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ORIGINAL ARTICLE

Soil bacterial community associated with twisted disease of shallot plants with the addition of casuarina and maize leaf litters

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Abstract

Twisted disease caused by *Fusarium* is one of the causes of the decline in shallot production in Indonesia. Disease management by adding Casuarina leaf litter and maize leaf litter during planting time has suppressed twisted disease incidence and increased shallot production in previous studies. Bacterial communities can influence disease suppression and increase plant production in soil. However, the effect of adding Casuarina and maize leaf litter on the soil bacterial community, and its impact on suppressing twisted disease is still unknown in shallot planting. In addition, no further studies have been conducted on the effectiveness of the application time of Casuarina and maize leaf litter. This study aimed to determine the best application time of leaf litter in suppressing twisted disease, the effect of changes in soil bacterial communities, and bacteria that act as core microbe, biomarkers, and keystone taxa. Results showed that adding Casuarina and maize leaf litter during planting time was more effective than adding Casuarina and maize leaf litter two weeks before planting. The diversity and abundance of bacteria in soil with the addition of Casuarina leaf litter (C) were higher than those of maize leaf litter (J). In addition, *Pseudarthrobacter* was found in group C, which acts as a core microbe, biomarker, and keystone taxa that may be able to suppress pathogens that cause twisted disease. *Paenibacillus* acts as a core microbe, biomarker, and keystone taxa in group J, which may increase shallot production. This study's results will help develop effective twisted disease management strategies to control disease development.

Keywords: 16s rRNA amplicon metagenomic, application time, core microbe, keystone taxa, *Paenibacillus, Pseudarthrobacter*

Introduction

Twisted disease is one of the barriers to shallot production in Indonesia. Lestiyani *et al.* (2021) reported that several *Fusarium* species, such as *Fusarium acutatum, F. solani,* and *F. oxysporum*, cause twisted disease in Indonesia. Twisted disease management with cultivation practices, namely, adding Casuarina leaf litter, Gliricidia leaf litter, peanut leaf litter, and maize leaf litter during planting has been carried out in previous studies. Based on the results, adding Casuarina and maize leaf litter during planting time was reported to suppress twisted disease and increase shallot production, respectively (Rahmawati 2024). According to Sloot *et al.* (2024), the application time of litter mixed into the soil can affect the suppression of soilborne diseases. A better reduction in disease caused by *F. oxysporum* can be caused by adding organic matter to the soil before planting (Gilardi *et al.* 2016). Therefore, based on the previous study, further studies must be conducted on the effectiveness of the application time of Casuarina and maize leaf litter.

Cultivation by applying litter as organic fertilizer can influence changes in soil microbial communities (Zhang *et al.* 2023). The soil microbial community helps with nutrient absorption, hormone production, nutrient transformation, stress reduction, protection of plants from pathogenic infections (Kumar *et al.* 2023). Suppression of disease and increased production after adding leaf litter involving changes in the soil bacterial community in shallot cultivation are still poorly understood. According to Lin *et al.* (2022), Casuarina leaf litter affects the high diversity of endophytic bacteria that can enhance the growth of Casuarina plants. These endophytic bacteria can inhibit *Fusarium*, thereby decreasing their numbers. According to Kwaśna *et al.* (2015) greater abundance and diversity of bacterial communities were often associated with less abundance and diversity of fungi. The application of maize leaf litter has also been reported to reduce the relative abundance of *Fusarium* sp., thus decreasing soilborne diseases (Zhang *et al.* 2023). The increased abundance of microbes is related to an increase in the suppression of soilborne diseases, such as *Fusarium* wilt disease on banana plants (Huang *et al.* 2019).

One approach that facilitates detailed characterization of microbial diversity and function is conducted through metagenomic analysis. This analysis has become an appropriate approach to maximize the benefits of microbes in the agricultural sector. Thus, this study aimed to determine the effect of the timing of the addition of Casuarina and maize leaf litter on the suppression of twisted disease on the soil bacterial community. In addition, this study aimed to identify core microbes, biomarkers, and keystone taxa in the soil by adding Casuarina and maize leaf litter that can suppress twisted disease.

Materials and Methods

Experimental design

This study was conducted in the Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada's greenhouse, from October to December 2024. The experiment used a completely randomized design with six treatments and four replications, which consisted of:

PC – application of Casuarina leaf litter two weeks before planting + inoculation of *F. acutatum*;

PJ – application of maize leaf litter two weeks before planting + inoculation of *F. acutatum*;

C – application of Casuarina leaf litter during planting + inoculation of *F. acutatum*;

J – application of maize leaf litter during planting + inoculation of *F. acutatum*;

K+ (K) – without application of leaf litter + inoculation of *F. acutatum*;

K- – without application of leaf litter + without inoculation of *F. acutatum*.

Preparation

Casuarina leaf litter was collected from fallen dry leaves, while maize leaf litter came from harvested crops. Both were sun-dried for 3–5 days and shredded into 1–1.5 cm pieces. The recommended application was 10 tons · ha^{-1}. In this study, each pot contained 4 kg of soil with 9.93% moisture and a bulk density of 1.609 g · cm^{-3}, requiring 15 g leaf litter · pot^{-1}. PC and PJ were mixed with soil two weeks before planting.

Shallot planting

The soil used for planting came from the soil of the shallot cultivation field. Four kg soil was put into each pot and planted with three bulbs of Tajuk variety. At 7 days after planting (dap), fertilization was conducted using urea 1 g/pot, fertiphos 0.75 g · pot^{-1}, KCl 0.6 g · pot^{-1}, and ZA 0.88 g · pot^{-1}.

Inoculation of *Fusarium acutatum*

The pathogen used for inoculation was the *F. acutatum* SkmBP isolate used in a previous study (Lestiyani *et al.* 2021; Rahmawati 2024). A suspension of 50 mL with a density of 10^6 conidia/mL was inoculated into each pot by pouring the suspension (Lestiyani *et al.* 2016). Inoculation of *F. acutatum* was conducted at 7 dap in PC, PJ, C, J, and K+ treatments.

Observation of disease and shallot production

Observations of twisted disease were conducted every 7 days by measuring the percentage of infected plants. Management effectiveness was assessed using the Abbott formula, which is commonly applied in phytopathology. Disease incidence data was used to calculate management effectiveness, categorized as high (75–95%), medium (50–74%), and low (< 50%) (Torguet *et al.* 2022).

$$E = \frac{Dic - Dit}{Dic} \times 100\% = \left(1 - \frac{Dit}{Dic}\right) \times 100\%$$

where:

E – effectiveness;

Dic – disease incidence on control;

Dit – disease incidence on treatments.

Production data was collected at harvest, 56 dap. Fresh weight and the number of bulbs were recorded,

followed by air-drying of fresh bulbs in a greenhouse for 14 days to determine dry weight. Data analysis was conducted using analysis of variance (ANOVA) with IBM SPSS Statistics version 27. Significant differences between treatments were further analyzed using Duncan's Multiple Range Test (DMRT) at 95% confidence level (Wibowo *et al.* 2023).

Soil sampling for metagenomic analysis

Soil samples for metagenomic analysis were taken from treatments C, J, and K+ (K), based on observations of twisted disease incidence. As there was no significant difference between treatments with application two weeks before planting and during planting, only the treatment with the best results was selected. Soil sampling was conducted at 49 dap from bulk soil at 8–10 cm depth. Samples (3–5 g) were collected near plants, placed in sterile plastic bags, stored in a cooler box, and preserved at –80°C.

DNA extraction and amplicon metagenomic sequencing

Following a modified protocol, DNA extraction was conducted using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany). The modifications involved increasing the soil sample quantity to 400 mg and incorporating liquid nitrogen, followed by grinding with a sterile mortar and pestle before extraction (Jayasinghe *et al.* 2024). DNA quality control (QC) was assessed using a Nanodrop spectrophotometer, 1% TBE agarose gel electrophoresis, and Qubit dsDNA HS assay. Library preparation included amplification of the 16S rRNA V4 region using the 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) primers, generating fragments of 290–300 bp (Zheng *et al.* 2021b). Samples that passed QC were sequenced using the Illumina MiSeq PE300 platform.

Bioinformatics analysis

Sequencing data processing

Paired-end reads and raw data quality were checked using Fastqc and Multiqc (Navya and Babu 2023). Unique read barcodes were cut by trimming the adapter and primer sequences using cutadapt and DADA2 (Kurniasari *et al.* 2024; Pratiwi 2024). Next, filtering was performed on the raw tags to eliminate low-quality amplicon sequences, resulting in clean tags (Widiastuti *et al.* 2024).

Amplicon sequence variants (ASV) denoise and taxonomic annotation

Amplicon analysis using the DADA2 algorithm produces ASV as an accurate representative sequences with 100% similarity. Furthermore, denoisedF and denoisedR data merged, and chimeric sequences were removed to obtain effective tags (reads) used in subsequent analysis (Kurniasari *et al.* 2024). Tags were compared with references in Silva138.1 database. Taxonomic reading was performed using Kraken2 and Bracken to obtain appropriate taxa information and its abundance distribution (Kurniasari *et al.* 2024).

Alpha and beta diversity

Metagenomic data quality for alpha diversity was assessed using rarefaction curves in Nephele. Alpha diversity indices included observed-species, Chao1, ACE, Shannon, Simpson, InvSimpson, and Fisher. Beta diversity was analyzed using Principal Coordinate Analysis (PCoA) based on Bray-Curtis dissimilarity (Zheng *et al.* 2021b; Jayasinghe *et al.* 2024). All diversity analyses were conducted in R using the vegan package and visualized with ggplot2 (Kurniasari *et al.* 2024).

Core microbe analysis

Core microbes were identified based on ASV in 100% of all samples (Dasgupta *et al.* 2024). The top 100 bacterial genera with 100% ASV occurrence were selected using a detection threshold of relative abundance > 0.01% and sample prevalence >60% (Navya and Babu 2023). Core microbe analysis and heatmap visualization were conducted using MicrobiomeAnalyst in the 'Marker Data Profiling' section (Navya and Babu 2023; Morni *et al.* 2025).

Differential abundance analysis

Differential abundance analysis among groups was performed using Linear Discriminant Analysis Effect Size (LEfSe) to identify statistically significant taxonomic and functional biomarkers (Zhang *et al.* 2023). The analysis involved a three-step approach: (1) the Kruskal-Wallis test ($p < 0.05$) to assess group differences, (2) the Wilcoxon test ($p < 0.05$) for pairwise comparisons between replicates, and (3) LDA scoring (> 2) to determine effect size (Huang *et al.* 2019; Jayasinghe *et al.* 2024). Analysis and visualization were conducted using MicrobiomeAnalyst, with results displayed in a horizontal bar plot.

Co-occurrence network and keystone taxa analysis

Co-occurrence network analysis based on ASV prevalence was conducted to examine bacterial correlations across different groups. The top 100 most abundant bacterial taxa were used to construct the network, with Pearson's correlation matrix assessing linear

relationships (Zheng *et al.* 2021a; Kumar *et al.* 2023). Positive and negative interactions among bacterial taxa were visualized using MicrobiomeAnalyst, applying a correlation threshold > 0.85 and p-value < 0.05 (Berry and Widder 2014; Navya and Babu 2023). Keystone taxa, representing central nodes, were selected from the top 10% of total nodes.

Results

Observation of twisted disease

The initial symptoms of twisted disease, such as pale leaves, yellowing, and bulb rot are illustrated in Figure 1. Early symptoms which appeared 5 days after inoculation (dai) and their development up to 21 dai are shown. Affected plants initially exhibited pale green leaves that turned yellow, followed by characteristic leaf twisting. Advanced symptoms included wilting, stunted growth, leaf drying, bulb rot, and plant death. The study observed that after planting on days 42 to 56, the disease incidence in the positive control (K+) continued to rise, while in Casuarina leaf litter treatment applied during planting (C), incidence remained stable until 56 dap (Fig. 2A). At 56 dap, disease incidence was significantly lower in treatments with Casuarina leaf litter applied 2 weeks before planting (PC) and during planting (C), with 6.67% and 13.33% incidence, respectively, compared to 40% in K+ (Fig. 2B). The effectiveness of twisted disease management calculated using Abbott's formula is shown in

Table 1. Effectiveness of twisted disease management with leaf litter addition

Treatment	Disease incidence [%]	Effectiveness of management [E]	
		E [%]	category
PC	13.33	66.67	medium effectiveness
PJ	26.67	33.33	low effectiveness
C	6.67	83.33	high effectiveness
J	20	50	medium effectiveness
K+	40	0	–

Management effectiveness refers to average disease incidence 56 dap and is calculated based on Abbott's formula supplemented with categories according to Torguet *et al.* (2022)

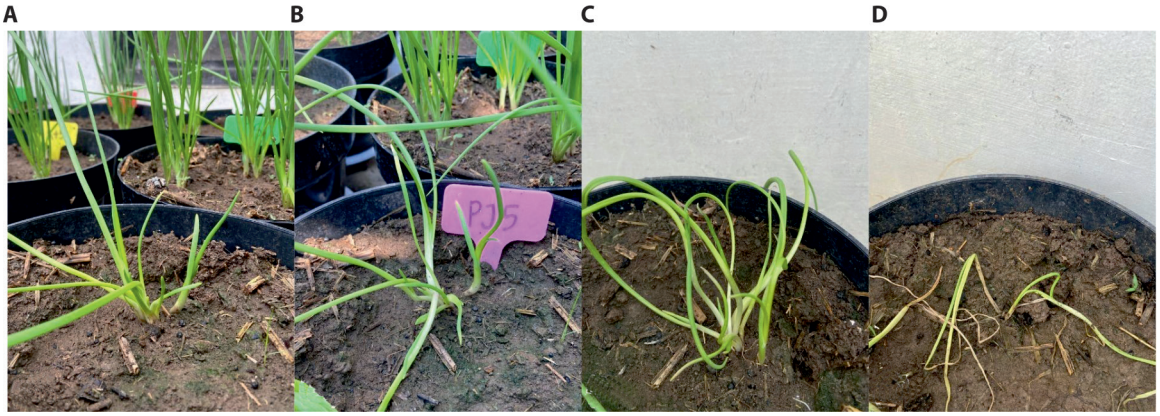

Fig. 1. Disease symptom development in the greenhouse. Twisted at 5 days after inoculation (dai) – A, twisted at 6 dai – B, twisted at 7 dai – C, bulb rot at 21 dai – D

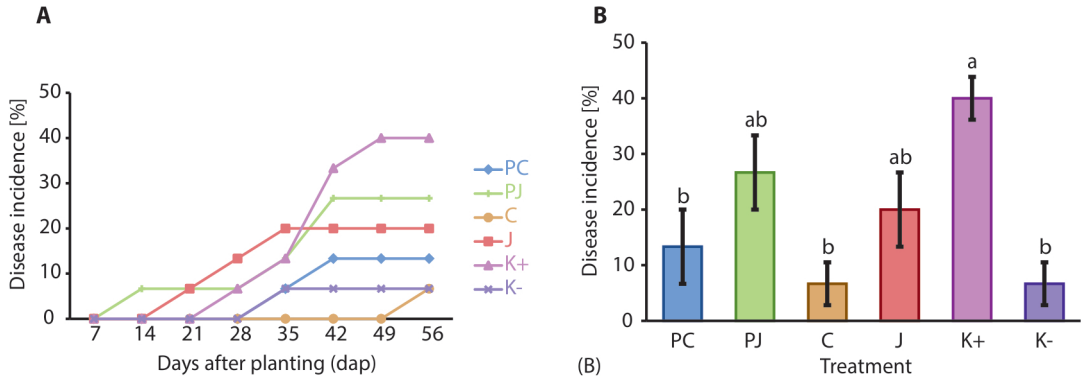

Fig. 2. Development of twisted disease incidence at 7–56 dap – A and average disease incidence at 56 dap – B. The letters on each bar represent significant differences

Table 1, where treatment C achieved the highest effectiveness among all treatments.

Observation of shallot production

The effect of leaf litter treatments on shallot production is demonstrated in Figure 3, showing the number of bulbs and both fresh and dry weights per treatment. Production results indicate that treatments with leaf litter addition during planting (C and J) resulted in a significantly higher number and weight of bulbs, than the K+ (Fig. 3). However, no significant difference was observed between treatments C and J. Similarly, the number of bulbs did not significantly differ between treatments where leaf litter was added 2 weeks before planting (PC and PJ) and during planting (C and J). Fresh and dry bulb weights showed significant differences between treatments PC and C and between PJ and J. Overall, treatments C and J effectively enhanced bulb production. Treatment J produced an average fresh weight of 8.76 g, dry weight of 6.86 g, and 11.83 bulbs per plant, while treatment C produced an average fresh weight of 7.96 g, dry weight of 6.12 g, and 11.79 bulbs.

Diversity of bacterial communities

The total sequencing reads across all samples amounted to 349,064, with 2,783 ASVs identified. The rarefaction curve indicated that species saturation was reached in all samples. As shown in Figure 4, Venn diagram analysis revealed 772 ASVs common to all

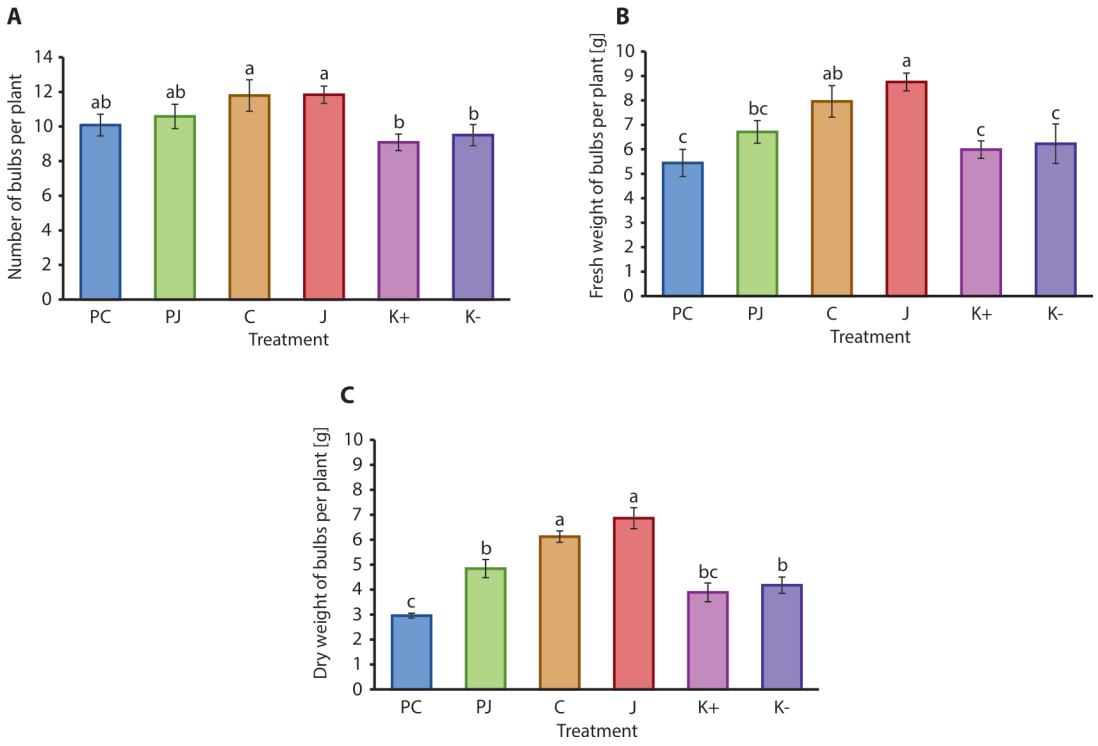

Fig. 3. Number of bulbs – A, fresh weight of bulbs – B, and dry weight of bulbs – C per plant. The letters on each bar represent significant differences

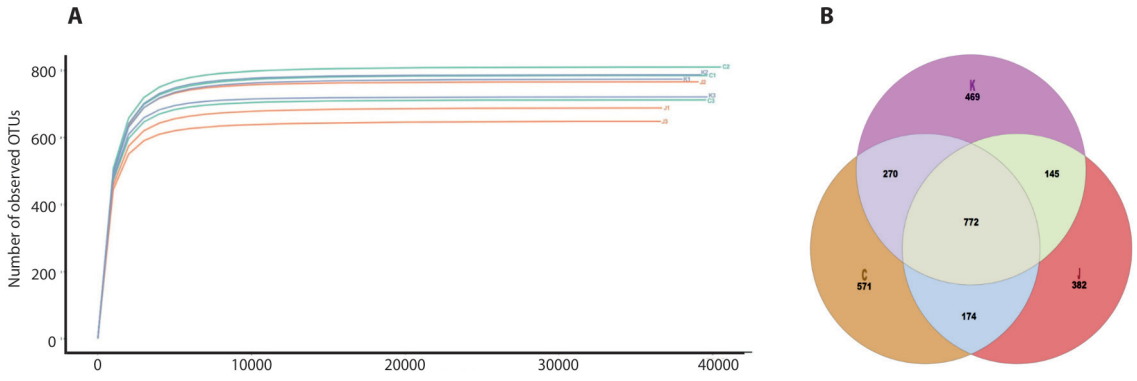

Fig. 4. Rarefaction curve – A and Venn diagram – B showing the distribution of common and specific ASV of bacterial communities in different groups

groups, with 571, 469, and 382 ASVs uniquely found in groups C, K, and J, respectively. Group C displayed the highest number of unique ASVs, indicating distinct microbial communities.

Figure 5 shows that the Alpha diversity analysis of bacterial richness was highest in group C with a significant difference observed in group J. However, bacterial evenness did not significantly differ between groups. This suggests that group C exhibited greater bacterial diversity than the other treatments. As shown in Figure 6, Beta diversity analysis with PCoA based on the Bray-Curtis matrix explained 21.3%, 15.39%, and 13.06% of the data variation through PCoA1, PCoA2, and PCoA3, respectively. Samples within the same treatment clustered together in the PCoA1 vs. PCoA2 plot, while in the PCoA1 vs. PCoA3 plot, samples K1 and J3 overlapped within the ellipse.

Core microbe

This study identified 25 bacterial taxa as core microbes in shallot cultivation soil and are presented in Figure 7. *Nocardioides*, *Gaiella*, and *Intrasporangium* exhibited high prevalence across all samples, even at higher detection thresholds, indicating their dominance in the soil microbiome. In contrast, *Pirellula, Tautonia,* and *Longispora* were not detected at higher thresholds due to their low abundance in the community.

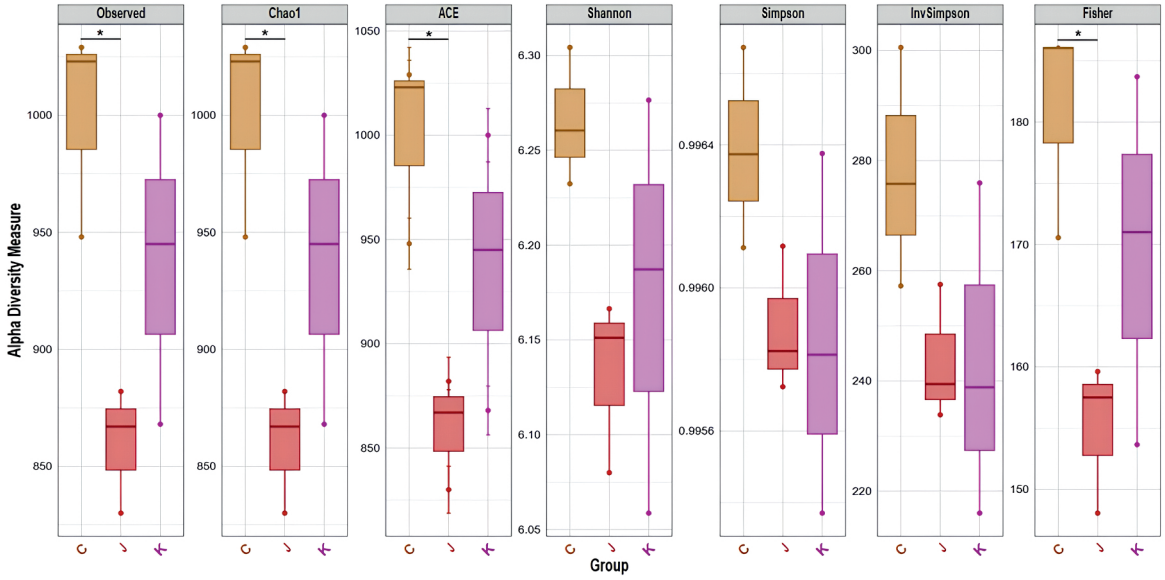

Fig. 5. Differences in bacterial alpha diversity indices were based on observed-species, Chao1, ACE, Shannon, Simpson, InvSimpson, and Fisher indices between different groups. The *sign on the bar represents a significant difference

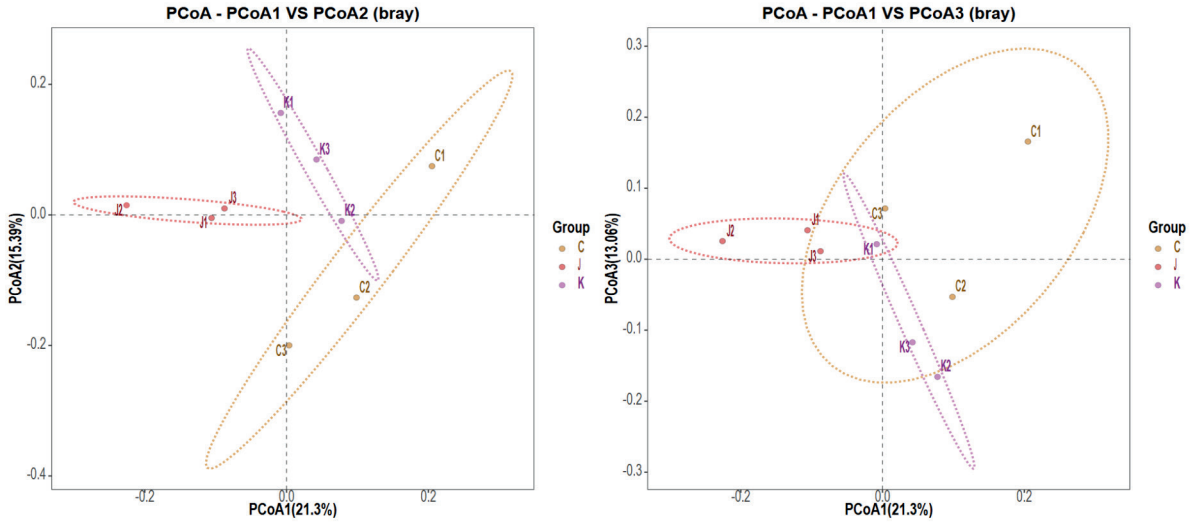

Fig. 6. Beta diversity using Principal Coordinates Analysis (PCoA) based on Bray-Curtis between different groups

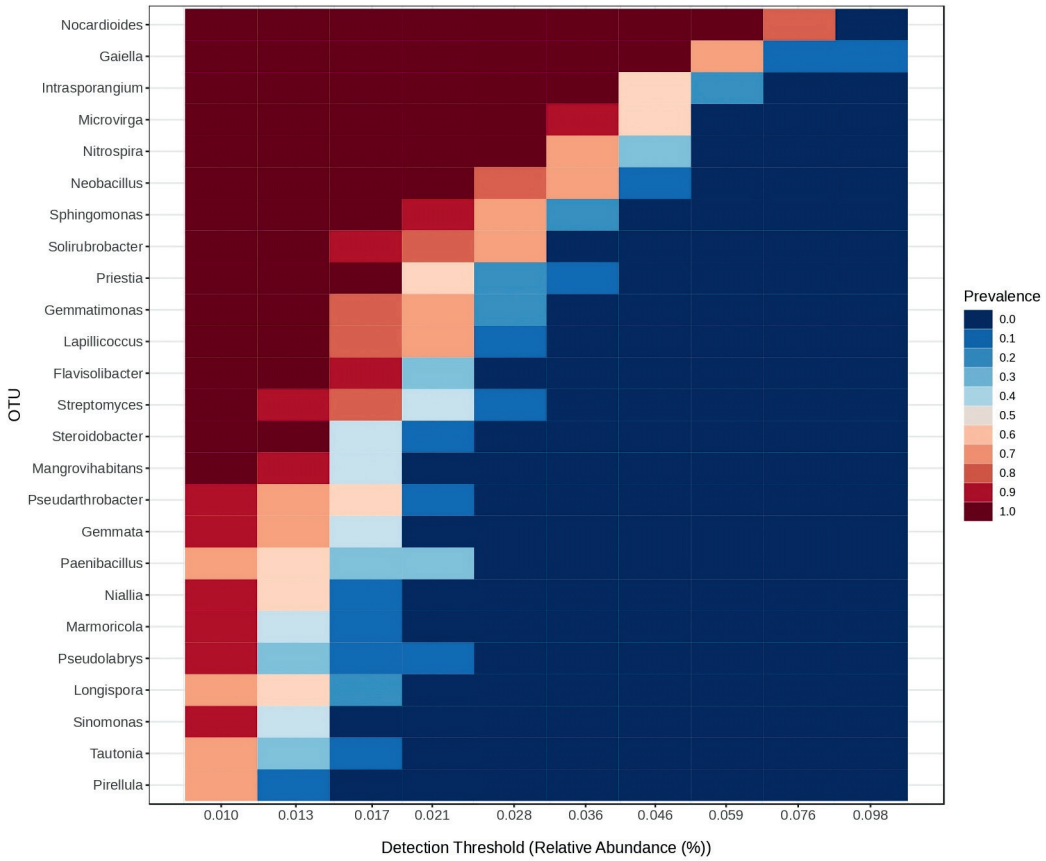

Fig. 7. Core microbe at genus level associated with shallot plants. The detection threshold used 10% (>0.01) for dominance and 0.1% for relative abundance

Differential abundance taxa (biomarkers)

The distinct bacterial biomarkers identified through LEfSe analysis are shown in Figure 8, with panels A-C comparing different treatment pairs (C vs K, J vs K, and C vs J), identifying taxa with significant LDA scores in each group. In group C from analyses C & K, *Sphingomonas, Lysobacter, Actinomadura, Bradyrhizobium, Phenylobacterium,* and *Baekduia* were detected as biomarkers with an LDA score > 2, while *Geodermatophilus, Pedomicrobium, Luteitalea,* and *Vicinamibacter* characterized group K (Fig. 8A). For analyses J and K, biomarkers in group J included *Gaiella, Paenibacillus, Anaeromyxobacter, Hyphomicrobium, Mycobacterium, Desulfitobacterium, Ammoniphilus, Baekduia, Bradyrhizobium,* and *Aquisphaera*. In contrast, group K contained *Microvirga, Pseudarthrobacter, Geodermatophilus,* and *Rhodoplanes* as biomarkers (Fig. 8B). Comparing groups C and J, group C was enriched with *Actinomadura, Pseudarthrobacter, Devosia, Niallia, Candidatus Alysiosphaera, Roseicella,* and *Chungangia.* In contrast, group J exhibited biomarkers such as *Gaiella, Paenibacillus, Anaeromyxobacter, Clostridium, Pedomicrobium, Ammoniphilus, Luedemannella, Hyphomicrobium, Desulfitobacterium, Aquisphaera,* and *Luteitalea* (Fig. 8C).

Co-occurrence network

The topological characteristics of the co-occurrence microbial networks are summarized in Table 2, indicating that groups C and K had the highest nodes and edges, suggesting stronger microbial interactions. Although groups C and J had fewer nodes, they exhibited more edges than the other groups (Table 2). Co-occurrence networks highlighting keystone taxa in each group comparison are shown in Figure 9. Key bacterial taxa with strong correlations included *Anaeromyxobacter, Archangium, Intrasporangium, Bradyrhizobium, Phenylobacterium, Rubrobacter, Lysobacter, Bryobacter,* and *Arboricoccus. Intrasporangium* was identified as

Table 2. Co-occurrence network topology of different groups

Topology	Group		
	C & K	J & K	C & J
Number of nodes	86	84	79
Number of edges	167	163	188
Number of positive edges	82	96	88
Number of negative edges	85	67	100
Average degree	1.9	1.9	2.4

Node – bacteria, edge – interaction, average degree – number of bacteria divided by number of interactions

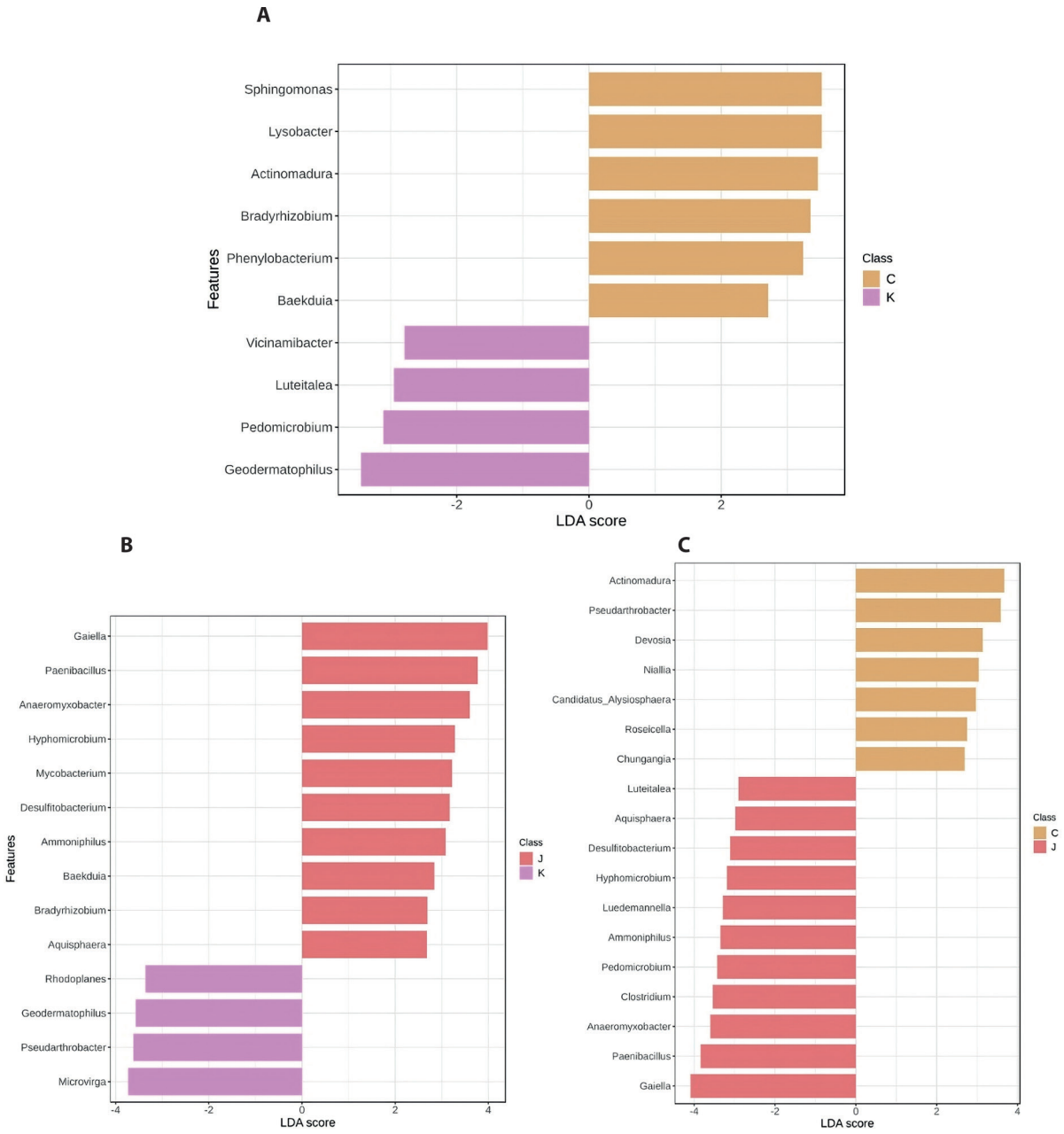

Fig. 8. LEfSe analysis of taxonomic abundance in groups C & K (A), J & K (B), and C & J (C). LDA score +/- does not have any meaning (it only indicates biomarkers in one of the groups

a keystone, forming positive interactions with several bacteria. Additionally, group C recognized *Bradyrhizobium, Phenylobacterium*, and *Lysobacter* as keystone and biomarkers (Fig. 9A).

The co-occurrence network analysis for groups J and K identified *Desulfitobacterium, Phenylobacterium, Gemmata, Steroidobacter, Ammoniphilus, Hyphomicrobium,* and *Paenibacillus* as key bacterial taxa. Among these, *Gemmata, Steroidobacter,* and *Paenibacillus* were classified as keystone taxa and core microbes, while *Desulfitobacterium, Ammoniphilus,* and *Hyphomicrobium* were identified as keystone taxa and biomarkers in group J. *Desulfitobacterium* exhibited the highest number of interactions, forming positive correlations with *Anaeromyxobacter, Clostridium, Gemmata, Hyphomicrobium, Paenibacillus,* and *Thermincola* (Fig. 9B).

The co-occurrence network analysis for groups C and J aimed to determine dominance among bacterial taxa in soils treated with different types of leaf litter. The results identified six central taxa with higher dominance in group C and four in group J.

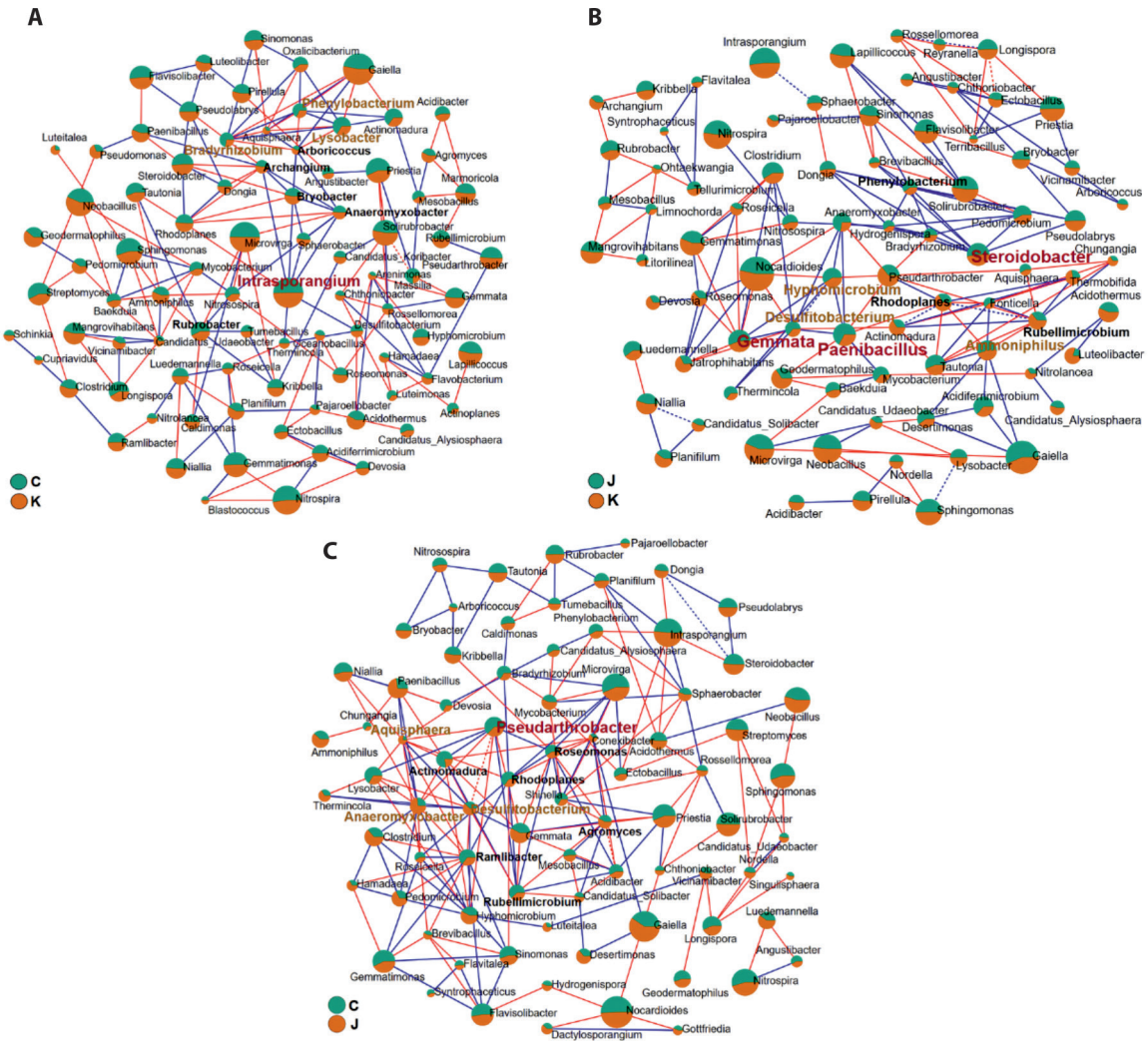

Fig. 9. Co-occurrence network of the bacterial genus in groups C & K – A, J & K – B, and C & J – C. Nodes are bacterial genera, and their size corresponds to relative abundance. The color dominance of the nodes indicates dominance of abundance of each group. Blue and red edges represent positive and negative relationships, respectively. Bold text – central taxa, red text – core microbe, and brown text – biomarker

Table 3. Bacterial genus found to play an important role in the group with leaf litter addition (C & J)

Genus bakteri	Core microbe	Bio-marker	Key-stone	Domi-nance
Pseudarthrobacter	√	√	√	C
Paenibacillus	√	√	√	J
Lysobacter	–	√	√	C
Actinomadura	–	√	√	C
Anaeromyxobacter	–	√	√	J
Hyphomicrobium	–	√	√	J
Ammoniphilus	–	√	√	J
Bradyrhizobium	–	√	√	C
Phenylobacterium	–	√	√	C
Desulfitobacterium	–	√	√	J
Baekduia	–	√	√	C
Aquisphaera	–	√	√	J

The dominance column shows the high abundance of a group. Sign (√) indicates the bacteria is a core microbe/biomarker/keystone, and sign (–) bacteria is not one of the three

Pseudarthrobacter emerged as a core microbe, biomarker, and keystone with greater abundance in group C, while *Anaeromyxobacter, Aquisphaera,* and *Desulfitobacterium* were dominant keystone biomarkers in group J (Fig. 9C). The integration of core microbes, biomarkers, and keystone taxa for each treatment group is summarized in Table 3. The findings revealed that *Pseudarthrobacter* and *Paenibacillus* were the most abundant taxa, acting as core microbes, biomarkers, and keystone taxa in groups C and J, respectively.

Discussion

Twisted disease, caused by *Fusarium* spp., is a significant plant disease in Indonesia, leading to symptoms such as leaf yellowing, twisting, bulb rot, and plant death (Sundari *et al.* 2023). This study showed

that early symptoms appeared 5 days after inoculation (dai). The early symptoms of twisted began to be observed at 14 days after planting (dap) and further developed at 42 dap (Wibowo *et al.* 2023). Among treatments, adding Casuarina leaf litter (C) resulted in the lowest disease incidence (6.67%) at 56 dap, demonstrating its potential for disease suppression. Adding Casuarina and maize leaf litter before planting was expected to be more effective in suppressing twisted disease, but treatments PC and PJ were less effective than C and J. Research suggests that shorter decomposition times enhance disease suppression, as seen in *Pythium ultimum* studies (Mayerhofer *et al.* 2021). Similarly, applying organic materials at planting is more effective in controlling soilborne diseases than pre-planting applications (Sloot *et al.* 2024).

Based on disease incidence data management effectiveness was evaluated, with treatment C being the most effective. *Casuarina equisetifolia* leaves contain antifungal compounds, including flavonoids such as kaempferol and quercetin, which disrupt fungal plasma membranes, inhibit nucleic acid and protein synthesis, and prevent biofilm formation (Abdallah *et al.* 2024). These compounds enhance plant resistance by modulating resistance-related genes and strengthening defense systems against pathogens (Ramzan *et al.* 2024). According to Bollina *et al.* (2010), kaempferol in barley plays a role as a metabolite associated with resistance to *Fusarium graminearum*.

This study also analyzed shallot production, showing that treatment J increased bulb production by 30–76%, while treatment C increased it by 30–57% compared to K+. The positive effects of Casuarina and maize leaf litter on production align with a previous study. Casuarina litter enhances production yields, while maize leaf litter boosts photosynthate accumulation, promoting bulb growth (Sarr *et al.* 2013). Additionally, kaempferol in *C. equisetifolia* leaves supports plant growth by improving stem length, leaf weight, and root weight in potato plants (Abdallah *et al.* 2024; Ramzan *et al.* 2024).

The metagenomic analysis aimed to compare bacterial communities in shallot soil with and without added leaf litter. Soil microbes influence plant pathogens by antagonism or improving plant health (Zhao *et al.* 2019). The study hypothesized that leaf litter increases bacterial diversity and complexity linked to keystone taxa and biomarkers (Dasgupta *et al.* 2024). Rarefaction curves confirmed sufficient sequencing depth, with the highest species count in sample C2 and the lowest in J3. The Venn diagram showed a high number of specific ASV in group C, correlating with a lower incidence of twisted disease (6.67%). Higher bacterial diversity is associated with reduced *Fusarium* abundance and improved pathogen suppression (Zhao *et al.* 2019).

The observed-species, Chao1, ACE, and Fisher indices indicate richness, while Shannon, Simpson, and InvSimpson indices indicate evenness (Navya and Babu 2023). Alpha diversity analysis revealed that bacterial richness and evenness were highest in group C, moderate in group K, and lowest in group J. Beta diversity analysis using PCoA showed that samples in group J were closely related and homogeneous, while group C displayed more significant heterogeneity and broader dispersion. Group K had a relatively narrow distribution, distinguishing it from other groups. These results suggest that leaf litter influences microbial diversity, with group C promoting the highest bacterial diversity.

Core microbes in shallot cultivation soil were identified, with 25 bacterial genera consistently present regardless of environmental changes, such as added leaf litter. These microbes play essential roles, including promoting plant growth, fixing nitrogen, producing antimicrobial compounds, decomposing organic matter, and contributing to nutrient cycling. Their stability in the soil ecosystem highlights their importance in supporting plant health and productivity.

Differential abundance analysis using LEfSe identified bacterial biomarkers in different soil treatment groups (Dasgupta *et al.* 2024). In group C from analyses C and K, *Sphingomonas* had the highest LDA score, likely due to its prevalence in Casuarina leaf litter and its role in suppressing *Fusarium* sp. (Lin *et al*. 2022). In group J, from analysis of J and K, *Gaiella* was the dominant biomarker, supported by its increased abundance with maize litter application and its antibiotic properties against *Fusarium oxysporum* (Zhao *et al.* 2019; Yu *et al.* 2024). *Actinomadura* was identified as a biomarker in group C from analyses C and J, known for its antimicrobial activity and role in enhancing plant growth (Wang *et al.* 2023).

Network analysis in bioinformatics helps in understanding microbial community interactions (Navya and Babu 2023). Keystone taxa enhance diversity through positive and negative relationships, which may be mutualistic, commensal, parasitic, or competitive. Nodes represent bacterial taxa, while edges indicate correlations, with node size reflecting abundance and edge thickness shows correlation strength (Morni *et al.* 2025). Groups C and J exhibited a more complex microbial network, with a higher number of edges and average degree than other group comparisons. Despite fewer nodes, these groups had stronger connectivity, indicating a more interconnected microbial community. A higher proportion of edges suggests increased interactions, influencing microbial composition and function (Dasgupta *et al.* 2024).

Intrasporangium, identified as a central taxa and core microbe in groups C and K, plays a crucial role in microbial community structure and function. As an

Actinobacteria, it is abundant in group C soils and is linked to a lower incidence of twisted disease. Actinobacteria produce secondary metabolites that inhibit pathogens and promote plant growth (Shivlata and Satyanarayana 2015). *Intrasporangium* exhibits positive interactions with *Anaeromyxobacter* and *Archangium* (Myxobacteria), which act as biocontrol agents. These bacteria produce bioactive compounds like Ambruticin and Soraphen A, known for their antifungal properties, further contributing to disease suppression (Bhat *et al.* 2021).

In groups J and K, *Gemmata, Steroidobacter,* and *Paenibacillus* are identified as keystone taxa and core microbes. *Gemmata* enhances plant utilization of organic material and degrades organic matter enzymatically, while *Steroidobacter* contributes to carbon and nitrogen cycling (Zhang *et al.* 2017; Tan *et al.* 2019). *Paenibacillus*, highly abundant taxa, plays a key role in microbial community interactions (Navya and Babu 2023). The keystone influence on microbial communities is shaped by its interaction properties with other microbes (Berry and Widder 2014). *Desulfitobacterium* exhibits the highest interactions, positively correlating with multiple bacteria, including *Anaeromyxobacter, Clostridium, Gemmata, Hyphomicrobium, Paenibacillus,* and *Thermincola*. As an antagonistic bacterium, its dominance helps suppress *Fusarium* communities (Pratiwi 2024). *Anaeromyxobacter* also contributes to nitrogen fixation, while *Hyphomicrobium*, an Alphaproteobacteria, mediates microbial community composition, particularly after adding maize leaf litter (Dasgupta *et al.* 2024).

Co-occurrence network analysis in groups C and J revealed that *Pseudarthrobacter* is a core microbe, biomarker, and keystone with high abundance in group C. This bacterium interacts with nine other bacteria, mainly from the Actinobacteria and Proteobacteria groups, which dominate Casuarina leaf litter (Lin *et al.* 2022). The dominance of *Pseudarthrobacter* is believed to suppress pathogens that cause twisted disease. Additionally, *Anaeromyxobacter* has the highest number of interactions with several other bacteria, including *Aquisphaera, Clostridium, Desulfitobacterium, Hyphomicrobium, Paenibacillus, Pedomicrobium,* and *Thermincola.*

The abundance and diversity of keystone taxa contribute to the complexity of microbial networks in healthy plants. Their presence is crucial, as their absence can alter network structure, composition, and microbial functions (Zheng *et al.* 2021a). Keystone taxa have a more significant impact when they are core microbes, emphasizing their essential role in the microbiome (Banerjee *et al.* 2018). This study identified *Pseudarthrobacter* and *Paenibacillus* as core microbes, biomarkers, and keystone taxa, with the highest abundance in groups C and J, respectively.

Pseudarthrobacter, a member of Actinobacteria, functions as a plant growth-promoting rhizobacteria (PGPR) by producing indole-3-acetic acid (IAA), which enhances plant growth (Chaiya *et al.* 2021). It also produces antimicrobial compounds that effectively suppress pathogens, correlating with the low incidence of twisted disease in treatment C. Additionally, *Pseudarthrobacter* generates siderophores, which inhibit pathogenic fungi by binding Fe^{3+}, colonizing roots, and creating conditions favorable for root growth while restricting fungal development (Sudewi *et al.* 2022).

Paenibacillus is a plant growth-promoting bacterium that enhances crop development by producing phytohormone IAA, utilizing atmospheric nitrogen and phosphorus, and degrading lignocellulose. *P. polymyxa* CR1 improves the growth of various plants, including maize, potato, tomato, cucumber, and chili. Beyond plant growth promotion, *Paenibacillus* suppresses *Fusarium* through the production of lipopeptides, hydrolytic enzymes, antifungal volatile compounds, and fusaricidins, as well as by inducing systemic resistance (Weselowski *et al.* 2016). This study suggested that adding leaf litter to soil can enhance beneficial soil bacteria, suppress disease incidence, and improve plant productivity by modifying microbial community structures.

Conclusions

This study showed that the addition of Casuarina and maize leaf litter during planting gave better results than the addition 2 weeks before planting in suppressing twisted disease. The addition of Casuarina leaf litter was able to increase the diversity and abundance of bacterial communities more than the addition of maize leaf litter. The greater abundance of bacterial communities in Casuarina leaf litter was suppressed by the low incidence of twisted disease. The addition of Casuarina leaf litter affected the role of *Pseudarthrobacter* as a core microbe, biomarker, and keystone that was able to suppress twisted disease. Furthermore, the addition of maize leaf litter affected the role of *Paenibacillus* as a core microbe, biomarker, and keystone that was able to increase shallot production. It would be wise to isolate *Pseudarthrobacter* and *Paenibacillus* as bacteria that were found to suppress twisted disease and increase production using selective media for bacterial growth.

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