MOLECULAR ANALYSIS OF MICROBIAL COMMUNITY CHARACTERISTICS IN SOILS WITH DIFFERENT LEVELS OF TOBACCO BACTERIAL WILT INDICES

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Abstract. Tobacco Bacterial Wilt (TBW) is a disease caused by the bacterium *Ralstonia solanacearum*. *R. solanacearum* is a soil-borne plant pathogen that can cause devastating losses in agricultural crops. However, there is limited information on the variation in the microbial community structure and molecular characteristics in soils with different index levels of tobacco bacterial wilt. Therefore, current study focused at undertaking molecular analysis of microbial community characteristics in soils of different index levels of TBW with the aim of providing insights on bio-control opportunities for bacterial wilt incidence in agricultural farms for improved yield. The findings indicated that the genus Enterobacteriaceae from the phylum Proteobacteria was more abundant under the control group (healthy soils), on the other hand genus *Ralstonia* of the phylum Proteobacteria was more abundant in the JYRS9 group (highly infected soils). The use of sample similarity analysis and COG function classification in relation to tobacco wilt disease index provided a comprehensive understanding of the microbial ecology of soil and the conditions that influence disease development and management. the use of KEGG pathway analysis in relation to tobacco wilt disease index provided a deeper understanding of the microbial ecology of soil and the factors that influence disease development and management.
Keywords: *bacterial community, bacterial wilt index, plant health, soil quality, Ralstonia solanacearum*

Introduction

Tobacco Bacterial Wilt (TBW) is a disease caused by the bacterium *Ralstonia solanacearum*. *R. solanacearum* is a soil-borne plant pathogen that can cause devastating losses in agricultural crops (Chang et al., 2022; Jiang et al., 2017). It is known to have a broad host range and can infect over 50 different plant families, including many economically important crops such as tomato, potato, and banana. This bacterium is capable of colonizing the xylem vessels of the host plant, resulting in wilting and plant death. The disease is spread by contaminated soil and water, as well as infected seedlings and plant debris. Effective control measures include the use of disease-free seed, crop rotation, and destruction of infected plants. The bacteria enter the plant through the roots and spreads to the xylem vessels, disrupting the flow of water and nutrients, leading to wilting and death. Prevention includes using disease-free seedlings, crop rotation, and avoiding movement of contaminated soil or plant material (Chang et al., 2022; Hu, Li, et al., 2021b; Seo et al., 2012). Chemical control methods are limited, and the most effective method is to implement cultural practices to reduce the spread of the disease. Bacterial wilt can have a negative impact on soil quality by

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reducing the fertility and organic matter of the soil, as well as by decreasing the populations of beneficial microorganisms. This can lead to reduced crop yields and decreased soil health over time. Bacterial wilt can have a negative effect on other soil microorganisms. The pathogen spreads rapidly in the soil, killing plants and reducing the available organic matter for other microorganisms to decompose. This can lead to a decline in the diversity and abundance of soil microorganisms, leading to changes in the soil ecosystem and potentially affecting nutrient cycling and plant growth. Bacterial wilt occurs mainly in tropical, semitropical and warm temperate zones. It is particularly severe in many Asian countries (China, Japan, Malaysia, Pakistan, Thailand, Vietnam), in America (USA, Brazil) and Africa (including South Africa). *R. solanacearum* is quite rare in Europe. It has been reported in Hungary, Italy and Yugoslavia (Jiang et al., 2017; Seo et al., 2012).

The TBW index is a scale used to assess the severity of the disease in a tobacco field. The index typically ranges from 0 (no disease symptoms) to 9 (complete wilting and death of the plant). The TBW index is used to determine the level of disease spread and to make decisions about disease management strategies, such as crop rotation, chemical treatment, and sanitation practices. The association between TBW index and other soil microorganisms is complex and depends on multiple factors such as soil type, climate, and agriculture practices (Li et al., 2010; Li et al., 2016; Li et al., 2021b). However, some studies have shown that the abundance of soil bacteria and fungi is influenced by the presence of tobacco. Tobacco cultivation has been found to reduce the number of beneficial microbes, while increasing the populations of pathogenic bacteria. This shift in the soil microbiome can have negative impacts on soil health and plant growth. On the other hand, some studies have also shown that the addition of tobacco residues to the soil can enhance the growth of certain beneficial bacteria and improve soil fertility. Overall, the relationship between tobacco bacterial index and other soil microorganisms is complex and multi-faceted (Chang et al., 2022; Janvier et al., 2007; Trivedi et al., 2022).

The ecological niche space for other bacterial species in the host roots may increase or decrease as a result of interactions between bacteria that coexist in the same environment, compete for the same resources, or depend on one another to survive. This rivalry between microbes may be a useful tool for biological control (Horita et al., 2014; Wang et al., 2022). Currently, beneficial bacteria that are mostly isolated from soil or plant rhizospheres provide the majority of biocontrols. using specialized antagonistic bacteria, such as Bacillus spp., to compete and induce systemic host resistance to the pathogens by creating antimicrobial metabolites (Buddenhagen and Kelman, 1964; Li et al., 2021a; Wen et al., 2020). Therefore, one of the areas that researchers need to focus on is finding bacterial communities that have inhibitory effects on tobacco bacterial wilt since this will be very helpful for biological control. Studies have shown that *R. solanacearum* can coexist with other pathogenic bacteria in the soil. For example, it had found been that the bacterium was frequently co-isolated with other plant pathogenic bacteria such as *Pseudomonas syringae* and *Xanthomonas campestris* from the rhizosphere of pepper plants. However, there is limited information on the soil bacterial community structures under different degrees of TBW (Berendsen et al., 2012a, 2018; Wen et al., 2020). Other studies have suggested that the presence of other bacteria in the soil can influence the virulence and survival of *R. solanacearum*. For example, a reported that the presence of Bacillus spp. in the soil can reduce the pathogenicity of *R. solanacearum* by producing antibiotics and competing for nutrients. However, the

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interactions between the *R. solanacearum* and other pathogenic bacteria in the soil are complex and not yet fully understood. Further research is needed to better understand these interactions and their implications for plant health and agricultural productivity (Berendsen et al., 2012a, 2018; Wen et al., 2020).

Soil physico-chemical properties may also affect bacterial community variation not only in rhizosphere soils of plants with different disease grades but also in soils from different locations, and the diversities were primarily driven by geography factors. *R. solanacearum* abundance has been found to be closely correlated with the soil pH value, status of the fertilizers and rhizobacterial community (Berendsen et al., 2018; Peeters et al., 2013; Wang et al., 2022). Therefore, proper adjusting the soil pH value, appropriate application of P and K fertilizers, and reducing the dosage of N fertilizer might be beneficial for suppressing bacterial wilt disease and promoting soil quality. Other studies have shown that the microbial diversities were quite different between *R. solanacearum* resistant and *R. solanacearum* susceptible soils. Moreover, soil quality played a key role in the difference in bacterial community distribution, and greater microbial diversity led to more extensive interactions (Berendsen et al., 2012a; Buddenhagen and Kelman, 1964; Genin and Boucher, 2002). Furthermore, several bacterial strains with excellent antagonistic ability to *R. solanacearum* have also been isolated from soil, which has provided a good foundation for future biological control of bacterial wilt (Compant et al., 2019; Li et al., 2021a; Peeters et al., 2013; Wen et al., 2020). Despite this knowledge, there is still limited information and understanding on the variation in the microbial community structure and molecular characteristics in soils of different index levels of tobacco bacterial wilt. Therefore, current study focused at undertaking molecular analysis of microbial community characteristics in soils of different index levels of TBW with the aim of providing insights on bio-control opportunities for bacterial wilt incidence in agricultural farms for improved yield.

Materials and methods

Description of the soil sampling site

The study analyzed soil samples taken from Jiangyong County, Yongzhou City, Hunan Province, China. The area has a climate typical of the Qinghai-Tibet plateau, which is characterized by cold, warm, and humid conditions with an annual range of sunshine hours between 2260 and 2740 h. The average annual temperature is 0.8℃, and the daily temperature fluctuates between 11.6 and 17.5℃. The yearly rainfall ranges from 530 to 560 mm, while the annual evaporation is between 1130 and 1340 mm. Based on the FAO classification, the soil type in the region is Kastanozems (Zhang et al., 2000).

Soil sampling and experimental design

Soil was sampled from tobacco growing fields in Jiangyong County, Yongzhou City, Hunan Province, China. Samples were obtained from different areas that were categorized based on the levels of tobacco bacterial wilt, with the cultivated tobacco having acquired different degree of the disease (Disease Index). There experiment was run on microorganism diversity between the different soil from different tobacco wilt disease index with the aim of analysing the relationship between soil microorganism with different Disease Index. The Disease Index were categories as below; (i) Healthy Tobacco that was marked as the Control, (ii) Occasionally chlorotic spots on tobacco

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stem, with less than 1/2 of the leaves of the diseased parts having experienced some wilting, this was marked as JYRS1 (where 1 means 1 degree of disease index) (iii) Stem with black stripes over covering 1/2 stem height, but not reaching the top of the stem, or more than 2/3 of the leaves on the diseased parts having experienced some wilting, this was marked JYRS5 (where 5 means 5 degree of disease index), and (iv) A whole tobacco tree at a state of complete death/wilt, this was marked JYRS9 (where 9 means 9 degree of disease index). The study collected soil samples from the root area at a depth of 30 cm in three separate instances, and five plants were randomly taken from each of the four plots, resulting in a total of 80 plant samples. The soil was mixed thoroughly, packed in a sealed bag, placed in an icebox, and transported to the laboratory under refrigeration at a temperature of -80°C for further analysis.

DNA extraction and PCR amplification of 16 S rRNA

To isolate the DNA, the study utilized the method outlined in (Han et al., 2017). In summary, the process involved extracting the genomic DNA directly from the soil samples using the E.Z.N.A.® Soil DNA kit from Omega Bio-Tec, Inc. (USA). The manufacturer's manual was followed closely to complete the extraction process. After completion of the genome DNA isolation, the drawn genomic DNA was detected using 1% agarose gel electrophoresis using Primers; 341F CCTACACGACGCTCTTCCGATCTNCCTAC- GGGNGGCWGCAG, and 805R GACTGGAGTTCCTTGGCACCCGAGAATTC-CAGACTACHVGGGTATCTAATCC as template for amplification of the V3–V4 hypervariable regions of 16S rRNA via PCR from the microbial genomic DNA (Claesson et al., 2009). To ensure that subsequent data analysis was precise and dependable, the study utilized low-cycle amplification and maintained a consistent number of cycles per sample amplification. To establish the appropriate concentration and achieve the least number of cycles, representative samples were randomly selected for pre-experimentation. Every sample underwent triplicate amplification in compliance with all experimental conditions. After the PCR products were mixed for the same sample, electrophoresis testing was performed using a 2% agarose gel, and the recovered PCR products were purified with the AxyPrep DNA gel recovery kit from AXYGEN Company. The gel material was cut, washed in Tris HCl, and then underwent electrophoresis testing. The PCR products were quantified using the QuantiFluor™-ST Blue Fluorescence Quantitative System from Promega Company and mixed proportionally based on the sequencing requirements for each sample. To create the Miseq library, the study added the official Illumina adapter sequence to the outer end of the target region using PCR. The PCR products were then recycled using a gel recovery kit, and the gels were cut and washed in Tris-HCl buffer. A swimming test using 2% agar was carried out, followed by sodium hydroxide denaturation to produce single-stranded DNA fragments. The TruSeqTM DNA Sample Prep Kit was used to carry out the procedure, followed by Miseq sequencing.

Bio-information analysis process

After Miseq sequencing, the PE reads were initially merged based on their overlap relationship, and their sequence quality was controlled and filtered. The samples were then distinguished, and OTU clustering and species taxonomy analysis were performed. This analysis included Diversity Index Analysis based on the results of OTU clustering.

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Sequencing depth detection was carried out at various classification levels of the community structure analysis based on taxonomic information. Based on these analyses, a range of in-depth statistical and visual analyses were performed on multi-sample community composition and system development information, such as multi-analysis and difference differentiation tests.

Taxonomic, species composition and variance analysis

The study utilized the OTU Taxonomics Comprehensive Information Table, which combined the results of the OTU analysis with taxonomic information, as well as the rank-abundance curve to assess the abundance and uniformity of species OTUs. Pan/Core Species Analysis, Alpha Diversity Analysis, and Dilution curve analysis were performed following the methods described in (Costa et al., 2020). To evaluate the diversity of microorganisms in the environment, richness and diversity of the microbiome were calculated within individual samples, using methods such as species Venn chart analysis and community composition analysis, as described in (Lam et al., 2016). The study utilized Circos diagrams, which utilize visual circles, to visually represent the correspondence between samples and species. These diagrams were used to show the dominant species composition of each sample or group and to identify the relationship between samples and species. The species variance analysis was conducted to identify differences in the abundance of the microbiome between different groups or samples. This was achieved by performing significant difference tests between groups and conducting multi-level species differential analysis using LEfSe (Linear discriminant analysis Effect Size).

Sample comparison and functional predictive analysis

The methods described in (Legendre and Condit, 2019; Maziarz et al., 2018) were used for the Beta Diversity Analysis and Sample grouping analyses. To cluster the samples hierarchically, the Partial least square's discriminant analysis (PLS-DA) was employed. The effect of the 16S marker gene in the genome of the species was predicted using PICRUSt, and the COG libraries were used for functional predictive analysis. The cluster of orthologous groups (COG) was determined according to the criterion of consistency of genome-specific best hits based on the results of an exhaustive comparison of all protein sequences from these genomes.

Quality control and statistical data analysis

In order to obtain high-quality sequencing data to improve the accuracy of subsequent biological information analysis, splicing was first done, followed by quality control and filtering to obtain effective data (Clean Data). This involved the use of vsearch's fastq_mergepairs command to splice the paired sequences obtained by double-terminal sequencing to obtain the merge sequence, use of CutAdapt software to remove primers from the sequence and then use vsearch's fastq_filter command to remove low-quality sequences, sequences containing N-bases, sequences less than 100 bp in length. The data was analyzed using various software including SPSS version 22.0 by IBM, QIIME version 2.0.5 and R version 4.2.3. Mean differences were calculated with a significance level of 0.05. Significant differences among the means were identified using the LSD test. T-tests and Metastats in Mothur were used to

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compare differences, and p-values were adjusted for false discovery rate using the BH method with the mt.rawp2adjp function in R.

Results

Quality control and orthogonal taxonomic units classification analysis

From the quality control, raw reads were 127004, 129628, 133078 and 125112 respectively for the control, JYRS1, JYR5 and JYRS9. The percentage of the raw rea that were clean were 68.67%, 68.93%, 68.7% and 70.42% respectively for the control, JYRS1, JYRS5 and JYRS9 groups. From the Orthogonal Taxonomic Units (OUT) analysis across groups, it was shown that genus Enterobacteriaceae from the phylum Proteobacteria was more abundant under the control group, on the other hand genus *Ralstonia* of the phylum Proteobacteria was more abundant in the JYRS9 group (*Fig. 1*). Generally, there was high abundance of more genera under the control group than the JYRS1, JYRS5 and JYRS9 groups (*Fig. 2*).

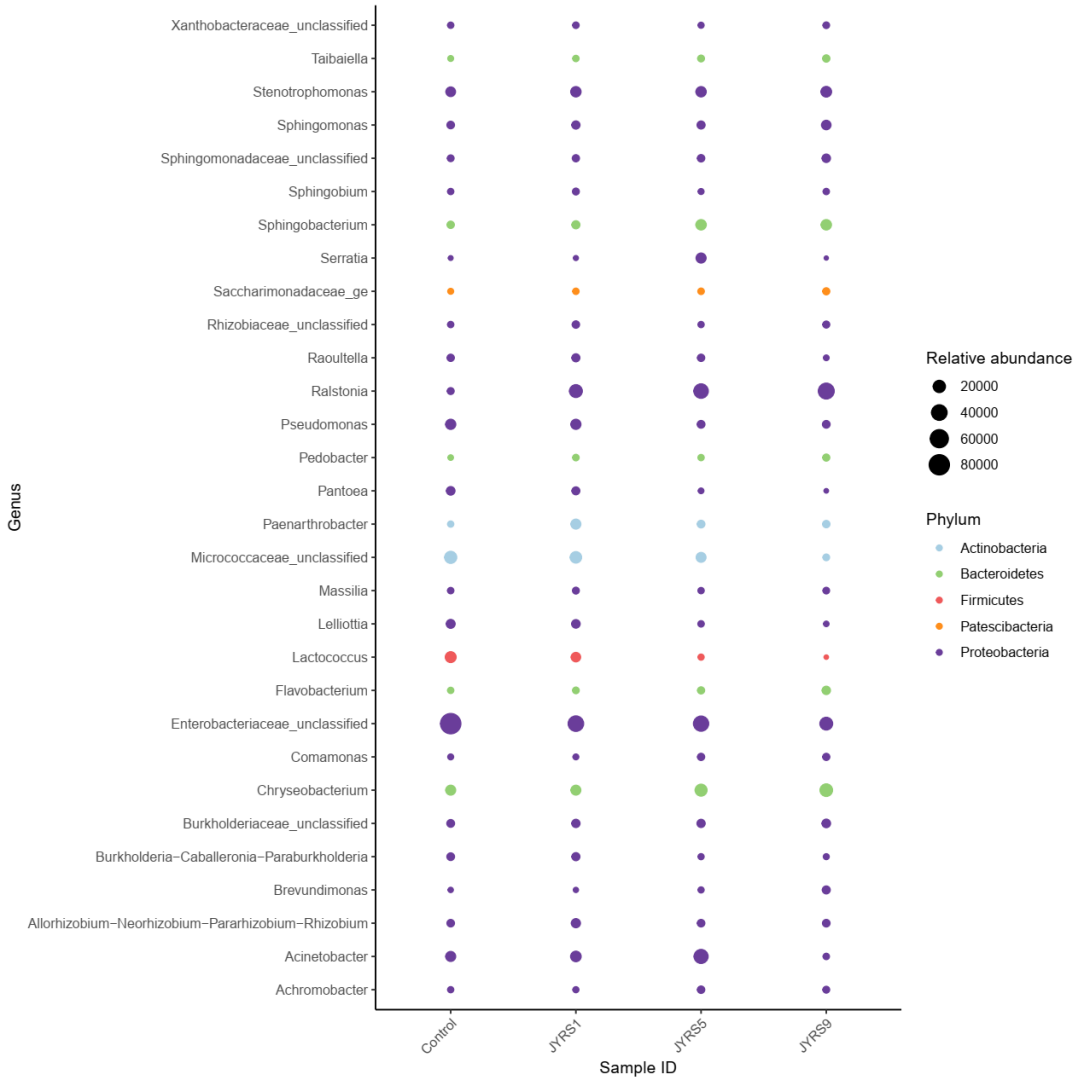

Figure 1. *The abundance of orthogonal taxonomic units (phylum and genus) across groups*

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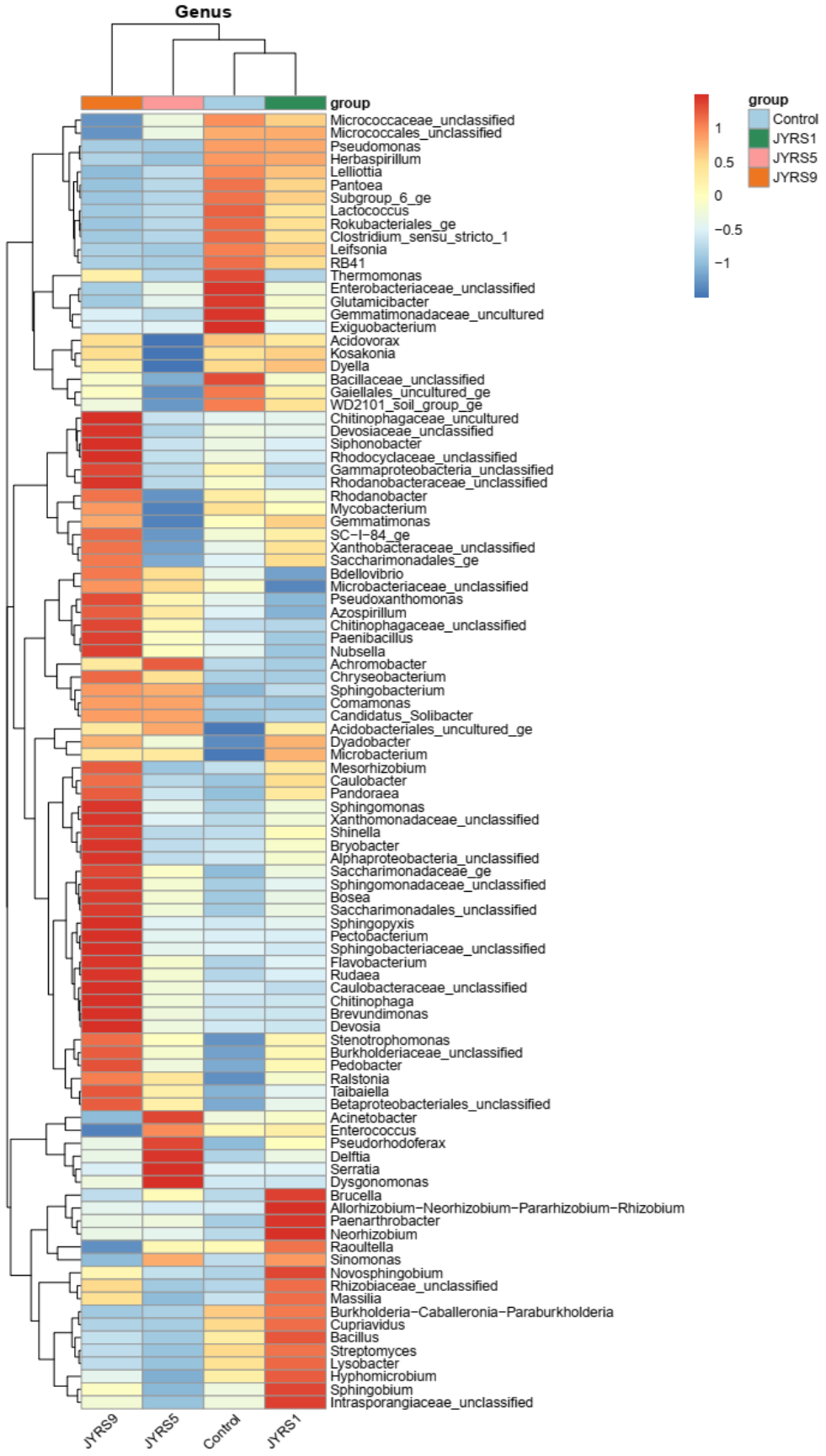

Figure 2. *Heatmap of the abundance genera across the groups*

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Alpha diversity analyses

To evaluate diversity across all the groups, various indices (ACE, CHAO, Shannon and Simpson) were applied. Through ACE index, JYRS1 and JYRS9 groups had higher values than the control and JYRS5 groups. Through the Chao index, JYRS1 and JYRS9 groups also had higher values than the control and JYRS5 groups. Through Shannon diversity index, JYRS9 groups had higher values than the control, JYRS1 and JYRS5, while through Simpson index, the control had higher values than the JYRS1, JYRS5 and JYRS9. However, the differences were not significant (*Fig. 3*).

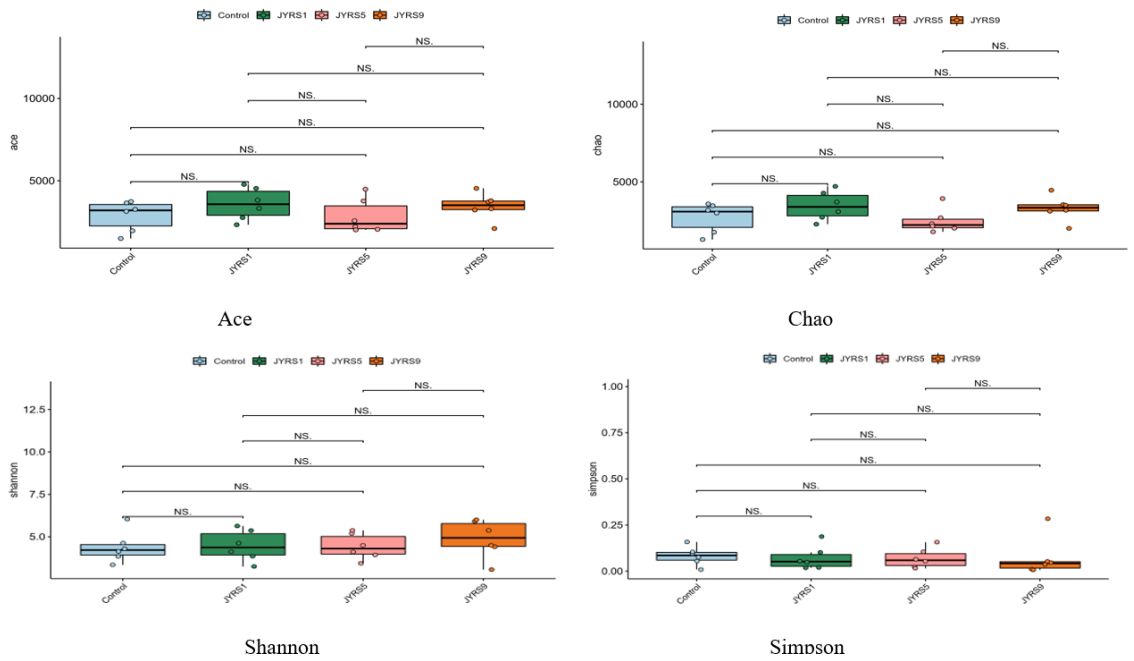

Figure 3. *Alpha diversity through ACE, CHAO, Shannon and Simpson indices. Areas with significant differences are marked as NS*

Beta diversity analyses

Through Principal Component Analysis (PCA) to determine the quantitative variability in diversity between group, it was shown that the control and the JYRS9 groups had very distinct genetic variability (*Fig. 4*), indicating the existing difference in the level of community bacterial characteristics. Through Principal coordinate Analysis (PCoA) to produce a set of uncorrelated (orthogonal) axes to summarize the variability in the data set (*Fig. 5*), it also showed that the control group was ordinated further away from the JYRS9 group. Through Venn diagram to represent relationships between genetic traits in different groups (*Fig. 6*), it was shown that the control group shared the least number of genes with JYRS5 and JYRS9 groups, as compared to the number of genes shared between the control group and JYRS1 group.

Sample similarities and clusters of orthologous groups function classification

To understand common ancestors and the characteristics of bacterial communities in various groups, linear discriminant analysis effect size (LEFSE) was run and presented

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in a cladogram (*Fig. 7*). To understand the high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, and for molecular-level information functional difference and molecular interactions in bacterial communities between the different groups, Kyoto Encyclopedia of Genes and Genomes (KEGG) was employed (*Fig. 8*). Through the heatmap output, it was shown that there were much higher expressions of KEGG pathways under the JYRS9 groups than the control group. Through PCA on KEGG pathways, there was distinct dissimilarity in the functions between groups (*Fig. 9*).

Discussion

Molecular analysis of microbial community characteristics in soils of different levels of tobacco bacterial wilt indices was pursued through advanced molecular techniques in investigating the diversity and abundance of microorganisms present in soil samples with varying degrees of tobacco bacterial wilt. The study aimed at understanding the changes in the microbial community affect the incidence and severity of tobacco bacterial wilt. From this basis, the study typically involved the collection of soil samples from different locations with varying degrees of tobacco bacterial wilt, ranging from healthy soils to severely affected soils. Thereafter, the extracted DNA were subjected to molecular analyses. The results therefore aimed at revealing important insights into the microbial ecology of the soil and the relationships between microorganisms and tobacco bacterial wilt.

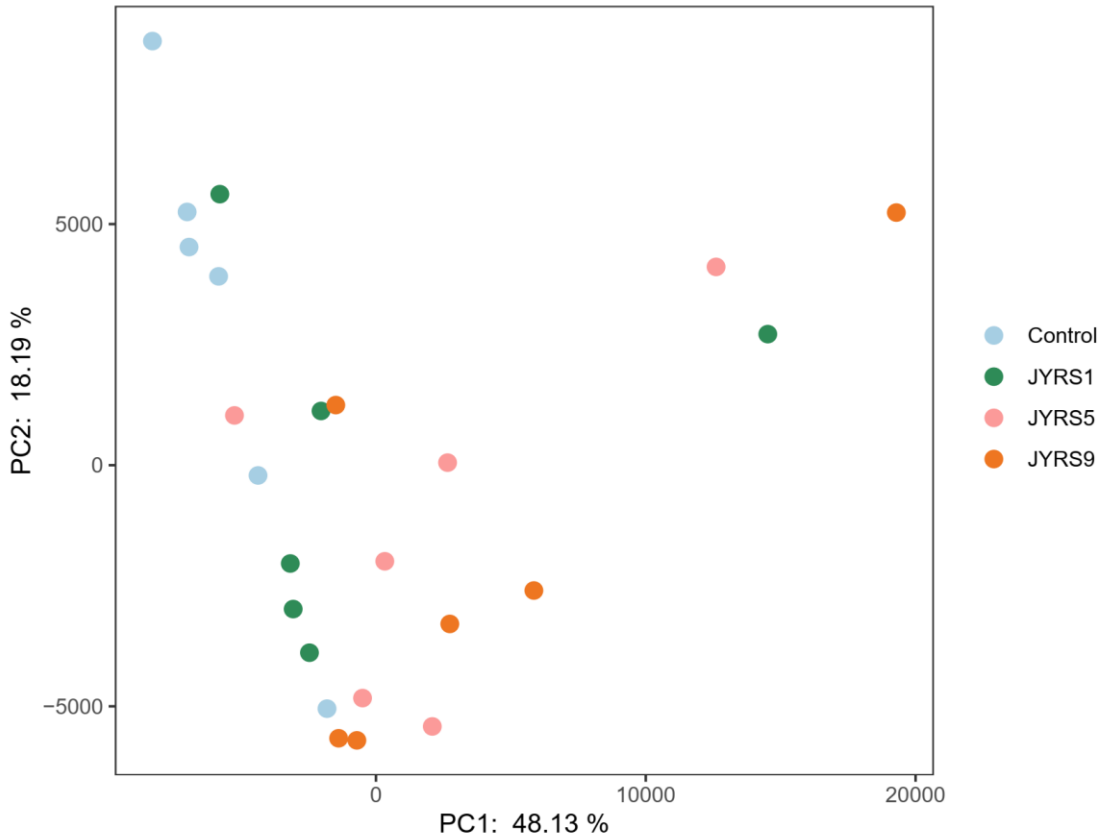

Figure 4. *Principal component analysis on quantitative variability in diversity between groups*

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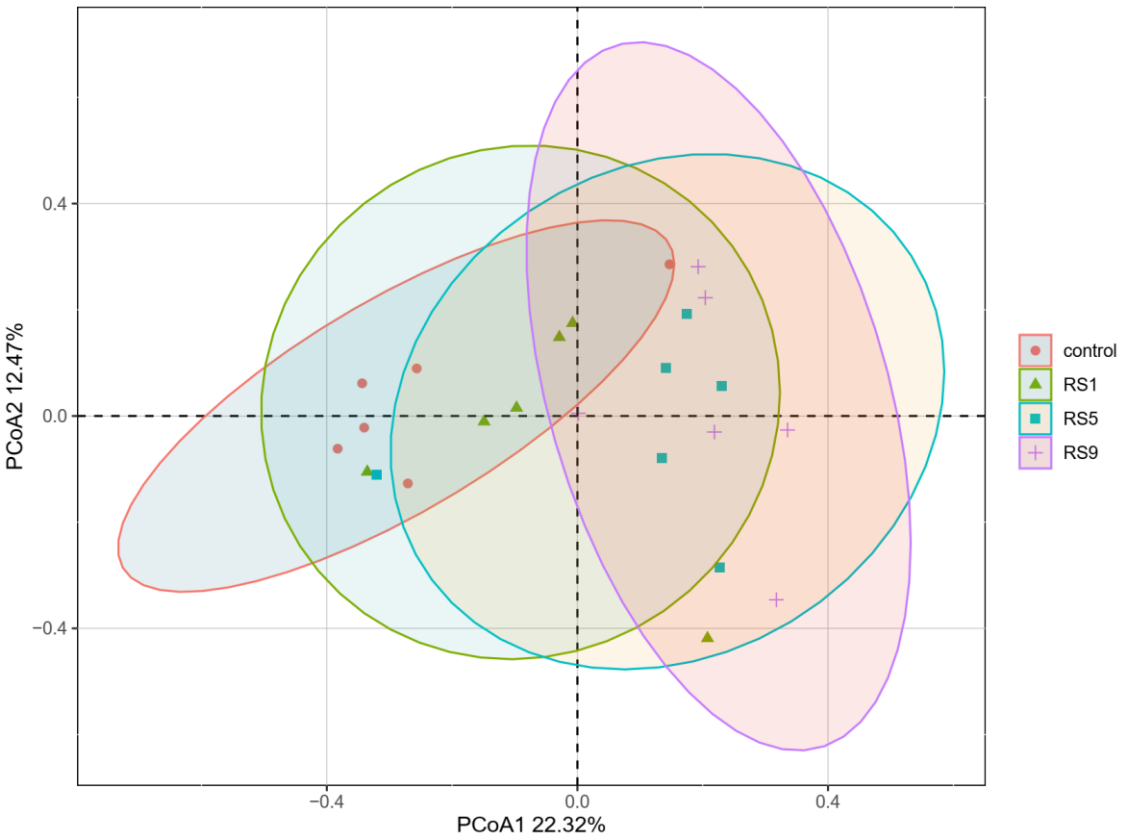

Figure 5. *Principal coordinate analysis to check on uncorrelated (orthogonal) axes and dissimilarity between groups*

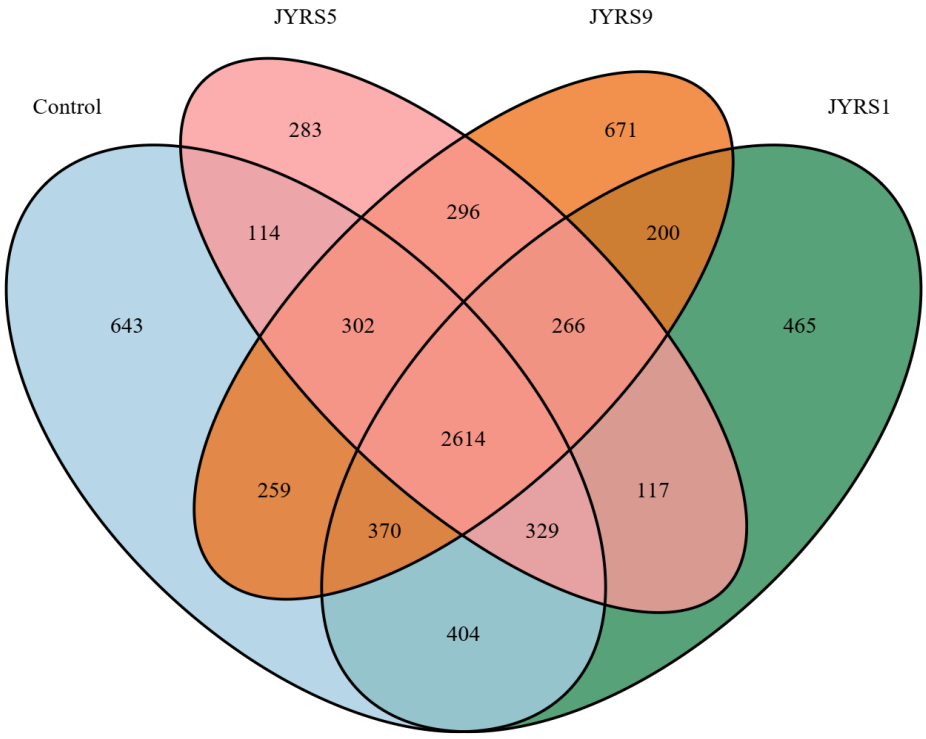

Figure 6. *Venn diagram on relationships between genetic traits in different groups*

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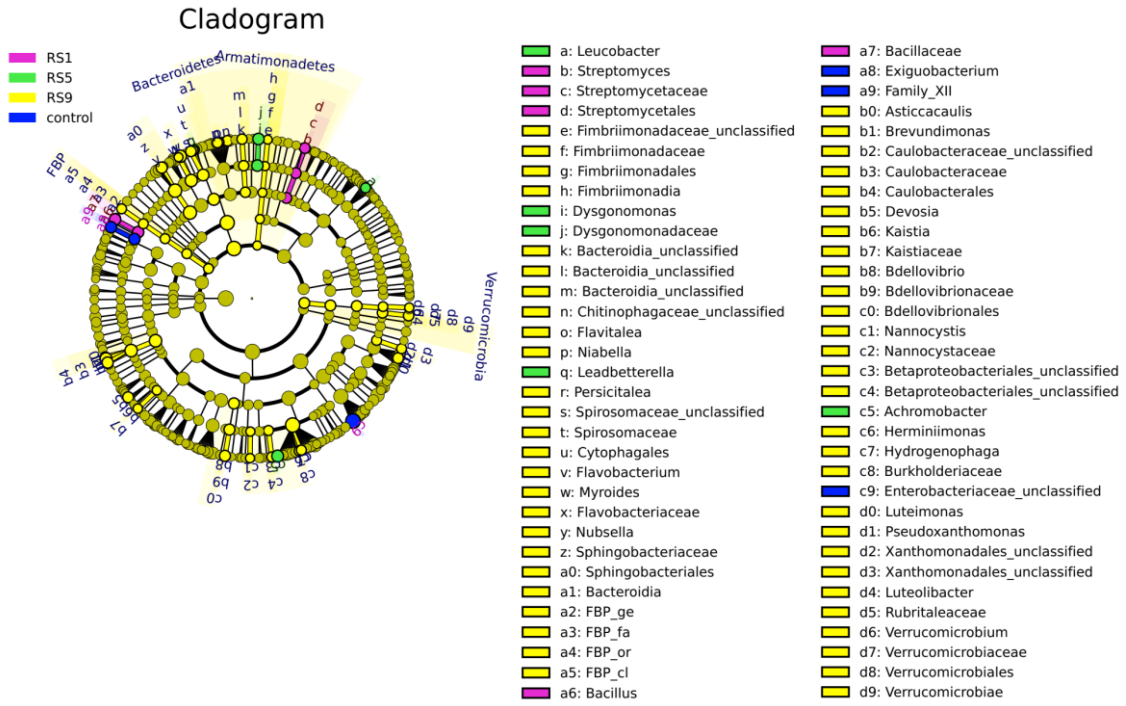

Figure 7. *Cladogram of linear discriminant analysis effect size (LEFSE) on ancestors and the characteristics of bacterial communities between the groups*

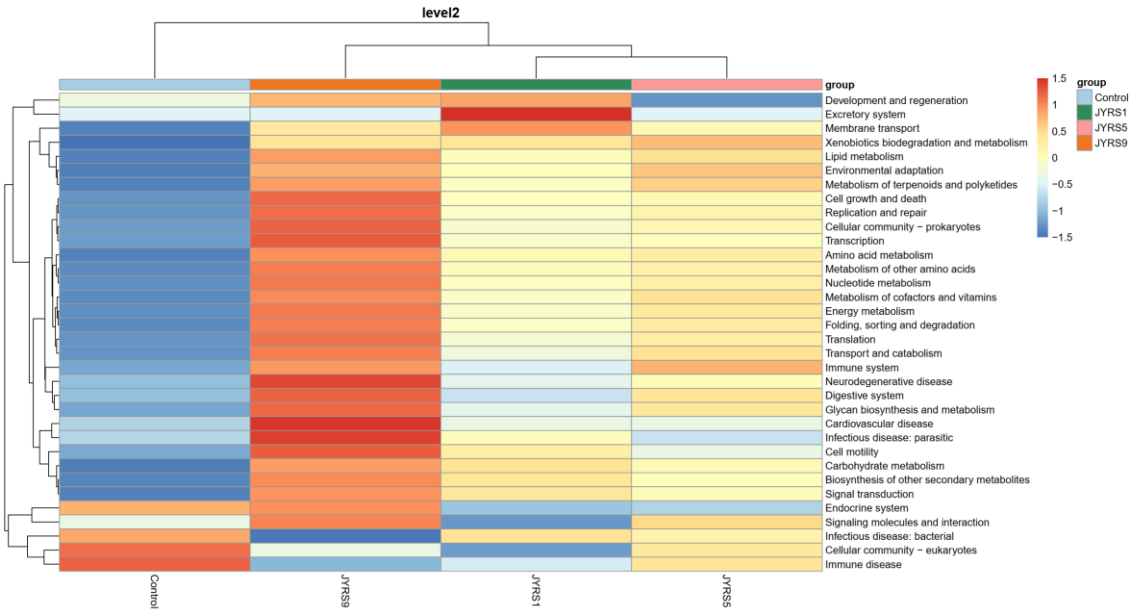

Figure 8. *Heatmap of Kyoto Encyclopedia of Genes and Genomes pathways*

Orthogonal taxonomic analysis is a statistical approach used to identify microbial taxa that are significantly associated with a particular environmental condition, such as the presence or absence of tobacco bacterial wilt (Faust and Raes, 2012; Mainali et al., 2017). This approach is important in the analysis of microbial community characteristics in soils of different levels of tobacco bacterial wilt indices, as it allows

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researchers to identify the specific microbial groups that are most strongly associated with the disease (Mainali et al., 2017). The significance of orthogonal taxonomic analysis in this context lies in its ability to provide a more detailed understanding of the relationships between microorganisms and tobacco bacterial wilt (Mainali et al., 2017). By identifying the specific microbial groups that are most strongly associated with the disease, researchers can gain insights into the mechanisms underlying disease development and identify potential targets for disease control. For example, if certain bacterial taxa are found to be more abundant in soils with high levels of tobacco bacterial wilt, this may suggest that these microorganisms are involved in the pathogenesis of the disease (Hu et al., 2021a; Tan et al., 2021). Conversely, if certain bacterial taxa are found to be less abundant in soils with high levels of tobacco bacterial wilt, this may suggest that these microorganisms have a protective effect against the disease. In addition to its significance in understanding the microbial ecology of soil, orthogonal taxonomic analysis can also have practical implications for agriculture. By identifying the specific microbial taxa associated with tobacco bacterial wilt, researchers can develop targeted interventions, such as the use of microbial inoculants or soil amendments, to promote the growth of beneficial microorganisms and suppress the growth of pathogenic microorganisms (Elnahal et al., 2022; French et al., 2021). The present study indicated that genus Enterobacteriaceae from the phylum Proteobacteria was more abundant in the control group (healthy soils), on the other hand genus *Ralstonia* of the phylum Proteobacteria was more abundant in the JYRS9 group (highly infected soils). Indeed, the disease main causative agent is a member of genus *Restonia*, and members of Enterobacteriaceae have been widely been applied in the control of tobacco wilt disease (Chang et al., 2022; Hu et al., 2021c).

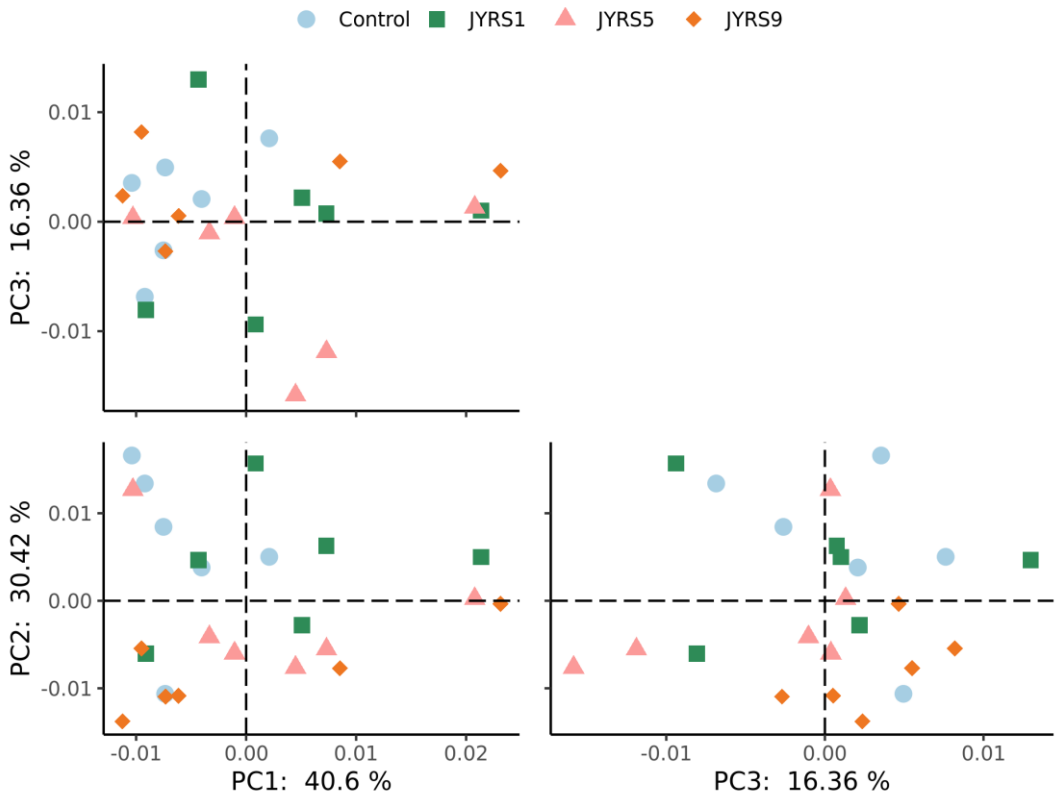

Figure 9. *PCA plots on abundance on KEGG pathways between different groups*

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Alpha diversity index is a measure of the diversity and richness of microbial communities within a single sample or habitat, such as a soil sample. It is typically calculated based on the number and abundance of different microbial taxa present in the sample (Roswell et al., 2021; Thukral, 2017; Willis, 2019). In the context of tobacco wilt disease, alpha diversity index can provide important insights into the relationship between microbial diversity and disease severity. Studies have shown that the alpha diversity index of microbial communities in soils is often negatively correlated with the severity of tobacco wilt disease (Cai et al., 2021; Kim et al., 2016). This means that as the disease severity increases, the diversity of microbial communities in the soil decreases. This negative correlation may be due to several factors, such as the selective pressure exerted by the disease on the microbial community or the disruption of microbial interactions caused by the disease (Cai et al., 2021; Hu et al., 2021c; Kim et al., 2016). However, the relationship between alpha diversity index and tobacco wilt disease is complex and may be influenced by other factors, such as soil type, crop management practices, and environmental conditions. For example, some studies have found that the negative correlation between alpha diversity index and tobacco wilt disease is stronger in soils with high clay content or low pH, while others have found no significant correlation between these factors (Han et al., 2017). Despite these complexities, the use of alpha diversity index in relation to tobacco wilt disease index is a useful tool for understanding the role of microbial diversity in the development and severity of the disease. By analyzing the alpha diversity index of microbial communities in soils with different levels of tobacco wilt disease index, researchers can gain insights into the factors that influence disease development and identify potential strategies for disease management, such as the promotion of microbial diversity through the use of cover crops or soil amendments. From the current study, most indices were higher in highly infected soils than the healthy soil, and the main dominant genera were the ones associated with promotion and survival and the disease environment. Moreover, the differences were not statistically significant.

Beta diversity index is a measure of the dissimilarity or variability in microbial community composition between different samples or habitats, such as soils with different levels of tobacco wilt disease index. It is often used to compare the overall composition of microbial communities and to identify the factors that contribute to differences in community composition (Tan et al., 2017). In the context of tobacco wilt disease, beta diversity index can provide important insights into the relationship between microbial community composition and disease severity (Shi et al., 2022a). Studies have shown that the beta diversity index of microbial communities in soils is often positively correlated with the severity of tobacco wilt disease. This means that as the disease severity increases, the dissimilarity in microbial community composition between different soil samples increases (Cha et al., 2016; Dong et al., 2013). This positive correlation between beta diversity index and tobacco wilt disease may be due to several factors. For example, the selective pressure exerted by the disease on the microbial community may lead to the proliferation of certain microbial groups that are better adapted to the disease environment. Alternatively, changes in soil properties caused by the disease may create niche opportunities for different microbial groups, leading to shifts in community composition (Berendsen et al., 2012b; Cai et al., 2021). However, as with alpha diversity index, the relationship between beta diversity index and tobacco wilt disease is complex and may be influenced by other factors. For example, soil type, crop management practices, and environmental conditions can all

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affect the composition of microbial communities and contribute to differences in beta diversity index between soil samples. Despite these complexities, the use of beta diversity index in relation to tobacco wilt disease index is a valuable tool for understanding the role of microbial community composition in the development and severity of the disease. By analyzing the beta diversity index of microbial communities in soils with different levels of tobacco wilt disease index, researchers can gain insights into the factors that contribute to disease development and identify potential strategies for disease management, such as the use of microbial inoculants or soil amendments to promote the growth of beneficial microorganisms (She et al., 2017a; Xiao et al., 2018; Yang et al., 2017). From the present study, it was noted that the heathy soil (control group) and the highly diseased soils (JYRS9 group) had very distinct variation based on PCA, PCoA and Venn analyses outputs.

Sample similarities and Clusters of Orthologous Groups (COG) function classification can provide important insights into the relationship between microbial community composition and function in soils with different levels of tobacco wilt disease index (Galperin et al., 2021). Sample similarity analysis can be used to visualize the similarities and differences in microbial community composition between different soil samples. In the context of tobacco wilt disease, sample similarity analysis can help identify patterns or clusters of microbial communities that are associated with different levels of disease severity (Qian et al., 2016). COG function classification is a bioinformatics approach that assigns functional categories to genes based on their homology to known genes. This approach can be used to identify the functional diversity of microbial communities in soils with different levels of tobacco wilt disease index. For example, COG function classification can identify genes involved in plant-microbe interactions, nutrient cycling, stress response, and other functions that may be important for disease development or management (Qian et al., 2016; Srivastava et al., 2021; Trivedi et al., 2020). By combining sample similarity analysis and COG function classification, researchers can gain insights into the relationship between microbial community composition and function in soils with different levels of tobacco wilt disease index. For example, if certain clusters of microbial communities are found to be associated with high levels of disease severity, COG function classification can be used to identify the functional characteristics of these communities and the genes that may be involved in disease development. Similarly, if certain functional categories are found to be more abundant in soils with low levels of disease severity, this may suggest that these genes or functions play a protective role against the disease (Liu et al., 2023; Trivedi et al., 2022; Vishwakarma et al., 2020). The present study indicated high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, and for molecular-level information on functional difference and molecular interactions in bacterial communities between the different groups.

KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways are a bioinformatics resource that provides a comprehensive understanding of the biological functions and pathways of genes and proteins. KEGG pathways can be used to identify the functional characteristics of microbial communities in soils with different levels of tobacco wilt disease index and to identify the pathways that may be involved in disease development or management (Janvier et al., 2007; Li et al., 2021b; Liu et al., 2016). In the context of tobacco wilt disease, KEGG pathway analysis can help identify the metabolic pathways and functional groups of microorganisms that are associated with different levels of disease severity (Shi et al., 2022b; Wieder et al., 2021). For example, KEGG pathway

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analysis can identify genes involved in plant-microbe interactions, nutrient cycling, stress response, and other functions that may be important for disease development or management. By analyzing the differences in KEGG pathways between soils with different levels of tobacco wilt disease index, researchers can gain insights into the factors that contribute to disease development and identify potential strategies for disease management. For example, if certain metabolic pathways or functional groups are found to be associated with high levels of disease severity, this may suggest that these pathways or groups of microorganisms are important for disease development and may be targeted for management. Similarly, if certain metabolic pathways or functional groups are found to be more abundant in soils with low levels of disease severity, this may suggest that these pathways or groups of microorganisms play a protective role against the disease (She et al., 2017b; Yang et al., 2017). The current study pointed out a much higher expressions of KEGG pathways under the highly diseased soils (JYRS9 groups) than the healthy soil (control group).

Conclusion

From the study, it was shown that the genus Enterobacteriaceae from the phylum Proteobacteria was more abundant under the control group (healthy soils), on the other hand genus *Ralstonia* of the phylum Proteobacteria was more abundant in the JYRS9 group (highly infected soils). The use of sample similarity analysis and COG function classification in relation to tobacco wilt disease index provided a comprehensive understanding of the microbial ecology of soil and the conditions that influence disease development and management. the use of KEGG pathway analysis in relation to tobacco wilt disease index provided a deeper understanding of the microbial ecology of soil and the factors that influence disease development and management.

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