Phyllosphere Microbial Community Structure and Diversity in Tobacco Wildfire Disease: Post-print

Authors: Guo Tao, Wang Hancheng, Yu Zhihe, Cai Liu Ti, Wang Feng, Chen Xingjiang, Yu Zhihe, Wang Feng

Date: 2024-06-02T00:00:00+00:00

Abstract

Phyllosphere microbial community stability is closely associated with tobacco plant health. To reveal the phyllosphere microecological characteristics of tobacco plants infected with wildfire disease, this study employed Illumina high-throughput sequencing technology to analyze the microbial community structure and diversity of lesion and healthy tissues from tobacco leaves at different disease severity levels. The results showed: (1) The dominant bacterial phyla in both lesion and healthy tissues were Proteobacteria and Ascomycota, with their relative abundances being significantly higher in lesion tissue than in healthy tissue; the dominant genera were Pseudomonas, Pantoea, Alternaria, Plectosphaerella, and Conocybe, with only Pseudomonas exhibiting significantly higher relative abundance in lesion tissue compared to healthy tissue. (2) Bacterial community diversity and richness in lesion tissue were higher than in healthy tissue, and with increasing disease severity, the diversity index displayed a trend of first decreasing then increasing, while the richness index showed a trend of first increasing then decreasing; fungal community richness and diversity indices in lesion tissue first increased then decreased with increasing disease severity, with fungal community diversity and richness in lesion tissues of mildly and moderately diseased leaves being higher than in healthy tissue, but lower than healthy tissue in severely diseased tissue. (3) The bacterial gene functional information and relative abundances were basically similar between lesion and healthy tissues, primarily comprising three categories: metabolism, genetic information processing, and environmental information processing; the dominant functional groups of fungi were plant pathogens, animal pathogens-endophytes-plant pathogens-wood saprotrophs, undefined saprotrophs, wood saprotrophs, and plant pathogens-wood saprotrophs. These results provide a scientific basis for understanding the evolution pattern of tobacco wildfire disease and the characteristics of phyllosphere microbial communities.

Full Text

Preamble

ChinaXiv DOI: 10.11931/guihaia.gxzw202312013
Title: Microbial Community Structure and Diversity of Leaf Phyllosphere in Tobacco Plants Infected with Wildfire Disease

Authors: Guo Tao[1,2], Wang Hancheng[2], Yu Zhihe[1], *Cai Liuti*[2], *Wang Feng*[2], Chen Xingjiang[2]

Affiliations:
1. College of Life Sciences, Yangtze University, Jingzhou 434025, Hubei, China
2. Guizhou Academy of Tobacco Sciences, Guiyang 550081, Guizhou, China

---

Abstract

The stability of phyllosphere microbial communities is intimately linked to plant health. To elucidate the microecological characteristics of tobacco leaves infected with wildfire disease, this study employed Illumina high-throughput sequencing technology to analyze the microbial community structure and diversity in both diseased spots and healthy tissues of tobacco leaves exhibiting varying disease severity. The results revealed three key findings. First, Proteobacteria and Ascomycota were the dominant bacterial and fungal phyla in both diseased and healthy tissues, with significantly higher relative abundance in diseased tissues. The dominant genera were *Pseudomonas*, *Pantoea*, *Alternaria*, *Plectosphaerella*, and *Conocybe*, though only *Pseudomonas* showed significantly elevated abundance in diseased tissues compared to healthy tissues. Second, bacterial community diversity and richness were higher in diseased spots than in healthy tissues. As disease severity increased, the diversity index exhibited a decreasing-then-increasing trend, while the richness index showed an increasing-then-decreasing pattern. For fungal communities, richness and diversity indices in diseased spots initially increased then decreased with disease progression. Mildly and moderately diseased leaves displayed higher fungal diversity and richness in diseased spots than in healthy tissues, whereas severely diseased tissues showed lower fungal diversity and richness compared to healthy tissues. Third, bacterial gene functional profiles and relative abundances were largely similar between diseased and healthy tissues, primarily encompassing metabolism, genetic information processing, and environmental information processing. The dominant fungal functional guilds included plant pathogens, animal pathogens-endophytes-plant pathogens-wood saprotrophs, undefined saprotrophs, wood saprotrophs, and plant pathogens-wood saprotrophs. These findings provide a scientific foundation for understanding the progression dynamics of tobacco wildfire disease and characterizing phyllosphere microbial community features.

Keywords: tobacco wildfire disease, high-throughput sequencing, diseased leaf,

community structure, diversity index, richness index

Introduction

Tobacco is a crucial economic crop in China, playing a significant role in the development of modern mountainous agriculture. In recent years, wildfire disease caused by *Pseudomonas syringae* pv. *tabaci* has evolved into a major bacterial leaf spot disease in tobacco cultivation, often co-occurring with angular leaf spot, brown spot, and *Corynespora* leaf spot, resulting in substantial economic losses to tobacco production (Zhang et al., 2002; Chen et al., 2018).

Plant disease occurrence is intimately associated with imbalances in the plant microecological environment, with phyllosphere microorganisms representing essential components of this ecosystem. Research demonstrates that the stability of phyllosphere microbial community structure is closely related to leaf health (Lindow & Brandl, 2003; Shakir et al., 2021). With the advancement and maturation of high-throughput sequencing technology, an increasing number of scholars have applied this technique to investigate relationships between plant diseases and microbial communities (Li et al., 2022). As tobacco is an important economic crop harvested primarily for its leaves, numerous studies have reported on the relationship between fungal leaf diseases and phyllosphere microbial communities. For instance, Huang et al. (2020) investigated tobacco powdery mildew and found that healthy tobacco leaves exhibited higher fungal richness and diversity than diseased leaves, with *Golovinomyces*, *Aspergillus*, and *Alternaria* as dominant fungi in both diseased and healthy tissues, though at higher abundances in infected tissues. Similarly, tobacco brown spot disease phyllosphere communities were dominated by Ascomycota and Proteobacteria, with *Alternaria* and *Pseudomonas* as the dominant fungal and bacterial genera, respectively, though healthy leaves showed higher microbial diversity and richness than diseased leaves (Liu et al., 2021; Dai et al., 2022). Sun et al. (2023) reported that pathogen abundance in tobacco target spot-infected leaves increased significantly with disease severity, while phyllosphere fungal diversity and richness were lower than in healthy leaves, and bacterial diversity was higher but richness lower than in healthy leaves. These findings collectively demonstrate that changes in phyllosphere microbial communities are closely associated with disease severity in tobacco fungal leaf diseases.

Tobacco wildfire disease, a bacterial disease that first emerged sporadically in Yunnan tobacco-growing regions in the late 1940s, has become increasingly problematic as tobacco cultivation has expanded. The disease frequently co-occurs with other tobacco leaf diseases, causing substantial losses during epidemic years. Current research on factors influencing wildfire disease has primarily focused on environmental variables such as climate and humidity, while in-depth understanding of phyllosphere microbial community structure and diversity remains limited. Cheng (2020) explored the relationship between tobacco phyllo-

sphere bacterial communities and wildfire disease occurrence but lacked investigation of fungal communities. Si et al. (2023) examined the effects of wildfire disease on cigar tobacco, characterizing differences in microbial communities and chemical factors between infected and healthy leaves, but did not further analyze leaves with varying disease severity. Notably, wildfire disease not only damages tobacco leaves in the field but continues to cause harm during post-harvest processing until flue-curing, causing lesions to expand into necrotic patches that lose curing value. Therefore, the relationship between disease prevalence and dynamic changes in phyllosphere microbial communities urgently requires investigation. To address this knowledge gap, this study utilized high-throughput sequencing technology to examine tobacco wildfire disease leaves with varying severity levels, specifically to: (1) characterize differences in microbial community structure and diversity between diseased spots and healthy tissues across different disease severities; (2) analyze microbial community changes in diseased spots with increasing disease severity; and (3) investigate functional changes in microbial communities across different disease severities. The objective is to provide a scientific basis for understanding the progression patterns of tobacco wildfire disease.

Materials and Methods

Sample Collection

In June 2019, tobacco leaf samples exhibiting wildfire disease were collected from the Shibing County tobacco production area in Guizhou Province (107°51 36 – 108°28 51 E, 26°49 47 –27°21 15 N), where the cultivated variety was Yunyan 87. Samples were harvested 60 days after transplanting and categorized based on lesion area proportion into mild (5%–10%), moderate (11%–20%), and severe (21%–50%) disease levels (Figure 1 [Figure 1: see original paper]), designated as A, B, and C, respectively. Diseased spots (b) and healthy tissues (j) were excised from sample leaves using sterile scissors, mixed separately, and placed in 50 mL sterile centrifuge tubes (3 tubes per sample, 5 g per tube). Samples were stored in a low-temperature container during transport and subsequently stored at -80°C until analysis.

Figure 1. Symptoms of diseased tobacco leaves. A. Mild; B. Moderate; C. Severe.

Genomic DNA Extraction, PCR Amplification, and High-Throughput Sequencing

Total genomic DNA from phyllosphere microorganisms was extracted using the Fast DNA® SPIN Kit (MP Biomedicals) following the manufacturer's instructions. DNA quality and concentration were assessed using a NanoDrop 2000 micro-spectrophotometer (Thermo Fisher Scientific), and samples were diluted to 1 ng $\cdot$ L^{-1} with sterile water.

Bacterial 16S rRNA genes and fungal internal transcribed spacer (ITS1) regions were amplified using primers 515F/806R (Yuan et al., 2020) and ITS5-1737F/ITS2-2043R (Wu et al., 2019), respectively. PCR amplification protocols followed Apprill et al. (2005). Amplified products were verified by 2% agarose gel electrophoresis, purified using the GeneJET Gel Extraction Kit (Thermo Fisher Scientific), and sent to Novogene Bioinformatics Technology (Beijing) for library construction using the Ion Plus Fragment Library Kit (Thermo Fisher Scientific) and sequencing on the Ion S5™ XL platform.

Data Quality Control and Community Structure Analysis

Raw sequencing data were processed using Cutadapt (https://cutadapt.readthedocs.io/en/v1.9.1/) to remove low-quality sequences and the UCHIME algorithm to eliminate chimeric sequences, yielding high-quality clean reads. Operational taxonomic units (OTUs) were clustered from clean reads at $ $97% similarity using UPARSE (http://www.drive5.com/usearch/). Representative sequences for each OTU were selected and annotated against the UNITE (v7.2) and Silva132 databases using the Mothur algorithm (Edgar & Robert, 2013; Urmas et al., 2013). Community bar charts, principal component analysis (PCA) plots, and Venn diagrams were generated using R. Alpha diversity indices (Shannon, Simpson, Chao1, etc.) were calculated using QIIME (https://docs.qiime2.org) after rarefying samples to the minimum sequence number. Differential analysis of relative abundances between groups was performed using DPS software (http://www.dpsw.cn/download.html). Functional guilds of fungal and bacterial communities were analyzed using Tax4Fun, FUNGuild, and the KEGG database (Sun et al., 2023).

Results

Sequencing Depth Assessment and Data Quality Control

Rarefaction curve analysis indicated that bacterial OTU numbers plateaued at a sequencing depth of 2,300 reads (Figure 2 [Figure 2: see original paper]A), while fungal OTU numbers plateaued at 40,000 reads (Figure 2B), demonstrating that the sequencing data captured the vast majority of microbial diversity in the samples. After quality control, an average of 80,092 high-quality bacterial sequences and 70,147 fungal sequences were obtained per sample group (Table 1). The GenBank Accession Numbers for the raw bacterial and fungal sequencing data are PRJNA1025832 and PRJNA1025370, respectively.

Figure 2. Species rarefaction curves. A. Bacteria; B. Fungi. The same below.

Table 1. Sample sequencing data and quality control metrics.

Phyllosphere Community Structure at the Phylum Level

Bacterial communities in both healthy and diseased tissues were dominated by Proteobacteria, Firmicutes, and Bacteroidetes, with Proteobacteria showing higher relative abundance in diseased tissues (Figure 3 [Figure 3: see original paper]A). In mild, moderate, and severe disease samples, Proteobacteria were the dominant bacterial phylum (49.44%, 72.39%, and 61.92%, respectively), followed by Firmicutes (0.01%, 1.54%, and 0.73%) and Bacteroidetes (0.06%, 0.19%, and 0.06%). Healthy tissues were similarly dominated by Proteobacteria (relative abundances of 2.89%, 8.76%, and 3.15% in Aj, Bj, and Cj, respectively). No significant differences in Proteobacteria abundance were observed between diseased and healthy tissues in mildly diseased leaves, but moderate and severely diseased leaves showed significantly higher Proteobacteria abundance in diseased spots. Firmicutes and Bacteroidetes showed no significant differences across all six sample groups (Table 2).

Fungal communities in healthy and diseased tissues were primarily composed of Ascomycota and Basidiomycota (Figure 3B), with higher relative abundances in diseased tissues. Significant differences in Ascomycota abundance between diseased and healthy tissues were observed only in severely diseased leaves. Ascomycota relative abundances in mild, moderate, and severe diseased spots were 10.25%, 25.86%, and 32.22%, respectively, while Basidiomycota abundances were 4.52%, 2.48%, and 4.35%. In healthy tissues, Ascomycota abundance decreased with disease severity (6.31%, 5.14%, and 0.47% in Aj, Bj, and Cj), whereas Basidiomycota abundance increased (0.19%, 0.30%, and 0.47%).

Figure 3. Community composition at the phylum level.

Table 2. Relative abundance and differential analysis of dominant communities at the phylum level.

Phyllosphere Community Structure at the Genus Level

At the genus level, bacterial communities in healthy and diseased tissues were dominated by *Pseudomonas* and *Pantoea* (Figure 4 [Figure 4: see original paper]A). *Pseudomonas* abundance in diseased spots (39.47%, 59.83%, and 46.05% in Ab, Bb, and Cb) was significantly higher than in healthy tissues (0.57%, 5.95%, and 1.10% in Aj, Bj, and Cj). *Pantoea* abundance in diseased spots increased progressively with disease severity but showed no significant differences from healthy tissues.

Dominant fungal genera in tobacco leaf tissues included *Alternaria*, *Plectosphaerella*, and *Conocybe*, with higher relative abundances in diseased spots than healthy tissues, though differences were not statistically significant (Figure 4B). Conversely, *Phoma* abundance was higher in healthy tissues than in diseased spots, with significant differences observed in mildly diseased leaves.

Figure 4. Heatmap of community composition at the genus level. Different

lowercase letters in the same row indicate significant differences in relative abundance between groups ($P < 0.05$).

OTU Analysis of Phyllosphere Communities

Venn diagram analysis revealed that diseased and healthy tissues shared 9 bacterial OTUs and 190 fungal OTUs. Shared bacterial OTUs belonged to *Pseudomonas*, *Pantoea*, Rickettsiales, etc., while shared fungal OTUs included *Alternaria*, *Conocybe*, *Plectosphaerella*, and *Phoma*. The moderately diseased leaf sample Bb exhibited higher numbers of unique bacterial OTUs than other samples (Figure 5 [Figure 5: see original paper]A), and also showed the highest number of unique fungal OTUs among all tissue samples (Figure 5B).

Figure 5. Common and unique OTU analysis across different samples.

Alpha Diversity of Phyllosphere Microbial Communities

Coverage indices for both healthy and diseased tissues exceeded 0.997, indicating that sequencing captured the vast majority of microbial communities (Table 3). No significant differences in bacterial Simpson, ACE, or coverage indices were observed among the six sample groups. However, the Shannon index of mildly diseased spots differed significantly from healthy tissues, and the Chao1 index of moderately diseased spots was significantly higher than that of healthy tissues from mild and severe disease groups. Bacterial diversity and richness were higher in diseased spots than healthy tissues, with Shannon and Simpson indices showing a decreasing-then-increasing trend as disease severity increased, while Chao1 and ACE indices exhibited an increasing-then-decreasing pattern. In healthy tissues, bacterial diversity and richness showed an increasing-then-decreasing trend with disease severity.

Fungal diversity and richness in mild and moderately diseased spots were higher than in healthy tissues, but severely diseased spots showed lower fungal diversity and richness than their healthy counterparts. The Shannon and Simpson indices of sample Bb were significantly higher than those of Cb, Aj, and Bj. No significant differences were observed in fungal Chao1, ACE, or coverage indices across the six sample groups. Fungal diversity and richness in diseased spots increased then decreased with disease severity, while healthy tissues showed increasing Shannon and Simpson indices but decreasing Chao1 and ACE indices with disease progression.

Table 3. Alpha diversity indices of phyllosphere microbial communities. Different letters in the same column indicate significant differences for fungal and bacterial samples ($P < 0.05$).

Functional Prediction of Phyllosphere Fungal and Bacterial Communities

PICRUSt functional prediction based on the KEGG database revealed that at the first functional level, bacterial communities in both healthy and diseased tissues were primarily involved in six metabolic pathways: metabolism, genetic information processing, environmental information processing, cellular processes, human diseases, and organismal systems (Figure 6 [Figure 6: see original paper]A). Gene functional profiles and relative abundances were largely similar between diseased and healthy tissues.

FUNGuild functional prediction indicated that fungal ecological guilds were similar between healthy and diseased tissues. Dominant guilds included plant pathogens, animal pathogens-endophytes-plant pathogens-wood saprotrophs, undefined saprotrophs, wood saprotrophs, and plant pathogens-wood saprotrophs. Plant pathogen abundance was higher in diseased spots, peaking in moderately diseased sample Bb (6.23%), followed by Ab (5.27%) and Cb (2.50%), while the highest abundance in healthy tissues was in Bj (0.91%). The animal pathogen-endophyte-plant pathogen-wood saprotroph guild was substantially more abundant in moderately diseased spot Bb (6.51%) than in other samples. Undefined saprotrophs and wood saprotrophs were also more abundant in diseased spots, with the former peaking in severely diseased sample Cb (4.66%) and the latter in moderately diseased sample Bb (1.04%). Plant pathogen-wood saprotroph abundance was higher in healthy tissues, reaching maximum levels in healthy sample Aj (1.84%) (Figure 6B).

Figure 6. Functional prediction analysis.

Discussion

Diverse phyllosphere microorganisms are intimately associated with host plant health. These microbes can facilitate nitrogen fixation, promote plant growth, and enhance stress and disease resistance, while pathogenic microorganisms can cause disease by invading leaf tissues and colonizing the phyllosphere (Abdelfattah et al., 2015; Yang et al., 2021). Using high-throughput sequencing technology, this study elucidated the similarities and differences in phyllosphere microbial communities between healthy and diseased tissues of wildfire disease-infected tobacco leaves, revealing community structure and diversity patterns across varying disease severities.

Our findings demonstrate substantial differences in microbial community structure between healthy and diseased tissues of wildfire disease-infected tobacco leaves. Dominant bacterial phyla and genera included Proteobacteria, *Pseudomonas*, and *Pantoea*, which are commonly found in the tobacco phyllosphere. These results align with previous studies on wildfire disease field control (Qin et al., 2019), target spot disease (Sun et al., 2023), and brown spot disease (Liu

et al., 2020). As wildfire disease is a bacterial disease that frequently co-occurs with fungal diseases, similarities in dominant bacterial groups are expected. *Pseudomonas*, the wildfire disease pathogen, showed significantly higher abundance in diseased spots, indicating that extensive pathogen colonization is the primary driver of disease spread. Although *Pantoea* abundance did not differ significantly between diseased and healthy tissues, its progressive increase with disease severity suggests a positive correlation with wildfire disease and a potential facilitative role in disease development.

Dominant fungal genera included *Alternaria*, *Plectosphaerella*, and *Conocybe*, consistent with findings from Liu et al. (2022) and Xiang et al. (2020). The presence of *Alternaria* suggests potential for mixed infections of brown spot and wildfire disease, which aligns with current field observations. The enrichment of *Alternaria*, *Plectosphaerella*, and *Conocybe* in diseased spots indicates these genera may synergistically promote disease development. Notably, *Phoma* abundance was higher in healthy tissues than diseased spots, with significant differences in mildly diseased leaves, suggesting a potential suppressive role against disease. These functionally significant dominant taxa warrant further investigation.

The highly complex relationships among microorganisms mean that changes in the abundance of certain pathogens or beneficial microbes can alter entire community compositions. Pathogen invasion and colonization during disease development often modify the microbial environment and community structure, thereby affecting microbial diversity (Liu et al., 2022). Zhang et al. (2018) found that *Podosphaera* abundance in pumpkin powdery mildew increased with disease severity. Sun et al. (2023) reported that phyllosphere pathogen abundance in tobacco target spot increased significantly with disease grade. Chen et al. (2019) observed that *Aspergillus* abundance was higher in severely moldy tobacco leaves than in mildly moldy ones. These studies collectively demonstrate associations between pathogen abundance and disease severity. However, our analysis of three wildfire disease severity levels revealed that *Pseudomonas* abundance exceeded 39% in mild, moderate, and severe disease stages, peaking in moderately diseased leaves. This pattern differs from previous predictions. Furthermore, microbial diversity indices in healthy and diseased tissues showed either increasing-then-decreasing or progressively decreasing trends with disease severity.

This phenomenon may be explained by the following mechanism: during early disease stages, tissue integrity disruption allows various environmental microbes, including pathogens, to invade and colonize damaged tissues, temporarily increasing microbial diversity and richness. As lesions expand, limited nutrients and space intensify microbial competition, ultimately reducing pathogen and other microbial populations. Huang et al. (2021) reported similar findings in *Didymella* leaf spot disease, where pathogen abundance increased proportionally with disease severity but declined during later stages. This parallels our observation of decreasing *Pseudomonas* abundance in severely diseased tissues.

Although phyllosphere microbial community changes are difficult to predict accurately due to environmental influences, the general trend is discernible. The disruption of phyllosphere microecological balance through extensive colonization by pathogens and associated microbes represents a key microscale mechanism of tobacco disease development. Consequently, tobacco disease management should focus on inhibiting pathogen and associated microbe colonization to achieve biological control. Liu et al. (2021) demonstrated effective wildfire disease control using antagonistic bacteria, which significantly altered the proportions of *Pseudomonas* and *Pantoea* while increasing beneficial *Bacillus* abundance 3.9-fold compared to controls.

Phyllosphere microorganisms perform specific physiological functions during colonization that are closely related to their community structure and composition (Gao et al., 2016). Our functional predictions revealed similar bacterial gene functional profiles between healthy and diseased tissues, predominantly involving metabolism, genetic information processing, and environmental information processing. These results align with functional predictions for bacterial communities in tobacco angular leaf spot and target spot (Wu et al., 2023; Sun et al., 2023), likely because *Pseudomonas* is a dominant group across these leaf diseases. The higher bacterial diversity and richness in diseased spots suggests that *Pseudomonas*, *Pantoea*, and other genera dominate nutrient utilization in the phyllosphere, proliferating extensively during colonization while their metabolic, genetic, and environmental information processing functions are stimulated. Additionally, fungal functional predictions indicated that pathogens, endophytes, and saprotrophs dominated both tissue types, with higher plant pathogen abundance in diseased spots. This pattern, consistent with Huang et al. (2021), further confirms that plant pathogens, endophytes, and saprotrophs are dominant fungal guilds in the tobacco phyllosphere, with plant pathogens being critical drivers of leaf diseases.

References

Abdelfattah A, Destri NMG, Cacciola SO, et al., 2015. Metabarcoding analysis of fungal diversity in the phyllosphere and carposphere of olive (*Olea europaea*) [J]. *PLoS ONE*, 10(7): e0131069.

Apprill A, McNally S, Parsons R, et al., 2005. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton [J]. *Aquat Microb Ecol*, 75(2): 129-137.

Chen QL, Li Z, Wang HC, et al., 2019. Fungal composition and diversity of tobacco phyllosphere from cured tobacco leaves [J]. *Acta Microbiol Sin*, 59(12): 2401-2409.

Cheng Q, 2020. Study on the relationship between the diversity of tobacco bacterial community and the occurrence of tobacco wildfire and its regulation

effect [D]. Chongqing: Southwest University: 23-24.

Chen T, Zhou W, Li HG, et al., 2018. Research progress in disease occurrence and integrated control of tobacco wild fire [J]. *Genomics Appl Biol*, 37(1): 469-476.

Dai YF, Wu XM, Wang HC, et al., 2022. Spatio-temporal variation in the phyllospheric microbial biodiversity of *Alternaria alternata*-infected tobacco foliage [J]. *Front Microbiol*, 13: 1096985.

Edgar H, Robert C, 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads [J]. *Nat Methods*, 10(10): 996-998.

Gao S, Liu XC, Dong Z, et al., 2016. Advance of phyllosphere microorganisms and their interaction with the outside environment [J]. *Plant Sci J*, 34(4): 654-661.

Huang Y, Wang HC, Cai LT, et al., 2021. Phyllospheric microbial composition and diversity of the tobacco leaves infected by *Didymella segeticola* [J]. *Front Microbiol*, 12: 699699.

Huang Y, Wang HC, Chen QL, et al., 2020. Community structure and diversity of fungi in leaves of tobacco plant infected with powdery mildew [J]. *Guizhou Agric Sci*, 48(5): 54-58.

Lindow SE, Brandl MT, 2003. Microbiology of the phyllosphere [J]. *Appl Environ Microbiol*, 69(4): 1875-1883.

Li PD, Zhu ZR, Zhang Y, et al., 2022. The phyllosphere microbiome shifts toward combating melanose pathogen [J]. *Microbiome*, 10(1): 56-56.

Liu C, Wang HC, Xie HL, et al., 2020. Biodiversity analysis of phyllosphere bacterial genus from tobacco leaves infected by brown spot disease [J]. *Tob Sci Technol*, 53(2): 8-14.

Liu TB, Teng K, Zhou XP, et al., 2021. Effects of antagonistic bacteria on tobacco wild fire and responses of phyllosphere microbiota [J]. *Microbiol Chin*, 48(8): 2643-2652.

Liu TT, Zeng YT, Wang HC, et al., 2021. Metabolic and microbial community structure analysis in the phyllosphere of tobacco leaves with different maturity during brown spot occurring season [J]. *Chin Tob Sci*, 42(6): 22-29.

Liu TT, Wang HC, Sun ML, et al., 2022. Effects of Bordeaux mixture on the community structure and metabolic function of tobacco phyllosphere microorganisms [J]. *Chin J Pestic Sci*, 24(6): 1446-1455.

Liu YX, Dong CB, Shao QY, et al., 2022. Advances on phyllosphere microorganisms and their association with plant health [J]. *J Microbiol*, 42(2): 88-98.

Qin C, Tao JM, Liu TB, et al., 2019. Responses of phyllosphere microbiota and plant health to application of two different biocontrol agents [J]. *AMB Express*, 9(1): 42.

Shakir S, Zaidi SS, Vries DTF, et al., 2021. Plant genetic networks shaping phyllosphere microbial community [J]. *Trends in Genetics*, 37(4): 306-316.

Si H, Cui B, Liu F, et al., 2023. Microbial community and chemical composition of cigar tobacco (*Nicotiana tabacum* L.) leaves altered by tobacco wildfire disease [J]. *Plant Direct*, 7(12): e551-e551.

Sun ML, Shi CH, Huang Y, et al., 2023. Effect of disease severity on the structure and diversity of the phyllosphere microbial community in tobacco [J]. *Front Microbiol*, 13(4): 1081576-1081576.

Sun ML, Shi CH, Xiao BQ, et al., 2023. Composition and diversity of phyllospheric microbial community in tobacco leaves infected by tobacco target spot disease [J]. *Tob Sci Technol*, 56(4): 1-9.

Urmas K, Nilsson RH, Kessy A, et al., 2013. Towards a unified paradigm for sequence-based identification of fungi [J]. *Mol Ecol*, 22(21): 5271-5277.

Wu XJ, Wang HC, Sun ML, et al., 2023. Composition and diversity of phyllosphere microbial community on tobacco leaves infected with angular leaf spot [J]. *Tob Sci Technol*, 56(10): 1-10.

Wu YY, Geng JK, Xu S, et al., 2019. Isolation and identification of intestinal fungi in *Spodoptera frugiperda* larvae of Chongqing area [J]. *J Southwest Univ (Nat Sci Ed)*, 41(9): 1-8.

Xiang LG, Wang HC, Zheng P, et al., 2020. Analysis of endophytic fungi and phyllosphere fungi of flue cured tobacco leaves with brown spot disease [J]. *Acta Tab Sin*, 26(4): 93-100.

Yang K, Wang HL, Ye KH, et al., 2021. Advances in research on phyllosphere microorganisms and their interaction with plants [J]. *J Yunnan Agric Univ (Nat Sci)*, 36(1): 155-164.

Yuan Z, Zhang CY, Peng XX, et al., 2020. Intestinal microbiota characteristics of mice treated with *Folium senna* decoction gavage combined with restraint and tail pinch stress [J]. *3 Biotech*, 10(4): 1-11.

Zhang GM, Lu JH, Kan GF, et al., 2002. Recent development in the study of tobacco wildfire disease [J]. *Acta Tabac Sin*, 8(2): 34-37.

Zhang Z, Luo LY, Tan XQ, et al., 2018. Pumpkin powdery mildew disease severity influences the fungal diversity of the phyllosphere [J]. *Peer J*, 6(2): e4559.

Note: Figure translations are in progress. See original paper for figures.