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RESEARCH ARTICLE

Microbial community and chemical composition of cigar tobacco (*Nicotiana tabacum* L.) leaves altered by tobacco wildfire disease

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Abstract

Tobacco wildfire disease caused by *Pseudomonas syringae* pv. *tabaci* is one of the most destructive foliar bacterial diseases occurring worldwide. However, the effect of wildfire disease on cigar tobacco leaves has not been clarified in detail. In this study, the differences in microbiota and chemical factors between wildfire disease-infected leaves and healthy leaves were characterized using high-throughput Illumina sequencing and a continuous-flow analytical system, respectively. The results demonstrated significant alterations in the structure of the phyllosphere microbial community in response to wildfire disease, and the infection of *P. syringae* pv. *tabaci* led to a decrease in bacterial richness and diversity. Furthermore, the content of nicotine, protein, total nitrogen, and Cl^- in diseased leaves significantly increased by 47.86%, 17.46%, 20.08%, and 72.77% in comparison to healthy leaves, while the levels of total sugar and reducing sugar decreased by 59.59% and 70.0%, respectively. Notably, the wildfire disease had little effect on the content of starch and K^+. Redundancy analysis revealed that *Pseudomonas*, *Staphylococcus*, *Cladosporium*, and *Wallemia* displayed positive correlations with nicotine, protein, total nitrogen, Cl^- and K^+ contents, while *Pantoea*, *Erwinia*, *Sphingomonas*, *Terrisporobacter*, *Aspergillus*, *Alternaria*, *Sampaiozyma*, and *Didymella* displayed positive correlations with total sugar and reducing sugar contents. *Brevibacterium*, *Brachybacterium*, and *Janibacter* were found to be enriched in diseased leaves, suggesting their potential role in disease suppression. Co-occurrence network analysis indicated that positive correlations were prevalent in microbial networks, and the bacterial network of healthy tobacco leaves exhibited greater complexity compared to diseased tobacco leaves. This study revealed the impact of wildfire disease on the microbial community and chemical compositions of tobacco leaves and provides new insights for the biological control of tobacco wildfire disease.

KEYWORDS
chemical composition, phyllophere microbial community, *Pseudomonas syringae* pv. *tabaci*, wildfire disease

1 | INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is an extensively cultivated economic crop with its economic value primarily derived from the quality of its leaves, which are processed into cigars, cigarettes, snuff, and so forth (Zappe et al., 2020). Cigars are a type of tobacco product crafted by rolling cigar tobacco leaves. In recent years, there has been an increasing cultivation area and yield of cigar tobacco leaves in China (Hu, Cai, et al., 2022). Tobacco wildfire disease is a highly problematic foliar disease of tobacco worldwide. First reported in the United States by Wolf and Foster in 1917, it has subsequently occurred in almost all tobacco-producing countries, primarily infecting mature tobacco leaves during the field period. The causal agent of the disease is *Pseudomonas syringae* pv. *tabaci*, which produces a toxin that results in a chlorotic halo around necrotic lesions, leading to a decline in the quality of tobacco leaves and reduced industrial use (Bender et al., 1999; Salch & Shaw, 1988; Xin et al., 2018). Considerable efforts have been made to control this disease, including the use of resistant varieties, chemical pesticide applications, and the utilization of microbial agents. Notably, biological control has gained increasing attention due to its environmental friendliness and safety compared to chemical pesticides in recent years (Liu, Gu, et al., 2021; Qin et al., 2019). Currently, the primary biological control agents for wildfire disease are *Bacillus subtilis* and *Pseudomonas fluorescens* (Chen, 2021; Sun et al., 2012).

Tobacco phyllosphere harbors a large number of microorganisms, including beneficial, pathogenic, or antagonistic microbes (Penuelas & Terradas, 2014; Ritpitakphong et al., 2016; Vannier et al., 2019). With the disease stress on plants, native antagonistic microbiome plays an important role in disease suppression (Bulgarelli et al., 2012). Gaining insights into the impact of disease infection on the microbial community of plants is valuable for understanding the pathogenic mechanisms of pathogens and advancing the development of innovative microbial agents (Perreault & Laforest-Lapointe, 2022). The advent of next-generation sequencing technology has facilitated the exploration of microbial diversity and structure in the plant ecosystem (Rastogi et al., 2013). Increasing studies have employed high-throughput sequencing to investigate microbial communities in the roots, soil, and phyllosphere of both healthy and diseased plants. For instance, Xiong et al. (2017) characterized microbial communities in soils with varying suppressive or conducive properties toward *Fusarium* wilt disease. Their findings revealed that the fungal genus *Mortierella* present in suppressive soil could serve as an indicator and enhancer of *Fusarium* wilt disease suppression. Similarly, Yang et al. (2020) demonstrated significant enrichment of *Pantoea*, *Pseudomonas*, and *Curtobacterium* in leaves affected by bacterial blight diseased leaves. They successfully isolated *Pantoea* strains from the diseased leaves, which exhibited the ability to inhibit the growth of *Xanthomonas oryzae* pv. *oryzae* in vitro.

The quantification of carbohydrate compounds (i.e., total sugar, reducing sugar, and starch) and nitrogenous compounds (i.e., nicotine, protein, and total nitrogen) in tobacco leaves is a critical parameter of leaf quality, as these substances also serve as nutrients for phyllosphere microorganisms (Chen et al., 2021; Markov & Laudet, 2022). In this study, we employed high-throughput sequencing to characterize the microbial communities in healthy and diseased cigar tobacco leaves. Furthermore, we measured the chemical compositions of different cigar tobacco leaves and analyzed the relationship between these compositions and microbial communities using redundancy analysis (RDA). Additionally, association networks were used to explore the interactions within the microbial communities associated with healthy and diseased tobacco leaves (DTL). These comprehensive analyses provided novel insights into the functional role of the leaf microbiome in tobacco protection.

2 | MATERIALS AND METHODS

2.1 | Sampling of tobacco leaves

The cigar tobacco leaves (cultivar Chuanxue No. 2) were collected from Dazhou, Sichuan Province, China (31°82′N, 107°68′E), on August 25, 2020. The field had a sandy loam soil texture with 20.28 g/kg organic matter, 20.32 mg/kg of rapidly available phosphorus, 98.99 mg/kg of rapidly available potassium, and a pH of 6.13. Chuanxue No. 2 plants were transplanted on April 25, 2020, with a row spacing of 110 cm and a plant spacing of 40 cm. During the field periods, few plots were naturally infected with wildfire disease. After harvesting, wildfire disease-infected leaves and healthy tobacco leaves (HTL) were separately air-dried in different shelters and cured for 28 days. The degree of disease infection was determined based on the Chinese National Standard GB/T 23222-2008. The scale is defined as follows: 0 (no disease symptoms on the entire leaf), 1 (lesion area < 1% of leaf area), 3 (lesion area 2∼5% of leaf area), 5 (lesion area 6∼10% of leaf area), 7 (lesion area 11∼20% of leaf area), and 9 (lesion area > 21% of leaf area). The DTL were selected from leaves with a Grade 9 infection, while the HTL were collected from asymptomatic in the shelter. Each sample consisted of five biological replicates, with 10 leaves randomly selected using 5-point sampling method. After sampling, the leaves were placed in sterilized plastic bags, immediately submerged in liquid nitrogen, and stored at −80°C for subsequent analysis.

2.2 | Determination of the chemical compositions of cigar tobacco leaves

The chemical compositions of cigar tobacco leaves were determined in accordance with industry standards established by the tobacco industry (Liu, Wu, et al., 2021). Various pretreatment methods were used to analyze the diverse chemical components in tobacco leaves, followed by the utilization of autoanalyzer to quantify their respective contents in the samples. The pretreatment methods utilized for the analysis of total sugar, reducing sugar, nicotine, and Cl^- and K^+ contents in tobacco leaves were as follows: a .25 g sample powder was accurately weighted and transferred into a 50 ml bottle, then mixed with 25 ml of 5% acetic acid, shaken for 30 min, and finally filtered

through a membrane filter. The pretreatment methods utilized for the analysis of starch content in tobacco leaves were as follows: a precisely weighted .1 g sample was placed into a G3 glass funnel, followed by the addition of 10 ml of 40% perchloric acid and 10 ml of deionized water to the funnel; the resulting liquid was then filtered and collected in a 100 ml volumetric flask, which was subsequently adjusted to the indicated level by adding deionized water. The pretreatment methods utilized for the analysis of total nitrogen content in tobacco leaves were as follows: a .1 g sample was accurately weighed into a digestion tube, followed by the addition of .1 g of anhydrous copper sulfate, 1.0 g of potassium sulfate, and 5.0 ml of concentrated sulfuric acid. The tube was then placed in the digester for digestion, after which the solution was adjusted to the indicated level with deionized water and filtering the mixture using filter paper to collect the resulting filtrate for subsequent analysis. The pretreatment methods utilized for the analysis of protein content in tobacco leaves were as follows: a .5 g sample was accurately weighted into a 100 ml erlenmeyer flask, followed by mixing the powder with 25 ml .5% acetic acid and heating it to a boiling point for 15 min; subsequently, the mixture was filtered using a filter paper and transferred to the digestion tube. The next steps were the same as for total nitrogen.

2.3 | DNA extraction, polymerase chain reaction amplification, and sequencing

DNA was extracted from .5 g samples with the E.Z.N.A. Soil DNA Kit (Omega Bio-tek, GA, United States) following the manufacturer's instructions. The primers 799F (5′-AACMGGATTAGATACCCKG-3′) and 1193R (5′-ACGTCATCCCCACCTTCC-3′) were used based on Wang et al. (2018) to amplify the V5–V7 region of the bacterial 16S rRNA gene. The fungal ITS gene was amplified using primers ITS1 (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′). The polymerase chain reaction products were extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, CA, United States). The amplification products were sequenced using the Illumina MiSeq PE300 platform at Majorbio.

2.4 | Statistical analyses

After removing the raw reads with low quality and short length, the operational taxonomic units (OTUs) were clustered with a ≥97% similarity using UPARSE (Version 7.1) (Edgar, 2013). The taxonomic information of bacterial and fungal OTUs was obtained using the Ribosomal Database Project Classifier against the SILVA 138 and UNITE 8.0 ITS database, respectively (Koljalg et al., 2013; Quast et al., 2013). Alpha diversity, including Sobs, Chao1, Shannon, and Simpson values, was used to analyze microbial community richness and diversity. Beta diversity analysis was performed based on the Bray–Curtis distance dissimilarity of the relative abundance of OTUs in Principal Coordinate Analysis (PCoA). Analysis of similarity (ANOSIM) and permutational multivariate analysis of variance (PERMANOVA) were used to detect the differences in microbial community composition between groups. Linear discriminant analysis effect size (LEfSe) analysis was conducted to identify key microbial groups that significantly influenced the microbiota structure at different taxonomic levels, using a linear discriminant analysis threshold of 3.5. Furthermore, the relationship between the microbiome and chemical factors was assessed using RDA. Co-occurrence networks of phyllosphere microorganisms were demonstrated using Spearman's rank analysis based on the absolute value of a correlation coefficient greater than .6 and a p value less than .05 (Xiang et al., 2022). All the analyses were conducted on the Majorbio cloud platform (Ren et al., 2022). The co-occurrence network was constructed and visualized using Gephi 0.9.7 (Bastian et al., 2009). A comparison of tobacco leaf chemical factors and alpha diversity indices was conducted using Student's t test in SPSS 22.0 (SPSS Inc., Chicago, United States).

3 | RESULTS

3.1 | Bacterial community diversity and structure

A total of 690,698 reads were obtained through Illumina MiSeq sequencing of two sample groups of samples after removing low quality and short length sequences. The dilution curve for each sample reached a plateau, indicating sufficient sequencing depth to capture most microorganisms present (Figure S1a). Based on a 97% nucleotide similarity level, a total of 1,141 OTUs were identified, corresponding to 22 phyla, 49 classes, 121 orders, 225 families, and 499 genera. At the phylum level, Proteobacteria was the predominant phylum, with relative abundances of 69.93% and 71.48% in HTL and DTL, respectively, followed by Firmicutes (20.17%, 18.04%), Actinobacteriota (8.26%, 9.30%), and Bacteroidota (1.29%, 1.04%) (Figure 1a). Notably, the relative abundances of these phyla displayed similarity between the two groups, suggesting minimal influence of wildfire disease at the bacterial community at the phylum level. In contrast, at the genus level, the relative abundance of bacteria exhibited considerable variation between HTL and DTL (Figure 1b). *Pseudomonas* was the most prevalent genus in both groups, with relative abundances of 34.43% and 54.78% in HTL and DTL, respectively. LEfSe analysis was employed to identify dominant microbial biomarkers in different groups (Figure 2a). *Pseudomonas*, *Brevibacterium*, *Brachybacterium*, and *Janibacter* were enriched in the diseased group, potentially playing crucial roles in disease suppression. Conversely, for HTL, *Sphingomonas*, *Methylobacterium_Methylorubrum*, *Aureimonas*, *Turicibacter*, *Delftia*, and *Corynebacterium* contributed to the observed differences. The proportion of *Sphingomonas* was significantly higher in HTL (7.28%) compared to DTL (.94%) (Figure 3a), and the relative abundances of other enriched bacteria were less than 5%.

Alpha diversity indices were employed to assess the impact of wildfire disease on the richness and diversity of microbial communities in tobacco leaves (Table 1). The Sobs and Chao1 indices for HTL

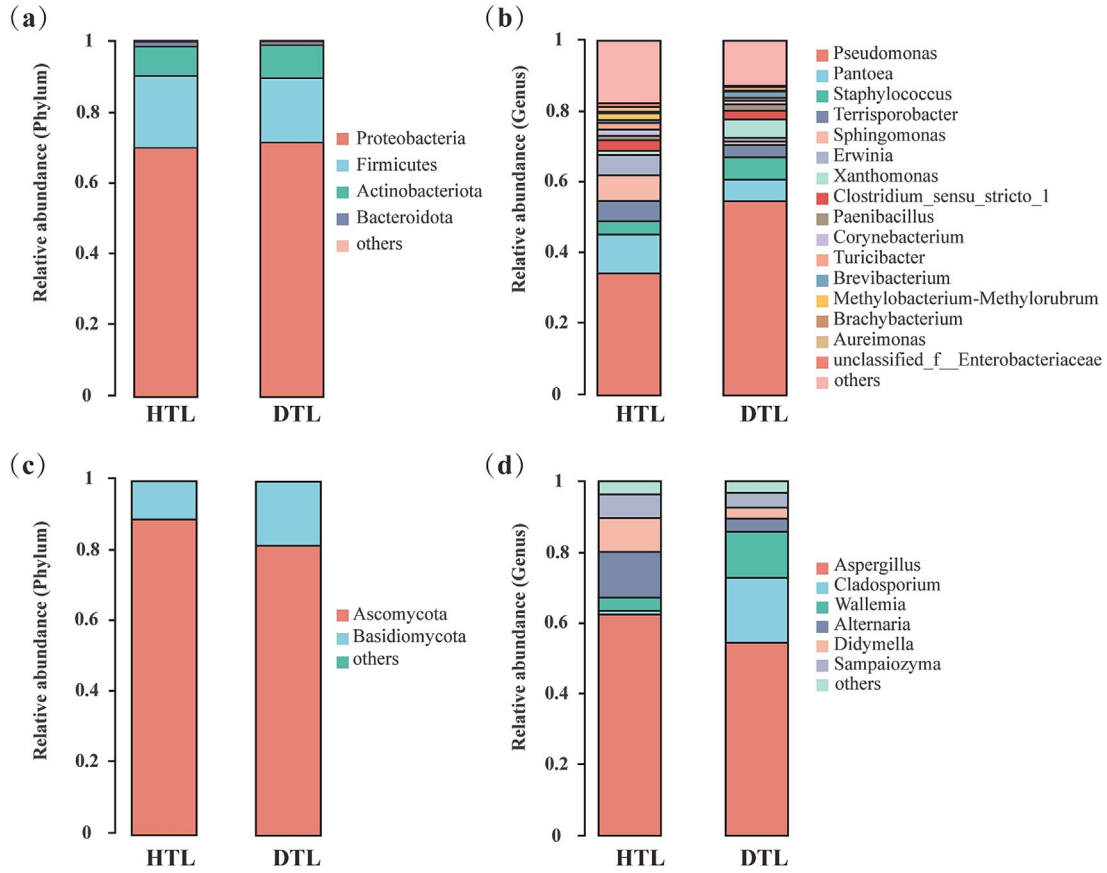

FIGURE 1 Bacterial community composition of healthy and diseased tobacco leaves at phyla level (a) and genus level (b); fungal community composition of healthy and diseased tobacco leaves at phyla level (c) and genus level (d). Microbes with relative abundance less than .01% were classified as "others." DTL, diseased tobacco leaves; HTL, healthy tobacco leaves.

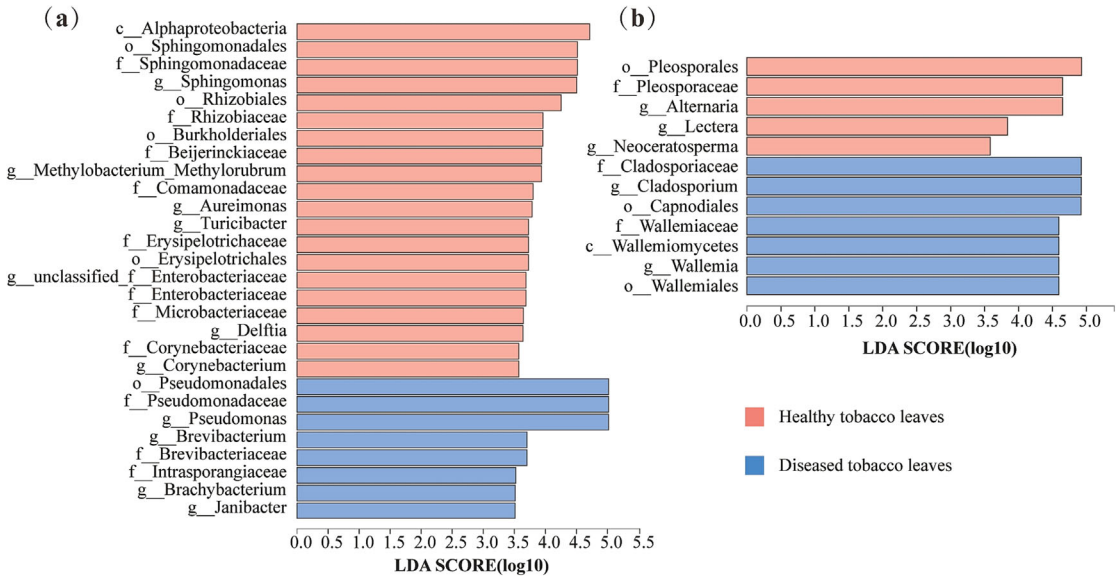

FIGURE 2 LEfSe analysis of microbial communities in tobacco leaves. (a) For bacterial communities and (b) for fungal communities.

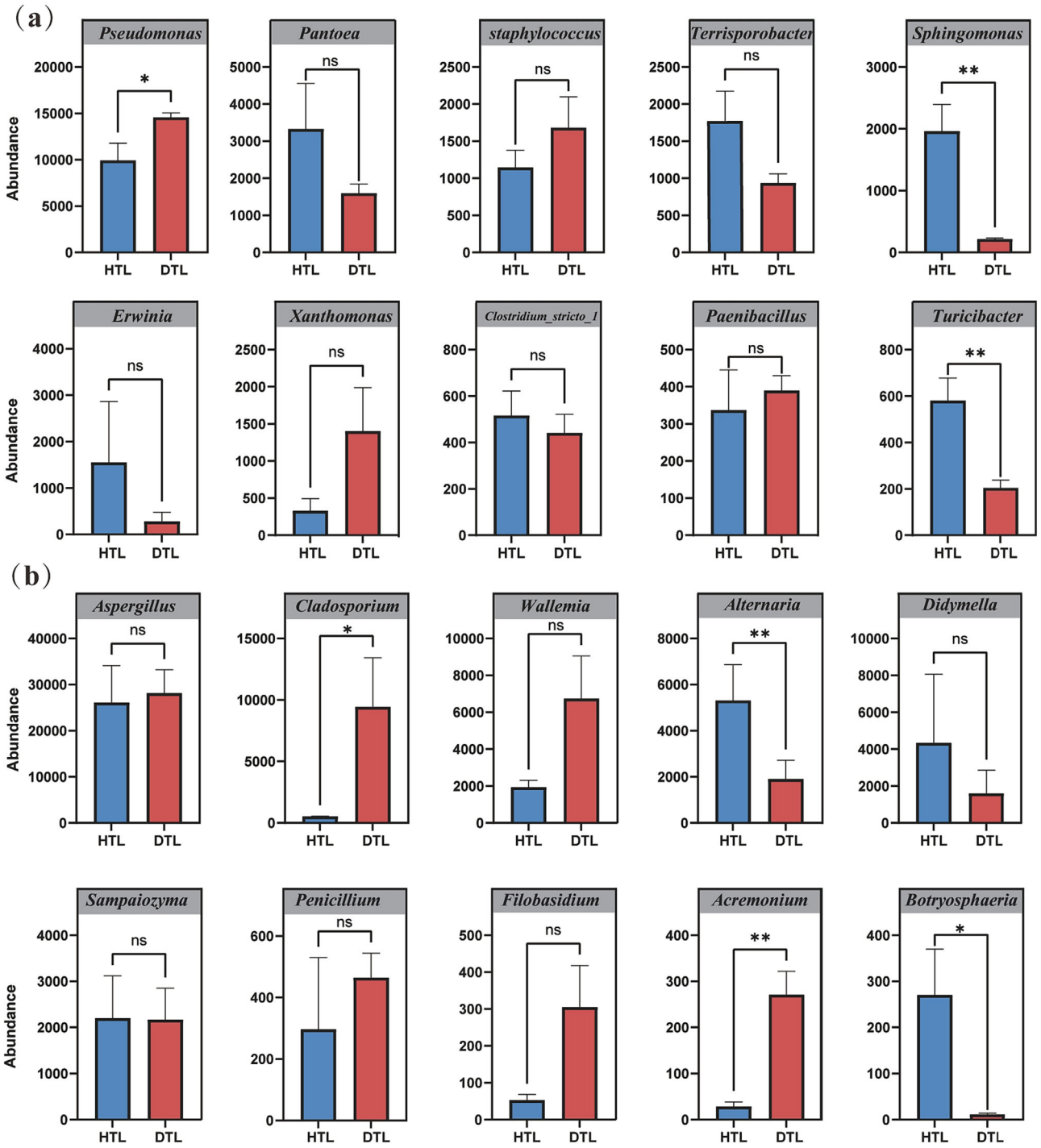

FIGURE 3 The observed abundance of the top 10 bacteria (a) and fungi (b) in tobacco leaves. $^{*}p < .05$, $^{**}p < .01$. DTL, diseased tobacco leaves; HTL, healthy tobacco leaves; ns, not significant.

were 545.6 and 626.65, respectively, exceeding those of DTL (512.4 and 601.36), indicating higher bacterial community richness in HTL. Moreover, the higher Shannon value of HTL (3.4) suggested greater bacterial community diversity compared to DTL (3.0), as supported by the Simpson index. However, the difference did not reach statistical significance. PCoA plots of beta diversity, computed using Bray–Curtis dissimilarity, exhibited distinct separation of the bacterial community at the OTU level ($R = .46$, $P = .004$) (Figure 4a). The first two principal coordinate axes explained 60.07% of the total variation in bacterial community, indicating a significant alteration in bacterial community structure due to wildfire disease. Non-parametric multivariate statistical tests, including ANOSIM and PERMANOVA, further confirmed significant differences in bacterial community structures between HTL and DTL (Table 2).

TABLE 1 Alpha diversity indices of microbial communities in tobacco leaves.

Samples	Microbe	Sobs	Shannon	Simpson	Chao1
HTL	Bacteria	545.60 ± 51.20	3.40 ± .46	.11 ± .05	626.65 ± 64.33
	Fungi	146.60 ± 12.72	1.44 ± .32	.44 ± .14	190.88 ± 30.92
DTL	Bacteria	512.40 ± 36.74	3.00 ± .27	.14 ± .04	601.36 ± 35.20
	Fungi	134.60 ± 30.22	1.78 ± .39	.30 ± .13	174.77 ± 38.09

Note: Mean ± SEM.
Abbreviations: DTL, diseased tobacco leaves; HTL, healthy tobacco leaves.

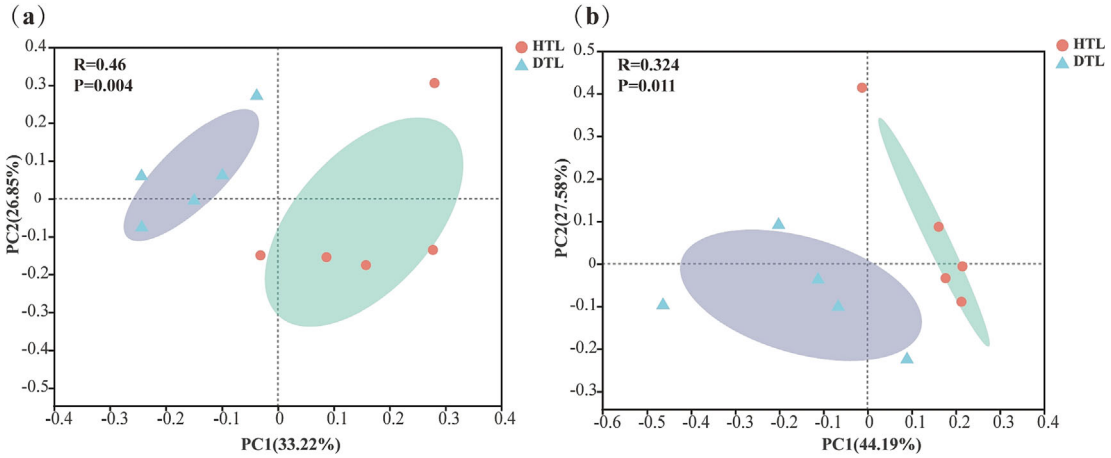

FIGURE 4 PCoA of the microbial communities in tobacco leaves. (a) For bacterial communities and (b) for fungal communities. DTL, diseased tobacco leaves; HTL, healthy tobacco leaves.

TABLE 2 The dissimilarity of bacterial and fungal communities in the healthy and diseased tobacco leaves.

	ANOSIM		PERMANOVA	
HTL and DTL	R	P	R	P
Bacterial community	.46	.004	.288	.012
Fungal community	.324	.011	.320	.015

Abbreviations: DTL, diseased tobacco leaves; HTL, healthy tobacco leaves.

3.2 | Fungal community diversity and structure

A total of 622,319 reads were obtained from all the samples. After clustering at 97% similarity, 304 OTUs were classified into 4 phyla, 15 classes, 40 orders, 94 families, and 155 genera. The phylum Ascomycota was found to be predominant, followed by Basidiomycota (Figure 1c). At the genus level, six genera showed relative abundances exceeding 1%, namely, *Aspergillus*, *Cladosporium*, *Wallemia*, *Alternaria*, *Didymella*, and *Sampaiozyma*. Importantly, their relative abundances differed greatly between HTL and DTL (Figure 1d). *Aspergillus* was the most abundant genus in both sample types, with relative abundances of 62.35% and 54.45% in HTL and DTL, respectively. LEfSe analysis indicated that the relative abundances of *Alternaria*, *Lectera*, and *Neoceratosperma* were enriched in the HTL, while the abundances of

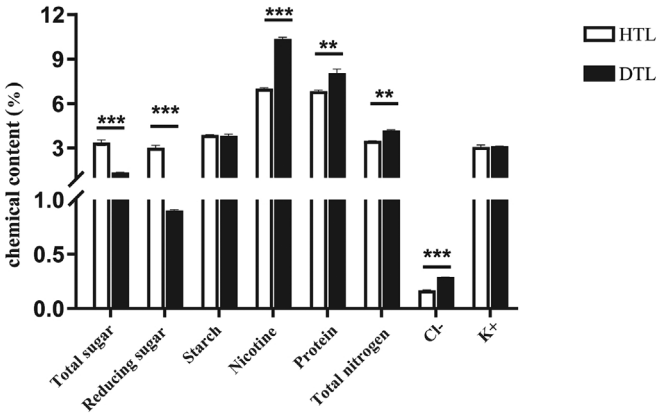

FIGURE 5 Different chemical factors in tobacco leaves. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$. DTL, diseased tobacco leaves; HTL, healthy tobacco leaves.

Cladosporium and *Wallemia* were enriched in the DTL, with *Cladosporium* reaching a significant level (Figures 2b and 3b). In contrast to bacterial diversity, the Shannon index suggested higher fungal diversity in DTL (1.78) compared to HTL (1.44) (Table 1). PCoA plots revealed significant clustering between HTL and DTL ($R = .324$, $P = .011$), demonstrating distinct differences in fungal community structure between the two sampling groups (Figure 4b).

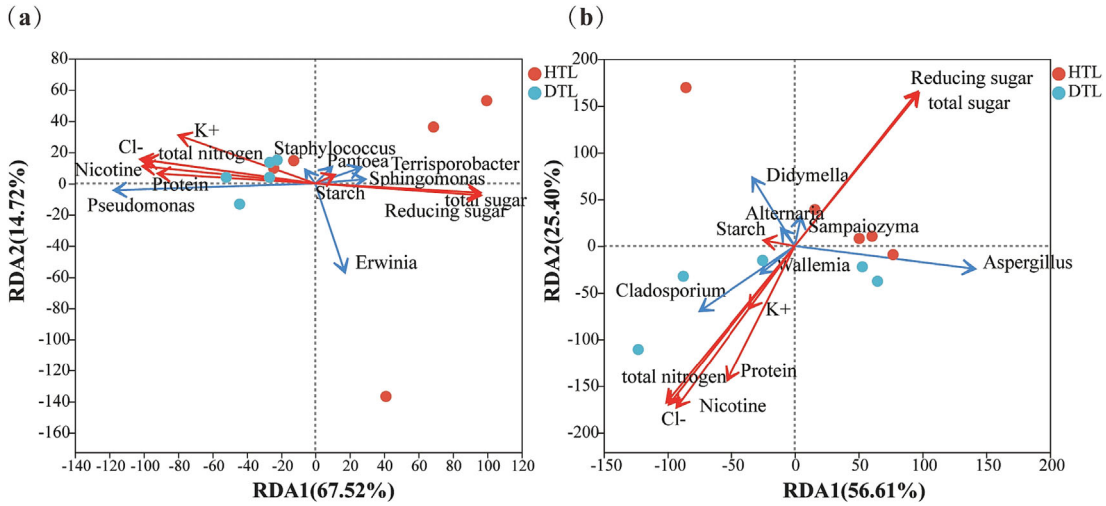

FIGURE 6 RDA of bacterial (a) and fungal (b) communities and chemical factors of tobacco leaves. The red arrow represents the chemical factors of tobacco leaves, while the blue arrow represents the predominant genus. DTL, diseased tobacco leaves; HTL, healthy tobacco leaves.

3.3 | Effects of wildfire disease on the chemical composition of cigar tobacco leaves

Carbohydrate compounds are known to be associated with the flavor and aroma of tobacco (Banozic et al., 2020), while nitrogenous compounds influence the physical characteristics and smoke concentration (Tso, 1990). Furthermore, K^+ and Cl^- are vital components that impact the flammability and hygroscopicity of tobacco. In this study, we analyzed total sugar, reducing sugar, starch, nicotine, protein, total nitrogen, and Cl^- and K^+ levels in different samples. Figure 5 depicts that DTL demonstrated significantly increased levels of nicotine, protein, total nitrogen, and Cl^- in comparison to HTL, exhibiting increases of 47.86%, 17.46%, 20.08%, and 72.77%, respectively. Conversely, the content of total sugar and reducing sugar in DTL was significantly lower (1.36% and .90%) in contrast to HTL (3.36% and 3.01%), experiencing reductions of 59.59% and 70.0%, respectively. There was minimal alteration observed in starch and K^+ content between the two sample groups.

3.4 | Correlation analysis of dominant microorganisms and chemical compositions of tobacco leaves

Tobacco leaves serve as a source of nourishment for phyllosphere microorganisms, and their growth and metabolic activities can impact the chemical composition of the leaves (Vorholt, 2012). In this study, RDA was conducted to investigate the influence of microorganisms on the chemical factors of tobacco leaves (Figure 6). The results revealed significant effects of microbial communities, comprising bacteria and fungi, on the contents of nicotine, total nitrogen, and Cl^-. Furthermore, the fungal community demonstrated a notable influence on the total sugar and reducing sugar content. Specifically, the abundance of *Pseudomonas*, *Staphylococcus*, *Cladosporium*, and *Wallemia* displayed positive correlations with nicotine, protein, total nitrogen, and Cl^- and K^+ contents, while the abundance of *Pantoea*, *Erwinia*, *Sphingomonas*, *Terrisporobacter*, *Aspergillus*, *Alternaria*, *Sampaiozyma*, and *Didymella* displayed positive correlations with total sugar and reducing sugar contents.

3.5 | Microbial co-occurrence network analysis

Network analysis of co-occurrence patterns among the 50 most abundant bacterial and fungal genera provided valuable insights into the relationships within phyllosphere microbial communities (Figure 7) (Deng et al., 2012). In bacterial networks, the average degree was 4.489 and 2.152 for HTL and DTL, respectively, and the average path length was 3.241 and 4.326, respectively (Table 3). Conversely, the fungal community network showed opposing results compared to the bacterial networks, with an average degree of 1.58 and 2.143 for HTL and DTL, respectively. Remarkably, positive correlations were prevalent across all networks. However, the invasion of bacterial pathogens resulted in a reduction in the proportion of positive links within the bacterial networks, while the fungal networks exhibited an increase in positive correlations. Furthermore, in the network of HTL, a unique negative correlation was observed between *Pseudomonas* and *Sphingomonas*. In contrast, in the network of DTL, a significantly positive correlation was observed between *Pseudomonas* and *Paenibacillus*, while *Dietzia* showed a negative correlation with *Pseudomonas*. In the HTL bacterial network, *Massilia*, *Janibacter*, and *Bacillus* exhibited the highest connectivity of 20. Their relative abundances were .40%, .31%, and .26%, respectively. In the bacterial network of DTL, *Nocardioides*, *Microbacterium*, and Clostridium_sensu_stricto_1 exhibited the highest connectivity, each scoring 10. Their relative abundances were .29%, .08%, and 2.4%, respectively. Within the fungal network, the predominant genus *Aspergillus* displayed a negative correlation with other fungi in both HTL and DTL. In the fungal network

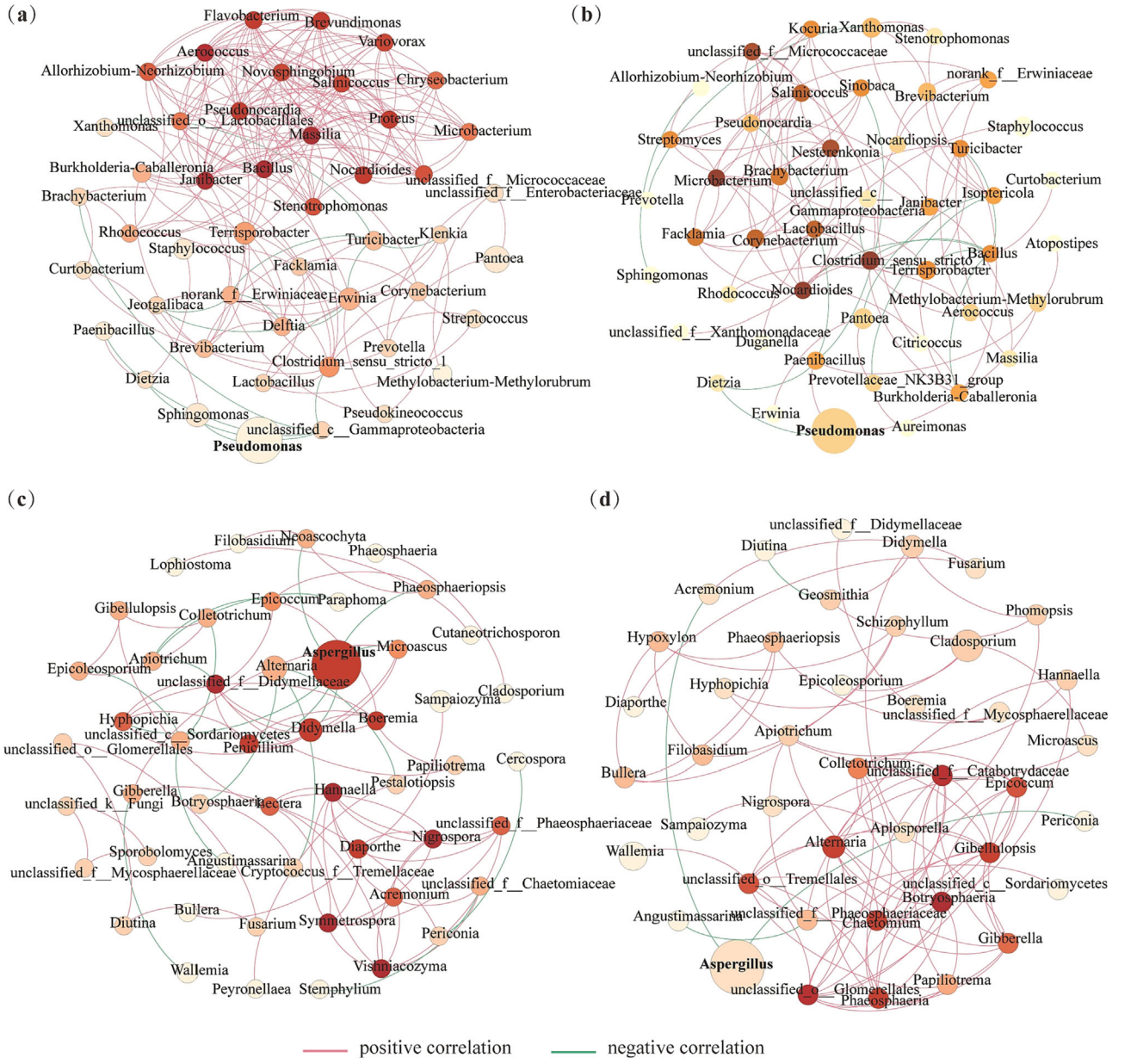

FIGURE 7 Network analysis of microbial communities in tobacco leaves. Bacterial communities in healthy (a) and diseased (b) tobacco leaves. Fungal communities in healthy (c) and diseased (d) tobacco leaves. Node size represents the relative abundance of the genus, and node color represents connectivity of the node. The higher the node connectivity, the darker the node color.

TABLE 3 Major topological properties of the phyllosphere microbial networks.

			Edges					
		Nodes	Positive	Negative	Average degree	Modularity	Average path length	Average clustering coefficient
Bacteria	Healthy	47	198 (93.84%)	13 (6.16%)	4.489	.372	3.241	.647
	Diseased	46	85 (85.86%)	14 (14.14%)	2.152	.551	4.326	.576
Fungi	Healthy	50	86 (77.22%)	4 (22.28%)	1.58	.707	2.769	.604
	Diseased	42	61 (95.56%)	18 (4.44%)	2.143	.542	2.266	.619

of HTL, *Hannaella* (.03%), *Nigrospora* (.05%), *Symmetrospora* (.15%), *Vishniacozyma* (.007%), and unclassified_f__Didymellaceae (.21%) had the highest connectivity (7), while *Botryosphaeria* (.02%) showed the highest connectivity of 12 in the DTL fungal network.

4 | DISCUSSION

In this study, we investigated the impact of wildfire disease on the microbial communities and chemical compositions of tobacco leaves, as well as the relationship between microbial community and chemical composition. Furthermore, we conducted a co-occurrence network analysis to explore the correlations among microorganisms. A total of 1,141 bacterial OTUs and 304 fungal OTUs were identified from all the samples, indicating a significantly higher abundance of bacteria compared to fungi within tobacco leaves. Four predominant bacterial phyla, namely, Proteobacteria, Firmicutes, Actinobacteria, and Basidiomycota, constituted more than 1% of the total microbial community composition, consistent with previous studies (Qin et al., 2019; Xiang et al., 2022). As expected, the DTL showed a significantly higher relative abundance of *Pseudomonas* compared to HTL, primarily due to the infection of *P. syringae* pv. *tabaci*. While most studies on the tobacco phyllosphere microorganisms have primarily focused on bacteria, with less emphasis on fungi (Hu, Liu, et al., 2022; Li et al., 2020; Xing et al., 2021; Zheng et al., 2022), our results revealed Ascomycota and Basidiomycota were the most prevalent fungal phyla in tobacco leaves, with the genus *Aspergillus* being highly abundant, in line with previous research (Liu et al., 2022; Zhang et al., 2020). The difference in alpha diversity index between HTL and DTL did not reach statistically significant. Moreover, Cheng (2020) reported that the bacterial diversity index of Yunyan 87 tobacco variety affected with wildfire disease Grades 1, 3, and 5 did not show significant differences when compared to HTL. We observed that the bacterial richness and diversity in DTL exhibit a reduction, which may be attributed to the competitive interactions for nutrients and niches between pathogenic bacteria and native microorganisms. Similar results have been reported in other studies. For example, Chinese chestnut leaves infected with yellow crinkle disease had lower bacterial diversity than asymptomatic leaves (Ren et al., 2021). Similarly, healthy *Euonymus japonicus* leaves harbored greater microbial abundances and diversity compared to powdery mildew diseased leaves (Zhang et al., 2019). Moreover, a highly diverse bacterial diversity tends to be conducive to resisting pathogen infection (Mallon et al., 2015; Wang et al., 2022). In addition, the investigation of phyllosphere microorganisms has received less attention in comparison to microorganisms inhabiting the belowground portions of plants, which have been extensively studied. Interestingly, bacterial diversity in the underground parts (i.e., roots, rhizosphere, and soil) of diseased plants is higher than that in healthy plants (Jiang et al., 2023; Wang et al., 2022). This phenomenon can be attributed to the "crying for help" strategy employed by plants, whereby they recruit beneficial microbes from soil to suppress disease, leading to increased community diversity (Dicke, 2009).

Phyllosphere microorganisms play a crucial role in plant disease defense. In response to pathogen infection, native microorganisms unite to combat the disease (Vannier et al., 2019). LEfSe analysis results showed that *Brevibacterium*, *Brachybacterium*, and *Janibacter*, which belong to the phylum Actinomycetes, were enriched in the diseased group, possibly contributing to the inhibition of wildfire disease. No previous reports of these genera inhibiting *P. syringae* pv. *tabaci* were found. Studies have demonstrated the efficacy of *Brevibacterium* in inhibiting various diseases, such as gray leaf spot disease in pepper (Son et al., 2014), root rot of sorghum (Idris et al., 2008), and tomato pathogens (On et al., 2015). Moreover, *Brachybacterium* spp. has been reported as a potential biological agent for decreasing the incidence of powdery mildew on flowering dogwoods (Mmbaga et al., 2008). Limited research is available on the *Janibacter* genus, mostly associated with human diseases. Recently, *Janibacter* sp. isolated from the rhizosphere of *Deschampsia antarctica* has demonstrated promising potential as a viable source of antimicrobial compounds (Oliveira et al., 2023). These results may present novel insights into the biological control of wildfire disease. In addition, our bacterial network analysis of HTL revealed a significant negative correlation between *Sphingomonas* and *Pseudomonas*. Notably, the genus *Pseudomonas* includes *P. syringae*, the causative agent of wildfire disease, while the genus *Sphingomonas* demonstrates a significant enrichment in healthy leaves. *Sphingomonas* is known for its capacity to promote plant growth and protect plants from pathogens (Rajkumar et al., 2009). Strains of *Sphingomonas* have been found to alleviate disease symptoms and suppress the growth of pathogenic *Pseudomonas syringae* DC3000 on *Arabidopsis thaliana* leaves (Innerebner et al., 2011). Transcriptional response studies have shown that *Sphingomonas melonis* Fr1 alters gene expression related to defense responses and contributes to plant protection against *P syringae* DC3000 (Vogel et al., 2016). Hence, compelling evidence from previous studies suggests that *Brevibacterium*, *Brachybacterium*, *Janibacter*, and *Sphingomonas* contain strains of antagonistic bacteria; further verification is required to determine the specific contribution of these bacteria in suppressing wildfire disease. The fungi *Cladosporium* and *Wallemia* were found to be enriched in DTL. *Cladosporium*, a genus from the family Cladosporiaceae, comprises plant pathogenic fungi known to induce diseases in various crops (Bensch et al., 2015; Xiang et al., 2022). For example, *Cladosporium cladosporioides* is one of the most common pathogens, capable of causing severe leaf spots and rot diseases (Swett et al., 2019). The genus *Wallemia*, classified under the family Wallemiaceae, comprises filamentous food-borne pathogenic fungi, including *Wallemia sebi*, *Wallemia mellicola*, and *Wallemia muriae*, which are associated with concerns regarding human health (Matheny et al., 2006; Zajc & Gunde-Cimerman, 2018; Zalar et al., 2005). Thus, the increased abundance of *Cladosporium* and *Wallemia* in diseased leaves poses risks to both plant and human health.

Upon the infection of tobacco leaves by wildfire disease, the microbial community experienced significant changes, leading to alterations in chemical composition and diminished leaf quality. Specifically, the levels of nicotine, protein, total nitrogen, and Cl^-

increased significantly, while total sugar and reducing sugar content showed a notable decrease. The effect of wildfire disease on the levels of nicotine, protein, total nitrogen, total sugar, reducing sugar, and Cl^- aligns with a previous study (Gu et al., 2017). However, different from their observations of increased K^+ content in flue-cured tobacco variety Yunyan 97 being infected with wildfire disease, our results indicate that K^+ content remained constant. Differences in these results could be attributed to varietal differences. Studies have shown that toxins produced by *P. syringae* pv. *tabaci* can interfere with the normal activity of glutamine synthetase. Consequently, this disruption leads to an increase in ammonia accumulation and a decline in soluble sugar levels (Popham et al., 1993; Xu & Zhang, 2006). The RDA analysis verified a strong positive correlation between *Pseudomonas* and the content of nicotine, protein, and total nitrogen in tobacco leaves. In addition, there was a strong negative correlation between *Pseudomonas* and total sugar, as well as reducing sugar. The current understanding of *P. syringae* pv. *tabaci* remains limited. Guo et al. (2017) reported that *P. syringae* metabolized 37.89% of carbon source while the efficiency of nitrogenous source utilization was 13.42%. Carbohydrates are the main carbon source for *P. syringae*, while amino acids are the primary nitrogen sources. Considering the dominant presence of *P. syringae* in *Pseudomonas*, it can be inferred that *P. syringae* may play a significant role in the accumulation of nitrogenous compounds and consumption of carbohydrates in DTL. In addition, our findings indicate that *P. syringae* has little effect on starch and K^+ content. However, a comprehensive understanding of the specific biotransformation mechanisms requires further investigation.

The infection of tobacco leaves by wildfire disease leads to significant alterations not only in the chemical composition but also in microbial interactions within the leaf ecosystem. Analysis of microbial community networks revealed a prevalence of positive correlations, indicating cooperation and mutualism among microorganisms (Marcel & Hartmann, 2016), consistent with previous findings (Qian et al., 2019; Sun et al., 2023). In DTL, a higher ratio of negative correlations among bacterial species indicates that the invasion of pathogenic bacteria enhanced competitive relationships. The HTL exhibited a more complex bacterial network with a greater number of nodes, edges, and average degree. Such a highly connected network may confer resistance to disturbance (Scheffer et al., 2012; Xiong et al., 2017; Zhang et al., 2018). Furthermore, *Massilia*, *Janibacter*, *Bacillus*, *Nocardioides*, *Microbacterium*, and Clostridium_sensu_stricto_1, among others, exhibited the highest connectivity within the networks, suggesting their potential role in maintaining the stability of tobacco phyllosphere microbial ecosystem (Xun et al., 2017). It is worth noting that most previous studies have primarily focused on abundant microorganisms with relative abundance exceeding 1%. However, our findings reveal that, apart from Clostridium_sensu_stricto_1 (2.4%), all other bacteria with the highest connectivity exhibited relative abundances lower than 1%. Hence, it is essential to direct attention toward these less abundant microorganisms in further studies (Peng et al., 2021; Zhao et al., 2022).

5 | CONCLUSION

In conclusion, our study revealed significant differences in the microbial community structure between healthy and wildfire disease-infected leaves. The presence of wildfire disease resulted in a decrease in bacterial richness and diversity, compared to healthy leaves. Furthermore, the disease caused a significant decrease in the levels of total sugar and reducing sugar, while elevating the levels of nicotine, protein, total nitrogen, and Cl^-. Notably, healthy leaves exhibited more complex bacterial networks characterized by a greater number of positive correlations in comparison to diseased leaves. These findings contribute to an enhanced understanding of the impact of wildfire disease on the microbial community and chemical compositions in tobacco leaves. Consequently, this study serves as a valuable resource for guiding the development of effective biological control strategies targeting wildfire disease.

AUTHOR CONTRIBUTIONS

Hongyang Si and Mingqin Zhao designed the research. Fang Liu analyzed the data. Hongyang Si performed the experiments and wrote the manuscript draft. Bing Cui edited the manuscript. All authors read the final manuscript.

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Not applicable.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available in the supporting information.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in Genebank under the accession number PRJNA 698768 (http://www.ncbi.nlm.nih.gov/bioprogect/PRJNA698768).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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