

Association of soil bacterial diversity and composition with *Fusarium* wilt disease of bananas in Yogyakarta Province, Indonesia

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Abstract. Kurniasari I, Wibowo A, Subandiyah S, Pattison AB. 2024. Association of soil bacterial diversity and composition with *Fusarium* wilt disease of bananas in Yogyakarta Province, Indonesia. *Biodiversitas* 25: 2264-2275. Infection of banana plants with *Fusarium oxysporum* f. sp. *ubense* Tropical Race 4 (*Foc*-TR4) leads to a cascade of changes in the rhizosphere, resulting in changes to the soil physicochemical properties and bacterial community composition. This study aimed to determine the core taxa associated with different soil physicochemical properties and disease statuses and to identify the factors exerting the greatest influence on the soil bacterial community composition of bananas in Special Region of Yogyakarta Province, Indonesia. Rhizospheric soil from healthy and infected plants was collected from four banana locations, including Kulon Progo, Gunungkidul, Bantul, and Sleman districts, at 10-30 cm depth between May and September 2021. Laboratory analysis was conducted for soil physicochemical properties, and the abundance of *Foc*-TR4 was undertaken. Additionally, 16S rRNA gene-based metagenomics analysis was used to quantify bacterial diversity in all samples. Soil type significantly influenced the abundance and composition of the bacterial community more than disease status did. Contrarily, disease status groups influenced the complexity of bacterial network interaction more than soil type. *Actinobacteriota*, *Proteobacteria*, *Chloroflexi*, *Acidobacteriota*, and *Bacteroidota* are the major phyla associated with all soils. This study identified 60 bacterial genera as core members of the banana rhizosphere. These core bacterial genera include various plant growth-promoting bacteria involved in nitrogen fixation, phytohormone production, siderophore production, and biocontrol mechanisms for producing antifungal compounds. Our work provides a basis for future research on the soil bacterial composition associated with banana plants to enhance plant health against *Fusarium* wilt disease.

Keywords: Bacterial communities, *Foc*-TR4, *Fusarium* wilt, soil physicochemical properties

INTRODUCTION

Bananas are a crucial fruit commodity in Indonesia, ranking 9th globally in banana production (FAOSTAT, 2024). However, the industry is plagued by *Fusarium* wilt disease, caused by the soil-borne pathogen *Fusarium oxysporum* f.sp. *ubense* (*Foc*). The Tropical Race 4 (TR4) of *Foc* remains one of the most virulent strains, with a wide host range, causing significant losses to banana farmers in Indonesia. This is due to many secreted genes in the xylem (SIX) responsible for virulence factors that can transfer genes horizontally (Maldonado Bonilla et al. 2018). Furthermore, 40 local banana varieties from 15 Indonesian provinces have been infected by *Foc*-TR4, causing annual losses of 1.21 trillion rupiahs (Hermanto et al. 2011). *Foc*-TR4 invades the vascular tissues of bananas through the roots and corm (Kaushal et al. 2020). The mycelia of *Foc*-TR4 block the xylem vessels, disrupting nutrient absorption and water transport to the pseudostem, causing the leaves to wilt and turn yellow (Li et al. 2017; Maldonado Bonilla et al. 2018).

The expression of *Fusarium* wilt disease symptoms is influenced by external factors like environment and bacterial community inhabiting the root and rhizome. The bacterial

community in the rhizosphere region of banana plants plays a vital role in attracting and initiating bacterial colonization on banana roots. It is known that the rhizosphere region of banana plants has the greatest diversity of microbial communities due to 5-20% of photosynthetic products released into the rhizosphere via root exudates (Turner et al. 2013). After pathogen infection, the host will change their metabolism, including recruiting specific bacteria, which helps the plants resist the disease, resulting in microbial structure changes. On the other hand, changes in the soil environment, including soil physicochemical properties, will impact the soil bacterial community structure. Several reports stated that soil physicochemical properties, including soil pH, soil texture, soil organic matter, N content, and P availability, significantly influenced microbial community structure (Zhang et al. 2018; Sopiarena et al. 2018; Xia et al. 2020; Obayomi et al. 2021; Hou et al. 2023).

The microbial community structures observed in this study are affected by various factors, including abiotic (soil physicochemical properties), biotic factors (*Foc*), and host factors (banana plants). According to Agler et al. (2016), three mechanisms generally contribute to microbial community structure: random colonization, species sorting

by local factors (e.g., nutrient availability, host availability, and microbial interactions), and isolating factors such as dispersion and distance. Agler also stated that specific bacteria are vital intermediaries between abiotic, biotic, and host factors through species-sorting mechanisms. However, the complex relationship among soil microbial species, *Foc*, soil physicochemical properties, and banana plants must be analyzed. Moreover, reports that reveal whether soil type or the presence of *Foc*-TR4 has the most influence on the composition of the bacterial community in the rhizosphere of banana plants in Special Region of Yogyakarta Province are still very limited.

This study used metataxonomic analysis to evaluate how various factors influence the bacterial community structure. This structure was visualized using a co-occurrence network and quantified through network topology. From the co-occurrence network, specific microbial 'hubs' within a complex microbial community can be identified due to their central positions. These hubs are disproportionately important in shaping microbial communities. The topological properties of the microbial network serve as good indicators of the stability and diversity of the microbial community. Understanding the soil bacterial composition associated with banana plants enhances plant health against *Fusarium* wilt disease, providing a basis for future research. Therefore, this study aimed to determine the core bacterial taxa associated with different soil physicochemical properties and disease status in banana plantations in Special Region of Yogyakarta Province, Indonesia, and identify the most influential soil bacterial community compositions.

MATERIALS AND METHODS

Sampling procedure

Furthermore, 8 soil samples were selected from four banana locations: Kulon Progo, Gunungkidul, Bantul, and Sleman districts in Special Region of Yogyakarta Province, Indonesia. The specific sampling site information is shown in (Table 1). Bananas have been planted continuously in mixed cultivar plantings for at least two years. All four districts have a tropical climate with a rainy season between November and April and a dry season from May to October 2021. In addition, a mean annual temperature of 17.3-28.7°C and an average rainfall of 2.8-699.2 mm. Soil samples were taken from the rhizosphere of healthy and infected mature banana plants in the development stage from 10-30 cm depth from May to September 2021. At each site, 5-8 individual plants were sampled to create composite soil

samples and separate samples collected from healthy and infected plants. After collection, soil samples were mixed and sieved with a 2-mm mesh; one portion was air-dried for physicochemical analysis, and the other was stored at -20°C for DNA extraction.

Physicochemical soil properties determination

Soil physical and chemical analyses were conducted at the Soil and Water Plant Laboratory, Institute for Agricultural Technology (BPTP) Yogyakarta. These analyses included soil texture determination using the hydrometer method, pH measurement with a pH meter, organic carbon content assessed by the Walkey & Black method, total nitrogen determined via the Kjeldahl method, available potassium and available phosphorous analyzed using the Olsen method, and calculation of the C/N ratio.

Disease incidence and *Foc*-TR4 quantification

Identification of plants infected by *Foc* was conducted through visual observation of typical wilt symptoms, including external wilting and yellowing symptoms on leaves and internal discoloration symptoms on pseudostem. The incidence of *Fusarium* wilt disease was calculated as follows (Wibowo et al. 2001):

$$\text{Disease incidence (\%)} = \frac{\Sigma (\text{Infected plants})}{\Sigma (\text{Total of observed plants})} \times 100$$

The abundance of *Foc*-TR4 was quantified by qPCR following a modified method (Ulilalbab et al. 2022). Standard curves were generated using tenfold serial dilutions of DNA from a *Foc*-TR4 single isolate. Quantitative PCR amplifications (BIO-RAD, USA) for standard and soil DNA samples were performed with a total volume of 10 µL in each reaction using the Thunderbird SYBR® qPCR Mix (Toyobo, Japan) using two set primer pairs. The primer *Foc*-Six1c with product length 96 bp was used with sequence Forward-CCAGAGGGGCAGGCTCAG and Reverse-GTAGACTTGTCGGTGGTAGCGAC. PCR reaction contained 1 µL of the target DNA, 5 µL of Thunderbird SYBR® qPCR Mix, 1 µL of primer *Foc*-Six1cF and *Foc*-Six1cR, and 3 µL sterile distilled water. Thermal cycling conditions consisted of 2 min at 95°C followed by 40 amplification cycles of denaturation at 95°C for 5 s, annealing at 55°C for 10 s, extension at 72°C for 5 s, and final extension at 72°C for 5 min. Each sample was performed with three parallel sub-samples, and the results were expressed as copy numbers per gram of dry soil.

Table 1. Basic information on the sampling site in Yogyakarta Province, Indonesia

Locations	Code	Longitude	Latitude	Altitude (m asl.)	Soil type
Nanggulan, Kulon Progo	KP	110.13.3	7.45.46	101	Clay
Wonosari, Gunungkidul	GK	110.34.22	7.58.16	191	Clay
Bambanglipuro, Bantul	BT	110.18.16	7.57.52	14	Sandy clay loam
Kalasan, Sleman	SM	110.27.55	7.46.11	133	Sandy clay loam

Total DNA extraction and sequencing

Total DNA was extracted using a ZymoBIOMICS™ DNA Miniprep Kit (United States of America) following the manufacturer's protocol. The final DNA concentration was measured using a nanodrop 2000 UV-vis spectrophotometer (NanoQuant Plate TECAN Spark) and then visualized using 1% agarose. Soil microbial analysis was carried out using Illumina HiSeq 2500 PE250 (Novogen, Korea) based on V3-V4 hypervariable region for 16S rDNA and amplified using 341-F (5'-CCTAYGGGRBGCAS CAG-3') and 806-R (5'-GGACTACNNGGGTATCTAAT-3') primer pair. The PCR product was run using 2% agarose. For PCR purification, the DNA bands were eluted using a DNA gel extraction kit (Qiagen, Germany), and the TruSeq® DNA PCR Kit was used to design the sequence library. The library quality was calculated using a Qubit 2.0 Fluorometer (Thermo Scientific, USA). Finally, the amplicon was sequenced on the Illumina paired-end platform to generate 250 bp paired-end raw reads performed at NovogeneAIT Genomics Singapore.

Quality control and host DNA removal

Quality control was performed to maximize the quality of the datasets using the QIIME2 pipeline version 2024.2 (Bolyen et al. 2019). Initially, adapters and primers were eliminated utilizing cutadapt (QIIME2 plugin), followed by denoising with DADA2 (another QIIME2 plugin). After denoising, the sequences were merged to obtain clean reads. The chimeric sequences in the clean reads were detected and removed to obtain effective reads, which can be used for subsequent analysis. Taxonomic clustering of the resulting ASVs (Amplicon Sequence Variance) was compared using the SILVA SSU database release 138.

Taxonomic profiling and diversity analysis

Effective reads were taxonomically assigned using Kraken2 version 2.0.8-beta (Wood et al. 2019) and Bracken version 2.5.0 (Lu et al. 2017) to obtain corresponding taxa information and taxa-based abundance distribution. The bacterial abundance table generated from taxonomic profiling was used as input for diversity analysis. Data normalization was executed in R version 4.2.1 (R Core Team 2022), employing the vegan R package version 2.6-4 (Oksanen et al. 2019). This process utilized the rarefying method, involving down-sampling reads to 50,000 reads per sample. Diversity analyses encompassing alpha and beta diversities were also performed using R version 4.2.1 (R Core Team 2022) and R packages vegan version 2.6-4 and visualized using ggplot2 version 3.4.2 (Wickham 2016).

Bacterial co-occurrence network analysis

Co-occurrence network analyses were constructed based on the Sparse Correlations for Compositional Data (SparCC) algorithm (Friedman and Alm 2012) using FastSpar version 1.0.0 (Watts et al. 2018) at the genus level. Visualization of the networks was achieved using Cytoscape version 3.10.1 (Shannon et al. 2003) with the following criteria: Strong correlation ($|r| > 0.8$) and p-value < 0.05 to reveal the distribution pattern of correlation coefficient and also

network topology parameters such as number of nodes, edges, positive edges, negative edges, and network density.

Core microbiome and differential abundance analysis

Core taxonomic microbiome analysis at the genus level was conducted using the microbiome R package version 1.18.0 (Lahti and Shetty 2019) with the following criteria: Detection = 0.001 and prevalence = 95%. Meanwhile, the differential abundance taxonomic analysis among samples was carried out using LEfSe version 1.1.2 and statistically examined using the LDA (Linear Discriminant Analysis) (Segata et al. 2011). LDA tests were performed to estimate the effect size of significantly abundant features between groups. This study's LDA score and the Wilcoxon p-value were at 2.0 and 0.05, respectively. The outcomes were visualized using the ggplot2 R package version 3.4.2 (Wickham 2016).

Statistical analysis

One-way Analysis of Variance (ANOVA) was conducted using SPSS version 26 to assess the significance of alpha diversity and the population of *Foc*-TR4. In comparison, the PERMANOVA (Permutational Multivariate Analysis of Variance) test determined beta diversity. This statistical technique analyzes differences in multivariate data among multiple groups or conditions.

RESULTS AND DISCUSSION

Soil physicochemical properties

The soil texture analysis revealed that the major soil texture classification in the Kulon Progo and Gunungkidul districts was clay based on the USDA classification. Bantul and Sleman districts had sandy clay loam (Table 2). The chemical properties of the soil samples from different districts were slightly different. Kulon Progo regions had an acidic pH, Gunungkidul had an alkaline pH, and Bantul and Sleman had a neutral pH. In our study, we found that clay soils were associated with a lower level of organic carbon (1.17 and 1.77%), total nitrogen (0.15 and 0.24%), and a C/N ratio (7.53 and 7.75). Additionally, it exhibits very high values of available potassium (213 and 873 ppm) and phosphorous content (197 and 725 ppm). Similarly, sandy clay loam soil is associated with low levels of organic carbon (1.17 and 1.96%), total nitrogen (0.14 and 0.18%), and a C/N ratio (8.15 and 10.88). It also exhibits high available potassium (504 and 1,292 ppm) and phosphorous content (96 and 128 ppm).

Disease incidence and *Foc*-TR4 quantification

The disease incidence in banana farms showed that Gunungkidul district recorded the highest, followed by Bantul, Kulon Progo, and the lowest in Sleman (Figure 2). The population of *Foc*-TR4 was analyzed in each sample using *Foc*-six1c primers, revealed the abundance of *Foc*-TR4 showed that in the healthy and infected rhizosphere ranged from 11.7 to 107.7 and 19 to 95.2 x 10¹⁰ copy numbers/g soil, respectively. The highest population of *Foc*-TR4 was detected in the Sleman healthy sample (107.7

x 10¹⁰ copy numbers/g soil) and the lowest (11.7 x 10¹⁰ copy numbers/g soil) in the Kulon Progo healthy sample. In general, the average population indicates that the population in the rhizosphere infected with *Foc*-TR4 is higher than in healthy rhizospheres. Moreover, clay soils tended to have a higher population of *Foc*-TR4 in infected rhizospheres than sandy clay loam soil. In contrast, in healthy rhizospheres, sandy clay loam soil has a higher population of *Foc*-TR4 compared to clay soil.

Diversity of bacterial communities

A total of 17,009 high-quality sequences were obtained after quality filtering. The average number of high-quality sequences in all samples is 2,126.5, with an average length of 417 bp. Good coverage exceeded 99%, indicating sufficient reads were obtained to analyze the entire bacterial community (Table 3) and rarefaction curves (Figure 3). The curve becomes steep on the left, indicating that a large fraction of the species diversity remains to be discovered, while the curve becomes flatter on the right, indicating that a reasonable number of individual samples have been taken. This means that only the scarce species remain to be sampled.

For the next analysis, we grouped samples based on soil type (clay and sandy clay loam) and disease status (healthy and infected) to determine the greatest influence on bananas' soil bacterial community compositions. Alpha diversity analysis was conducted to determine the Richness (Chao1 Index), Diversity (Shanon and Simpson Index), and Evenness

(Pielou Index) of bacterial communities in each group. The Chao1 Index of clay soil was higher than that of sandy clay loam soil, and there were significant differences between the two soil types. The comparison of the Shanon and Simpson Diversity indices showed no significant difference in bacterial diversity between clay and sandy clay loam soil, as well as the Pielou Evenness Index (Figure 4.A). Furthermore, there were no significant differences between healthy and infected samples in the disease status group in all alpha diversity indices (Figure 4.B). In line with the result of the PERMANOVA test conducted to estimate beta diversity, soil type ($P = 0.038$) showed more significant differences than disease status ($P = 0.876$) (Table 4).

Table 2. Soil physicochemical properties in four different districts

Properties	Locations			
	Kulon Progo	Gunungkidul	Bantul	Sleman
Sand (%)	33	8	60	69
Silt (%)	48	30	36	29
Clay (%)	19	62	4	2
pH	5.6	7.8	7.0	7.6
Total N	0.24	0.15	0.18	0.14
C-organic (%)	1.77	1.17	1.96	1.17
P (ppm)	725	197	128	96
K (ppm)	873	213	504	1,292
C/N ratio	7.53	7.75	10.88	8.15

Note: Each 5-8 tress sample was mixed as a composite soil sample

Table 3. Alpha diversity indices of the soil bacterial community at the ASVs level in different samples

Samples	Observed species	Chao1	Shanon	Simpson	Pielou	Good coverage
Clay soil	2,195 a	2,227 a	7.05 a	0.27 a	0.92 a	99.4
Sandy clay loam soil	2,058 b	2,073 b	7.04 a	0.28 a	0.92 a	99.4
Healthy rhizosphere	2,098 a	2,125 a	7.01 a	0.27 a	0.92 a	99.4
Infected rhizosphere	2,155 a	2,175 a	7.08 a	0.29 a	0.92 a	99.4

Note: Clay soil: KP and GK, Sandy Clay Loam soil: BT and SM. Healthy and Infected rhizosphere consist of: KP, GK, BT, and SM

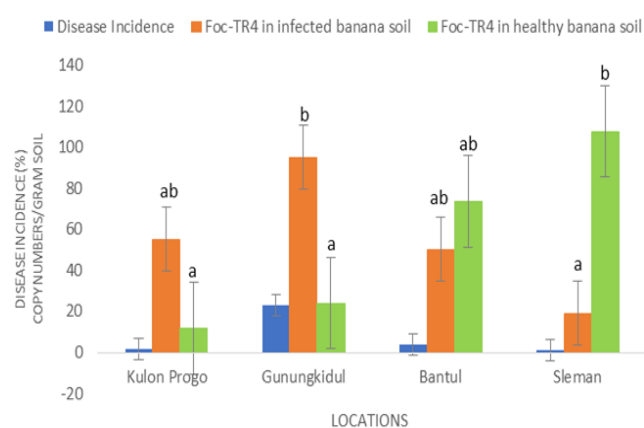


Figure 2. The percentage of disease incidence and the population of *Foc*-TR4 are expressed in the copy numbers/gram soil in four districts. The letter on each bar represents a significant difference at the 5% level based on the Duncan test

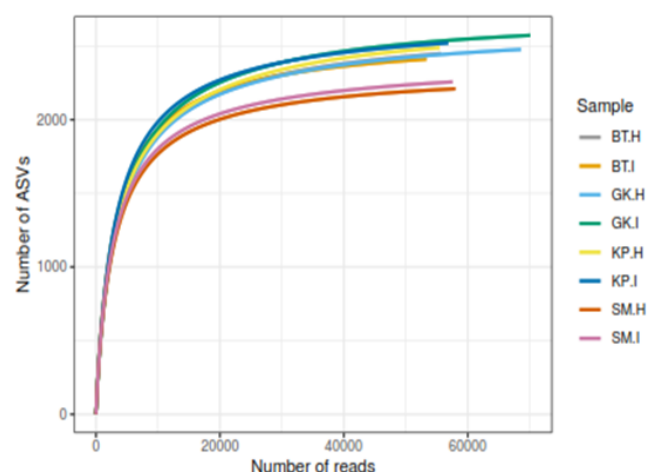


Figure 3. Rarefaction curves, drawn based on the abundance of bacterial communities in different sample

Taxonomic composition of bacterial communities

More bacterial ASVs existed in the infected rhizosphere than in the healthy rhizosphere. Furthermore, clay soil also exhibited a higher number of ASVs than sandy clay loam soil. The annotation result showed that taxa with a higher relative abundance and proportion were assigned at the phylum level for each group. *Actinobacteriota* was the dominant bacterial phylum in all groups (21.5%), followed by *Proteobacteria* (15.5%), *Chloroflexi* (13.5%), *Acidobacteriota* (9.6%), and *Bacteroidota* (8.9%) as major phyla associated with all soils (Figure 5.A, 5.B). A few minor phyla, such as *Firmicutes* (6.5%), *Gemmatimonadota* (6.5%), *Myxococcota* (5.9%), *Verrucomicrobiota* (4.9%), *Planctomycetota* (1.3%), *Nitrospirota* (0.7%), *Patescibacteria* (0.7%), *Elusimicrobiota* (0.6%), *Cyanobacteria* (0.5%), *Bdellovibrionota* (0.4%),

Armatimonadota (0.3%), and *Thermoplasmata* (0.3%), were also identified, including a small percentage of unknown sequences (0.2%).

Table 4. PERMANOVA test based on Bray-Curtis distance

	Df	Sum of Squares	R ²	F	Pr(> F)
Soil type	1	0.000679	0.235872	1.783682	0.038*
Disease status	1	0.000264	0.091781	0.694058	0.876
Soil type:	1	0.000413	0.143393	1.084347	0.346
Disease status					
Residual	4	0.001523	0.528954		
Total	7	0.00288	1		

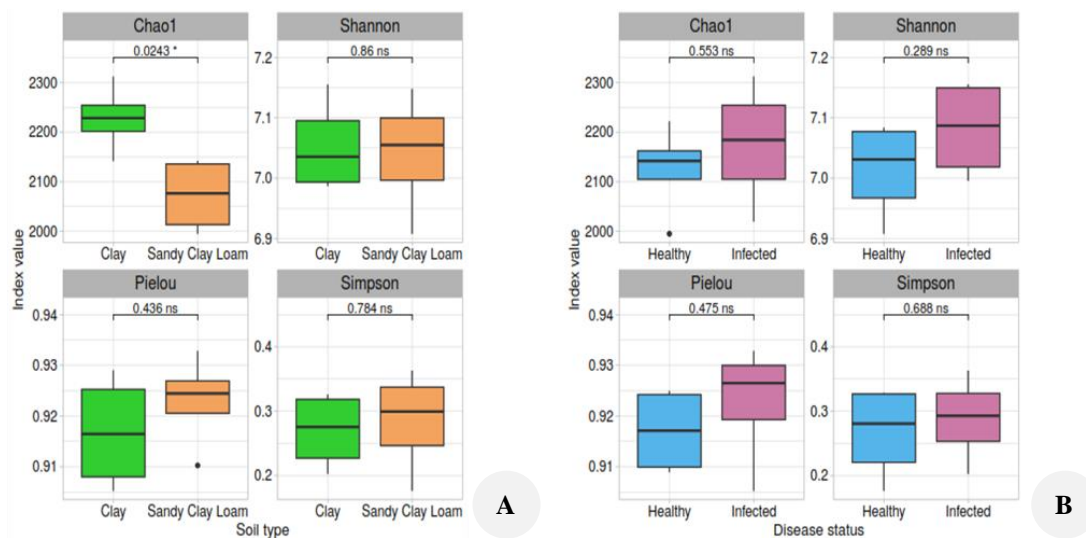


Figure 4. The comparison of alpha diversity indices using Chao1, Shanon, Simpson, and Pielou index between groups. A. Soil type; B. Disease status. Statistically analyzed using a t-test

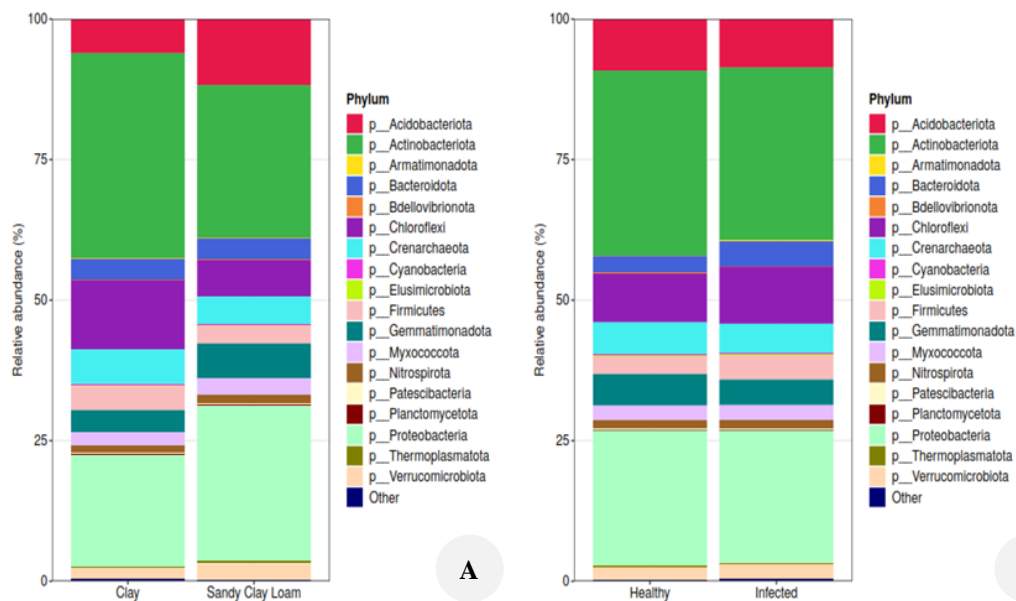


Figure 5. Relative abundance at phylum level. A. Based on soil type (clay and sandy clay loam); B. Based on disease status (healthy and infected)

Co-occurrence network analysis

Co-occurrence network analysis revealed that clay soil exhibited higher network connectivity, number of edges, and network density than sandy clay soil (Figure 6). Similarly, infected soil also exhibited higher network connectivity, number of edges, and network density than healthy soil (Table 5). In this study the hubs of both of soil type and disease status networks belonged to 21 different bacterial phyla. Among them, *Proteobacteria* were the more dominant phyla (23%), followed by *Actinobacteria* (22%), *Bacteroidota* (11.7%), *Chloroflexi* (9.2%), *Firmicutes* (7.8%). *Proteobacteria* are very common in soil environments and related to a wide range of functions involved in carbon, nitrogen, and sulfur cycling (Mhete et al. 2020), while *Actinobacteria* are known to secrete a large number of secondary metabolites to inhibit the growth of pathogens while promoting plant growth (Shivlata and Satyanarayana 2015).

Core microbiome and differential abundance analysis

The core microbiome was identified by selecting frequently detected taxa in a high proportion across all samples. We observed 60 bacterial genera as core members of the banana rhizosphere. These core bacterial genera include various plant growth-promoting bacteria such as (i.e *Sphingomonas*, *Sphingomonadaceae* (Maquia et al. 2021), *Dongia* (Nditasari et al. 2023), *Pedomicrobium* (Ridge et al. 2007), *Rubrobacter* (Egas et al. 2014), *Nocardioideae*, *Devosia*, (Anshari et al. 2024), *Candidatus nitrososphaera* *Candidatus solibacter*, *Candidatus udaeobacter*, *Candidatus alysiosphaera* (Gu et al. 2022), *Alphaproteobacteria* (Gu et al. 2022), *Gemmatimonadaceae* (Zeng et al. 2022), *Vicinamibacteraceae* (Świątczak et al. 2023), *Ammoniphillus*,

Gemmatimonas, *Chitinopagaceae* (Shen and Lin 2021), *Pseudarthrobacter* (Ham et al. 2022), *Mycobacterium* (Hakim et al. 2021), *Acidobacteriales*, *Nocardioides* (Ma et al. 2023), *Reyranella* (Jia et al. 2022), *Acidimicrobia* (Jerez 2009). The function associated with these genera includes nitrogen fixation (i.e., *Azospirillales*, *Xanthobacteraceae* (Di Benedetto et al. 2017), *Nitrospira*, *Nitrososphaeraceae* (Gu et al. 2022), *Roseiflexaceae* (Nam et al. 2023), *Micromonosporaceae*, *Microtrichales*, *Rhizobiaceae*, *Bryobacter*, production of antimicrobial compound (i.e., *Gaiella*, *Gaiellales* (Nditasari et al. 2023), *Pseudonocardia* (Riahi et al. 2022), *Haliangium* (Anshari et al. 2024), *Phenylobacterium*, *Streptomyces* (Kulshreshta et al. 2023), phytohormone production (i.e. *Microvirga* (Safronova et al. 2017), production of antifungal metabolites (i.e. *Paenibacillus* (Mousa and Raizada 2015), *Myxococcaceae* (Yang et al. 2023), and siderophore production (i.e *Bradyrhizobium* (Di Benedetto et al. 2017).

Table 5. Co-occurrence network topology in different soil types

Topology	Soil type		Disease status	
	Clay	Sandy Clay Loam	Healthy	Infected
Number of nodes	202	198	370	388
Number of edges	1,061	840	2,358	2,893
Number of positive edges	616	471	1,485	1,689
Number of negative edges	445	369	873	1,204
Average number of neighbors	10,505	8,485	12,746	14,912
Network density	0.052	0.043	0.035	0.039

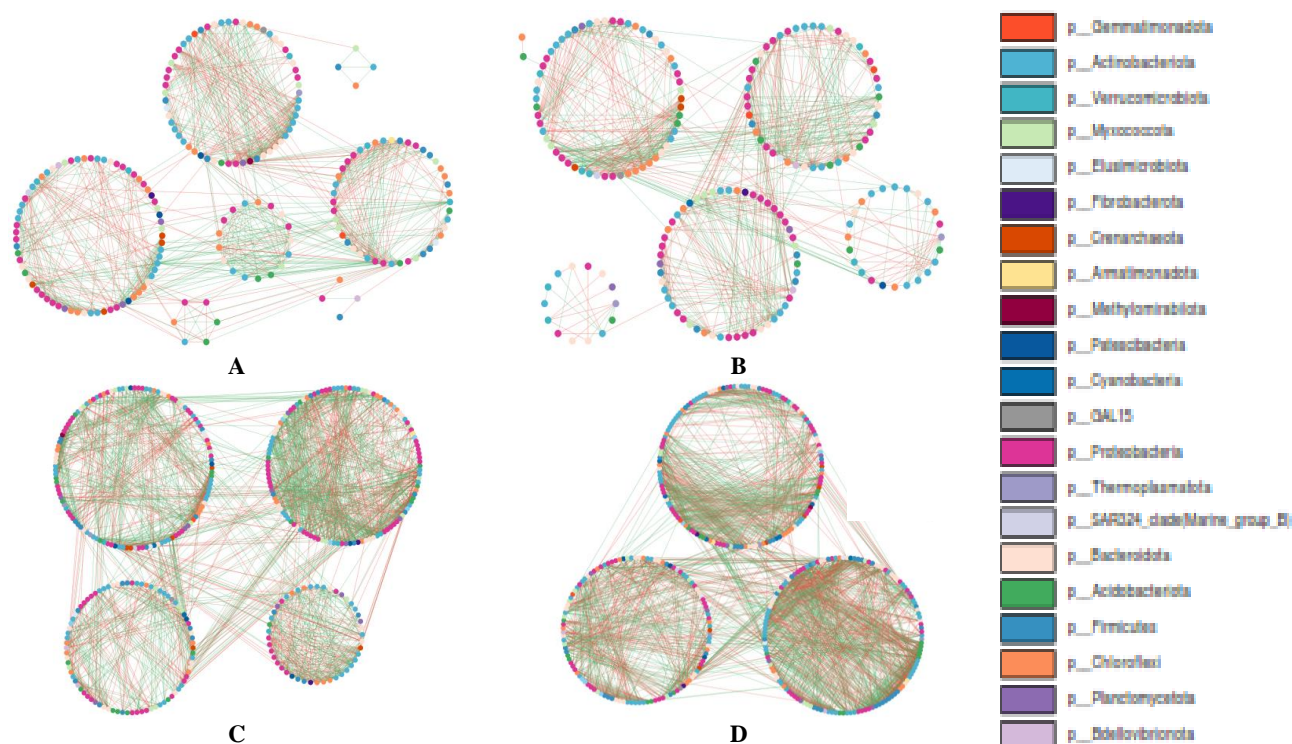


Figure 6. Bacterial co-occurrence network in different soil, A: Clay soil, B: Sandy clay loam soil, C: Healthy soil, D: Infected soil. Edges are color-coded, with green representing positive and red representing negative relationships. The color of nodes corresponds to their respective phylum

The identification of differential abundance taxa (biomarkers) using LEfSe analysis (LDA score >2) can describe specific abundance differences among soil type and disease status groups. Clay soil exhibited lower unique bacteria than sandy clay loam soil, with 21 and 31 unique bacteria, respectively. Clay soil exhibited 21 unique bacterial genera, including *Microtrichales*, JG30_KF_CM45, *Micromonosporaceae*, TK10, AKYG1722, *Kribbella*, *Tepidimicrobium*, *Caldicoprobacter*, *Luedemannella*, *Dactylosporangium*, *Actinoplanes*, *Anaerosalibacter*, M55_D21, *Agromyces*, *Roseomonas*, *Micavibrionales*, *Clostridium_sensu_stricto_15*, *Clostridium_sensu_stricto_12*, *Nostocaceae*, *Brevibacterium*, and *Caloramatoraceae*. Sandy clay soil exhibited 31 unique bacterial genera consist of *Parvibaculaceae*, *Pontibacter*, 37_13, possible_genus_04, *Verrucomicrobia*, DEV008, SJA_28, *Beijerinckia*, *Fimbrimonadales*, *Ferruginibacter*, *Stenotrophobacter*, *Candidatus_Xiphinematobacter*, *Labrys*, FFCH5858, *Sandaracianaceae*, *Aliihoeflea*, DS_100, *Edaphobaculum*, Subgroup_11, 0319_7L14, JGI_0001001_H03, *Nitrospira*, *Terrimonas*, *Arthrobacter*, *Crossiella*, *Methyloligellaceae*, 11_24, *Bradyrhizobium*, *Pedomicrobium*, *Vicinamibacteraceae*, and *Gemmatimonadaceae* (Figure. 7). Healthy soil consist of *Acidobacteriaceae*, *Minicystis*, *Sorangium*, *Sphaerobacter*, *Sphingobacterium*, *Oscillospiraceae*, and *Ohtaekwangia*. The unique genera in all networks were dominated by plant growth-promoting bacteria (36%), followed by nitrogen-fixing bacteria (21.8%), production of antimicrobial compound (10.9%), production of secondary metabolite (3.1%), and unknown metabolic function (28%).

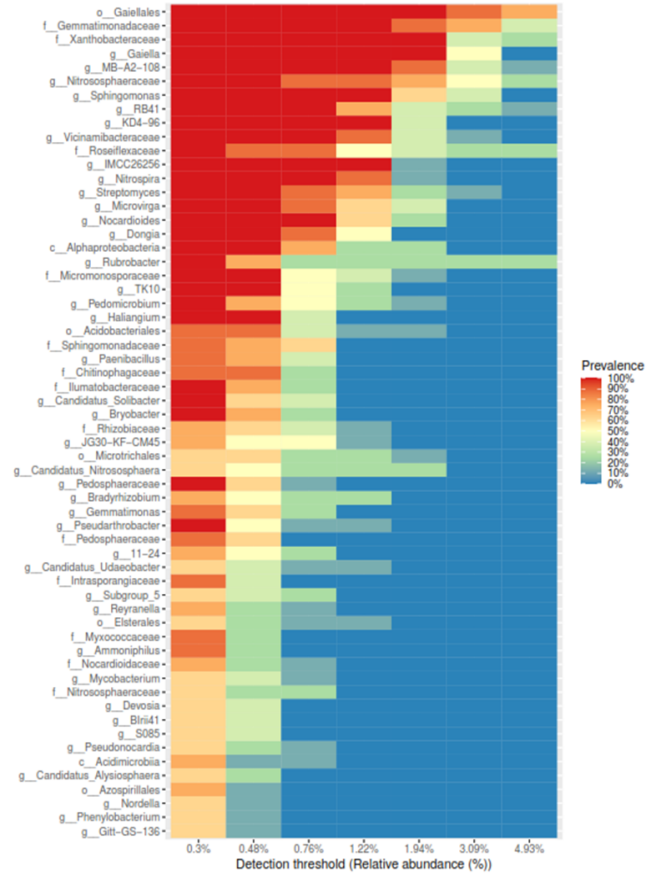


Figure 7. Core microbiome at the genus level associated with banana plants

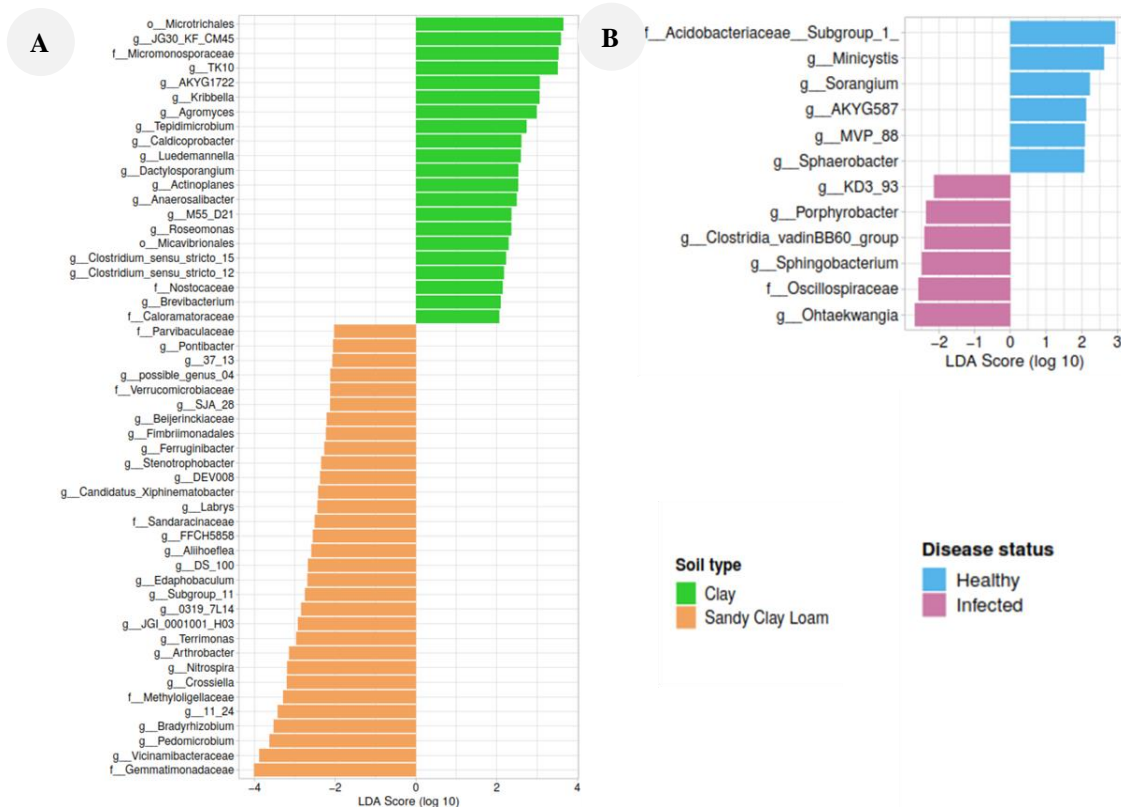


Figure 8. LEfSe analysis of taxonomic abundance in different groups. A. Soil type; B. Disease status

Discussion

Soil is the habitat for soil-borne diseases, such as *Fusarium wilt*. Therefore, changes in the soil environment may lead to this disease's suppression or severity. Several factors contribute to the development of *Fusarium wilt* disease in the soil; the rhizosphere bacterial community can significantly influence these factors. It is known that root exudates released by banana plants contain several organic acids, including oxalic, malic, and fumaric acids (Yuan et al. 2015). Furthermore, Yuan et al. (2015) also stated that organic acids play an important role in attracting and initiating bacterial colonization on banana roots via a chemostatic response and the induction of biofilm formation. Additionally, plants recruit specific beneficial bacteria after pathogen infection, which helps plants resist changes in root exudates. Not only the pathogen population but also the soil, as the primary source of root-colonizing bacteria, could drive microbial community composition, diversity, and abundance between different soil environments. In our study, we investigated the composition of soil bacterial communities in the rhizosphere of banana plants associated with *Foc*-TR4 in different soil types. Understanding the soil bacterial composition associated with banana plants can enhance plant health against *Fusarium wilt* disease, providing a basis for biological control strategies. The composition genera in this study include plant growth-promoting bacteria (55%) and biocontrol agents (15%).

Influence of soil physicochemical properties on soil bacterial diversity

This study emphasizes soil physicochemical properties, especially soil texture, by revealing the greatest influence on bananas' soil bacterial community composition, especially soil texture. A previous study highlighted that soil texture is the most important factor in shaping microbial communities (Xia et al. 2020; Obayomi et al. 2021). Based on USDA classification, Kulon Progo and Gunungkidul districts have clay soil, while Bantul and Sleman districts have sandy clay loam soil. Soil texture is related to the distribution of pores and adsorption of mineral particles, ultimately determining the soil's water-holding capacity, air movement speed, and fertility. In line with (Kusumandari 2014), sand is well-aerated but does not hold much water and is low in nutrients, while clay soil generally holds more water and is better at supplying nutrients. According to the observed species, alpha and beta diversity indices showed that clay soil also exhibited a higher number of bacterial ASVs than sandy clay loam soil, and there was a significant difference between them (Table 3). Obayomi et al. (2021) found that bacterial diversity in clay and silt fractions was higher than in sand due to higher nutrient availability in fine particles. The more detailed mechanism is that clay fractions have a large surface area and negative charges, allowing them to attract and hold positively charged ions, including calcium, magnesium, and potassium. Additionally, clay acts as a binding agent between soil particles, providing strong soil elasticity and cohesion of soil particles, resulting in higher pore connectivity. The higher pore connectivity would likely promote enhanced spatial interaction and niche homogeneity, leading to sustainable bacterial richness and diversity

(Obayomi et al. 2021). In addition, the clay fraction retains high moisture due to the presence of many micropores, increasing oxygen availability, which can enhance microbial diversity in the soil. The availability of nutrients in the soil also encourages variations in soil bacteria. High levels of phosphorous (P) and potassium (K) in the soil can increase the diversity of soil bacteria. However, in this study, the availability of P and K showed inconsistent results. Furthermore, more diverse microbial communities would potentially increase the host plant's resistance to pathogen invasion by supporting beneficial microbial symbionts (Kinkel et al. 2011).

Influence of *Foc*-TR4 population on soil bacterial diversity

According to the observed species, alpha and beta diversity indices (Table 3) showed that the infected rhizosphere had a higher number of bacterial ASVs than the healthy rhizosphere in all districts even though there were no significant differences, indicating that the species structures were not the same in the two rhizospheres. In contrast, the *Foc*-TR4 population showed inconsistent numbers in all healthy and infected rhizosphere districts. In general, the population of *Foc*-TR4 was detected higher in the infected rhizosphere than in the healthy rhizosphere. A detailed study showed that clay soil has a higher population of *Foc*-TR4 in infected soil than in healthy soil (Figure 2). However, sandy clay loam soil also exhibited a higher *Foc*-TR4 population in healthy soil than in clay soil. This is in line with the report by (Fan et al. 2023), which also found the population of *Foc*-TR4 to be higher in infected soils than in healthy soils. Higher *Foc*-TR4 numbers might likely induce more antagonistic microorganisms via direct interactions with invading *Foc*-TR4, resulting in higher bacterial ASVs numbers in infected soils. Indirectly, in response to *Foc*-TR4 infection, banana plants alter their rhizospheric bacterial community via altered plant root exudation changes in plant physiology, which could also explain the higher abundance of bacterial ASVs in the infected rhizosphere soil. Moreover, regarding the pathogen perspective, an anaerobic condition due to low soil permeability in clay fraction increases the solubility of essential elements for *Foc*, including iron and manganese, causing *Foc* to be more virulent (Olivares et al. 2020). The clay fraction having a high moisture due to the presence of many micropores, increases the availability of oxygen and nutrients, which are also important for *Foc* growth. Both of these reasons, as mentioned above, could explain the higher population of *Foc*-TR4 in clay soil (Gökbülak and Özcan 2008; Jiang et al. 2021). In addition, the high available K and P content in sandy clay loam soil likely induces nutritional imbalance in banana plants due to excessive adsorption of K and P concentration, leading to the disruption of the plant defense response to pathogen infection (Campos-Soriano et al. 2020; Guimarães et al. 2020). It is possible that banana plants are stressed due to high levels of K and P in the soil but still appear as healthy plants externally. This could be why sandy clay loam in a healthy rhizosphere has a higher population of *Foc*-TR4 than in an infected rhizosphere (Figure 2).

According to the alpha and beta diversity analysis, soil type impacts bananas' soil bacterial community more than the disease status group. This study performed a Co-occurrence network analysis to gain deeper insights into the interaction pattern and structure of bacterial communities associated with bananas based on soil type and disease status communities. Co-occurrence networks represent the spatial interaction patterns and help determine the potential relationships between individual taxa, represented by nodes and edges. Nodes represent unique taxa, while edges represent the connectivity between them. Co-occurrence patterns can be measured using network topology metrics such as the clustering coefficient, which represents the density of connection between nodes, and the edge ratio, which represents the degree to which the community has potentially synergistic or competitive interaction (Osborne et al. 2024). In addition to nodes and edges, network hubs are a useful feature of network analysis. Network hubs are highly connected taxa responsible for the structure of specific microbial communities.

Co-occurrence network analysis

Co-occurrence network analysis revealed that the clay soil network was more complex than sandy clay loam soil. Clay soil exhibited more nodes and edges than sandy clay loam soil, with counts of (202 and 1,061) and (198 and 840) nodes and edges, respectively (Table 5). Consistent with bacterial diversity findings, clay soil showed higher diversity than sandy clay loam soil, increased diversity with greater connectivity within the bacterial community. In the disease status group, infected soil was more complex than healthy soil, with counts of (388 and 2,893) compared to (370 and 2,358) nodes and edges, respectively (Table 5). Previous reports have indicated that phytopathogens reduce microbial diversity and alter community organization (Xiao et al. 2024). This decline in diversity is often attributed to competition between soil microbes and phytopathogens, involving toxins production or nutritional competition. This study revealed the contrary result that infection by *Foc*-TR4 increased bacterial abundance and complexity of bacterial communities, as evidenced by an enlarged network scale. It is plausible that *Foc*-TR4 infection induces the proliferation of antagonistic bacteria and other beneficial microbial symbionts, which may contribute to the suppression of *Fusarium* wilt disease. Network analysis revealed that the disease status group exhibited more complex interaction within the bacterial network than soil type groups (Table 5, Figure 6). This suggests that the presence of *Foc*-TR4 may elevate bacterial abundance, leading to increased complexity in bacterial network interactions, although these differences were not significant compared to soil-type groups.

Network topology analysis revealed that clay soil exhibited significantly greater positive and negative edge numbers than sandy clay loam soil. Similarly, infected soil displayed more positive and negative edges than healthy soil (Table 5). Both soil type and disease status groups showed higher-positive edges than negative edge numbers, indicating greater microbial community collaboration or niche sharing in soil than competition. According to Ma et

al. (2020), negative edges may arise from various co-exclusion mechanisms, such as direct competition, toxin production, environmental modification, and differential niche adaptation. Furthermore, sandy clay loam soil had fewer negative edges than clay soil, suggesting collaboration or niche sharing in sandy clay loam soil. Conversely, competition or niche differentiation seemed more prevalent in clay soil. This may be due to higher nutrient availability in clay soil's fine particles, inducing higher bacterial diversity and resulting in a lower population of *Foc*-TR4. Similarly, infected soil exhibited more negative edges than healthy soil, indicating competition or niche differentiation in infected soil, possibly due to competition between soil bacteria and the pathogenic fungus *Foc*-TR4. In line with Durán et al. (2018), negative interactions are prominent among members of different kingdoms (bacteria, fungi, and oomycetes) in the plant root endosphere.

In our study, the hubs are predominantly dominated by the *Proteobacteria*, *Actinobacteria*, *Bacteroidota*, *Chloroflexi*, and *Firmicutes* phyla (Figure 6). According to Agler et al. (2016), not all hubs are keystone taxa since overall interaction in the network depends on them rather than defining keystones. The specific hubs identified by Agler were *Dioszegia* (a yeast fungus) and *Albugo* (an oomycete) from various kingdoms, describing how microbial communities are formed through species sorting mechanism carried out by hub taxa. These hub taxa also act as an intermediary between abiotic, temporal, and host factors. Furthermore, the contribution of keystone taxa will be higher if they are part of the core microbial community and consistently present in an environment, highlighting the importance of such taxa for microbial functioning (Banarjee et al. 2018). According to (Osborne et al. 2024), the two most common microbial community assembly processes are stochastic or niche assembly processes. Stochastic microbial assembly occurs when microbes are randomly introduced from the environment into a community. In contrast, niche assembly occurs when microbes are incorporated based on their ecological role and existing community members. In this study, plants may recruit keystone taxa based on their ecological role to enter the rhizosphere communities to help plants resist the disease.

Core microbial and differential abundance analysis

The core microbial community, comprising taxa consistently associated with and evolving within the banana plant rhizosphere, was investigated in this study. It was revealed that 60 bacterial genera were consistently associated with banana plants, most of which are considered taxa. These keystone genera include plant growth-promoting bacteria, such as N_2 -fixing, antibiotic-producing, phytohormone-producing, and siderophore-producing. A meta-analysis reported that many genera have been cultivated in vitro as synthetic communities, serving as both plant growth-promoting bacteria and biocontrol agents against *Fusarium* wilt disease in various commodities (Birt et al. 2022). In our study, these genera include: *Sphingomonas*, *Dongia*, *Pedomicrobium*, *Rubrobacter*, *Nocardioidaceae*, *Devosia*, *Candidatus Solibacter*, *Alphaproteobacteria*, *Gemmatimonadaceae*, *Nocardioides*, *Vicinamibacteraceae*,

Ammoniphillus, *Azospirillales*, *Reyranella*, *Gemmatimonas*, *Chitinopagaceae*, *Bryobacter*, *Gaiella*, *Pseudarthrobacter*, *Mycobacterium*, *Microvirga*, *Nitrospira*, *Acidobacteriales*, *Acidimicrobia*, *Xanthobacteraceae*, *Nitrososphaeraceae*, *Roseiflexaceae*, *Micromonosporaceae*, *Microtrichales*, *Rhizobiaceae*, *Haliangium*, *Paenibacillus*, *Phenylobacterium*, *Pseudonocardia*, *Myxococcaceae*, and *Bradyrhizobium*. Differential abundant analysis revealed that soil type groups influenced the abundance of unique taxa more significantly than disease status groups. A detailed study showed that 31 unique taxa characterized sandy clay loam, whereas clay soil had 21 unique taxa. It was also observed that the composition of unique taxa differed greatly between clay and sandy clay loam. In contrast, disease status groups exhibited only 6 unique genera in both infected and healthy rhizosphere, which showed notable differences between the two communities. Therefore, soil type appears to have a greater influence on the composition of unique taxa than disease status groups.

In this study, the mechanism to reveal the greatest influence on soil bacterial community composition is mediated by the complex interaction between abiotic factors (soil physicochemical properties) and microbes, biotic factors (*Foc*) and microbes, and host factors (banana plants) and microbes. These factors act directly on the microbial community structure via hubs, through which microbe-microbe interactions transmit their effects. Hubs act as intermediaries between abiotic, biotic, and host factors via species-sorting mechanisms. The variations in species sorting mechanisms include seasonal and locational effects. Furthermore, sampling time (season) correlates with 10% of community variation, while sampling location correlates with 25% to 35% (Agler et al. 2016). It is also possible that sampling techniques can lead to variations in the communities formed, resulting in diversity in the microbial community. Although the impact of microbial diversity on plant growth has not been conclusively demonstrated, we expect that microbial diversity in soil is also an important factor in managing soil health and quality. Various modes of action and their effects in promoting plant growth or biocontrol effects have been proven very beneficial for plants' health. Understanding the composition of soil bacteria associated with plants has been reported to enhance plant growth against *Fusarium* wilt in several commodities (Nisrina et al. 2021; Yang et al. 2023).

Our findings revealed that soil type significantly influenced the abundance and composition of the bacterial community more than disease status did. These results suggest that soil physicochemical characteristics influence bacterial community composition mainly through the pore and particle-scale distribution of organic matter. On the other hand, the presence of *Foc*-TR4 in the soil causes more complexity in bacterial network interaction than soil type does. The interaction between bacteria and the fungus *Foc*-TR4 may cause an enlarged network scale. Regarding soil type, there was a higher abundance of plant growth-promoting bacteria involved in the degradation of numerous organic substances compared to the bacterial composition in the disease status group. This study provides a basis for future research on the soil bacterial composition associated

with banana plants to enhance plant health against *Fusarium* wilt disease.

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