but declined sharply as disease severity increased, allowing pathogenic microbes to dominate in moderate- to high-incidence fields. Although these isolates were not identified as *G. boninense*, several exhibited pathogenic traits commonly associated with plant-pathogenic microorganisms, suggesting that their presence in the rhizosphere may compromise host health and potentially facilitate *Ganoderma* infection. Similar synergistic interactions between secondary pathogens and primary infections have been documented in other systems, such as rust diseases caused by *Puccinia* sp. (Fauzi and Murdan 2009).

Collectively, these findings indicate that both microbial abundance and functional diversity synergistically contribute to the suppression of BSR disease. The significant relationships between VOC-producing, antagonistic, and nitrogen-fixing microbes and BSR parameters suggest that disease regulation is a multifactorial process involving competitive, biochemical, and nutrient-mediated mechanisms. However, the positive association between nitrogen-fixing microbes and BSR severity should be interpreted with caution. Because this study was based on correlation analysis, the observed patterns do not imply causation. It is possible that nitrogen-fixing microbes do not directly promote disease but instead proliferate in soils where BSR has already altered nutrient availability and redox balance. Decomposition of infected palm roots could increase ammonium and dissolved organic nitrogen, creating favorable conditions for diazotrophic bacteria (Tanikawa et al. 2023; Duan et al. 2025). Alternatively, both increased nitrogen-fixing activity and disease severity may result from a third, unmeasured factor, such as reduced organic carbon or soil oxygen imbalance (Orr and Nelson 2018; Wang et al. 2023; Zhou et al. 2024).

Table 6. Correlation between rhizosphere microbial attributes and basal stem rot incidence and severity

Rhizosphere microbial attribute	Disease incidence		Disease severity	
	r	p-value	r	p-value
Pathogenic microbes	0.36	0.18	0.31	0.26
Antagonistic microbes	-0.10	0.72	-0.05	0.85
VOC-producing microbes	-0.60*	0.02	-0.44	0.10
Total microbial diversity	-0.12	0.68	-0.16	0.58
Fungal diversity	0.03	0.90	-0.01	0.97
Bacterial diversity	0.09	0.75	0.10	0.72
Microbial evenness (total)	0.26	0.34	0.13	0.63
Fungal evenness	0.28	0.29	0.21	0.43
Bacterial evenness	0.30	0.26	0.25	0.34
Microbial dominance (total)	-0.06	0.83	-0.04	0.88
Fungal dominance	-0.00	0.99	-0.03	0.91
Bacterial dominance	0.13	0.63	0.06	0.83
Phosphate-solubilizing microbes	0.17	0.52	0.22	0.41
Nitrogen-fixing microbes	0.47	0.08	0.63*	0.01
Total microbes	-0.29	0.28	-0.21	0.46
Total bacteria	-0.26	0.33	-0.32	0.22
Total fungi	-0.29	0.28	-0.22	0.43

Note: Correlation coefficients (r) marked with (*) are significant at $p<0.05$

These ecological hypotheses warrant further testing through field-scale experiments and metagenomic studies to clarify whether nitrogen-fixing microbes play an adaptive or incidental role in the ecology of BSR-affected soils. Future work using molecular or metagenomic approaches is recommended to identify key taxa involved in these interactions. From an applied perspective, identifying the interactions between soil nutrients-particularly potassium (K), magnesium (Mg), and sodium (Na)-and microbial dynamics provides a scientific basis for developing microbiome-informed fertilization strategies. Integrating nutrient management with beneficial microbial inoculants could form the foundation of a sustainable and integrated BSR management system for oil palm plantations. Such approaches not only enhance plant nutrition and resistance but also promote long-term soil health and ecosystem resilience. The study revealed that estimates of microbial diversity, evenness, and dominance likely represent only the dominant culturable fraction of the rhizosphere community, as culture-based and morphotype-based approaches tend to underestimate total microbial diversity and exclude non-culturable taxa. Future studies integrating soil nutrient indices (e.g., K, Mg, and Na) with culture-independent approaches such as amplicon sequencing or metagenomic analyses would provide a more comprehensive understanding of suppressive soil characteristics and rhizosphere microbial functions.

Overall, this study demonstrates that variations in Basal Stem Rot (BSR) incidence under field conditions are more closely associated with the functional composition of rhizosphere microbial communities than with overall microbial abundance alone. Consistent with the study hypothesis, soils with low BSR incidence were characterized by a greater prevalence of antagonistic and VOC-producing microbes, which were repeatedly identified as key microbial attributes across both correlation and multivariate analyses. These findings suggest that disease suppression in oil palm soils is linked to functional microbial traits related to antibiosis, volatile-mediated interactions, and nutrient dynamics, rather than to single dominant microbial groups. This functional perspective provides a conceptual framework for understanding BSR-suppressive soils and highlights the importance of targeting microbial functions, rather than solely microbial abundance, in the development of sustainable BSR management strategies.

In conclusion, this study demonstrates that Basal Stem Rot (BSR) incidence in oil palm is closely associated with shifts in the abundance, diversity, and functional composition of rhizosphere microbial communities. Soils with low BSR incidence contained significantly higher total microbial, bacterial, and fungal populations (4.99×10^7, 4.94×10^7, and 5.18×10^5 CFU g^{-1} soil, respectively) than soils with moderate and high incidence. These low-incidence soils also exhibited greater microbial and bacterial diversity (H'=1.07 and 0.74) and lower dominance, indicating a more balanced community structure. Functional microbial groups were strongly associated with disease suppression patterns, as antagonistic and Volatile Organic Compound (VOC)-producing microbes were most abundant in low-incidence soils and showed stronger inhibition of *G. boninense* (51.5% and 45.1% inhibition, respectively). Correlation analysis revealed a significant negative association between VOC-producing microbes and BSR incidence ($r=-0.60$, $p=0.02$), while nitrogen-fixing microbes were positively associated with disease severity ($r=0.63$, $p=0.01$). LASSO regression further identified antagonistic and VOC-producing microbes as key predictors explaining 63.3% of the variability in BSR incidence, highlighting the relevance of functional microbial traits in BSR-suppressive soils. Collectively, these findings indicate that BSR-suppressive soils are characterized by functionally active rhizosphere microbial communities, particularly those capable of antibiosis and volatile-mediated inhibition.

This study was based on a single sampling period and culture-dependent approaches, which may underestimate total microbial diversity and temporal dynamics. Future research should incorporate longitudinal sampling, molecular and metagenomic analyses to identify key taxa, and controlled field experiments to clarify causal relationships between functional microbes and BSR suppression. Integrating microbial functional indicators with soil nutrient management may further support the development of sustainable, microbiome-informed strategies for BSR management in oil palm plantations.

ACKNOWLEDGEMENTS

This study was supported by a research grant under contract number 1/E1/KP.PTNBH/2021 (2021) from the Directorate General of Higher Education, Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia.

REFERENCES

Che Y, Jin G. 2024. Plant-soil microbial diversity and structural attributes jointly dominate the multifunctionality of the temperate forest. Ecol Indic 166: 112282. https://doi.org/10.1016/j.ecolind.2024.112282.

Chen W, Modi D, Picot A. 2023. Soil and phytomicrobe for plant disease suppression and management under climate change: A review. Plants 12 (4): 2736. https://doi.org/10.3390/plants12142736.

Danaei M, Baghizadeh A, Pourseyedi S, Amini J, Yaghoobi MM. 2014. Biological control of plant fungal diseases using volatile substances of *Streptomyces griseus*. Eur J Exp Biol 4 (1): 334-339.

Di K, Yuanyuan S, Shishi F, Qianmin L, Shuzhen Z. 2025. Microbial community diversity and assembly processes in the aridification of wetlands on the Qinghai-Tibet Plateau. iScience 28 (5): 112494. https://doi.org/10.1016/j.isci.2025.112494.

Duan P, Zhao D, Yang S, Chen J, Chen Z, Cao L. 2025. Soil native C/N ratio affects diazotrophic bacterial composition and N fixation by regulating SOC distribution in soil particles after residue. Microorganisms 13 (5): 1104. https://doi.org/10.3390/microorganisms13051104.

Fauzi MT, Murdan M. 2009. The role of secondary fungal pathogens in increasing biocontrol ability of a rust fungus (*Puccinia* sp.) on purple nutsedge (*Cyperus rotundus*). J Crop Agro 8 (2): 152-157.

Gilligan PH. 2013. Identification of pathogens by classical clinical tests. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (eds). The Prokaryotes. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-30144-5_90.

He L, Sun X, Li S, Zhou W, Chen Z, Bai X. 2023. The vertical distribution and control factor of microbial biomass and bacterial

community at macroecological scales. Sci Total Environ 869: 161754. https://doi.org/10.1016/j.scitotenv.2023.161754.

Iqbal B, Li G, Alabbosh KF, Hussain H, Khan I, Tariq M, Javed Q, Naeem M, Ahmad N. 2023. Advancing environmental sustainability through microbial reprogramming in growth improvement, stress alleviation, and phytoremediation. Plant Stress 10: 100283. https://doi.org/10.1016/j.stress.2023.100283.

Istifadah N, Septiandini A, Hartati S, Widiantini F. 2022. Inhibition effects of culture filtrates and volatile compounds of antagonistic microbes isolated from vermicompost and compost teas on the growth of *Alternaria solani* Sor. in vitro. Cropsaver J Plant Prot 5 (2): 98-105. https://doi.org/10.24198/cropsaver.v5i2.43278.

Karunasinghe TG, Maharachchikumbura SSN, Velazhahan R, Al-Sadi AM. 2020. Antagonistic activity of endophytic and rhizosphere fungi isolated from sea purslane (*Sesuvium portulacastrum*) against *Pythium* damping-off of cucumber. Plant Dis 104 (8): 2158-2167. https://doi.org/10.1094/pdis-01-20-0003-re.

Levic J, Stankovic S, Ivanovic D, Petrovic T. 2015. Pathogenicity of *P. terrestris* on maize seedlings. Pestic Phytomed 27 (3): 213-217. https://doi.org/10.2298/PIF1203213L.

Magurran AE. 2004. Measuring Biological Diversity. Wiley-Blackwell, Oxford, UK.

Mustapha JC, Mazliham S, Boursier P. 2011. Temporal analysis of basal stem rot disease in oil palm plantations: An analysis on peat soil. Intl J Eng Technol 11 (3): 96-101.

Ning X, Lin M, Hung G, Mao J, Gao Z, Wang X. 2023. Research progress on iron absorption, transport, and molecular regulation strategy in plants. Front Plant Sci 14: 1190768. https://doi.org/10.3389/fpls.2023.1190768.

Niu B, Wang W, Yuang Z, Sederoff RR, Sederoff H, Chiang VL, Borriss R. 2020. Microbial interactions within multiple-strain biological control agents impact soil-borne plant disease. Front Microbiol 11: 585404. https://doi.org/10.3389/fmicb.2020.585404.

Odum EP. 1971. Fundamentals of Ecology (3rd ed). W.B. Saunders Co., Philadelphia, US.

Orr R, Nelson PN. 2018. Impact of soil abiotic attributes on *Fusarium* Wilt, focusing on bananas. Appl Soil Ecol 132: 20-33. https://doi.org/10.1016/j.apsoil.2018.06.019.

Pirttila AM, Tabas HMP, Baruah N, Kossimaki JJ. 2021. Biofertilizers and biocontrol agents for agriculture: How to identify and develop new potent microbial strains and traits. Microorganisms 9 (4): 817. https://doi.org/10.3390/microorganisms9040817.

Rahman KA, Othman R. 2020. Influence of pH levels on disease development in oil palm seedling roots infected with *Ganoderma boninense*. Rhizosphere 13: 100181. https://doi.org/10.1016/j.rhisph.2019.100181.

Ramdan EP, Hartono A, Giyanto G, Hidayat SH, Widodo W. 2023. The relationship between soil fertility and basal stem rot disease in oil palm plantations. Agrosainstek Jurnal Ilmu dan Teknologi Pertanian 7 (1): 32-39. https://doi.org/10.33019/agrosainstek.v7i1.384.

Ramdan EP, Hartono A, Giyanto G, Hidayat SH, Widodo W. 2024. Effects of soil physicochemical properties on basal stem rot disease (*Ganoderma boninense*) in oil palm. Pak J Phytopathol 36 (1): 39-44. https://doi.org/10.33866/phytopathol.036.01.1029.

Ramdan EP, Perkasa AY, Munif A, Astuti D, Hanif A, Wati C, Afriani A, Nurholis N. 2020. Abundance of soil microbial communities and plant growth in agroecosystems and forest ecosystems. Eur J For Sci 8 (2): 123-128. https://doi.org/10.3195/ejejfs.712478.

Schaad NW, Jones JB, Chun W. 2001. Laboratory Guide for Identification of Plant Pathogenic Bacteria. APS Press, St. Paul.

Schlatter DC, Kinkel LL, Thomashow LS, Weller DM, Paulitz TC. 2017. Disease-suppressive soils: New insights from the soil microbiome. Phytopathology 107 (11): 1284-1297. https://doi.org/10.1094/phyto-03-17-0111-rvw.

Shannon CE. 1948. A mathematical theory of communication. Bell Syst Tech J 27 (3): 379-423. https://doi.org/10.1002/j.1538-7305.1948.tb01338.x.

Simpson EH. 1949. Measurement of diversity. Nature 163: 688. https://doi.org/10.1038/163688a0.

Siregar BA, Giyanto G, Hidayat SH, Siregar IZ, Tjahjono B. 2020. Epidemiologi of Bacterial Wilt Disease on *Eucalyptus pellita* F. Muell. in Indonesia. IOP Conf Ser Earth Environ Sci 468: 012033. https://doi.org/10.1088/1755-1315/468/1/012033.

Spooren J, van Bentum S, Thomashow LS, Pieterse CMJ, Weller DM, Berendsen RL. 2024. Plant-driven assembly of disease-suppressive soil microbiomes. Ann Rev Phytopathol 62: 1-30. https://doi.org/10.1146/annurev-phyto-021622-100127.

Sun T, Wang Y, Lucas-Borja ME, Jing X, Feng W. 2021. Divergent vertical distributions of microbial biomass with soil depth among groups and land uses. J Environ Manag 292: 112755. https://doi.org/10.1016/j.jenvman.2021.112755.

Tanikawa T, Maie N, Fujii S, Sun L, Hirano Y, Mizoguchi T, Matsuda Y. 2023. Contrasting patterns of nitrogen release from fine roots and leaves driven by microbial communities during decomposition. Sci Total Environ 855: 158809. https://doi.org/10.1016/j.scitotenv.2022.158809.

Wafa A. 2017. Sebaran Vertikal Mikroba Fungsional pada Perakaran Kelapa Sawit dan Potensinya Sebagai Agens Pengendalian Hayati *Ganoderma boninense* Pat. [Thesis]. Institut Pertanian Bogor, Bogor. [Indonesian]

Wahyudi AT, Pinem MI, Pangestiningsih Y. 2017. Kemampuan cendawan tanah supresif terhadap *Ganoderma boninense* pada kebun kelapa sawit. Jurnal Agroteknologi 90: 707-715. [Indonesian]

Wang W, Shi F, Du J, Li L, Bai T, Xing X. 2023. Soil factors that contribute to the abundance and structure of the diazotrophic community and soybean growth, yield, and quality under biochar amendment. Chem Biol Technol Agric 10: 54. https://doi.org/10.1186/s40538-023-00429-0.

Widiantini F, Yulia E, Kurniawan A. 2020. Penghambatan pertumbuhan *Rhizoctonia oryzae* dan *Cercospora oryzae* oleh senyawa volatil yang dihasilkan bakteri endofit padi. Agrikultura 31 (1): 61-67. DOI: 10.24198/agrikultura.v31i1.27323. [Indonesian]

Zain NMM, Mohamad RB, Sijam K, Awang Y. 2014. Isolation and identification of microorganisms from soil in a young oil palm plantation. J Food Agric Environ 12 (3): 443-447. https://doi.org/10.1234/4.2014.5426.

Zhao X, Zhou J, Tian R, Liu Y. 2022. Microbial volatile organic compounds: Antifungal mechanisms, applications, and challenges. Front Microbiol 13: 922450. https://doi.org/10.3389/fmicb.2022.922450.

Zhou S, Buteschoen O, Handa T, Berg MP, McKie B, Huang C, Hattenschwiler S, Scheu S. 2024. Litter mixture effects on nitrogen dynamics during decomposition predominantly vary among biomes but little with litter identity, diversity, and soil fauna. Soil Biol Biochem 199: 109602. https://doi.org/10.1016/j.soilbio.2024.109602.