NARDI FUNDULEA, ROMANIA ROMANIAN AGRICULTURAL RESEARCH, NO. 42, 2025
www.incda-fundulea.ro; doi.org/10.59665/rar4270 First Online: July, 2025. DII 2067-5720 RAR 2025-182

The Influence of Different Rotation Patterns on Soil Microbial and Production Quality of Flue-Cured Tobacco

Qin Yan[12#], Guoyou He[1#], Zhenchong Wang[2], Tao Yang[3*], Hafiz Ghulam Muhu Din Ahmed[3,4*], Sajid Hussain[5], Madeha O. I. Ghobashy[6,7], Rasha M. Alzayed[8], Chuisi Kong[1*]

[#]*These authors have contributed equally to this work*

[1]Institute of Agricultural Environment and Resources, Yunnan Academy of Agricultural Sciences, Kunming, 650205, China

[2]School of Agriculture and Life Sciences, Kunming University, Kunming, Yunnan 650214, China

[3]Institute of Biotechnology and Germplasm Resources, Yunnan Academy of Agricultural Sciences, Kunming, 650205, China

[4]Department of Plant Breeding and Genetics, Faculty of Agriculture & Environment, The Islamia University of Bahawalpur, 63100, Pakistan

[5]Department of Agronomy, Faculty of Agricultural Sciences and Technology (FAST), University of Layyah 31200, Pakistan

[6]Department of Biology, Faculty of Science, University of Tabuk, 71491 Tabuk, Saudi Arabia

[7]Department of Microbiology, Faculty of Science, Ain Shams University, Cairo 11566, Egypt

[8]Biology Department, College of Science, Jouf University, Sakaka 41412, Saudi Arabia

[*]Corresponding authors. E-mail: yt52279076@163.com, ahmedbreeder@gmail.com, kes@yaas.org.cn

ABSTRACT

This study explores the effect of crop rotation on soil quality and the yield and quality of flue-cured tobacco. Using a 10-year tobacco field (L1) as a control, nine rotation patterns were tested, including flue-cured tobacco-corn-tobacco (L2), tobacco-sorghum-marigold-tobacco (L4), and tobacco-tobacco-soybean-tobacco (L5). The goal was to optimize soil structure, reduce pests and diseases, alleviate nutrient imbalances, and improve tobacco yield and quality. Results showed that crop rotation significantly influenced soil microbial community structure. Rotation increased both bacterial and fungal OTUs, enhancing microbial diversity. The maize (L2) and marigold (L4) rotations had the most diverse and abundant soil bacterial communities. While fungal community richness and diversity showed no significant differences, community composition varied significantly with rotation, indicating sensitivity to specific soil factors. Regarding economic traits, except for L7 (pepper), L9 (Chinese cabbage), and L10 (sweet potato), crop rotation increased tobacco yield and output value. Specifically, L2 and L4 rotations resulted in an 18.21% increase in yield and a 20.86% increase in output value compared to continuous cropping. Additionally, L2 and L4 rotations had the highest proportion of superior quality tobacco.

Keywords: flue-cured tobacco, crop rotation, microbial community, physicochemical properties, economic traits.

INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is one of the most important cash crops in China (Wang et al., 2022). According to the data, Yunnan Province is not only the province with the largest flue-cured tobacco planting area in China, but also the main producing area of high-quality flue-cured tobacco in the world. Its tobacco output accounts for about 45% of the national total (Tang et al., 2020). With the optimization and adjustment of agricultural industrial structure and the innovation of planting system in Yunnan Province, the flue-cured tobacco planting industry has ushered in a rapid development, but at the same time, the problem of continuous cropping of flue-cured tobacco has become increasingly prominent. Long-term continuous planting the same crop or its relative species can lead to abnormal soil nutrient accumulation or excessive depletion, promote the rapid reproduction of pathogenic microorganisms, and destroy the balance of soil microbial population, and cause crop yield decline, quality deterioration and

Received 16 May 2025; accepted 18 July 2025.

frequent plant diseases and insect pests, the sustainable development of flue-cured tobacco industry constitutes a serious threat to (Chen et al., 2022). Therefore, how to reasonably and effectively alleviate the continuous cropping obstacles of Yunnan flue-cured tobacco, balance the soil fertility level and improve the soil microbiological environment has become an important problem to be solved urgently.

Rotation can inhibit the production of continuous cropping obstacles in some aspects and play a positive role in crop growth. Crop rotation, through the method of land cultivation, while providing normal facility agricultural production, using the difference in soil nutrient absorption and utilization of different crops, using different crops for rotation cultivation, which is beneficial to balance the soil.

The nutritional elements in China promote the healthy development of soil ecosystem, so as to achieve the purpose of land resource conversion and maintaining soil health (Ball et al., 2005). Rotation is more conducive to change the composition of soil microbial communities, enhancing the stability of microbial network structure, and enhance the potential ecological function of soil (Yan et al., 2024). Studies have shown that compared with flue-cured tobacco pattern, different crop rotation patterns can improve the yield and quality of flue-cured tobacco, in the autumn, respectively, eggplant, soybean, peanuts, sweet potato, late rice, corn, garlic and double cropping rice, after crop harvest, planting tobacco in the soil shows that different crop rotation has a significant impact on the growth of tobacco (Fang et al., 2011). Therefore, reasonable crop rotation can effectively balance soil nutrients, improve soil environment, and contribute to good circular planting of flue-cured tobacco.

Although there are relevant reports on the rational rotation of flue-cured tobacco, there are few studies on flue-cured tobacco rotation in Yunnan. Because different regions are affected by various factors such as light, temperature, water, gas and human activities, their planting environment and soil microenvironment are different. In view of this, in order to solve the flue-cured tobacco crop rotation selection unreasonable soil quality degradation, we selected the corn, sorghum, marigold, soybean, potato, pepper, peanuts, cabbage, sweet potato the nine crops and flue-cured tobacco rotation experiment, analytical flue-cured tobacco rotation patterns of soil microbial diversity, flue-cured tobacco production quality and regulation, provide theoretical basis and practice for flue-cured tobacco crop rotation and reasonable support.

MATERIAL AND METHODS

The experiment was conducted in Malong District, Qujing City, Yunnan Province (25°18′37″N, 103°22′29″E; elevation 1979.3 m), a major tobacco-growing region characterized by a low-latitude plateau monsoon climate with moderate temperatures, abundant rainfall (1032 mm annually), and over 1985 sunshine hours per year. The mean annual temperature is 13.6°C. The tested flue-cured tobacco variety, Yunyan 121 (a hybrid of Y97 × PY5), was raised in a unified nursery and transplanted on April 16, 2024. A randomized block design was used with 10 rotation treatments and 3 replicates (30 plots; 60 m² each). Treatments included a fallow control (L1) and nine crop rotations: maize (L2), sorghum (L3), marigold (L4), soybean (L5), tomato (L6), pepper (L7), peanut (L8), cabbage (L9), and sweet potato (L10). Post-rotation crops were planted in September 2023 and harvested in March 2024. Tobacco was then transplanted with a 1.2 m row spacing and 0.7 m plant spacing. Standard water and field management were applied throughout.

Soil and Yield Measurements

Soil samples (0-20 cm) were collected in spring and autumn using an S-pattern method (5 subsamples per plot, mixed to 1.0 kg). Samples were divided for microbial (stored at 4°C) and physicochemical analysis (air-dried). Soil properties measured included pH (pH meter), organic matter (potassium dichromate oxidation), available nitrogen (alkaline diffusion), phosphorus (colorimetry), and potassium (NH_4OAc extraction with flame photometry). Tobacco yield was measured at physiological maturity. Leaves were graded, and economic traits (yield, output value, average price, upper-grade leaf ratio, and grade index) were calculated based on 2024 local market prices. Single leaf weight was determined by averaging 2-4 replicates of 10 leaves each.

Data Analysis

Data were processed using WPS Office and IBM SPSS Statistics 27. Means and standard deviations were calculated, and one-way ANOVA ($p<0.05$) was used to test significance. Microbial diversity was assessed via 16S rRNA (bacteria) and ITS (fungi) sequencing using the SILVA and UNITE databases, respectively. OTUs and diversity indices (Chao1, Shannon, and phylogenetic diversity) were computed with MOTHUR software.

RESULTS AND DISCUSSION

Effect of crop rotation on the chemical composition of flue-cured tobacco

This study analysed the microbial community structure and production quality of flue-cured tobacco soil under various rotation modes to assess their impact on long-term tobacco soil quality. Results show that crop rotation significantly enhances the microecological environment, increasing the diversity and richness of bacterial and fungal communities. Economically, crop rotation boosted the yield and value of flue-cured tobacco, improving the industry's economic benefits. Among the rotation modes, the flue-cured tobacco-corn-flue-cured tobacco and flue-cured tobacco-marigold-flue-cured tobacco rotations were the most effective, providing both theoretical and practical insights for tobacco production in regions like Yunnan (Wang et al., 2023). Regarding chemical composition, rotation modes altered tobacco’s chemical properties, improving sweetness and taste. The highest total sugar content was observed in flue-cured tobacco-corn-flue-cured tobacco (L2), while potassium oxide levels in L6 and L7 rotations enhanced combustion. Chloride levels in some rotations were high, potentially affecting combustion. Overall, L2 and L4 rotations improved tobacco quality. Studies by Feng et al. (2023) and Huang et al. (2024) support these findings, showing similar improvements in sugar content, nitrogen, and potassium levels under crop rotation.

Table 1. Effects of crop rotation on the chemical composition of flue-cured tobacco

Handle	Total sugar (%)	Reducing sugar (%)	Total nitrogen (%)	Total phytobase (%)	Burnt potash (%)	Chloridion (%)
L1	40.34±2.79a	26.82±1.41ab	2.29±0.21a	2.65±0.53a	1.75±0.01d	0.04±0.02b
L2	45.64±5.23a	30.97±1.41a	2.00±0.41a	1.97±0.88a	1.85±0.04bcd	0.05±0.03b
L3	40.08±4.37a	27.82±1.08ab	2.23±0.43a	2.41±0.79a	2.06±0.28bc	0.11±0.02ab
L4	44.18±5.95a	28.29±1.89ab	2.08±0.42a	2.24±0.74a	2.04±0.12bc	0.10±0.07ab
L5	41.36±2.33a	27.18±0.18ab	2.39±0.51a	2.39±0.90a	1.83±0.02cd	0.04±0.03b
L6	38.86±2.39a	26.22±0.75ab	2.39±0.44a	2.41±0.82a	2.12±0.24b	0.22±0.10a
L7	37.46±2.03a	26.22±2.24ab	2.42±0.43a	2.74±1.13a	2.47±0.37a	0.14±0.07ab
L8	39.41±0.56a	26.24±2.85ab	2.27±0.29a	2.36±0.77a	2.10±0.08bc	0.22±0.12a
L9	39.14±4.26a	25.47±1.33b	2.18±0.26a	2.53±0.81a	1.99±0.03bcd	0.08±0.05b
L10	43.23±4.00a	28.69±2.48ab	1.87±0.23a	1.93±0.65a	1.42±0.10e	0.08±0.06b

Effect of crop rotation on the physiochemical properties of the rhizosphere of flue-cured tobacco

As shown in Table 2, crop rotation improved soil pH, organic matter, total nitrogen, and other indicators compared to winter idle (L1). The flue-cured tobacco-corn-flue-cured tobacco (L2), flue-cured tobacco-sorghum-tobacco (L3), and flue-cured tobacco-soybean-flue-cured tobacco

(L5) rotations had higher soil organic matter and total nitrogen, enhancing soil fertility and nutrient absorption. These rotations also slightly increased soil pH, improving soil acid-base balance. The flue-cured tobacco-marigold-tobacco (L4) and flue-cured tobacco-pepper-tobacco (L7) rotations had higher total phosphorus and potassium, likely due to the nutrient uptake and root secretion characteristics of these crops. Overall, L2, L3, and L5 improved the physical and chemical properties of the rhizosphere soil. Previous studies by (Zhou et al., 2023; Qin et al., 2024) support these findings, showing that crop rotation enhances soil organic matter, nitrogen, and phosphorus, while reducing potassium levels in some cases.

Table 2. Effect of crop rotation on physicochemical properties of the rhizosphere of flue-cured tobacco

Handle	pH price	Organic matter (g/kg)	Total nitrogen (g/kg)	Total phosphorus (g/kg)	Total potassium• (g/kg)	Hydrolytic nitrogen (mg/kg)	Quick-acting potassium (mg/kg)
L1	4.67±0.12b	33.48±2.46b	1.78±0.19a	0.94±0.08a	11.93±0.26a	170.61±19.38b	390.49±59.37a
L2	4.75±0.13b	36.55±2.96b	2.06±0.10a	0.86±0.09a	11.35±2.74a	161.65±18.20b	242.97±52.64ab
L3	4.84±0.16ab	39.44±3.06a	1.77±0.11a	0.83±0.08a	11.06±0.76a	155.14±15.91bc	235.87±68.92ab
L4	4.37±0.05b	35.62±3.04b	1.64±0.06a	0.85±0.07a	13.72±1.42a	144.55±13.29bc	351.59±21.69ab
L5	5.04±0.19a	37.38±3.75b	1.76±0.09a	1.22±0.10a	12.79±0.58a	192.24±27.59a	394.62±61.12a
L6	5.26±0.24a	43.01±2.95a	2.09±0.17a	1.10±0.09a	12.93±0.87a	189.13±35.26a	326.44±43.83ab
L7	4.92±0.13ab	36.86±3.22b	1.76±0.13a	1.10±0.09a	14.86±0.12a	148.62±14.72bc	414.92±24.96a
L8	4.78±0.13b	38.75±4.39ab	1.85±0.16a	0.95±0.09a	12.13±0.91a	157.58±14.17bc	383.12±60.01a
L9	4.57±0.09b	37.73±3.61b	1.78±0.12a	0.96±0.08a	11.62±0.81a	163.28±20.01b	332.17±32.86ab
L10	4.75±0.12b	34.04±4.29b	1.65±0.18a	0.85±0.08a	11.68±0.49a	135.59±11.86c	289.60±42.53ab

Effect of crop rotation on the economic traits of flue-cured tobacco

As shown in Table 3, different rotation patterns had varied impacts on the economic traits of flue-cured tobacco. Compared with the winter fallow (L1) treatment, most rotation treatments improved both yield and output value, except for pepper (L7), cabbage (L9), and sweet potato (L10), which showed a reduction. Notably, marigold (L4) rotation resulted in the highest yield and output value, increasing by 18.21% and 20.86%, respectively. Similarly, the corn (L2) rotation also showed considerable improvements.

These findings align with previous studies indicating that crop rotation enhances agronomic traits, boosts root activity, and improves both yield and economic returns of flue-cured tobacco (Shen et al., 2022). Previously author (Liu et al., 2022) observed that rotations with wheat, rapeseed, and potato optimized soil microbial communities and nutrient profiles, enhancing yield and leaf quality. Zhong et al. (2024), and Wang et al., (2024a) demonstrated that intercropping with medicinal herbs like patchouli, perilla, and isatis increased the proportion of high-quality tobacco leaves and their market value. Similarly, Svotwa and Mahiya (2017) reported that rotations with basil and fennel promoted plant growth and reduced disease incidence, ultimately improving yield and output value. These results collectively suggest that rotation and intercropping systems contribute to a more balanced nutrient supply, disease suppression, and enhanced economic performance of flue-cured tobacco (Biao et al., 2015).

Table 3. Effect of crop rotation on the economic traits of flue-cured tobacco

Handle	Acre yield (kg)	Acreen output value (yuan)	Average price (yuan/kg)	Better smoke than (%)
L1	165.32±13.21abc	5100.89±243.24b	30.91±0.84ab	0.67±0.02b
L2	174.19±9.35ab	5642.28±235.46ab	32.42±0.52a	0.74±0.01a
L3	179.89±5.68ab	5634.29±354.35ab	31.64±0.73ab	0.67±0.03b
L4	192.15±11.64a	6060.91±464.36a	31.56±0.79ab	0.73±0.02a
L5	165.26±7.14abc	5058.42±394.17b	30.84±0.48ab	0.67±0.01b
L6	167.88±6.41abc	5034.81±556.28b	30.75±0.69ab	0.56±0.04c
L7	145.49±9.85c	4276.57±476.59c	29.49±0.73ab	0.61±0.01c
L8	180.33±6.95ab	5648.72±310.14ab	30.14±0.28ab	0.67±0.03b
L9	164.91±9.37abc	5002.62±111.92b	30.31±0.26ab	0.66±0.03b
L10	165.72±13.12abc	5103.48±249.12b	30.92±0.87ab	0.67±0.02b

Diseases of flue-cured tobacco in a crop rotation mode

The incidence of flue-cured tobacco as shown in Table 4, the incidence of sorghum (L3)> sweet potato (L10)> winter (L1)> cabbage (L9)> pepper (L7)> potato (L6)> soybean (L5)> peanut (L8)> corn (L2), marigold (L4) (L4), the highest sorghum (L3) rotation pattern than corn (L2), marigold (L 4) rotation incidence. Compared with the winter leisure block (L1), except for the high incidence and disease index of flue-cured tobacco in sorghum (L3) and sweet potato (L10) plots, the incidence and disease index of flue-cured tobacco were mild, and the incidence of flue-cured tobacco in marigold (L4) was the least. (Biao et al., 2015) studied the single cropping of flue-cured tobacco and the intercropping of 1:2 (line ratio, same below) between flue-cured tobacco and ginger, 1:3 For, 1:4 intercropping four treatment of flue-cured tobacco yield and quality, soil physical and chemical properties, the results show that flue-cured tobacco intercropping ginger can significantly promote tobacco growth, significantly improve tobacco canopy form, with flue-cured tobacco and ginger 1:2 intercropping red star disease best effect, and flue-cured tobacco intercropping ginger tobacco chemical composition more harmonious, flue-cured tobacco smoke proportion, average price and output value are increased. (Qin et al., 2023) showed that the incidence of flue-cured tobacco wilt blight, black tibia, mosaic disease, red star disease and other diseases decreased under the rotation of flue-cured tobacco and red star disease, and the disease index also decreased. The rotation of flue-cured tobacco and other crops can effectively alleviate the obstacles of continuous cropping, reduce the occurrence of diseases (especially root diseases), improve the yield and quality of tobacco leaves, increase the income of tobacco farmers, and improve the land utilization rate and soil fertility (Irshad et al., 2024).

Table 4. Diseases of flue-cured tobacco root black rot in crop rotation mode

Handle	Attack (%)	Disease index
L1	13.19±2.41a	11.04±1.75a
L2	9.03±4.33a	8.26±3.54a
L3	21.53±10.69a	19.83±8.97a
L4	9.03±3.18a	8.03±2.76a
L5	11.11±7.32a	9.88±6.17a
L6	12.07±5.32a	11.23±4.90a
L7	12.50±3.61a	11.57±3.64a
L8	9.72±3.18a	8.41±3.16a
L9	12.51±2.09a	10.03±2.93a
L10	13.20±7.89a	12.58±7.79a

Analysis of the sequencing results of soils with different rotation patterns

The dilution curve is a curve constructed based on the number of individuals and species number by randomly selecting a certain number of individuals from the sample and counting the number of species represented by these individuals. Dilution curves can be used to measure whether sampling size is reasonable and to compare the richness of sample species with varying amounts of sequencing. The community dilutions curve of bacteria (A) and fungi (B) in soil samples gradually flattened out with the deepening of sequencing data, indicating that the further increase of sequences will not cause the emergence of more new OTU. It can be seen that the soil microbial community has high confidence, which can comprehensively reflect the real situation of the microbial community and structure of the rhizosphere soil samples, which has certain research significance and lays a foundation for further analysis (Lynn et al., 2021; Bui et al., 2023). Therefore, the sequencing depth and amount of data in this study are reasonable (Figure 1).

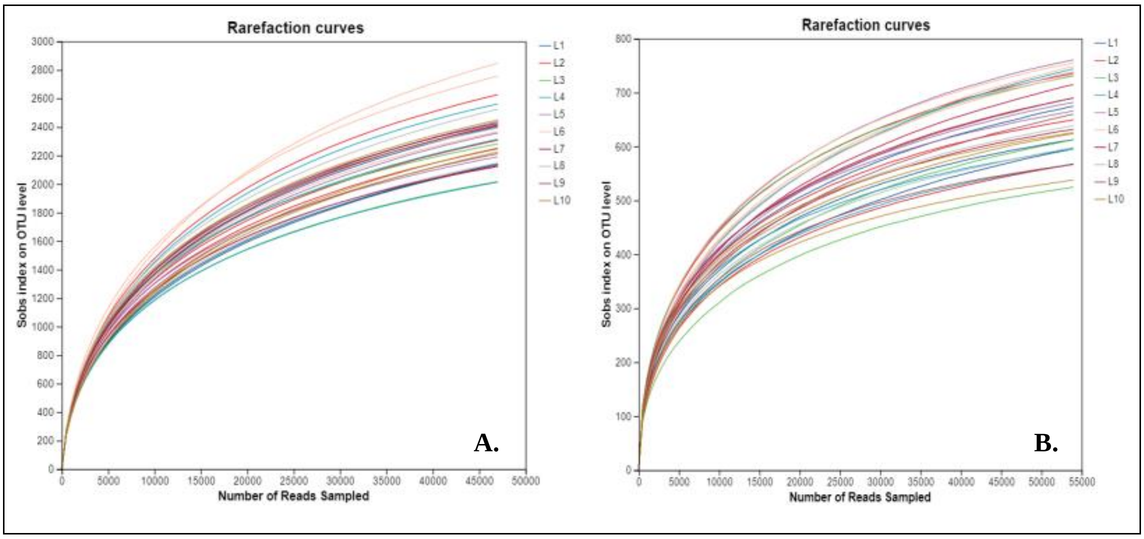

Figure 1. Dilution curves of bacteria (A) and fungi (B) with different rotation patterns

Effect of different rotation patterns on the number of soil microbial OTUs

Figure 2 shows the number of unique OTUs for bacteria and fungi in different rotation modes. The total number of OTUs was 1653, with L1 having 198 unique bacterial OTUs and L2, L3, L4, L5, L6, L7, L8, L9, and L10 having 269, 155, 399, 202, 573, 219, 217, 243, and 250, respectively. Fungal OTUs totalled 373, with L1 having 74 unique OTUs and other rotation modes showing higher diversity (L2: 99, L3: 80, L4: 136, etc.). Soil bacterial numbers were significantly higher than fungal numbers, with L4, L5, and L6 showing the greatest increase in bacterial OTUs. These rotations enhanced soil microbial communities, promoting nutrient cycling and crop growth (Lynn et al., 2021). Rotation systems like rice-soybean and flue-cured tobacco-Salvia miltiorrhiza increased microbial abundance and beneficial flora, improving soil health and flue-cured tobacco yield, consistent with this study's findings.

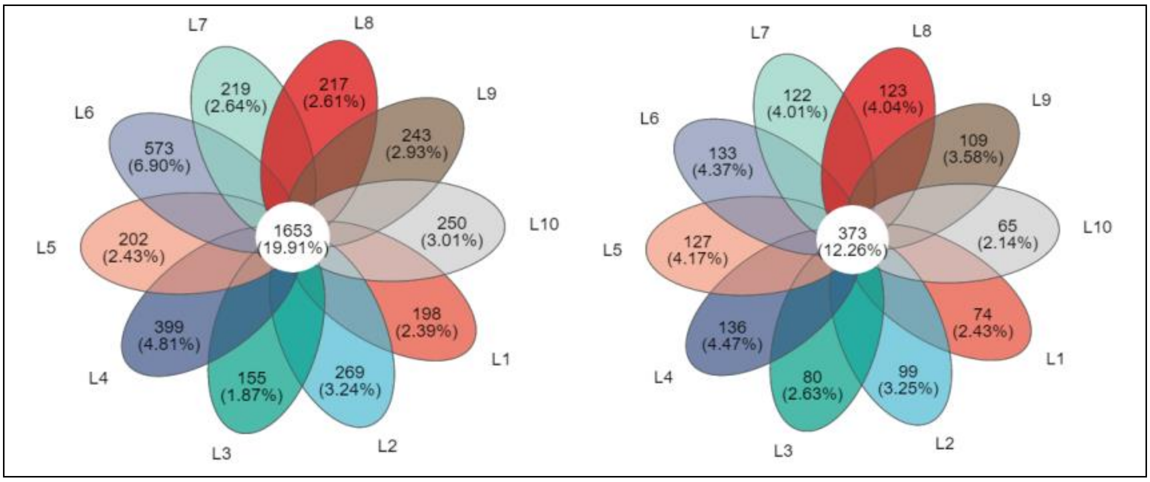

Figure 2. Number of common and unique OTUs in different rotation patterns

The effect of different rotation patterns on soil microbial α diversity

In the Alpha diversity index table, we used the Ace, Chaol, Shannon, and Simpson indices to assess soil microbial diversity. The Ace and Chaol indices reflect species richness, while the Shannon index indicates community diversity. Table 5 shows that maize (L2) and marigold (L4) rotations had the highest Ace and Chaol indices, indicating the richest and most abundant species. Maize (L2) and sorghum (L3) rotations had the lowest richness. Previous studies by (Wu et al., 2016; Paranavithana et al., 2021) found that crop rotations can enrich microbial populations and improve bacterial diversity, consistent with our results. For soil fungi, there were no significant changes in richness or diversity across the rotation patterns. Fungal content is crucial for soil fertility and root vitality, as fungi help balance soil ecosystems, preventing the overgrowth of pathogenic species and reducing crop diseases (Akpor et al., 2023; Zhou et al., 2023).

Table 5. Analysis of α diversity of soil microbial communities in different rotation patterns

Class	Handle	Enrichment index	Diversity indices	The Shannon index	The Simpson index
		Ace index number	Chaol index number		
Thin fungus	L1	2887.82±165.37a	2814.16±138.12a	5.93±0.15a	0.0081±0.0021a
	L2	3378.13±379.29a	3264.31±319.23a	6.16±0.16a	0.0056±0.0004a
	L3	2808.86±285.75a	2762.06±282.79a	6.09±0.14a	0.0059±0.0011a
	L4	3029.61±220.03a	2955.24±179.87a	6.15±0.11a	0.0055±0.0004a
	L5	2953.85±187.42a	2906.27±144.66a	6.08±0.08a	0.0063±0.0011a
	L6	2875.69±313.40a	2813.94±289.08a	5.99±0.15a	0.0080±0.0024a
	L7	2879.78±252.99a	2801.79±226.62a	6.09±0.05a	0.0061±0.0005a
	L8	2985.00±271.19a	2910.11±299.76a	6.10±0.11a	0.0061±0.0009a
	L9	2839.94±166.17a	2777.84±156.14a	6.09±0.04a	0.0070±0.0021a
	L10	2963.41±100.70a	2889.88±95.72a	6.00±0.20a	0.0071±0.0021a
True fungus	L1	771.44±61.72a	767.11±71.14a	3.89±0.14a	0.0603±0.0106a
	L2	833.34±69.92a	823.14±63.02a	4.09±0.03a	0.0476±0.0027a
	L3	742.34±119.53a	746.65±124.04a	3.89±0.40a	0.0621±0.0386a
	L4	820.38±87.58a	808.38±97.55a	4.07±0.35a	0.0542±0.0340a
	L5	907.49±30.23a	889.27±22.73a	4.10±0.14a	0.0407±0.0052a
	L6	769.21±90.09a	757.51±86.94a	3.85±0.37a	0.0664±0.0352a
	L7	767.99±146.06a	764.97±128.84a	3.98±0.14a	0.0483±0.0054a
	L8	799.51±99.83a	803.82±92.13a	3.85±0.22a	0.0675±0.0250a
	L9	769.32±105.93a	768.63±94.68a	3.95±0.28a	0.0543±0.0193a
	L10	701.53±65.06a	696.23±56.28a	3.83±0.47a	0.0849±0.0651a

The effect of different rotation patterns on soil microbial β diversity

β-diversity analysis was used to compare differences in microbial communities across rotation patterns. Based on sequencing data, relative abundance and genetic distances were calculated to assess community composition. Principal Component Analysis (PCA) revealed that Axis 1 and Axis 2 explained 22.23% of the variance in bacterial (Figure 3A) and 14.81% in fungal communities (Figure 3B), indicating significant structural differences, particularly among fungal populations. The dispersion of points and lines in the PCA plots demonstrates that crop rotation altered microbial communities, with notable differences in marigold (L4) and soybean (L5) rotations compared to the winter fallow control (L1). The r values were 0.161 (bacteria) and 0.196 (fungi), highlighting greater sensitivity and variability in fungal communities under different rotations, likely due to shifts in soil pH and organic matter. These findings align with previous studies. (Liu et al., 2020) reported cultivation systems significantly affect soil microbial structure. (Gai et al., 2023) found that marigold-tobacco rotation enhanced microbial diversity indices, improving the rhizosphere environment and mitigating continuous cropping effects. Similarly, Jiao and Yuan (2019), showed that crop rotations involving grain amaranth and black beans increased microbial diversity while reducing bacterial richness in grain stubble.

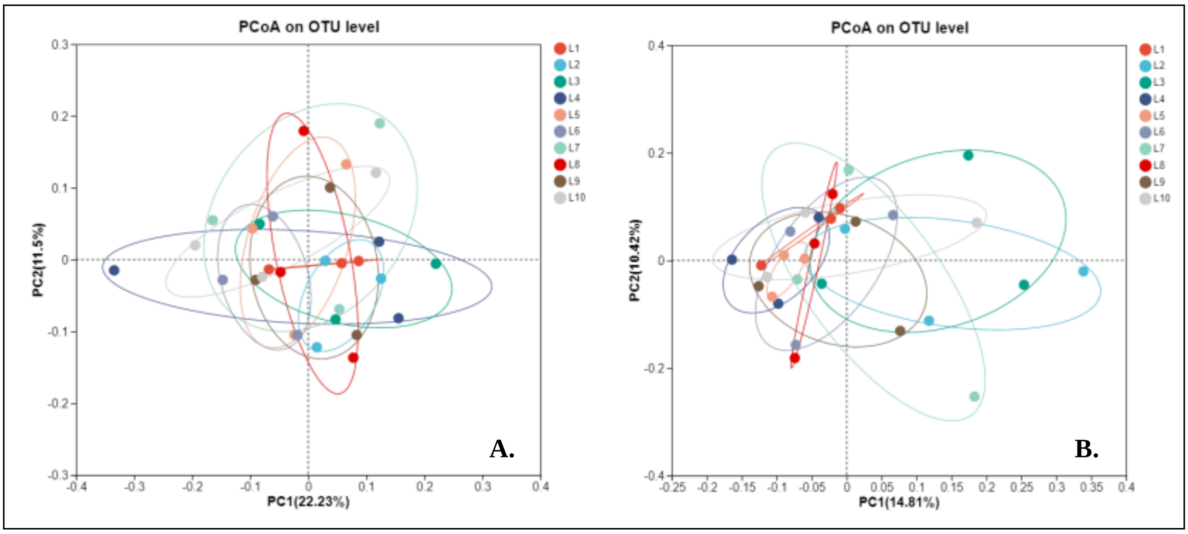

Figure 3. PCoA analysis of soil microbial communities with different rotation patterns

Effect of different rotation patterns on soil microbial community structure

Effect of different rotation patterns on community structure at the soil bacterial phylum level

A total of 32 bacterial phyla were identified across treatments, with higher relative abundances observed for Chloroflexi (22.28-28.16%), Proteobacteria (18.95-25.39%), Actinobacteriota (18.36-21.82%), Acidobacteriota (7.37-10.61%), Gemmatimonadota (2.65-4.56%), and Firmicutes (2.39-4.69%) (Figure 4). Chloroflexi abundance was lowest under soybean rotation (L5), suggesting soybean may inhibit its growth. Proteobacteria, key in nitrogen cycling, varied across treatments, indicating altered soil nutrient dynamics. Acidobacteriota, common in acidic soils, showed changes likely due to pH shifts, while Firmicutes, which form spores, may reflect environmental stress. Despite low relative abundance, taxa like Patescibacteria, Planctomycetota, and Myxococcota may play critical roles in organic matter decomposition and nutrient cycling (Wang et al., 2024b). Soybean rotation (L5) significantly affected the community, notably reducing Chloroflexi and increasing Actinobacteria. These shifts are likely influenced by rhizosphere secretions and nitrogen fixation by soybean-rhizobia symbiosis. (Fierer et al., 2007) noted that carbon/nitrogen enrichment increases eutrophic Proteobacteria and reduces oligotrophic taxa, consistent with our results. (Chen et al., 2025) also reported dominant phyla like Proteobacteria and Acidobacteria across treatments, echoing our findings. Flue-cured tobacco rotations with crops like corn, marigold, and pepper (L2-L4, L6-L10) altered bacterial community composition by modifying soil conditions. Overall, all treatments except L5 increased Chloroflexi and decreased Actinobacteria, indicating rotation patterns significantly shape soil bacterial communities.

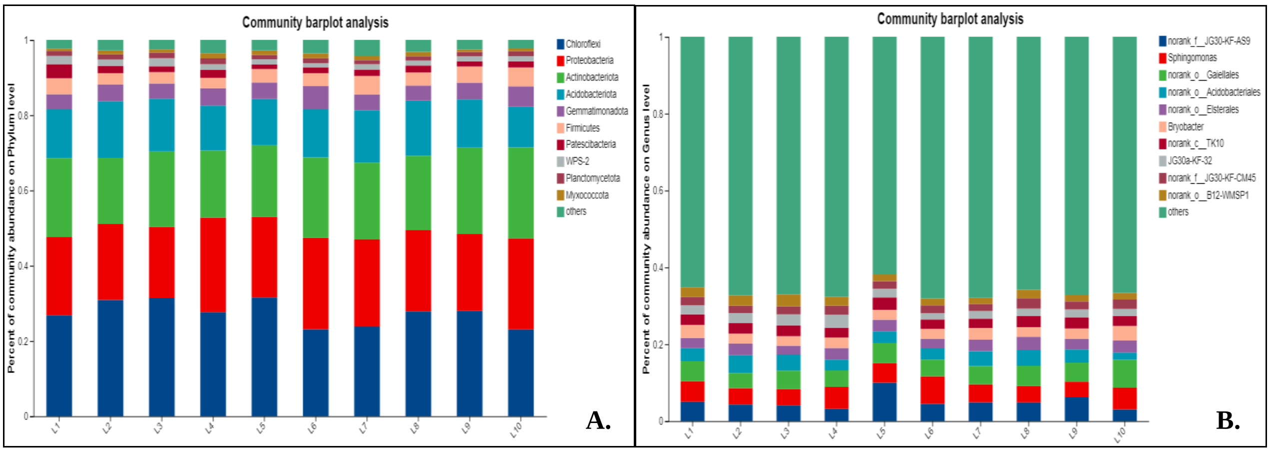

Figure 4. Relative abundance of major colonies at the level of soil bacterial phyla in different rotation patterns

Effect of different rotation patterns on community structure of soil bacterial genera

Soil Bacterial Community Composition at the Genus Level under Different Rotation Patterns.

A total of 1,153 bacterial genera and undetermined taxa were identified (Figure 4B). Among these, norank_f_JG30-KF-AS9 (2.71-9.91%), Sphingomonas (3.83-5.82%), norank_o_Gaiellales (4.35-5.41%), and Bryobacter (2.16-3.87%) exhibited high relative abundance. The soybean rotation treatment (L5) notably increased the abundance of these genera, suggesting that soybean root exudates or rhizosphere interactions may favor their proliferation during flue-cured tobacco growth. Sphingomonas, involved in organic matter decomposition and transformation, likely benefited from L5-induced changes in soil chemical properties. Similarly, the increase in norank_o_Gaiellales, known for its role in nutrient cycling, indicates improved soil nutrient dynamics under L5.

The significant enhancement of key bacterial genera under L5 is likely attributed to soybean's nitrogen-fixing ability and the selective influence of its root exudates. (Yan et al., 2024) reported that field rotation with crops like sweet wormwood significantly promotes nitrogen-transforming bacteria such as Nitrospira, enhancing nitrogen availability for subsequent crops. Feng et al. (2023) also observed improved soil nutrients and microbial diversity, including increased actinobacteria, under flue-cured tobacco-colored wheat rotation, aligning with our findings. While treatments L2-L4 and L6-L10 did not show specific changes in these dominant genera, differences in overall bacterial community structure suggest that crop-specific traits - such as exudate composition, growth cycle, and nutrient uptake - drive microbial variability across rotation patterns.

Effect of different rotation patterns on horizontal community structure in soil fungal phylum

A total of 15 fungal phyla and undetermined taxa were identified across treatments (Figure 5). Dominant phyla included Ascomycota (53.38%-69.71%), Basidiomycota (18.82%-30.21%), and

Mortierellomycota (8.93%-11.27%). Ascomycota, containing many plant pathogenic fungi, was reduced by 6.92% in the flue-cured tobacco–maize–flue-cured tobacco rotation (L2), suggesting a suppression of soil-borne pathogens. Basidiomycota and Mortierellomycota are involved in organic matter decomposition and nutrient cycling, with their abundance reflecting soil condition changes. Although fungal species composition remained stable, rotation patterns altered the abundance of dominant taxa, affecting soil nutrient transformation and plant nutrient uptake efficiency. The L2 rotation was particularly effective in reducing Ascomycota, and other rotations likely influenced fungal phyla through changes in soil properties. Previous studies by Meng et al. (2022) and Gong et al. (2024) reported similar findings, showing that rotation improves microbial richness, reduces soil-borne pathogens, and enhances agricultural productivity.

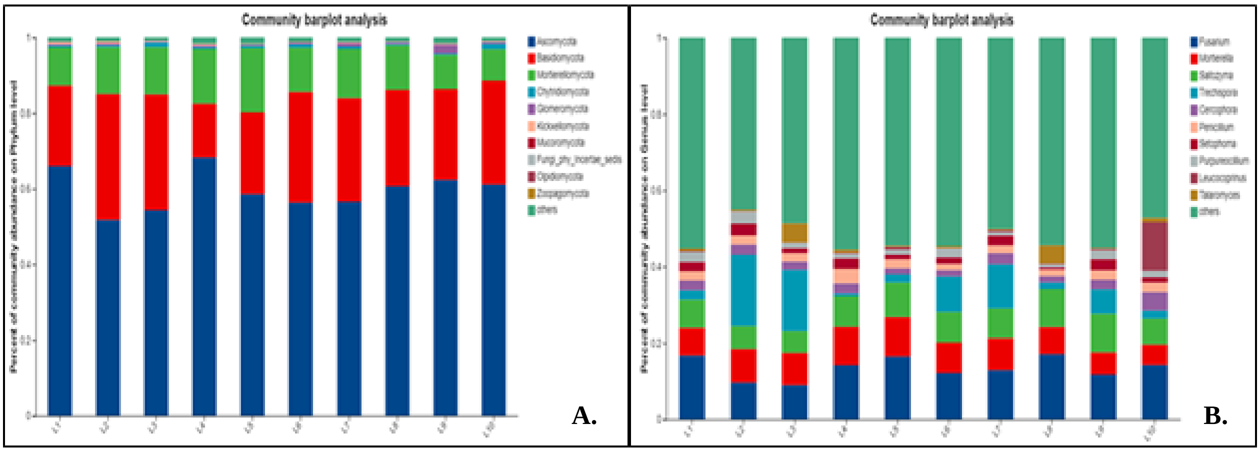

Figure 5. Relative abundance of major colonies at soil fungal genus level in different rotation patterns

Effect of different rotation patterns on soil fungal genus-level community structure

A total of 340 fungal genera, including unidentified groups, were detected (Figure 5B). Dominant genera included Fusarium, Mortierella, Saitozyma, and Trechispora. Fusarium, a known tobacco pathogen causing root rot, showed significantly reduced abundance under maize (L2) and sorghum (L3) rotation treatments, indicating their potential to lower soilborne disease risk. Interestingly, some Fusarium strains may also enhance disease resistance and improve tobacco yield (Rui et al., 2023; Xie et al., 2024).

Mortierella, associated with nutrient-rich soils, can decompose organic matter, enhance nutrient cycling, and suppress pathogens (Hammad et al., 2017; Gonçalves Diniz et al., 2020). Its consistently high abundance highlights its ecological importance. Although fungal species composition remained relatively stable across treatments, crop rotations notably reduced the abundance of dominant genera, especially Fusarium. These results suggest that flue-cured tobacco rotations can improve soil microbial health by reducing pathogens and promoting beneficial fungi.

CONCLUSIONS

This study through comparative analysis of different rotation patterns of flue-cured tobacco soil microbial community structure, flue-cured tobacco production quality, found that crop rotation as an effective agricultural measures, can significantly improve the micro ecological environment of tobacco soil, improve soil microbial community diversity and richness, optimize the soil structure, and contribute to the growth of flue-cured tobacco and yield quality. Specifically, maize and marigold perform well as crop rotation crops, which can not only significantly improve the diversity of soil bacterial communities, but also increase the yield and output value of tobacco leaves. In contrast, rotation crops such as pepper, cabbage and sweet potatoes contribute less to the growth and economic traits of flue-cured tobacco, and even perform poorly in some aspects. Therefore, from the perspective of optimizing soil structure, improving the yield, quality and economic value of tobacco leaves, it is suggested to adopt the rotation mode of flue-corn-flue-cured tobacco and flue-cured tobacco-marigold-flue-flue-cured tobacco in major flue-cured tobacco producing areas such as Yunnan. These crop rotation modes can not only alleviate the continuous cropping obstacles but also provide strong support for the sustainable production of flue-cured tobacco. The results of this study provide theoretical basis and practical guidance for rational rotation of flue-cured tobacco, which is of great significance for promoting the healthy development of flue-cured tobacco industry.

ACKNOWLEDGEMENTS

This work was funded by Yunnan Province Cai Peng Expert Workstation (Grants No. 202405AF140079), the Yunnan Major special research project "Creation and application of special bio-fertilizer for Plateau characteristic economic crops in Yunnan Province" (Grants No. 202202AE090015). This research was supported by the Research, Development, and Innovation Authority (RDIA), Kingdom of Saudi Arabia (Grant No. 13445-Tabuk-2023-UT-R-3-1-SE), and the Deputyship for Research & Innovation, Ministry of Education, Saudi Arabia (Project No. 0011-1442-S).

REFERENCES

Akpor, O., Ezekudo, E., Sobajo, O., Edoh, P., Mabayoje, S., 2023. *Optimization and antimicrobial properties of biosurfactant production by four indigenous soil bacterial species*. Asian J. Agric. Biol., 2023: 2022146.

Ball, B., Bingham, I., Rees, R., Watson, C., Litterick, A., 2005. *The role of crop rotations in determining soil structure and crop growth conditions*. Canadian Journal of Soil Science, 85(5): 557-577.

Biao, T., Xi-zhou, Z., Xian-bin, Y., 2015. *Effects of tobacco garlic crop rotation and intercropping on tobacco yield and rhizosphere soil phosphorus fractions*. Yingyong Shengtai Xuebao, 26(7): 1977-1984.

Bui, V.H., Nguyen, H.C., Ngo, Q.H., 2023. *Establishment of rice yield prediction model using soil compaction*. Asian J. Agric. & Biol., 2023(2), 202109327.

Chen, D., Zhou, Y., Wang, M., Mujtaba Munir, M.A., Lian, J., Yu, S., Dai, K., Yang, X., 2022. *Succession pattern in soil micro-ecology under tobacco (Nicotiana tabacum L.) continuous cropping circumstances in Yunnan province of southwest China*. Frontiers in Microbiology, 12: 785110.

Chen, W., Zhang, S., Wang, B., Zhang, M., Ding, D., He, J., 2025. *The Combined Application of Biofertilizer Alleviates the Continuous Cropping Obstacles of Replanted Zanthoxylum bungeanum: A Preliminary Study*. Plants, 14(6): 854.

Fang, S.-M., Tang, L.-N., Chen, S.-H., Gu, G., Chen, Y.-S., 2011. *Influence of crop rotation on tobacco bacterial wilt number and pathogenesy*. Chinese Journal of Eco-Agriculture, 19(2): 377-382.

Feng, J., Xia, T., Wu, C., Chen, Z., Li, Z., Bai, Y., Yang, L., Gong, Y., Zhai, N., Tang, Z., 2023. *Current research and perspective of soil nutrients spatial variation characteristics in flue-cured tobacco planting soil in China*. Eurasian Soil Science, 56(10): 1432-1441.

Fierer, N., Bradford, M.A., Jackson, R.B., 2007. *Toward an ecological classification of soil bacteria*. Ecology, 88(6): 1354-1364.

Gai, X.-T., Lu, C.-H., Xia, Z.-Y., Huang, C.-J., Hu, Y.-X., Wang, J.-M., Fang, D.-H., 2023. *Crop rotation suppresses tobacco black shank disease incited by Phytophthora nicotianae and influenced the structure of rhizosphere bacterial communities*. Journal of Plant Pathology, 105(3): 673-682.

Gonçalves Diniz, A., Barbosa, L.F.S., Santos, A.CdS., Oliveira, N.Td., Costa, A.Fd., Carneiro-Leão, M.P., Tiago, P.V., 2020. *Bio-insecticide effect of isolates of Fusarium caatingaense (Sordariomycetes: Hypocreales) combined to botanical extracts against Dactylopius opuntiae (Hemiptera: Dactylopiidae)*. Biocontrol Science and Technology, 30(4): 384-395.

Gong, B., He, Y., Luo, Z., Peng, H., Cai, H., Zhu, Y., Bin, J., Ding, M., 2024. *Response of rhizosphere soil physicochemical properties and microbial community structure to continuous cultivation of tobacco*. Annals of Microbiology, 74(1): 4.

Hammad, I., Abdel-Razik, A., Soliman, E., Tawfik, E., 2017. *Transgenic potato (Solanum tuberosum) expressing two antifungal thionin genes confer resistance to Fusarium spp*. J. Pharm. Biol. Sci., 12: 69-79.

Huang, J., Wang, X., Yang, L., Li, Y., Xia, B., Li, H., Deng, X., 2024. *Analysis of tobacco straw return to the field to improve the chemical, physical, and* *biological soil properties and rice yield*. Agronomy, 14(5): 1025.

Irshad, M., Ondrasek, G., Iqbal, A., 2024. *Influence of anaerobic digestate type on nitrogen dynamics and leaching losses across two soils*. Asian J. Agric. & Biol., 2024(4): 2024041.

Jiao, Y., and Yuan, L., 2019. *Positive effects of increasing crop diversity in land use on soil microbial biomass, enzyme activity and bacterial community composition*. Soil Research, 57(7): 779-787.

Liu, J.-Q., Peng, G.-J., Zheng, Z.-Y., Tang, C.-G., Zhou, Q.-M., Li, Q., Li, J., 2022. *Effects of vertical rotary tillage and straw decomposed organic fertilizer on soil nutrients, yield and quality of flue-cured tobacco in Hunan province*. Southwest China Journal of Agricultural Sciences, 35(2): 397-404.

Liu, X., Li, Y., Ren, X., Chen, B., Zhang, Y., Shen, C., Wang, F., Wu, D., 2020. *Long-term greenhouse cucumber production alters soil bacterial community structure*. Journal of Soil Science and Plant Nutrition, 20: 306-321.

Lynn, T.M., Zhran, M., Wang, L.F., Ge, T., Yu, S.S., Kyaw, E.P., Latt, Z.K., Htwe, T.M., 2021. *Effect of land use on soil properties, microbial abundance and diversity of four different crop lands in central Myanmar*. 3 Biotech, 11(4): 154.

Meng, X.-J., Liu, Y., Li, Y.-Y., Xu, R.-B., Li, C.-R., Zhou, Y., Sun, Z.-X., Li, X.-H., 2022. *Effects of tobacco-buckwheat rotation on regulation of tobacco black shank prevention and control and soil microenvironment*. Journal of Southern Agriculture, 53(6): 1525-1535.

Paranavithana, T., Marasinghe, S., Perera, G., Ratnayake, R., 2021. *Effects of crop rotation on enhanced occurrence of arbuscular mycorrhizal fungi and soil carbon stocks of lowland paddy fields in seasonaly dry tropics*. Paddy and Water Environment, 19: 217-226.

Qin, F., Rao, D., Yu, H., Han, Y., Pan, G., Hu, Z., Teng, Y., Lyu, F., Yan, P., Yang, H., 2023. *Reductive soil disinfestation to improve soil properties in long-term tobacco cultivation*. Archives of Agronomy and Soil Science, 69(14): 3284-3299.

Qin, P., Wang, D., Yang, C., Chen, G., Yang, Y., Fu, C., Wang, Z., Fu, W., Liu, G., Yan, J., 2024. *Tobacco-Grain and Economic Crop Intercropping Mode and Its Application in Chongqing*. Plant Health And Medicine, 1: 79-85.

Rui, Q., Xiaojie, L., Juan, L., Chang, L., Yuguo, C., Chengjun, L., Xiaobing, H., Pu, M., Jingke, B., Dongsheng, L., 2023. *Identification of pathogens causing tobacco Fusarium root rot in Henan tobacco-growing area and their infections to tobacco root system*. Tobacco Science & Technology, 56(7).

Shen, M.-C., Zhang, Y.-Z., Bo, G.-D., Yang, B., Wang, P., Ding, Z.-Y., Wang, Z.-B., Yang, J.-M., Zhang, P., Yuan, X.-L., 2022. *Microbial responses to the reduction of chemical fertilizers in the rhizosphere soil of flue-cured tobacco*. Frontiers in Bioengineering and Biotechnology, 9: 812316.

Svotwa, E., and Mahiya, T., 2017. *Assessing flue-cured tobacco crop growth and biomass response to nitrogen application levels using canopy reflectance*. App. Sci. Reports, 18(2): 47-55.

Tang, Z., Chen, L., Chen, Z., Fu, Y., Sun, X., Wang, B., Xia, T., 2020. *Climatic factors determine the yield and quality of Honghe flue-cured tobacco*. Scientific Reports, 10(1): 19868.

Wang, G., Zheng, Y., Suo, W., Zhou, D., Xu, J., He, Y., Wang, Z., Zhang, L., 2024a. *Comparative peptidomics analysis reveals the peptides involving tobacco seed ageing*. Asian Journal of Agriculture and Biology, 2024(04): 2023367.

Wang, J., Pan, Y., Liu, L.-P., Wu, C., Shi, Y.-Z., Yuan, X.-L., 2023. *Identification of key volatile flavor compounds in cigar filler tobacco leaves via GC-IMS*. Asian J. Agric. & Biol., 2023(3): 2023013.

Wang, Y.R., Hu, Z., Zhong, J., Chen, Y., Zhu, J.Z., 2022. *First report of Colletotrichum cliviicola causing leaf spot on tobacco (Nicotiana tabacum) in Hunan province of China*. Plant Disease, 106(1): 316.

Wang, Z., Guo, X., Cao, S., Yang, M., Gao, Q., Zong, H., Shang, X., Gao, Y., Zhang, L., Yang, L., 2024b. *Tobacco/Isatis intercropping system improves soil quality and increase total production value*. Frontiers in Plant Science, 15: 1458342.

Wu, Y., Huang, F., Zhang, C., Jia, Z., 2016. *Effects of different mulching patterns on soil moisture, temperature, and maize yield in a semi-arid region of the Loess Plateau, China*. Arid Land Research and Management, 30(4): 490-504.

Xie, Z., Gao, C., Zhang, X., Du, M., Wang, J., Wang, X., Lu, B., Chen, C., Yang, L., Zhang, Y., 2024. *Identification and biological characteristics of Fusarium tobaccum sp. nov., a novel species causing tobacco root rot in Jilin Province, China*. Microbiology Spectrum, 12(12): e00925-00924.

Yan, H., Wu, S., Li, P., Jin, X., Shi, D., Tu, D., Zeng, W.-A., Tan, L., 2024. *Tobacco crop rotation enhances the stability and complexity of microbial networks*. Frontiers in Microbiology, 15: 1416256.

Zhong, J., Pan, W., Jiang, S., Hu, Y., Yang, G., Zhang, K., Xia, Z., Chen, B., 2024. *Flue-cured tobacco intercropping with insectary floral plants improves rhizosphere soil microbial communities and chemical properties of flue-cured tobacco*. BMC Microbiology, 24(1): 446.

Zhou, W., Guo, X.-H., Han, D., Shao, H.-F., Xu, R., Wang, X.-L., Niu, H.-W., 2023. *Effects of intercropping Pinellia ternata on growth, yield and quality of flue-cured tobacco*. Journal of Agricultural Science and Technology, 25(7): 161-169.