Pak. J. Bot., 58(8) August 2026.

ARTICLE DOI: http://dx.doi.org/10.30848/PJB2026-8(6)

BACTERIAL BIODIVERSITY OF THE LEAF PHYLLOSPHERE OF DIFFERENT VARIETIES OF TOBACCO (*NICOTIANA TABACUM* L.) AND METABOLIC PATHWAYS

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Abstract

The phyllosphere is a distinctive habitat that hosts diverse microbial communities. Plant species and environmental factors determine the microbial diversity, which subsequently regulates the growth and metabolism of host plants. This study analyzed the relationship between tobacco leaves and their phyllosphere microbiome by determining the bacterial biodiversity of various tobacco varieties and evaluating its impact on metabolic pathways. For this purpose, 16S rRNA gene sequencing was conducted, and in silico functional annotation was performed. The results indicated significant variations in the α-diversity of bacterial communities among seven tested tobacco varieties. *Proteobacteria* were identified as the dominant phylum, with a relative abundance ranging from 74.4% to 98.2% across all varieties. *Pseudomonas* emerged as a predominant genus at the genus level, comprising 16.7% to 77.7% of the microbial communities. Fifty-four genera (22.7% of the detected genera) were shared among all tobacco varieties. The number of unique operational taxonomic units (OTUs) ranged from 16 to 130 across tobacco varieties, with 68 OTUs (13.7%) shared among all 7 varieties. The results also revealed that carbohydrate metabolism was the predominant pathway, accounting for 19.0% to 20.0% of all metabolic pathways. These findings highlight the intricate relationship between tobacco varieties and their phyllosphere microbiome, providing insights into microbial diversity and functional potential.

Key words: Phyllosphere microbiome; Tobacco leaves; 16S rRNA sequencing; Metabolic pathways; Microbial diversity

Introduction

The phyllosphere of plants is a vital habitat for diverse microbial communities (Muhammad *et al*., 2017; Müller & Ruppel, 2014). As part of the phyllosphere, the leaf surface is recognized as one of the largest habitats for bacteria, archaea, and fungi (Senthilkumar, 2021 & Legein *et al*., 2020). However, bacteria found in the phyllosphere (Martirosyan *et al*., 2016) have more beneficial effects on plants- such as recycling nutrients, producing growth hormones, preventing pathogen attacks, and performing bioremediation of harmful chemicals (Gu *et al*., 2010; Hu *et al*., 2025). For example, methylobacterium provides growth-promoting substances, including vitamin B12, cytokinins, and auxins, which enhance seed germination and root development, thereby boosting plant productivity (Senthilkumar *et al*., 2021). In recent decades, bacterial communities in the plant phyllosphere have garnered significant attention from researchers worldwide (Liu *et al*., 2020). This is because the interactions among microorganisms in the phyllosphere can impact sustainable plant production and health (Niu *et al*., 2016; Sang *et al*., 2012). Previous studies have shown that the structure of bacterial communities in the phyllosphere is influenced by abiotic and biotic factors, including environmental conditions (climate and soil properties), geographic location, agricultural practices, microbial interactions, leaf traits, and the host plant's genotype, among others (Awad *et al*., 2023).

Tobacco (*Nicotiana tabacum* L.) is a non-food economic crop. It is still widely cultivated worldwide (Zappe *et al*., 2020), including some regions of China, despite the recognition that tobacco smoking is harmful to health (Li *et al*., 2020). Besides its economic importance, tobacco is used as a model plant for studies related to plant biotechnology and plant-microbe interactions. For instance, tobacco plants are employed to examine the effects of gene modifications on photosynthetic efficiency (South *et al*., 2019), to conduct RNA interference (RNAi) experiments to control pests (Burke *et al*., 2019), to induce resistance to viral pathogens (Konakalla *et al*., 2021), to explore interactions between cropping systems and soil bacterial communities (Chen *et al*., 2018), and to screen for new antimicrobial agents (Ameya *et al*., 2017). To date, several studies have reported on the bacterial community of the tobacco phyllosphere based on 16S rRNA sequencing. Chen *et al*., (2021) found that Proteobacteria dominate the tobacco phyllosphere after the application of a broad-spectrum fungicide. Xing et al. (2021) analyzed the effects of environmental and host genetic factors on the bacterial community in the tobacco phyllosphere. The results from Xing et al. (2021) showed that Proteobacteria and Cyanobacteria were the dominant bacterial phyla in the tropical region (Hainan Province, China). They suggested this was due to the greater influence of site-specific factors compared to genotype-specific factors. Additionally, the community of phyllosphere bacteria was significantly affected by environmental conditions (Tang *et al*., 2020).

Received: 03-07-0000 | Revised: 04-12-2025 | Accepted: 11-12-2025 | Online: 01-04-2026

As a broadleaf plant, the primary component of tobacco biomass is the leaves, which provide microbes with a unique habitat called the phyllosphere. Tobacco leaves are rich in alkaloids (nicotine, nornicotine, neonicotine, anatabine, myosmine), polyphenols (rutin, quercetin), and coumarin (scopoletin). These compounds are found in varying amounts depending on the growth conditions and different tobacco varieties (Xia *et al*., 2014), and they may have antimicrobial functions (Camlica & Yaldiz, 2021). Tobacco leaves are mainly used for smoking, and the bacterial community on their surface plays a key role in affecting leaf quality, including flavor and yield. This study examines the bacterial community structure in the phyllosphere of 7 tobacco varieties grown in the same region. Using 16S rRNA gene sequencing, we assessed the diversity and composition of these microbial communities and explored differences among the seven tobacco varieties.

Materials and Methods

Sample collection, transportation, and storage: Leaf samples were collected from 7 varieties of tobacco (*Nicotiana tabacum* L.) grown at Xiponao village of Zhangmao Township, Shanzhou District, Sanmenxia City of Henan Province, Central China (111.37ºE, 34.70ºN). The tobacco varieties labelled as YY13, Y2001, and LY1306 were local to Henan Province; the QY96 and YA1 varieties were from Shanxi Province (northwestern China); YY87 originated from Yunnan Province (southern China); and the seventh variety, NC89, was sourced from America. The salient features of the above mentioned tobacco varieties are summarized in the supplementary Table S1. Three mature, healthy leaves were randomly selected from each variety of tobacco plant. The selected leaves were harvested one week before shearing, immediately transported to the laboratory in sterilized plastic bags, and stored on ice packs. The leaf samples were placed separately in the sterilized bags and stored at -80°C until further processing.

DNA extraction and PCR amplification: Leaf samples were ground after removal from −80°C storage. Microbial genomic DNA was extracted using the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, USA). DNA concentration and purity were measured with a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA), and quality was confirmed via 0.8% agarose gel electrophoresis.

The V5–V7 hypervariable regions of the bacterial 16S rRNA gene were amplified through PCR. The first 27 cycles used primers 799F (5′-AACMGGATTAGATACCCKG-3′) and 1392R (5′-ACGGGCGGTGTGTRC-3′), followed by 27 cycles with primers 799F and 1193R (5′-ACGTCATCCCCACCTTCC-3′). PCR conditions included: 95°C for 3 min; 27 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s; ending with a final extension at 72°C for 10 min. Reactions were carried out in triplicate in 20 µL volumes containing 4 µL 5× buffer, 2 µL 2.5 mM dNTPs, 0.8 µL of each primer (5 µM), 0.4 µL polymerase, and 10 ng template DNA.

PCR products were extracted from 2% agarose gels, purified using the AxyPrep DNA Gel Extraction Kit (Axygen, USA), and measured with QuantiFluor™-ST (Promega, USA).

Illumina Miseq sequencing and data analysis: Purified 16S rRNA amplicons were pooled in equal molar amounts and sequenced using paired-end (2 × 300 bp) Illumina MiSeq, following Majorbio Bio-Pharm Technology Co. Ltd. protocols. Raw reads (SRA accession: PRJNA699872) were demultiplexed, quality-filtered with Trimmomatic, and merged with FLASH based on these criteria: truncation at a quality score <20 over a 50 bp window, removal of reads shorter than 50 bp or containing ambiguous bases, and merging of overlapping reads longer than 10 bp with ≤0.2 mismatch. Barcodes and primers were matched exactly and with up to 2 bp mismatches, respectively (Hu *et al*., 2023).

OTUs were clustered at 97% similarity using UPARSE, and chimeras were removed with UCHIME in MOTHUR (Hu *et al*., 2025b). Taxonomic classification of representative sequences was performed using the RDP Classifier against the Silva SSU128 database (confidence threshold = 0.7) (Tarakanov *et al*., 2022). Alpha diversity metrics (Chao1, ACE, Shannon, Coverage) and heat maps of taxonomic composition were generated in MOTHUR and R, respectively (Candry *et al*., 2020). Beta diversity was assessed using UPGMA clustering and NMDS (Bray–Curtis distance) via the vegan package in R, supported by ANOSIM. Functional annotation was performed using BLASTP (e-value < 1e-5) against the NCBI NR and KEGG databases. LEfSe analysis (LDA >3.0, $p<0.05$) was conducted using the Huttenhower Galaxy platform to identify significantly enriched taxa.

Statistical analysis

Each group included a minimum of three biological replicates. Data are presented as mean ± SEM. Statistical significance was assessed using one-way ANOVA with Duncan's post hoc test ($p<0.05$).

Supplementary Table S1. Characteristics of different tobacco varieties.

Varieties	Origination	Reducing sugar	Total sugar	Nicotine	Total nitrogen
QY96	Shanxi Province, China	21.23%	24.98%	2.01%	1.70%
YY13	Henan Province, China	25.12%	26.86%	2.67%	1.77%
YY87	Yunnan Province, China	24.05%	31.14%	2.28%	1.65%
YA1	Shanxi Province, China	26.02%	28.67%	1.94%	1.67%
NC89	USA	20.11%	22.14%	2.59%	1.77%
Y2001	Henan Province, China	ND	ND	ND	ND
LY1306	Henan Province, China	24.98%	27.34%	1.95%	1.41%

ND: Not detected

Results

Sequence analysis: This study analyzed 21 samples from 7 varieties of tobacco leaves, yielding a total of 396,770 16S rRNA gene sequences. The rarefaction curves across all samples are shown in Fig. S1. The average length of sequences was 377 bp. A total of 30 phyla, 70 classes, 166 orders, 263 families, 451 genera, 648 species, and 843 OTUs of bacteria were identified from all samples. The indices of diversity of leaf bacterial communities were estimated from data presented in Table 1. The Shannon index of studied phyllosphere microbiota revealed the highest average value (2.56) in the sample NC89 and the lowest value (1.06) in the sample Y2001 (Table 1). The Shannon indices of samples NC89 and YY13 were significantly higher than Y2001 (p<0.05).

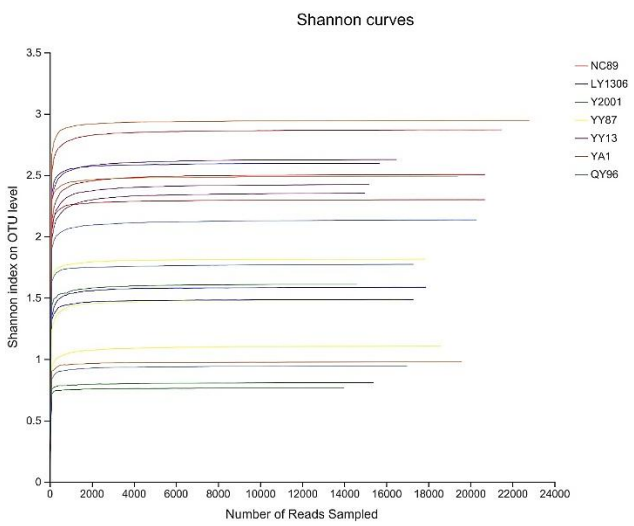

Fig. S1. The rarefaction curves to confirm sufficient sequencing depth across all samples.

Evolution of microbial communities: To examine the overall variability in community composition, we conducted non-metric multidimensional scaling (NMDS) analysis, which revealed the microbial community composition in different leaf samples of tobacco (stress=0.0-06) (Fig. 1). The phyllosphere bacterial communities from the same tobacco variety generally clustered in all triplicates, except YY87 and QY96, which were sourced from Shanxi and Yunnan provinces, respectively (Fig. 1). Two of the three replicates from YY87 and QY96 were very close, while the other replicates aligned with the cluster of LY1306 and YA1, respectively. Overall, statistical analysis demonstrated that the microbial community composition on the surface of different tobacco leaves was significantly different (p<0.05).

The results in Fig. 2a showed that Proteobacteria was the most common and abundant phylum across all varieties, with relative amounts ranging from 74.4% in YY13 to 98.2% in Y2001. In comparison, it was 75.5% for NC89, 86.4% for LY1306, and over 90% for QY96, YA1, and YY87. Actinobacteriota was the second most abundant phylum across all varieties, ranging from 21.3% in NC89, followed by 18.1% and 11.9% in YY13 and LY1306, respectively, and 3.2% to 5.8% in QY96, YA1, and YY87, while it was only 1.2% in Y2001. The results indicate that the composition of the phyllosphere bacterial community at the phylum level does not depend on the geographic origin of the varieties. Instead, it appears to be a characteristic of the variety itself, as YY13 from Henan and NC89 from America showed similar levels of Proteobacteria and Actinobacteria.

The results in Fig. 2b showed that the most abundant genus varied depending on the tobacco varieties rather than their geographic origins. Pseudomonas ranged from 77.7% for Y2001 to 16.7% for YA1. Afipia had the highest content (35.7%) for YY87 and the lowest (0.3%) for NC89. Pantoea made up 22.9% of the bacterial community in YY87 and less than 0.1% in YY13. Variations in Ralstonia (25.4% and 32.0%), Rhodococcus (14.5% and 14.7%), and Bacillus (9.4% and 12.1%) contents were also observed among the varieties. The results in Fig. 2c display community phylogenetic analysis at the phylum level across all three replicates. Some tobacco varieties, such as YY13, Y2001, and LY1306, clustered well, while YY87 replicates were separated into three phylogenetic branches.

Differences in bacterial diversity among the varieties of tobacco: The comparison of bacterial OTUs shared by different varieties showed that the number of unique OTUs ranged from 16 in Y2001 to 130 in YY13 (both varieties were from Henan). In contrast, 68 OTUs were shared by all varieties (Fig. 3a). At the phylum level, all varieties shared 10 common phyla. Meanwhile, the number of unique phyla varied from 0 to 2 depending on the array (Fig. 3b). At the genus level (Fig. 3c), all varieties shared 54 genera; however, 4 unique genera were found in Y2001 and 62 in YY13 among the 7 varieties. These results suggested that the phyllosphere bacteriome might differ more among varieties with the exact geographic origin than among those from different regions.

Table 1. Alpha diversity of bacteria was analyzed by ANOVA analysis and the significant analysis using the method of Duncan test (n=3).

Samples	Alpha diversity index*				
	Simpson	Shannon	Chao	Ace	Coverage (%)
YY87	0.42 ± 0.10 ab	1.47 ± 0.21 ab	173.88 ± 23.00 ab	176.37 ± 24.21 a	99.80 ± 0.06 bc
YY13	0.21 ± 0.03 b	2.47 ± 0.08 a	226.16 ± 11.31 a	224.80 ± 13.02 a	99.88 ± 0.01 abc
YA1	0.24 ± 0.11 b	2.14 ± 0.59 ab	130.26 ± 23.26 b	147.26 ± 9.73 a	99.90 ± 0.01 abc
Y2001	0.54 ± 0.06 a	1.06 ± 0.28 b	114.35 ± 41.10 b	156.91 ± 43.60 a	99.79 ± 0.06 c
QY96	0.37 ± 0.11 ab	1.62 ± 0.35 ab	176.51 ± 36.59 ab	183.28 ± 35.70 a	99.79 ± 0.01 c
NC89	0.16 ± 0.02 b	2.56 ± 0.17 a	175.84 ± 30.56 ab	173.90 ± 29.58 a	99.96 ± 0.01 a
LY1306	0.32 ± 0.10 ab	1.89 ± 0.35 ab	136.90 ± 2.31 ab	135.06 ± 2.78 a	99.91± 0.03 ab

*Numbers are mean value ± standard deviation. Different letters following the numbers in the same column present a significant difference (P=0.05)

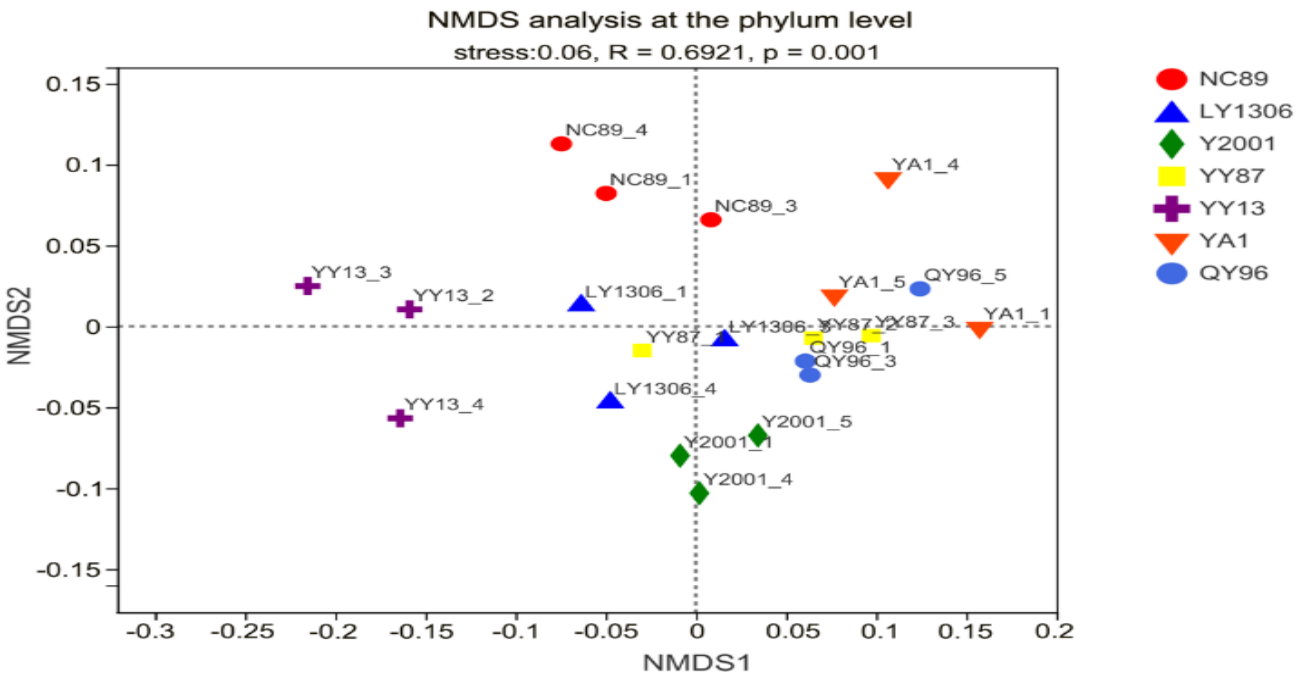

Fig. 1. Non-metric multidimensional scaling (NMDS) analysis of bacterial communities based on the Bray-Curtis similarity index. The six groups are primarily separated according to the host tobacco variety.

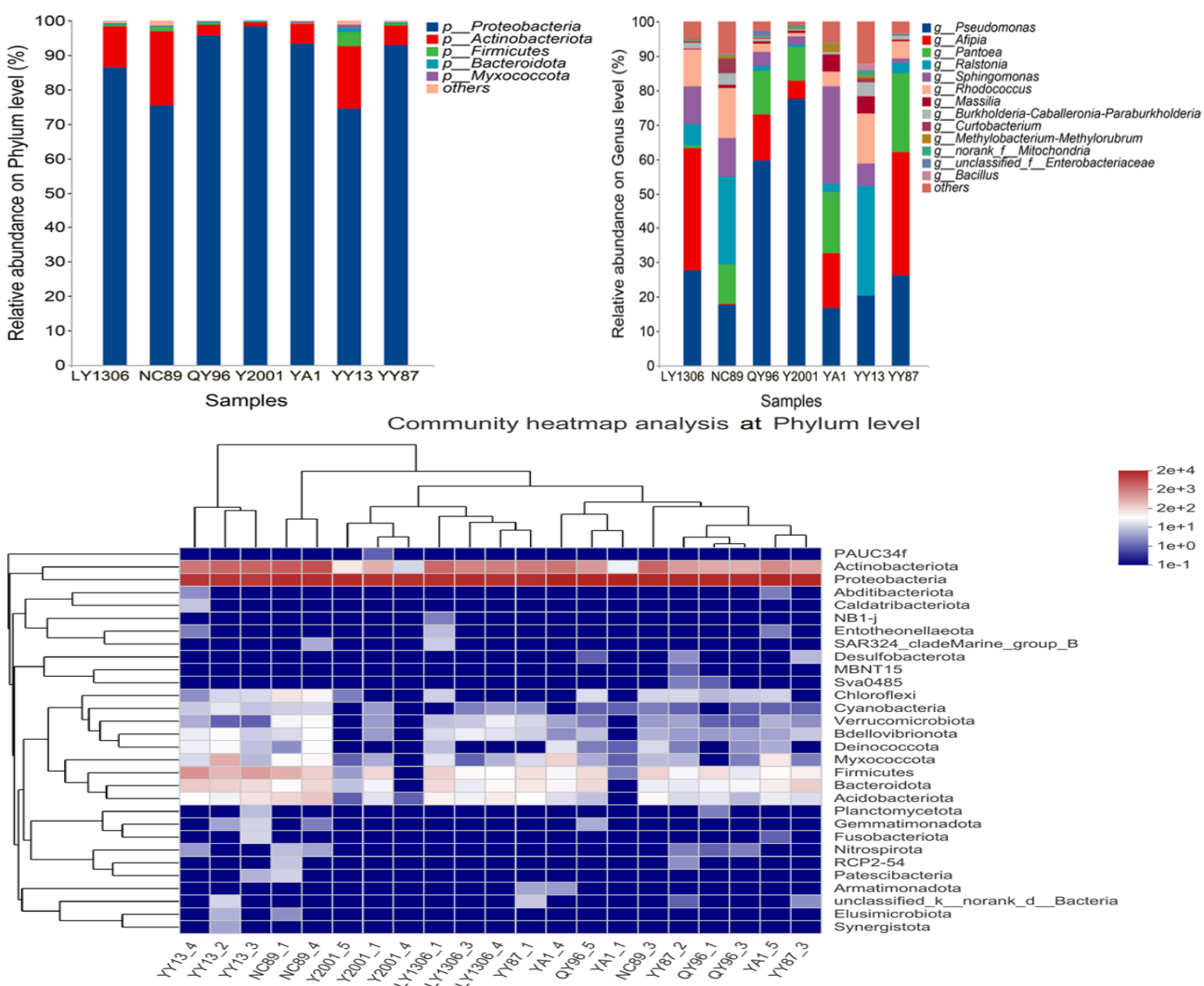

Fig. 2. Taxonomic composition of the bacterial microbiota on tobacco leaves at the phylum level (a), genus level (b), and community heatmap analysis at the phylum level (c).

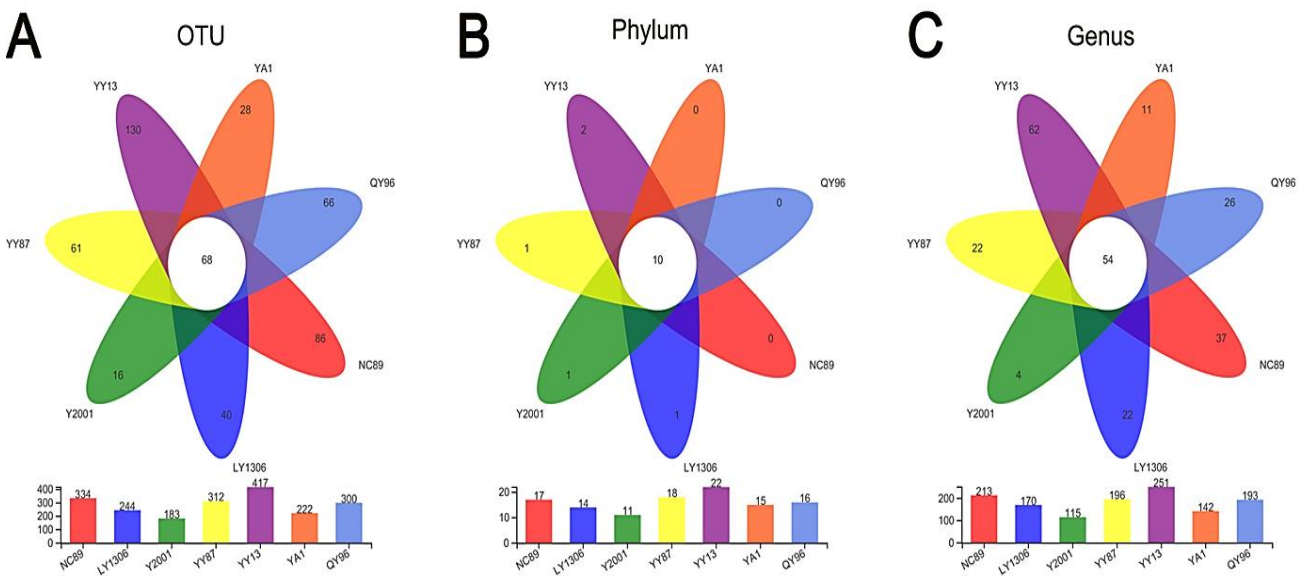

Fig. 3. Venn diagrams showing the number of shared bacterial operational taxonomic units (OTUs) (a), phyla (b), and genera (c).

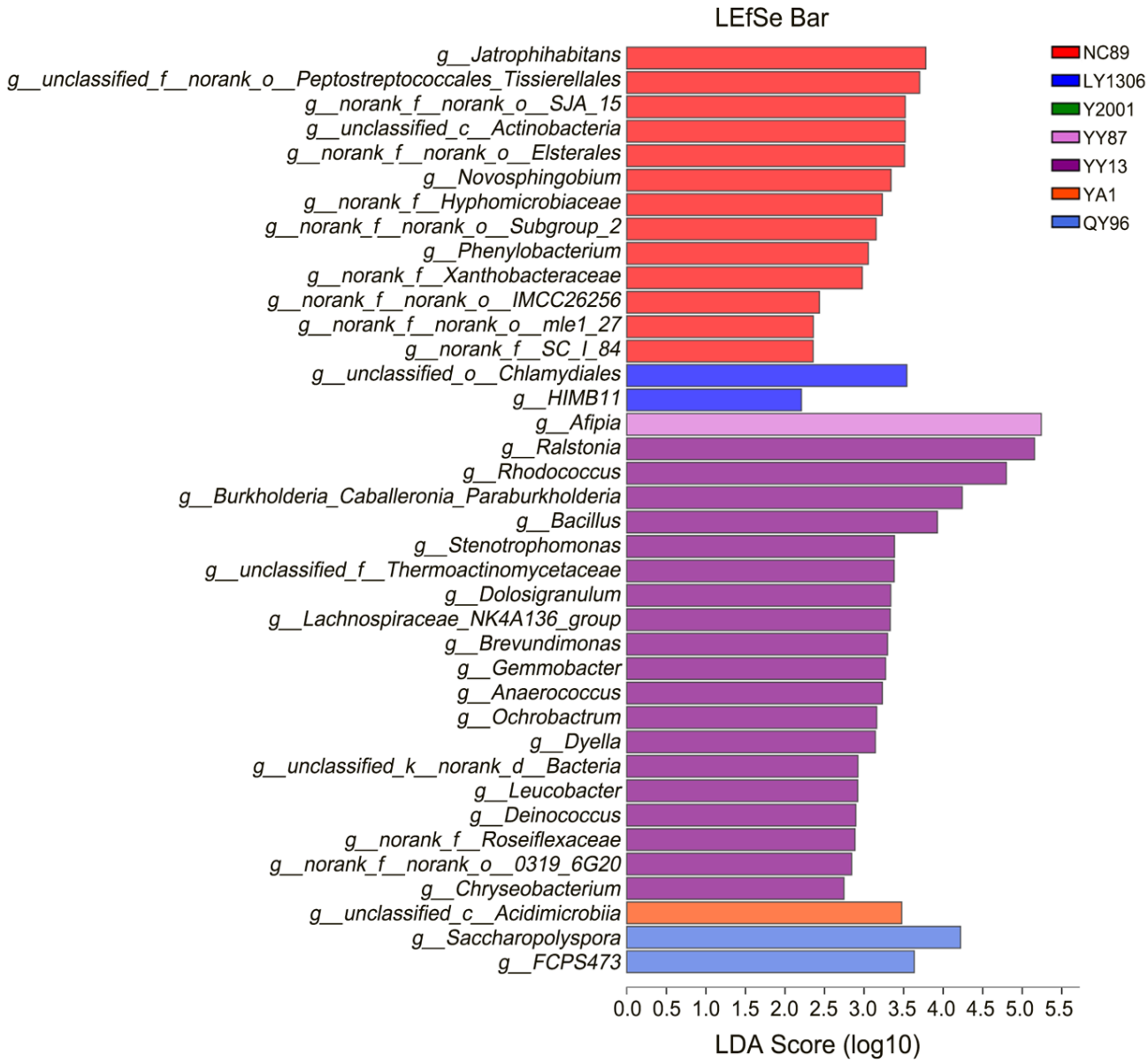

Fig. 4. Taxonomic differences at the genus level among bacterial microbiota associated with tobacco leaves of different varieties, analyzed using the linear discriminant analysis (LDA) Effect Size (LEfSe) method, with an LDA threshold of 2.0.

Supplementary Table S2. Fun KEGG pathway analysis at level 1 (n=3).

Pathway level 1	Average occupation for each pathway						
	LY1306	NC89	QY96	Y2001	YA1	YY13	YY87
Metabolism	66.30%	68.20%	63.80%	62.90%	65.40%	67.90%	64.20%
Environmental Information Processing	10.50%	9.90%	12.50%	13.20%	10.20%	10.20%	12.00%
Cellular Processes	7.70%	7.30%	8.70%	9.20%	8.00%	7.50%	8.40%
Genetic Information Processing	7.40%	7.30%	7.30%	7.20%	7.80%	7.20%	7.40%
Human Diseases	6.10%	4.90%	5.70%	5.40%	6.40%	4.90%	6.20%
Organismal Systems	2.00%	2.40%	2.10%	2.10%	2.10%	2.40%	1.90%

Supplementary Table S3. Metabolism pathway analysis at level 2 (n=3).

Pathway level 2	Average occupation for each pathway						
	LY1306	NC89	QY96	Y2001	YA1	YY13	YY87
Carbohydrate metabolism	19.30%	19.80%	19.40%	19.10%	20.00%	19.40%	19.00%
Amino acid metabolism	16.40%	17.40%	17.30%	17.90%	16.30%	17.50%	16.30%
Global and overview maps	16.50%	16.50%	16.40%	16.40%	16.40%	16.50%	16.60%
Energy metabolism	10.30%	9.10%	9.40%	9.00%	9.90%	9.20%	10.30%
Metabolism of cofactors and vitamins	8.50%	8.00%	9.10%	9.30%	8.70%	8.00%	8.90%
Xenobiotics biodegradation and metabolism	6.90%	7.10%	5.90%	5.70%	5.90%	7.30%	6.40%
Nucleotide metabolism	5.70%	5.70%	6.20%	6.30%	6.20%	5.70%	6.00%
Lipid metabolism	5.80%	5.90%	5.40%	5.30%	5.40%	6.00%	5.60%
Metabolism of other amino acids	3.80%	3.60%	3.80%	3.80%	3.90%	3.50%	4.00%
Metabolism of terpenoids and polyketides	2.60%	2.80%	2.60%	2.60%	2.50%	2.80%	2.30%
Biosynthesis of other secondary metabolites	2.20%	2.30%	2.40%	2.50%	2.50%	2.20%	2.30%
Glycan biosynthesis and metabolism	1.90%	1.80%	2.10%	2.20%	2.20%	1.80%	2.10%

A biomarker analysis using the linear discriminant analysis (LDA) Effect Size (LEfSe) method was conducted to identify taxa with significant differences in abundance among various tobacco varieties (Fig. 4). Applying an LDA threshold of 2.0, 38 bacterial genera showed statistically substantial variations across the 7 tobacco varieties. Specifically, 19 genera were enriched in YY13, 13 in NC89, 2 in both LY1306 and QY96, and 1 in YY87 and YA1, with no biomarkers detected in Y2001. The 32 most abundant bacterial genera differed between NC89 and YY13. In the YY87 sample, only one genus, Afipia, showed a significant increase in abundance, the highest among all enriched genera (Fig. 4). These findings suggested that different bacterial genera were associated with distinct tobacco varieties grown in the exact location.

Metabolic potential of leaf phyllosphere microbes of different varieties of tobacco: To demonstrate the physiological capabilities of bacterial communities and link taxonomic changes with functional roles, the differential abundances of KEGG orthologs (KOs) were analyzed to identify key genotypic features of microorganisms across 7 tobacco varieties. All samples showed detection of KOs, which were categorized into 6 primary metabolic pathways at level 1 (Suppl Table S2), 46 pathways at KEGG level 2, and 144 pathways at level 3 within the bacteriomes. These basic metabolic pathways include metabolism, genetic information processing, environmental information processing, cellular processes, human diseases, and organismal systems. The metabolic pathway was the most dominant, accounting for 62.9% to 68.2% of all pathways analyzed. Environmental information processing was the second most abundant, comprising 9.9% to 13.2%, while organismal systems had the lowest abundance, at 1.9% to 2.4%. The abundance of metabolic pathways from different systems is detailed in Suppl. Table S2, with pathways at level 2 highlighting carbohydrate metabolism, amino acid metabolism, and global overview maps as the top three, occupying 19.0% to 20.0%, 16.3% to 17.9%, and 16.4% to 16.5%, respectively. (Suppl. Table S3).

Discussion

Numerous studies indicate that both biotic (Fungi and archaea) and abiotic environmental factors (Temperature and humidity) shape the bacterial community in the phyllosphere (Muhammad *et al*., 2017; Legein *et al*., 2020; Gu *et al*., 2010; Awad *et al*., 2023). In this study, we investigated how different tobacco varieties affected the composition and diversity of leaf surface bacterial communities through 16S rRNA gene sequencing. Our findings showed no significant differences in alpha diversity among bacterial communities associated with various tobacco varieties. However, differences in tobacco varieties significantly impacted beta diversity and community structure. Previous study reported that tree species possess a distinctive bacteriome composition on their leaves, which remains consistent regardless of where they are planted globally (Ed-Dahmani *et al*., 2024). In our research, the plant variety clearly influenced the community structure of phyllosphere bacteria, as all samples were grown in the same area under similar environmental conditions. This consistent environment likely explains the similar alpha diversity, while variations in tobacco variety and origin may cause differences in beta diversity among phyllosphere bacterial communities.

Proteobacteria were the most prevalent phylum across all seven tobacco varieties, aligning with previous research

on phyllosphere bacteria in crops like spinach (Senthilkumar *et al*., 2021), flue-cured tobacco (Wu *et al*., 2025a), tea (Cernava *et al*., 2019), cigar tobacco (Xing *et al*., 2021), and rice (Thapa *et al*., 2017). These bacteria are known to colonize various niches, including the rhizosphere, which may explain their dominant presence (Xiong *et al*., 2021). The proportion of Proteobacteria differed among the varieties, ranging from 74.4% in YY13 to 98.2% in Y2001, suggesting that plant variety may influence bacterial composition within this phylum. The possible reasons are as follows: firstly, the chemical components in different tobacco leaves vary. Second, environmental changes in different periods have indirect effects on the composition of microbial communities. Although YY13 and Y2001, both from Henan Province, showed distinct levels of Proteobacteria, this indicates that the tobacco variety is a key factor shaping the bacterial community and relative abundance in the phyllosphere. The core bacterial phyla associated with tobacco leaves include Proteobacteria, Actinobacteria, and Bacteroidetes, though their abundances vary among different varieties. Previous studies have shown that these core phyla are present in Arabidopsis roots regardless of soil type or genotype (Bulgarelli *et al*., 2012; Lundberg *et al*., 2012; Yuan *et al*., 2015; Zhang *et al*., 2014a and 2014b; Schlaeppi *et al*., 2014), indicating that bacteria do not colonize randomly. Instead, specific phyla tend to preferentially colonize certain parts of the plant.

Additionally, the genetic background of tobacco varieties may influence the diversity and structure of bacterial communities on the phyllosphere (Chen *et al*., 2020). Lambais & Crowley (2015) found that similarities in phyllosphere bacterial communities were related to different tree species and were shaped by the phylogeny of the host plant. More closely related host plants hosted more similar bacterial communities. Xiong *et al*., (2021) showed that the host plant's selection pressure reduced bacterial diversity. Another study reported that tobacco phyllosphere bacterial communities were highly resilient to environmental changes (Chen *et al*., 2021). These findings suggest that the same host plant can support similar bacterial community structures. Overall, our results indicate that different tobacco varieties significantly impact the structure of phyllosphere bacterial communities, independent of their geographic origin (Fig. 1-4).

Some bacteria might have evolved specific mechanisms to survive in the plant phyllosphere. In this study, *Pseudomonas* is the main genus (16.7% to 77.0%) found in the tobacco phyllosphere, and its abundance variation may be related to different tobacco varieties. Members of the *Pseudomonas* genus can adapt to and survive in diverse environments with the help of their flagella, produce biosurfactant osmoprotectants to retain water on leaf surfaces, and release effectors to cause water to leak from cells into the apoplast. In addition, previous studies have also shown that the *Pseudomonas* genus can degrade nicotine (Chen *et al*., 2020). Yu *et al*., 2011 observed that *Pseudomonas* dominates nicotine degradation via the pyrrolidine pathway (Yu *et al*., 2011) and uses nicotine as a unique source of carbon and nitrogen (Li *et al*., 2010; Tang *et al*., 2012). In this study, the relative abundance of *Pseudomonas* varied significantly across different tobacco varieties, suggesting that members of this genus have other mechanisms to adapt to the phyllosphere of various tobacco types.

This study's second most abundant genus is *Sphingomonas* (1.2% to 28.4%), a highly competitive plant leaf colonizer, although its abundance varies greatly. Experiments have shown that leaf bacteria, such as *Sphingomonas* spp., protect plants from leaf pathogens through substrate competition (Ryffel *et al*., 2016). Carbon partitioning is important in the development of *Sphingomonas* spp. effective antagonists in the phyllosphere (Delmotte *et al*., 2009). *Sphingomonas* spp. also promote crop growth by producing plant-growth-stimulating factors (Abdallah et al., 2016). These bacteria can degrade nicotine and may be suitable for reducing nicotine in tobacco (Wang *et al*., 2011; Zhu *et al*., 2016). *Pseudomonas* and *Sphingomonas* can be used to minimize harmful substances in cigarettes and play a crucial role in tobacco fermentation; thus, they can be used as inoculants in this process (Li *et al*., 2020). Afipia was most abundant in samples LY1306 and YY87, with ratios of 35.6% and 35.7%, respectively. In contrast, the QY96 and YA1 samples showed ratios of 13.5% and 16.0%, respectively, due to differences in tobacco varieties. A previous study reported that Afipia could degrade endosulfan in tea fields, but at a lower concentration (100 μg/L) (Lian *et al*., 2023). Conversely, another study identified it as a Cd-resistant bacterium (Duan *et al*., 2020). The sample YY87 exhibited the highest content of *Pantoea* (22.9%) compared to other samples, possibly due to variations in tobacco varieties. *Pantoea* has been isolated from a wide range of ecological niches, where it plays diverse biological roles, such as plant epiphytes or endophytes, biocontrol agents, or growth promoters (Shetty *et al*., 2024). Some *Pantoea* can produce N-acyl-homoserine lactone (AHL), plant-growth hormone (indole-3-acetic acid (IAA)) (Abdallah *et al*., 2016), fix nitrogen from the atmosphere (Loiret *et al*., 2004), and establish quorum-sensing systems on leaves, enabling them to suppress pathogen growth on the leaves (De *et al*., 2022; Habibi *et al*., 2024).

Venn analysis (Fig. 3) and LEfSe analysis (Fig. 4) showed that the YY13 and NC89 tobacco varieties hosted quite different bacterial communities compared to other varieties. The results suggest that the leaf phyllosphere of these two varieties is enriched with distinct bacterial communities, with YY13 coming from Henan and NC89 originating from America. All samples displayed six basic metabolic pathways, with the dominant pathway accounting for 62.9% to 68.2% of the total. Additionally, carbohydrate and amino acid metabolism pathways were predominant, indicating that although different bacterial communities are present in the phyllosphere of various varieties, they share similar metabolic functions, likely due to the unique chemical composition of the tobacco leaves.

Conclusion

The leaf phyllosphere of various tobacco varieties displayed distinct bacterial community structures, yet shared similar metabolic pathways across all varieties, including the core microbiota. Proteobacteria are the most common and abundant phylum across all varieties. KEGG analysis showed that carbohydrate metabolism was the predominant pathway, accounting for 19.0% to 20.0% of all metabolic pathways.

Acknowledgements

The authors would like to thank every researcher who contributed to a better understanding of the bacterial biodiversity of the leaf phyllosphere of different tobacco varieties and the metabolic pathways involved.

Authors Contribution: Zongcan Yang and Wei Hu: participated in the design of this study and analyzed the data; Robina Manzoor, Jincun Yang, Shenshen Zhao and Yongfeng Yang: performed all the experiments, Duobin Mao, Junjie Zhang and Qiuling Wang: supervision, writing-review and editing, and funding acquisition. All authors contributed to the article and approved the submitted version.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding: This work was supported by the China National Tobacco Corporation Key R&D Project (110202202006) and the Henan China Tobacco Industrial Co., Ltd. Science and Technology Project (AW2022017).

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