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The effects of tobacco straw on the rhizosphere soil microbial community structure of bobacco (healthy tobacco and black shank tobacco)

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Abstract

As a vital agricultural management strategy, straw returning enhances soil fertility and promotes crop growth. Tobacco straw shows promise as an organic amendment for soil improvement; however, its impact on soil microbial diversity and community structure remains unclear. This investigation focused on the effects of tobacco straw on the rhizosphere soil microbial community structure of healthy tobacco and black shank tobacco, utilizing high-throughput sequencing technology. The results indicated that the abundance and diversity of microbial communities decreased following the addition of tobacco straw. In terms of community structure, the abundance of species such as Proteobacteria, Bacteroidetes, Flavobacterium, and Pseudomonas increased after the addition of tobacco straw, potentially inhibiting the onset of black shank disease. Conversely, the abundances of Actinobacteria, Ascomycota, and Fusarium decreased following straw addition. Random forest model analysis revealed that the most representative genera in the healthy tobacco rhizosphere soil were Acidibacter and Luteimonas, while Herbinix served as the corresponding biomarker in the black shank tobacco rhizosphere soil.

Keywords: Straw returning; Microbial diversity; Black shank

Introduction

Soil microbial diversity is a key element in maintaining ecosystem functions, playing an irreplaceable role in ensuring ecosystem stability and enhancing resistance to environmental stressors (Xiang *et al.,* 2022; Scherzinger *et al.,* 2024). Furthermore, the soil microbial community structure is sensitive to environmental changes and responds rapidly to alterations in soil properties and the introduction of exogenous additives (Jia *et al.,* 2016; Wang *et al.,* 2016). Consequently, soil microbial community diversity is frequently employed as a key indicator for assessing soil quality (Tang *et al.,* 2022).

In China, the annual production of crop straw ranges from 700 to 800 million tons, which is rich in organic matter, essential nutrients, and trace elements necessary for plant growth (Akhtar *et al.,* 2019; Su *et al.,* 2020). Improper disposal of this straw not only harms the ecological environment but also represents a waste of valuable resources. In recent years, straw returning has emerged as a primary method for resource utilization, effectively addressing the waste of agricultural resources and mitigating issues related to soil degradation and agricultural contamination caused by chemical fertilizers over-application (Tao *et al.,* 2024). This practice has garnered significant attention due to its potential benefits.

Straw returning can directly or indirectly influence the soil microbial community by enhancing soil structure and providing a carbon source. It has been reported that straw returning significantly increases the diversity of soil bacteria and fungi while also affecting the relative abundance of soil nitrogen cycle microorganisms and plant pathogenic microorganisms (Tang *et al.,* 2023). Furthermore, straw returning enhances the biomass of soil microorganisms, improves soil organic carbon content and contributes to soil structure and fertility (Sheng *et al.,* 2016). From a long-term perspective, repeated straw returning alters the soil bacterial community structure (Chen *et al.,* 2017); and further enhances soil fertility by promoting the decomposition of organic matter

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such as cellulose and lignin by soil microorganisms, which releases nutrients (Huang *et al.,* 2024).

Our previous studies demonstrated that the mineralization characteristics of soil organic carbon and the humus content significantly changed following the addition of tobacco straw; the total organic carbon (TOC) and humus components in soil with straw were significantly elevated, indicating that the addition of tobacco straw enhances both the organic carbon content and the degree of humification in the soil (Lijuan *et al.,* 2019). This raises the following questions: Has the addition of straw reshaped the structure of soil microbial communities? What ecological role do soil microorganisms play in the decomposition process of tobacco straw? Furthermore, tobacco black shank is a typical soil-borne disease caused by *Phytophthora nicotianae*, which has attracted widespread attention (Du *et al.,* 2022). Therefore, this study aimed to evaluate how the addition of tobacco straw alters the diversity and structure of bacterial and fungal communities in healthy and black shank-affected tobacco soils using high-throughput sequencing.

Materials and Methods

The experimental soil was collected after the harvesting of tobacco leaves from the flue-cured tobacco-rice crop rotation experiment field at the scientific research base of Hunan Agricultural University. The rhizosphere soil from five healthy flue-cured tobacco plants and five infected flue-cured tobacco plants was thoroughly mixed and designated as C1 and C2, respectively. The basic physical and chemical properties of the tested soil are presented in Table S1. The tobacco straw was sourced from the teaching and research base of Hunan Agricultural University. Fresh tobacco straw was washed, air-dried, segmented into 2-3 cm pieces, dried at 60°C to a constant weight, and then crushed and screened through a 60-mesh sieve for use. The basic properties of the test crop straw are detailed in Table S2.

The experiment was conducted in the greenhouse of the Tobacco Research Institute of Hunan Agricultural University, maintaining a constant temperature of 26°C using the nylon net bag method (Tian *et al.,* 2019). A sample of 20 g of tobacco straw was weighed and sealed into a 200-mesh nylon net bag, which was then inserted vertically into a plastic basin containing test soil. As shown in Table 1, each treatment was replicated three times. In this experiment, four treatments were established: C1 (healthy soil, no straw added), C2 (black shank disease soil, no straw added), T1 (Tobacco straw treatment 1, consisting of 2% tobacco straw mixed with healthy soil), and T2 (Tobacco straw treatment 2, consisting of 2% tobacco straw mixed with black shank disease soil). Samples were collected on days 0, 7, and 30

Table 1: Experimental design and sample identifiers of tobacco straw treatments in healthy and black shank disease soils across time points

Treatment Group	Treatment Description	0 d Samples	7 d Samples	30 d Samples
C1	Healthy Soil	C1	C1W	C1M
C2	Black Shank Disease Soil	C2	C2W	C2M
T1	Healthy Soil + 2% Straw	-	T1W	T1M
T2	Black Shank Disease Soil + 2% Straw	-	T2W	T2M

Table S1: The basic physical and chemical properties of soil samples

Soil Types	pH	OM ($g\ kg^{-1}$)	N ($g\ kg^{-1}$)	AN ($mgkg^{-1}$)	P (gkg^{-1})	AP ($mgkg^{-1}$)	K ($g\ kg^{-1}$)	AK ($mgkg^{-1}$)
C1	5.71	24.73	1.42	125.95	0.6	30.21	6.31	147.12
C2	5.47	28.57	1.64	122.35	0.78	37.13	7.03	169.34

OM: Soil Organic Matter, AN: Available Nitrogen ,AP: Available Phosphorus,AK: Available Potassium

Table S2: Basic properties of crop straw

OC	N	P	K	C/N
$435.51g{\cdot}kg^{-1}$	$10.27g{\cdot}kg^{-1}$	$1.26g{\cdot}kg^{-1}$	$15.72g{\cdot}kg^{-1}$	42.41

OC: Organic Carbon,C/N: Carbon - Nitrogen Ratio

(Ning *et al.,* 2022). As indicated in Table 1, the samples collected on day 0 were labeled as C1 and C2. After 7 days, the samples without straw were labeled as C1W and C2W, while those with added straw were labeled as T1W and T2W. At the 30 day-mark, the samples without straw were labeled as C1M and C2M, while those with added straw were labeled as T1M and T2M.

Detection of microbial diversity and bioinformatics analysis

The soil samples were immediately stored at -80°C after collection, and sent to Shanghai Euoyi Biotechnology for testing. Genomic DNA was extracted using a kit, and the concentration was evaluated through gel electrophoresis and NanoDrop2000. The V3-V4 region of the bacterial 16S rRNA was amplified using specific primers 343F (5'-TACGGRAGGCAGCAG-3') and 798R (5'-AGGGTATCTAATCCT-3'), while the ITS region of fungi was amplified using primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3'). Paired-end sequencing was performed using the Illumina MiSeq sequencing platform.

The original sequences were processed sequentially using Trimmomatic, Flash, QIIME's split_libraries, and UCHIME to complete assembly, filter out low-quality sequences and chimeras, and obtain effective tags. V search was employed to cluster these into Operational Taxonomic Units (OTUs) at 97% similarity, with the sequence exhibiting the highest abundance in each OTU being selected as the representative sequence for database comparison and OTU annotation. Based on the OTU classification information, both α diversity and β diversity were calculated using QIIME.

Results and Discussion

Alpha diversity analysis of soil microbial community

The rarefaction curves exhibited asymptotic stabilization in both bacterial (Shannon index) and fungal communities across treated samples (Figure S1), confirming that the sequencing depth was sufficient to capture the majority of microbial diversity in the processed specimens.

Table S3: The alpha diversity index of the different treatment groups of bacteria

Samples	Chao1	Shannon	Observedspecies	Simpson
C1	7021.26±193.40 ab	10.30±0.17 ab	5182.93±210.25 a	1
C2	7164.92±155.66 a	10.52±0.03 a	5470.6±41.56 a	1
C1W	6991.37±179.33 ab	10.47±0.04 a	5212.93±80.52 a	1
C2W	6831.13±143.06 ab	10.53±0.05 a	5277.33±29.29 a	1
T1W	6031.27±183.42 c	9.55±0.27 c	4247.63±161.07 bc	0.99
T2W	6113.03±226.72 c	9.63±0.34 c	4422.53±170.17 c	0.99
C1M	6638.79±303.88 b	10.26±0.06 b	4915.03±153.51 a	1
C2M	6920.76±106.88 ab	10.47±0.06 a	5212.83±84.32 a	1
T1M	6231.84±290.92 c	10.12±0.16 c	4526.23±228.65 a	1
T2M	6101.17±246.53 c	9.94±0.23 c	4399.50±259.96 ab	1

Table S4: The alpha diversity index for the different treatment groups of fungi

Samples	Chao1	Shannon	Observedspecies	Simpson
C1	1140.6±216.88 a	4.68±2.65 bcd	790.47±236.36 b	0.72±0.39
C2	1209.92±103.54 a	5.81±1.22 abc	911.43±122.88 ab	0.89±0.13
C1W	1287.54±15.09 a	6.75±0.29 a	984.8±22.25 ab	0.97±0.01
C2W	1258.97±61.15 a	6.56±0.41 ab	993.17±60.63 ab	0.96±0.02
T1W	645.95±92.77 b	3.21±0.74 d	352.83±66.09 c	0.74±0.14
T2W	654.39±96.69 b	3.14±0.36 d	352.93±83.56 c	0.73±0.04
C1M	1173.32±53.43 a	5.56±0.7 abc	872.97±85.42 ab	0.89±0.07
C2M	1318.58±111.66 a	6.88±0.11 a	1044.6±81.16 a	0.97±0.01
T1M	804.94±151.93 b	3.88±0.83 cd	492.87±118.09 c	0.8±0.07
T2M	612.72±88.64 b	3.09±0.77 d	349.1±74.76 c	0.77±0.08

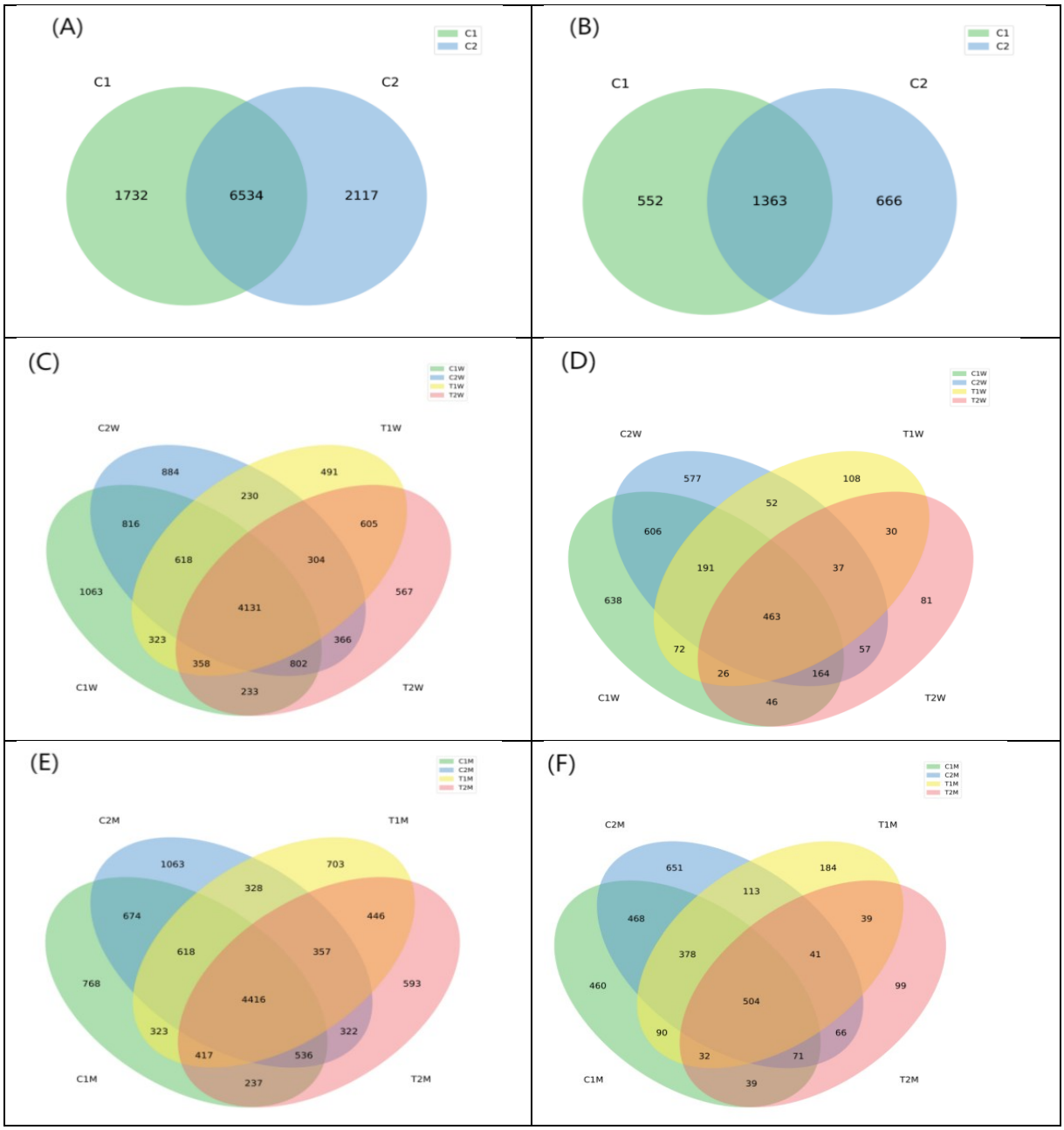


Figure 1: Venn plot depicting the distribution of bacterial and fungal OTUs in soil subjected to various treatments

Note: Figure (A) presents the Venn diagram showing the distribution of bacterial OTUs between healthy soil (C1) and diseased soil (C2) without straw addition. Figure (B) displays the Venn diagram for fungal OTUs distribution under the same conditions. Figure (C) represents the Venn diagram of bacterial OTUs distribution between healthy soil (C1) and diseased soil (C2) on day 7 following straw addition. Figure (D) illustrates the Venn diagram for fungal OTUs distribution on the same day. Figures (E) and (F) depict the Venn diagrams for bacterial and fungal OTUs distribution, respectively, between healthy soil (C1) and diseased soil (C2) on day 30 after straw addition.

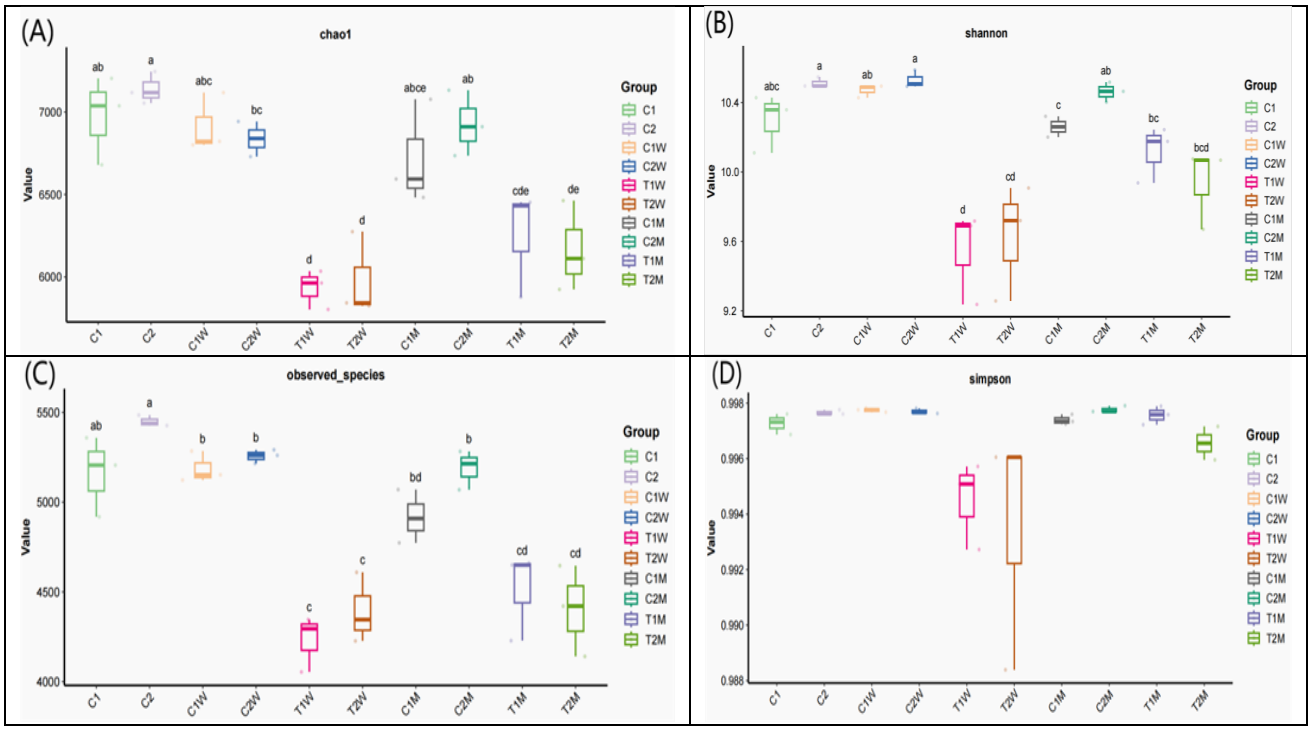


Figure 2: Comparison of the α diversity index of bacteria between groups

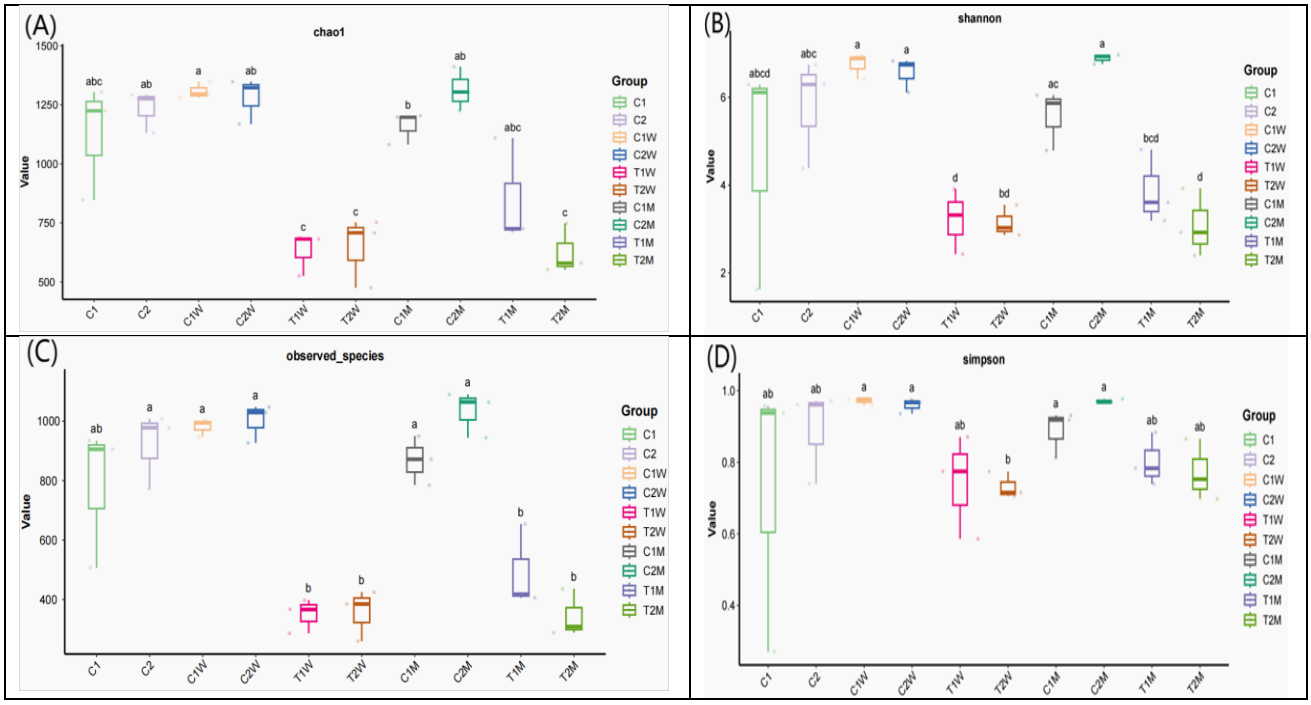


Figure 3: Comparison of the α diversity index of fungi between groups

As illustrated in figure 1, the total number of bacterial and fungal OTUs, as well as the number of unique OTUs in C2 soil, were both greater than those in C1, indicating that the sick soil itself possesses higher species richness. After the addition of straw for 7 days, the number of bacterial and fungal OTUs decreased in both types of soil. Notably, the number of bacterial OTUs in black shank disease soil (7366) remained higher than that in healthy soil (7060), while the number of fungal OTUs (904) was lower than that in healthy soil (979). After 30 days, the OTU number in black shank disease soil was still lower than that observed at 7 days, indicating that the microbial community in healthy soil exhibited a significantly better recovery ability compared to that in black shank disease soil.

According to figure 2, Table S3 and Table S4, in the initial state, the diversity indices of both bacteria and fungi in C2 were higher than those in C1, with the exception of the Simpson index. Following the addition of tobacco straw, the diversity indices of both bacteria and fungi initially decreased before showing an increase. In black shank disease soil, only the Shannon index of bacteria exhibited a similar pattern of decrease followed by an increase, whereas the diversity indices of fungi recovered slowly, demonstrating a decreasing trend. From a stage-specific perspective, at 7 days of treatment, the bacterial and fungal diversity indices in black shank disease soil (excluding the Shannon index for fungi) were higher than those in healthy soil. However, after 30 days, this trend reversed, with all diversity indices in healthy soil surpassing those in infected soil.

According to Zhang's research, bacterial diversity decreased after straw addition for 7 days, while it increased after 118 days of straw addition (Zhao *et al.,* 2021). This finding aligns with the results of this study, which also shows a similar trend: after straw addition, bacterial richness and diversity initially decreased but subsequently increased. This phenomenon may be due to the decomposition of straw, which consumes nutrients such as nitrogen and phosphorus in the soil, leading to nutrient depletion and reduced soil fertility. Consequently, this decline in soil fertility may negatively affect plant growth and reduce plant diversity. Li et al. found that incorporating organic matter from straw can enhance bacterial growth in soil, potentially increasing the abundance of specific bacterial species (Li *et al.,* 2021).

Beta diversity analysis

Bacterial Principal Coordinate Analysis (PCoA) revealed that the first principal component (PC1) accounted for 36.62% of the variance in the original variables, while the second principal component (PC2) accounted for 16.49%, together explaining 53.11% of the total variance (Figure 4). Similarly, fungal PCoA analysis indicated that PC1 explained 39.15% of the variance, and PC2 explained 14.06%, cumulatively accounting for 53.21% of the total variance. The bacterial and fungal treatments were distinctly separated along PC1 and PC2. Notably, the treatments without straw clustered closely together, whereas the treatments with straw formed separate clusters, highlighting a significant difference between samples with and without tobacco straw addition. Furthermore, over time, the 7-

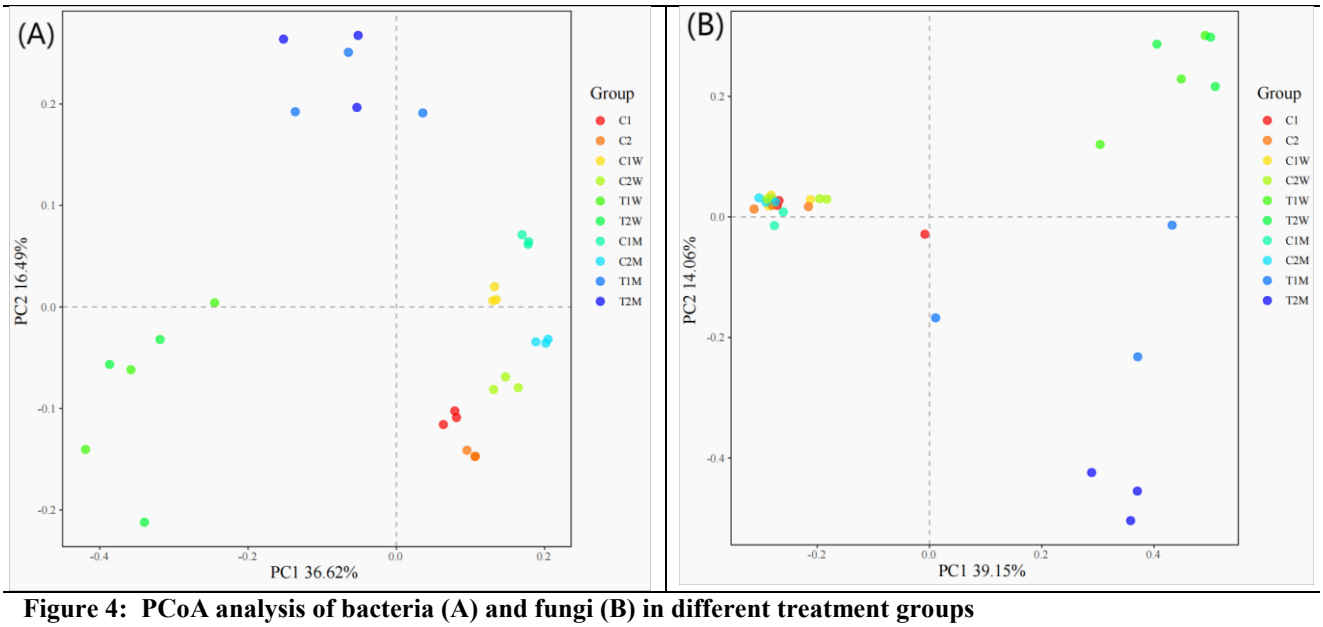


Figure 4: PCoA analysis of bacteria (A) and fungi (B) in different treatment groups

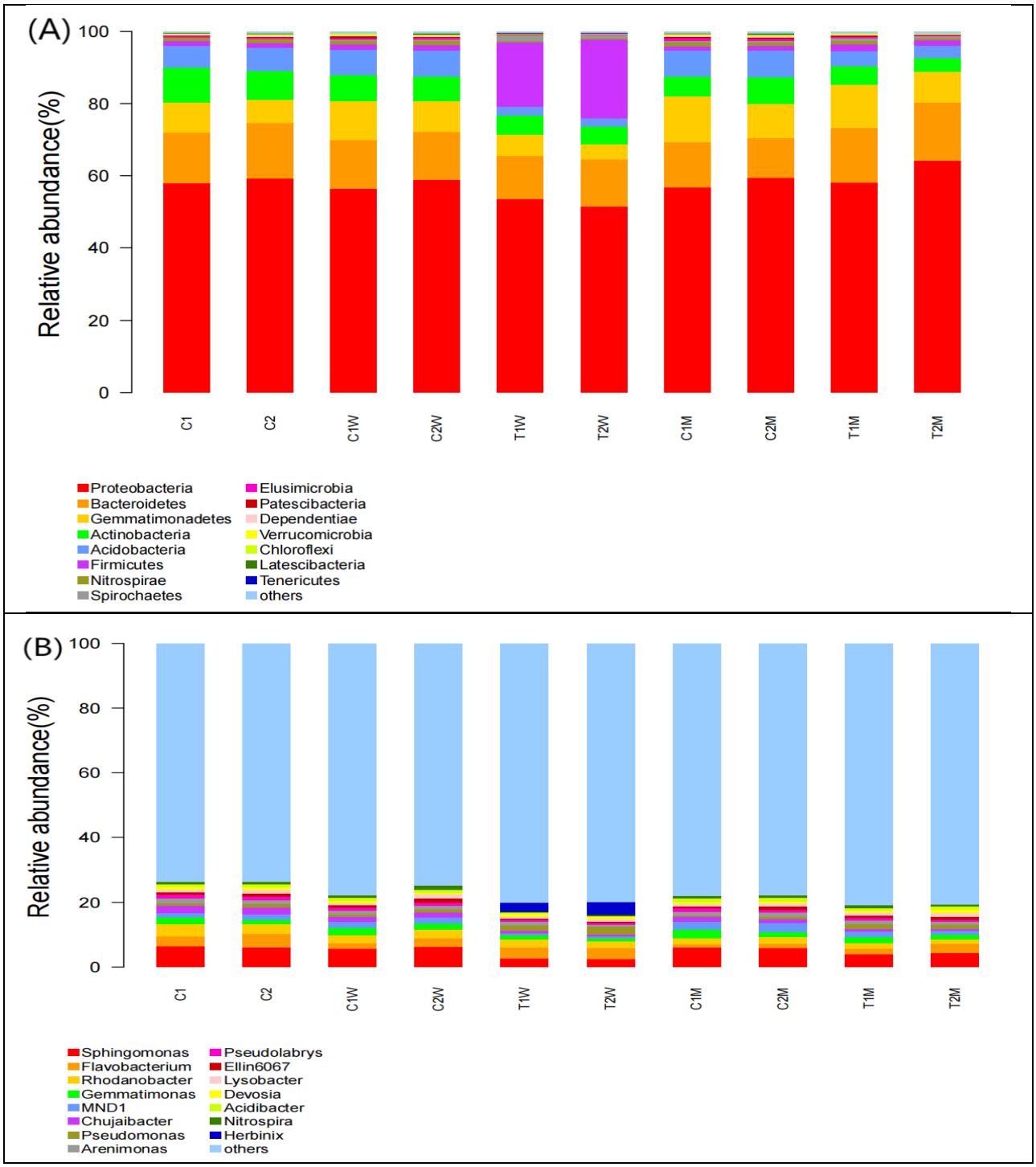


Figure 5: Composition and relative abundance of bacterial communities at the phylum (A) and generic (B) level

day and 30-day treatment groups exhibited significant differences in bacterial and fungal community structure.

Analysis of microbial community structure

Analysis of bacterial community structure

At the phylum level (Figure 5A), Proteobacteria exhibited the highest relative abundance, ranging from 51.54% to 64.22%, suggesting its significant role within the bacterial community. Following straw addition, the relative abundance of Proteobacteria exhibited an increasing trend over time, likely due to their ability to utilize organic matter in the soil as a carbon source and energy (Yang AnNa *et al.,* 2019;Shi *et al.,* 2020). Additionally, Bacteroidetes, the next most abundant phylum, accounted for 10.99% to 15.95% and also demonstrated an increasing trend with straw addition, which aligns well with previous studies (Liu *et al.,* 2023). It has been reported that Bacteroidetes can decompose various complex organic substances in straw, including cellulose, hemicellulose, and pectin (López-Mondéjar *et al.,* 2016). In each treatment, the change in Firmicutes was pronounced, with its performance significantly increasing from 1.43% to 21.57% within 7 days following straw addition. Firmicutes, a group of microorganisms known for their efficiency in decomposing soil organic matter, can degrade lignocellulose, chitin, cellulose, lignin, and other recalcitrant carbon sources (Chen *et al.,* 2016;Yang AnNa *et al*. 2019). Conversely, the relative abundance of Actinobacteria decreased with straw addition, with its abundance in healthy soil being higher than that in black shank diseased soil. This decline may be attributed to the rapid growth and high relative abundance of Proteobacteria and Bacteroidetes, which may have suppressed Actinobacteria, resulting in its relatively low representation in the samples (Ning *et al*. 2022;Tilei *et al.,* 2024).

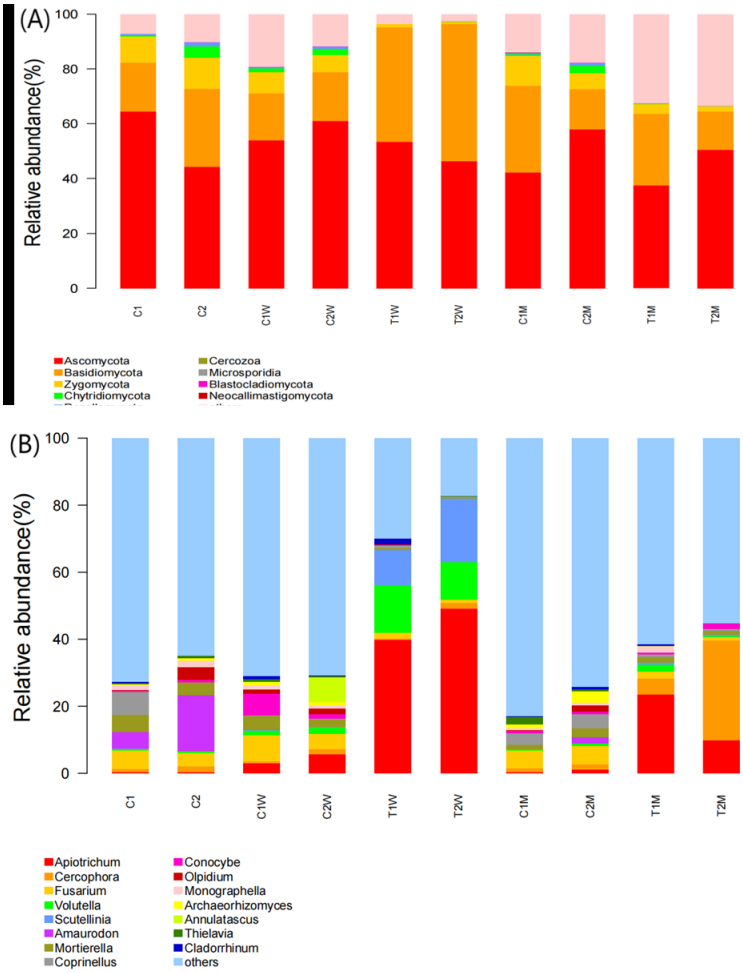


Figure 6: Composition and relative abundance of fungal communities at the phylum (A) and (B)level

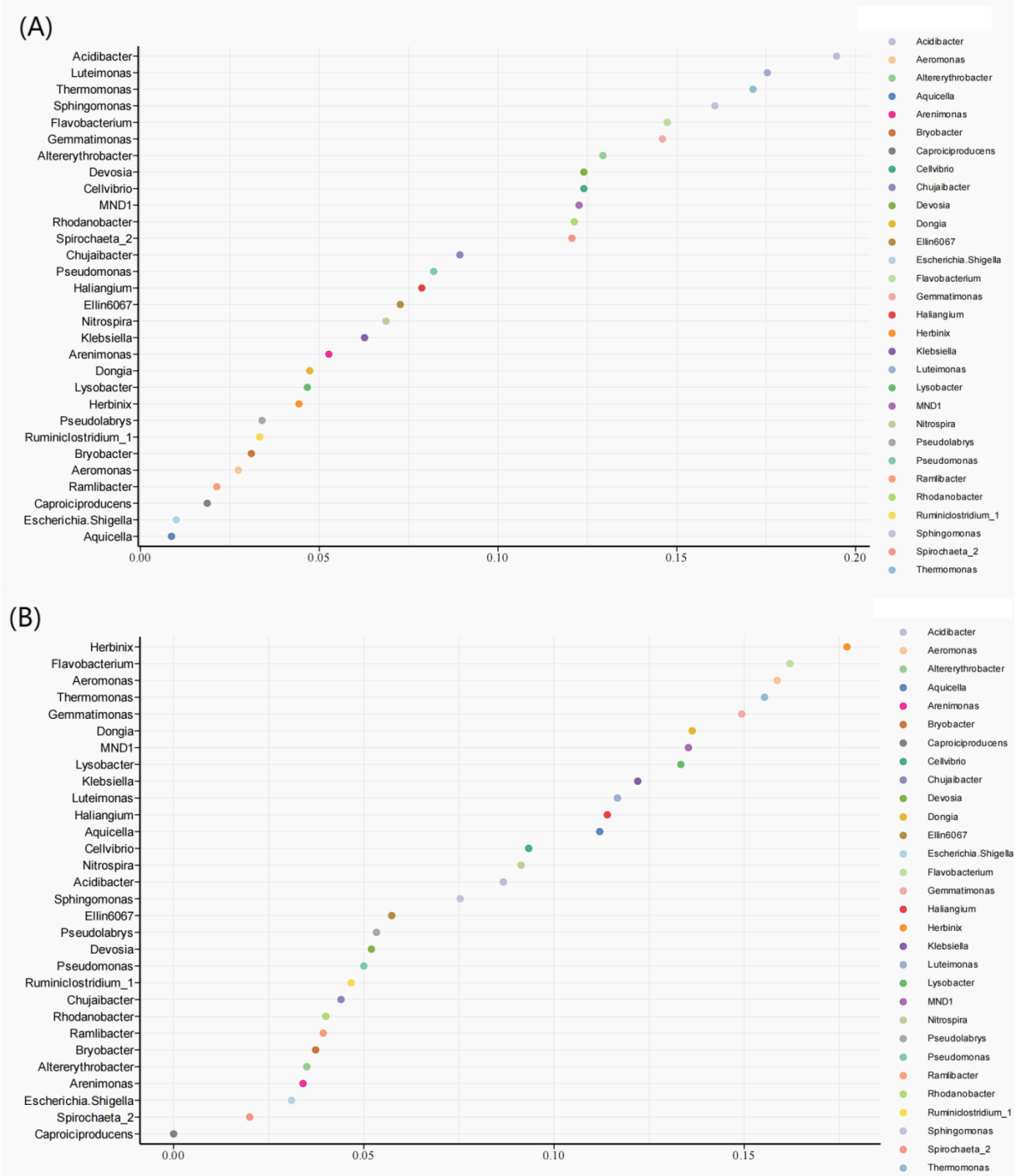


Figure 7: Random Forest model of characteristic bacterial genera in straw-free healthy soil and black leg-infected soil

Note: A represents the random forest model of bacteria in healthy soil without straw; B represents the random forest model of bacteria in soil with black leg disease without straw.

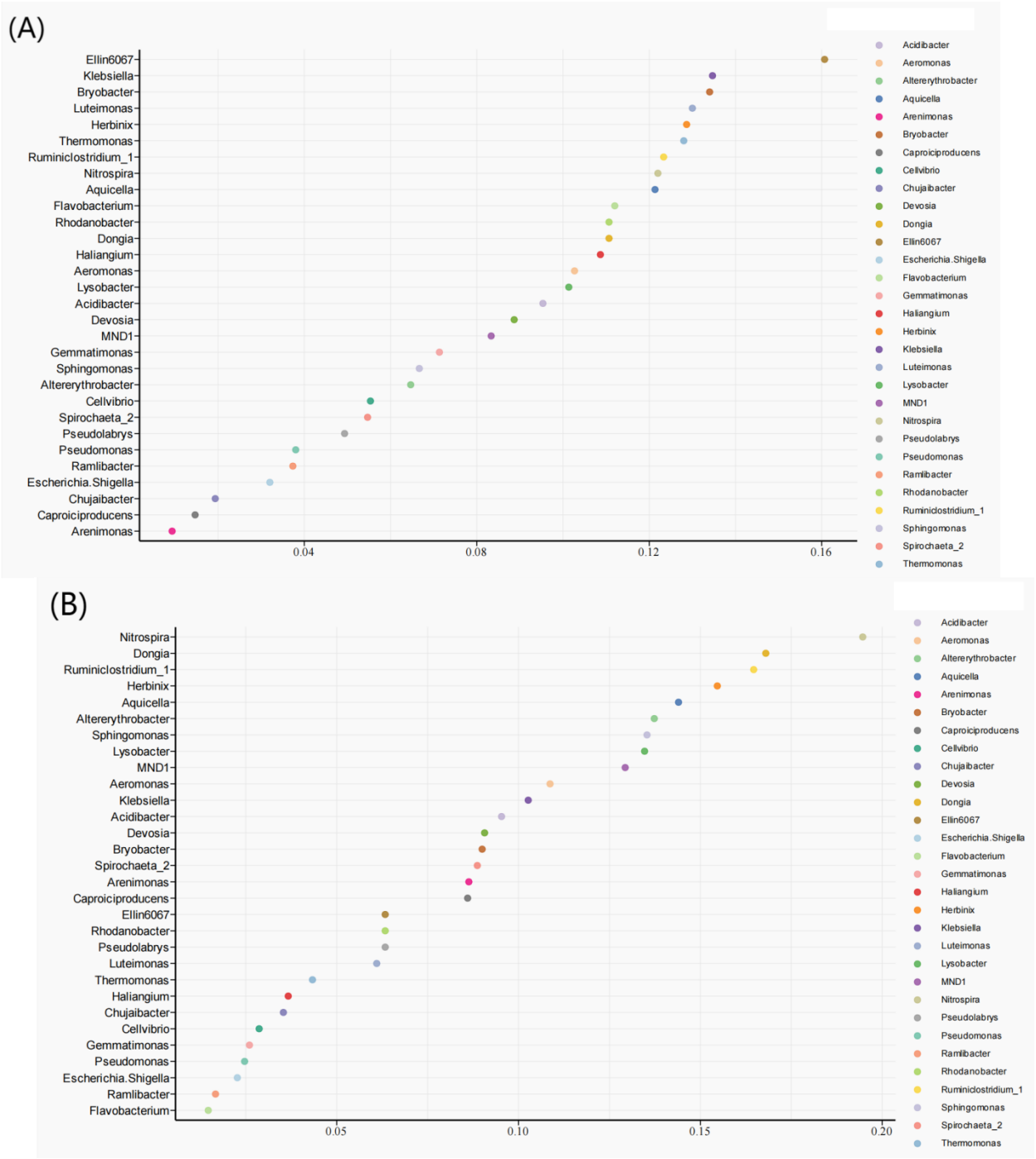


Figure 8: Random Forest model of characteristic bacterial genera in straw-added healthy soil and black leg-infected soil

Note: A represents the random forest model of bacteria in healthy soil with straw addition; B represents the random forest model of bacteria in soil with black leg disease with straw addition.

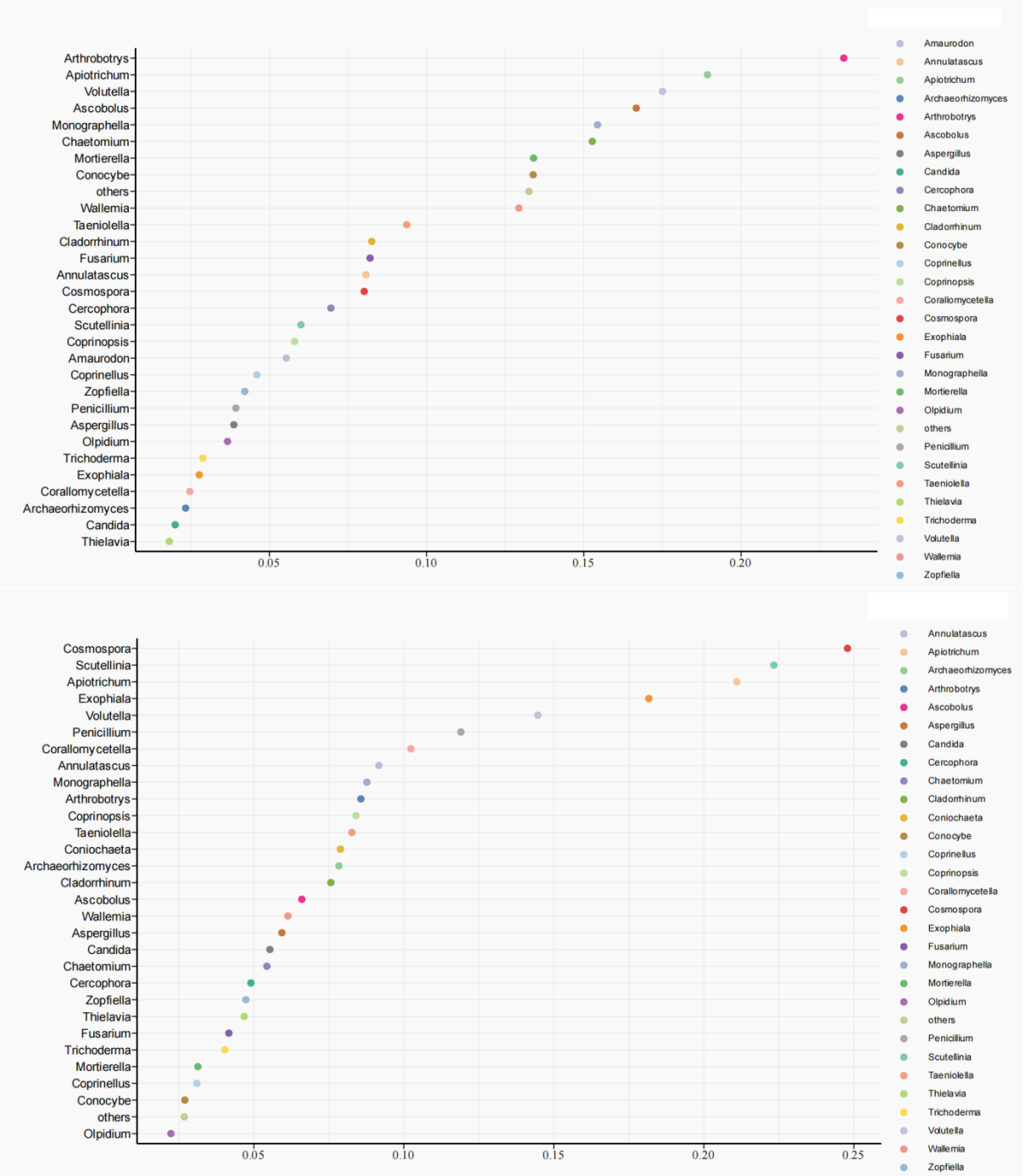


Figure 9: Random Forest model of characteristic fungal genera in straw-free healthy soil and black leg-infected soil

Note: A represents the random forest model of fungi in healthy soil without straw; B represents the random forest model of fungi in soil with black leg disease without straw.

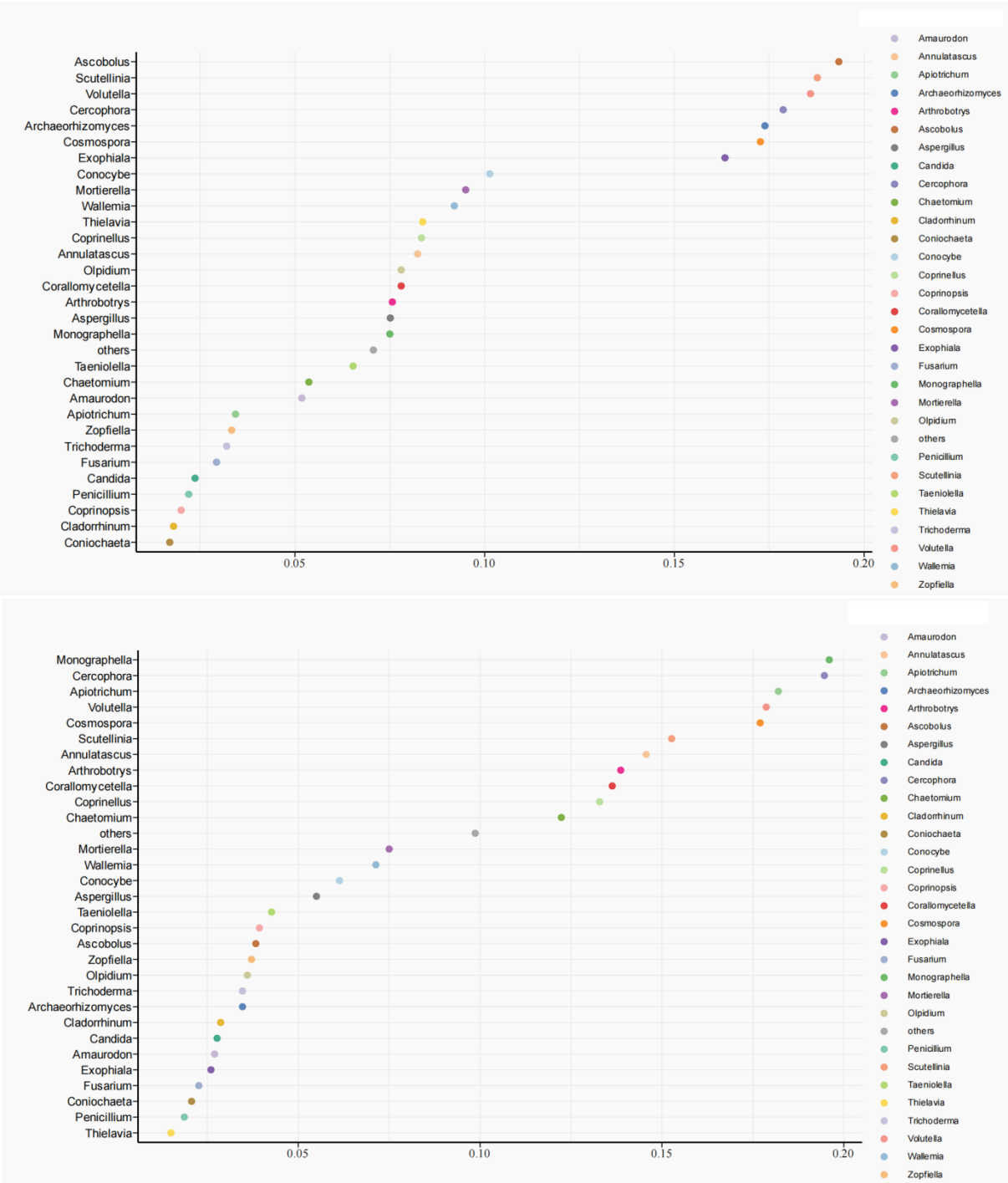


Figure 10: Random Forest model of characteristic fungal genera in straw-added healthy soil and black leg-infected soil

Note: A represents the random forest model of fungi in healthy soil with straw addition; B represents the random forest model of fungi in soil with black leg disease with straw addition.

At the genus level (Figure 5B), the increase in abundance of *Sphingomonas* following straw addition, can be attributed to the rich organic carbon source provided by the straw. Furthermore, the relative abundance of *Sphingomonas* in black shank diseased soil was higher than that in healthy soil. Previous studies have indicated that *Sphingomonas* represents one of the most effective microorganisms for degrading toxic substances in soil.This genus not only facilitates nutrient absorption but also effectively mitigates the detrimental effects of various pathogens (Asaf *et al.,* 2020). Thus, its abundance in black shank diseased soil is expected to positively impact soil conditions. Over time, the relative abundances of *Pseudomonas* increased after straw addition. 30 days after straw addition, the relative abundance of *Pseudomonas* in healthy soil was higher than that in black shank diseased soil. *Pseudomonas* constitutes a primary group of environmental microorganisms that metabolize nicotine and can synthesize various plant hormones through nitrogen fixation, thereby promoting the absorption of water and mineral elements by tobacco plants (Ma *et al.,* 2012). Moreover, *Pseudomonas* exhibited a strong inhibitory effect on tobacco black shank, playing a vital role in suppressing *Phytophthora*, the pathogen responsible for tobacco black shank. The addition of straw resulted in a significant increase in the amount of *Herbinix*, and its relative abundance in black shank disease soil was higher than that in healthy soil. This phenomenon is attributed to the straw's provision of a rich carbon source and cellulose, which are essential for *Herbinix*'s role in the degradation of cellulose and hemicellulose (Koeck *et al.,* 2015). Therefore, it could be effectively demonstrated that incorporating tobacco straw not only enhances the inhibitory effects of tobacco fields on soil-borne diseases but also improves soil structure and increases soil organic matter.

Analysis of fungal community composition

At the phylum classification level (Figure 6A), Ascomycota and Basidiomycota were the dominant phyla. After straw was added, the relative abundance of Ascomycota gradually decreased. This may be because at the initial stage of returning straw to the field, a large amount of recalcitrant lignin and cellulose in it will inhibit the growth and reproduction of the Ascomycota phylum. The relative abundance of Basidiomycota increased by the 7th day after straw addition. In the first seven days following straw addition, the relative abundance of Zygomycota was low, however, by the thirtieth day, its relative abundance not only gradually increased, surpassing the level observed on the seventh day, but also constituted a higher proportion in healthy soil. Ascomycota and Basidiomycota, both saprophytic fungi, play a crucial role in the decomposition of complex compounds, effectively decomposing both plant and straw residues. Among them, Ascomycota are prevalent fungal decomposers in soil and dominate in most soil types (Kõljalg *et al.,* 2013), exhibiting strong capabilities to decompose cellulose and recalcitrant organic matter alongside Zygomycota (Wu *et al.,* 2020).

At the genus level, straw addition significantly influenced the composition of fungal community structures (Figure 6B). Seven days post-straw addition, the relative abundances of *Apiotrichum*,*Scutellinia* and *Volutella* increased in both soil types. *Apiotrichum* is notable for containing numerous carbohydrate-degrading enzymes and is involved in the degradation of various complex substrates (Aliyu *et al.,* 2020). *Scutellinia*, as a saprophytic fungus, primarily thrives in moist, decayed wood or soil environments, contributing to soil nutrient transformation and organic matter mineralization through the decomposition of organic substances (Charlotte 2021). *Volutella* plays a vital role in forest ecosystems by decomposing plant residues and facilitating nutrient cycling (Babu *et al.,* 2015). Furthermore, straw addition significantly affected other genera; thirty days post-addition, the abundance of *Cercophora* increased markedly, exhibiting relatively high abundance in diseased soil. In contrast, the relative abundance of *Fusarium* displayed a decreasing trend after straw addition. Given that *Fusarium* encompasses various pathogens capable of infecting crops such as soybeans and wheat, this change suggests that straw application may mitigate the presence of potential plant pathogens by inhibiting the growth and reproduction of Fusarium, thereby positively impacting soil health (Yang AnNa *et al*. 2019).

Correlation analysis of soil bacteria and fungi

Random Forest analysis indicated that in the straw-free healthy soil treatment group (comprising C1W and C1M treatments), the most representative genera of bacteria were *Acidibacter* and *Luteimonas* (Figure 7A). Conversely, the most representative genus in the straw-free diseased soil treatment group (encompassing C2W and C2M treatments) was *Herbinix* (Figure 7B). In the healthy soil treatment group with tobacco straw addition (including T1W and T1M treatments), the most representative genus was *Ellin6067* (Figure 8A), while *Nitrospira* was the most representative genus in the diseased soil treatment group with tobacco straw addition (including T2W and T2M treatments) (Figure 8B).

For fungi, the most representative genera in the tobacco straw-free healthy soil treatment group (including C1W and C1M treatments) were *Arthrobotrys*, *Apiotrichum*, and

Volutella (Figure 9A). In the tobacco straw-free diseased soil treatment group (including C2W and C2M treatments), the most representative genera were *Cosmospora*, *Scutelinia*, *Apiotrichum*, and *Exophiala* (Figure 9B). The healthy soil treatment group with tobacco straw addition (including T1W and T1M treatments) featured *Ascobolus*, *Scutelinia*, and *Volutella* as the most representative genera (Figure 10A), while the diseased soil treatment group with tobacco straw addition (including T2W and T2M treatments) included *Monographella*, *Cercophora*, *Apiotrichum*, *Volutella*, and *Cosmospora* as the most representative genera (Figure 10B).

Ellin6067 is reported to be involved in processes such as the soil carbon cycle, organic matter decomposition, and pollutant degradation (Tang *et al*. 2023). *Nitrospira* plays a crucial role in the soil nitrogen cycle, with its abundance closely linked to the concentrations of nitrate nitrogen and ammonium nitrogen in the soil (Yousuf *et al.,* 2025). *Ascobolus*, *Scutelinia*, and *Cercophora* are associated with organic matter decomposition (van Brummelen 1967;Doveri 2016), while *Monographella* is known to infect various crops, including barley, leading to disease (Du et al. 2022)

Conclusion

This study elucidates the differences in microbial community structures of bobacco rhizosphere soil, comparing healthy tobacco and black shank tobacco, both with and without the addition of tobacco straw. Following the incorporation of tobacco straw, microbial diversity was observed to decrease in both soil types. Among all samples, the relative abundance of Proteobacteria was found to be the highest. Conversely, the relative abundances of Actinobacteria, Ascomycota and Fusarium exhibited a declining trend. After the addition of tobacco straw, the signature bacterial genera identified in healthy and diseased soils were *Ellin6067* and *Nitrospira*, respectively. The signature fungal genera in healthy soil included *Ascobolus*, *Scutelinia*, and *Volutell*a, while those in diseased soil comprised *Monographella*, *Cercophora*, *Apiotrichum*, *Volutella*, and *Cosmospora*. In conclusion, the judicious return of tobacco straw to fields has the potential to enhance soil health by regulating microbial community structures, thereby creating a favorable microecological environment conducive to crop growth. This study provides a theoretical foundation for improving the quality of diseased soils and promoting sustainable agricultural production through straw return technology.

Author Contributions

Conceptualization, Yougen Zhao; Data curation, Xiaodong Gu; Formal analysis, Jinxiu Zhang; Investigation, Yi Zhang; Methodology, Donghua Hu; Resources, Lijuan Chen; Software, Guirong Su; Writing—original draft, Lijuan Chen; Writing—review and editing, Jinxiu Zhang. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

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