Bangladesh J. Bot. **51**(4): 829-838, 2022 (December) Special DOI: https://doi.org/10.3329/bjb.v51i40.63824

EFFECTS OF INTERCROPPING AROMATIC PLANTS ON THE SOIL MICROBIOTA IN TOBACCO FIELD

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Keywords: Tobacco, Aromatic plants, Row intercropping, Microbial diversity

Abstract

By utilising high-throughput sequencing technology, we systematically analysed microbial flora changes in tobacco fields intercropping various Lamiaceae plants that are cultivated mainly for extracting the aromatic compounds. The results showed that the number of soil microorganisms in the rhizosphere changed during the resettling, flourishing, and harvesting periods. Among these periods, the number of actinomycetes in the flourishing period increased, which played a role in enhancing the disease resistance of tobacco plants to a certain extent. The soil microbial diversity analyses revealed that the amount, species and Alpha diversity index of rhizosphere soil microbes were the highest at the resettling and flourishing stages. Bacterial floras belonging to*Actinobacteria, Firmicutes, Proteobacteria and Nitrospirae*were the dominant ones, playing a certain role in the soil nitrogen cycle and supply. For both bacteria ~~or~~ and fungi, the difference between communities of the soils with the intercropping pattern was greater than that of the control.

Introduction

Tobacco is one of the important economic crops in China, and it is difficult to reasonably rotate this crop due to the large amount of planting. The reasonable application of intercropping measures can maximize the physiological resistance of crop groups to stresses, control or reduce the occurrence of certain diseases and insect pests, and improve the soil structure. As a result, intercropping measures may achieve the effect of applying pesticides or even better (Li *et al*. 2007). Aromatic plants are the general term for cultivated plants and wild plants that are rich in aromatic components and can be used to extract aromatic oils. It is a group of plants that share the attributes of medicinal plants and spice plants (Sun *et al*. 2007). Xie (2009) showed that aromatic plants are the natural fragrant raw materials for tobacco plants, and that it is feasible to use their volatiles to prevent and control crop diseases and insect pests through the cultivation of aromatic plants and flue-cured tobacco. Peng *et al*. (2014) carried out a study on the cultivation of flue-cured tobacco K326 and 4 kinds of aromatic plants in Shilin, Yunnan, and they found that the flue-cured tobacco with planting treatments was superior to the monocropping control in terms of agronomic and economic properties. Gao *et al*. (2014) conducted pot experiments to study the effects of planting rosemary and mint plants on soil nutrients in flue-cured tobacco fields. The results suggested that planting aromatic plants in flue-cured tobacco fields do not have insignificant effects on soil alkaline nitrogen content, but may increase the content of available phosphorus and potassium in the soil during the middle and late maturity stages of flue-cured tobacco. At present, there are few reports on the model of planting aromatic plants in flue-cured

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tobacco fields. Different crops planted together result in different planting methods, and there are mutual benefits or competition between crops with regard to the absorption of soil nutrients. Therefore, this planting model and the related technologies warrant further investigation.

The quantity and diversity of soil microorganisms are important biological indicators to measure soil fertility (Zou *et al.* 2020). When flue-cured tobacco plants are co-planted with other crops, and the litter of mature crops and their interacting root activities may make a significant contribution to soil improvement. Li *et al*. (1999) have shown that plant litter can effectively increase soil's organic matter content, enrich soil microbial activity, promote soil enzymes' activity, enhance fertility, and boost the transformation and renewal of active substances in soil. In the past, researchers have studied intercropping and rotating tobacco with many other plant species, such as rice (Wang *et al*. 2015), corn (Fang *et al.* 2011), garlic (Yang *et al*. 2016), green manure crops (Liu *et al.* 2013), which generally showed a good effect on improving the microbial composition of tobacco soil.

Zhang *et al*. (2017) have shown that for intercropping of S. ningpoensis and tobacco, crops and planting patterns had far-reaching influence on soil microbes, and the crops effect on bacteria was stranger than that on fungi. For the whole of intercropping soil, there were two microbial community structures because of crops.

Cigarette experts generally opine that natural aroma materials such as lavender, rosemary, geranium, rose, and mint can enrich the aroma of flue-cured tobacco and give rise to added comfort (Li *et al*. 2014). In this study, we intercropped three Lamiaceae aromatic plant species with tobacco, and studied the influence of these planting models on rhizosphere soil microorganisms in tobacco fields. Our results may provide theoretical support for further research and application of biodiversity technology to improve soil health in tobacco-growing areas, which may be beneficial to developing green flue-cured tobacco agriculture.

Materials and Methods

The experiment was conducted in 2020 to establish an intercropping model of aromatic plants and flue-cured tobacco in the Yunnan Shilin Experimental Centre. Three Lamiaceae species: *Ocimumbasilicum*, *Monardafistulosa*, *Agastacherugosa* and flue-cured tobacco variety K326 were used. One flue-cured tobacco plant was planted with each of the three Lamiaceae species; thus, for every 12 ditches of tobacco plants, 4 ditches of each Lamiaceae species were planted. The spacing between adjacent ditches was 1.2 m.

During different growth stages of tobacco plants, soils were sampled according to the planting mode. A 5-point sampling method (centre and four corners) was used, and 200 g soil (1 cm in depth) was extracted from the rhizosphere zone (around 15 cm in depth). The soil samples were transferred to the laboratory in ice boxes, and the 5 soils of one treatment were pooled and mixed thoroughly.

Soil sample dilution method: 5 test tubes containing 9 ml sterile water were prepared, numbered '10^{-1}', '10^{-2}', '10^{-3}', '10^{-4}', and '10^{-5}'. For dilution, 1 g mixed soil sample was added to the '10^{-1}' test tube. After being shaken thoroughly, 1 mL of the mix was transferred to the '10^{-2}'test tube using a pipette. The similar step was repeated until the solution was diluted to the required concentration. For bacteria and actinomycetes, the final diluted concentration was 10^{-5}, and for fungi, it was 10^{-4}.

To cultivate the microorganism, 0.1 mL of the diluted solution was added to the surface of the culture medium, and evenly spread using a spreading rod. The plates were cultured in an incubator in an inverted position. For bacteria, they were cultivated in a constant 37°C incubator, and fungi and actinomycetes were cultivated in a constant 28°C incubator. Single bacterial colonies appeared

after the bacteria were cultured for 24 h, and single fungal colonies appeared after the fungi were cultured for 48 h-72 h. After recording the total number of colonies on each plate, the average number of colonies on the plates of the same dilution was calculated, and the number of colonies per gram (or ml) in the soil sample was obtained. The bacterial isolation medium was beef extract peptone medium, the fungal isolation medium was potato dextrose agar (PDA) medium, and the actinomycete isolation medium was Gauze's synthetic medium No.1.

Three sampling points were selected in the soil in the intercropping mode and the control mode at different growth periods of tobacco, and 3 well-growing tobacco plants were collected at each sampling point. The soil attached to the roots was gently shaken to self-sealing bags, mixed well in the bag, and stored at 4℃ to keep fresh (Table 1). Genomic DNA from soil samples were extracted less than 24 hours after sampling using MP FastDNA® Spin Kit for Soil (MP Biomedical, USA).

Table 1. Soil sampling information and sample IDs.

Sampling Information	Sample ID	Sample group
Intercropping①resettling	MT1-1	
Intercropping②resettling	MT1-2	MT1
Intercropping③resettling	MT1-3	
Intercropping①flourishing	MW1-1	
Intercropping②flourishing	MW1-2	MW1
Intercropping③flourishing	MW1-3	
Intercropping①harvesting	MHT1-1	
Intercropping②harvesting	MHT1-2	MC1
Intercropping③harvesting	MHT1-3	
CK① (resettling)	CKT-1	
CK② (resettling)	CKT-2	CKT
CK③ (resettling)	CKT-3	
CK① (flourishing)	CKW-1	
CK② (flourishing)	CKW-2	CKW
CK③ (flourishing)	CKW-3	
CK① (harvesting)	CKC-1	
CK② (harvesting)	CKC-2	CKC
CK③ (harvesting)	CKC-3	

For each sample, the DNA was diluted to a final concentration of 1 ng/μl in a centrifuge tube with sterile water, and the diluted genomic DNA was used as the template. Specific primers with barcodes were designed according to the selected sequencing region. Phusion ® High-Fidelity PCR Master Mix with GC Buffer was used to ensure amplification efficiency and accuracy. Primers for each region: 16S V4–V5 region primers were 515F-907R; ITS1 region primers were ITS5-1737F and ITS2-2043R. PCR system (30 μl): Phusion Master Mix (2x) 15 μl, Primer (2 μM) 3 μl for each (6 μl in total), gDNA (1 ng/μl) 10 μl (5–10 ng), H_2O 2 μl. Reaction program: 98°C pre-denaturation for 1 min; 30 cycles (including 98°C for 10 sec, 50°C for 30 sec; 72°C for 30 sec); and 72°C for 5 min.

The PCR products were detected by electrophoresis with agarose gel (2%). According to the concentration, the PCR products were mixed in equal amounts. After thorough mixing, the PCR products were purified by using agarose gel electrophoresis (2%, prepared using 1×TAE), and the target band was cut and recovered, using the Gene JET gel recovery kit (Thermo Scientific).

Ion Plus Fragment Library Kit 48 rxns (Thermo Fisher Scientific) was used to construct the library. After the constructed library was qualified by using Qubit and passed the quality control, sequencing was conducted on the Ion S5(TM)XL high-throughput sequencing platform. The whole process from DNA samples to final data acquisition included: sample quality control, PCR, purification, library construction, and sequencing.

Ion S5(TM)XL output data were in the fastq format. After filtering and dividing samples according to the barcode using Cutadapt software (Martin 2011), clustering of operational taxonomic units (OTUs) and species classification analysis were performed using Uparse software (Edgar *et al.*2010).

Alpha diversity mainly concerns the number of species in a local uniform habitat, so it is also called within-habitat diversity. This index is usually used to measure the species abundance of a community in an ecosystem. It is a composite index that reflects the abundance and uniformity of species. It has two types of community richness indices, including three indicators, namely, ace, Chao1, and observed otus. Chao is an indicator that uses the chao1 algorithm to estimate the number of OTUs in a community; chao1 is commonly used in ecology to estimate the total number of species and was first developed by Chao (1984). Ace is an indicator used to estimate the number of OTUs in the community, and it was also proposed by Chao. It is one of the commonly used indicators for estimating the total number of species in ecology, and it is different from the Chao I algorithm.

The community diversity index is a composite indicator that reflects species richness and uniformity, including indicators—Shanon and Simpson 2. Simpson is one of the indicators used to estimate the diversity of microorganisms in a sample. It was proposed by Edward Hugh Simpson (1949), and it is commonly used in ecology to quantitatively describe the biodiversity of an area. The larger the Simpson value, the lower the community diversity level is. Shannon is another indicator used to estimate the diversity of microorganisms in the sample. Both Shannon and Simpson diversity indices are commonly used to reflect alpha diversity. The larger the Shannon value, the higher the community diversity level is.

Results and Discussion

Tobacco plants grown in the intercropping mode gave rise to significantly higher numbers of three types of soil microorganisms in the resettling stage than the plants in the control mode. In the flourishing and harvesting stages, only the number of actinomycetes was significantly higher than the control; while the numbers of bacteria and fungi were higher than the control, the differences were not significant (Table 2).

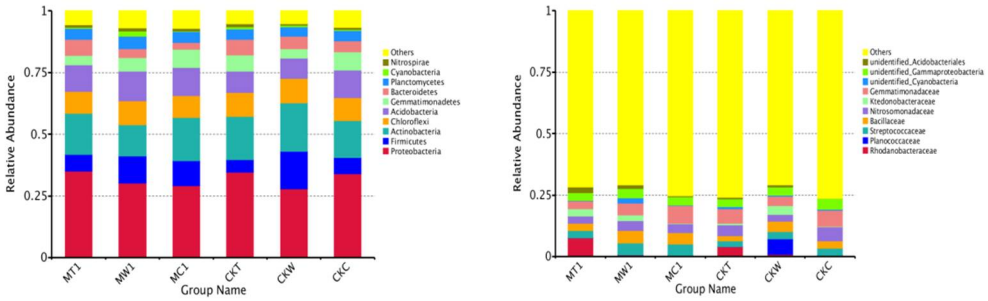
A total of approximately 40 different bacterial phyla were found. The top ten (according to the average abundance) bacterial phyla accounted for more than 90% of all 18 samples, and the total abundance of the top five phyla was greater than 70%. These advantages phyla were: *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Chloroflexi*, *Acidobacteria*, *Gemmatimonadetes*, *Bacteroidetes*, *Planctomycetes*, *Cyanobacteria*, and *Nitrospirae*. The abundance of the top ten tobacco soil bacterial communities differed slightly between tobacco fields with different planting methods and at different tobacco growth stages, but there was little difference in composition (Fig. 1a).

Table 2. Microbial quantity in soils from the tobacco (at various growth stages) fields in the intercropping mode.

Growth stage	Treatment	Bacteria (10^6cfu/g)	Fungi (10^4cfu/g)	Actinomycetes (10^5 cfu/g)
Resettling	Intercropping	8.29±0.05a	6.44±0.77a	7.25±0.12a
	Control	6.21±0.33b	5.39±0.45b	6.54±0.09b
Flourishing	Intercropping	7.23±0.01a	6.55±0.13a	7.08±0.19a
	Control	7.08±0.05a	5.87±0.14a	5.76±0.11b
Harvesting	Intercropping	7.45±0.02a	7.11±0.03b	7.55±0.11a
	Control	7.24±0.12a	6.35±0.11b	6.54±0.12b

The values in the table are the converted logarithmic values of the number of microorganisms. Different letters marked after the values (mean ± SE) in the same column indicate that they are significantly different ($P< 0.05$).

At the family level, the first seven dominant families were *Rhodanobacteraceae*, *Planococcaceae*, *Streptococcaceae*, *Bacillaceae*, *Nitrosomonadaceae*, *Ktedonobacteraceae*, and *Gemmatimonadaceae*, and their average abundance in the sample was around 20% (Fig. 1b).

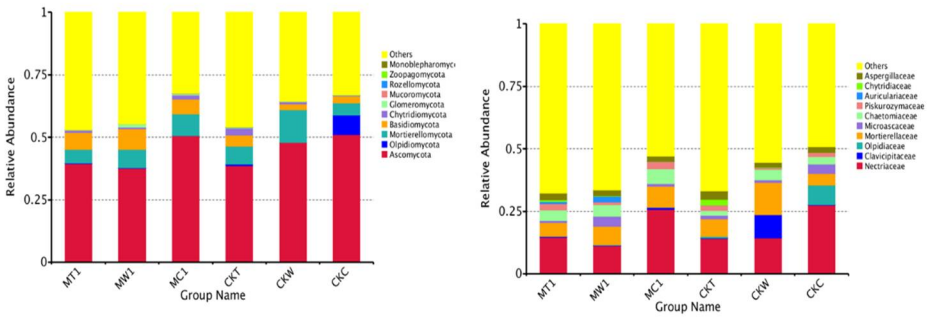


a b

Fig. 1. The composition of rhizosphere bacterial communities in the intercropping tobacco field during different growth stages. (a) Soil bacterial community composition in the tobacco field at the phylum level. (b) Soil bacterial community composition in the tobacco field at the family level.

There was little difference between the dominant fungal phyla in the intercropping and control rhizosphere soils. The average abundance of the top ten dominant fungal phyla in the samples was around 50-70%. These dominant phyla were *Ascomycota*, *Olpidiomycota*, *Mortierellomycota*, *Basidiomycota*, *Chytridiomycota*, *Glomeromycota*, *Mucoromycota*, *Rozellomycota*, *Zoopagomycota*, and *Monoblepharomycota* (Fig. 2a).

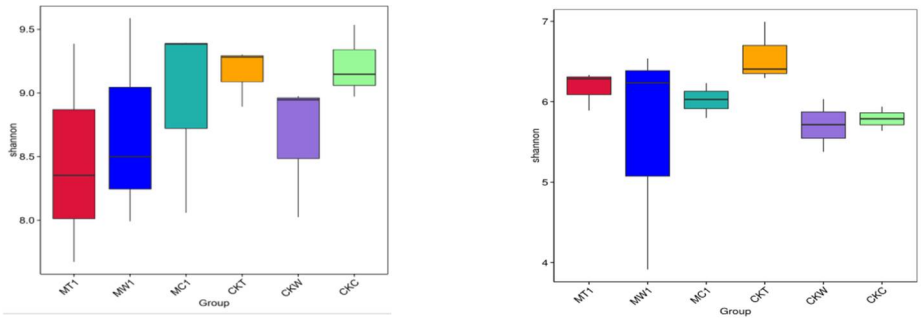
At the family level, the top ten dominant families were *Nectriaceae*, *Clavicipitaceae*, *Olpidiaceae*, *Mortierellaceae*, *Microascaceae*, *Chaetomiaceae*, *Piskurozymaceae*, *Auriculariaceae*, *Chytridiaceae*, and *Aspergillaceae*, and their average abundance in the sample was around 20–30% (Fig.2b).



(a) (b)

Fig. 2. The composition of rhizosphere fungal communities in the intercropping tobacco field during different growth stages. (a) Soil fungal community composition in the tobacco field at the phylum level. (b) Soil fungal community composition in the tobacco field at the family level.

The Shannon index of the soil bacterial community was slightly higher in the control group, but the difference between the two groups was not significant (Welch test, P = 0.65). The Shannon index of soil fungal community was not different between the groups neither (Welch test, P = 0.20) (Fig. 3). This is consistent with the microbial quantity data shown in Table 2 - in the intercropping mode, the amount of bacteria was the highest at the resettling and flourishing stages, and the amount of actinomycetes was the highest at the resettling stage.

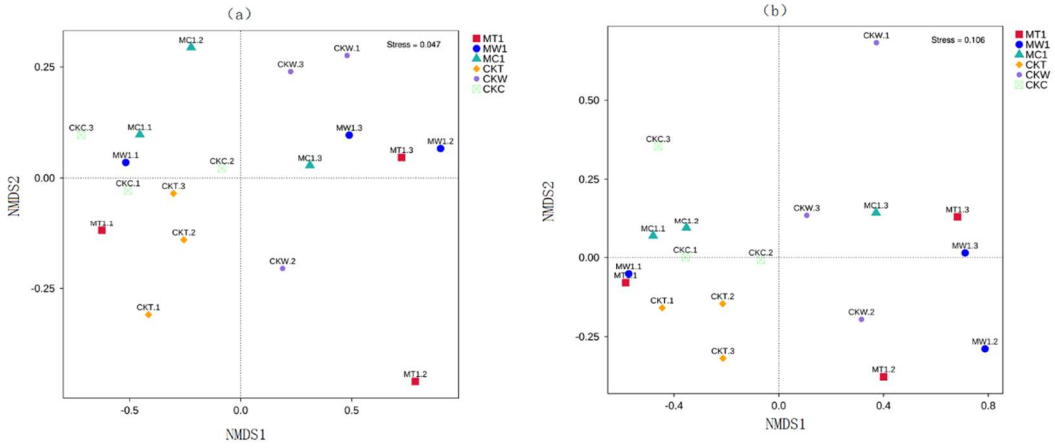


(a) (b)

Fig. 3. Alpha diversity of soil bacterial and fungal communities in the intercropping tobacco field during different growth stages different growth stages. (a) Soil bacterial community Alpha diversity in the tobacco field. (b) Soil fungal community Alpha diversity in the tobacco field.

Alpha diversity index is usually used to measure the abundance of species in the community ecology. It is a composite index reflecting the richness and evenness of species. The higher the index, the better the species richness and uniformity are.

Beta diversity is used to compare biodiversity between different samples. Non-metric multidimensional scaling (NMDS) analysis is one type of Beta diversity analyses. It utilises dimensionality reduction sorting and a nonlinear model to reflect the differences between different sample groups, which are visualised as the distances between dots. The principle is similar to principal component analysis (PCA). NMDS plots can visually represent the clusters or differences between samples. The NMDS plots show that our soil samples from the tobacco field were not differentiated significantly (Fig. 4), and Adonis analysis also revealed that the differences between the groups were not significant.



(a) (b)

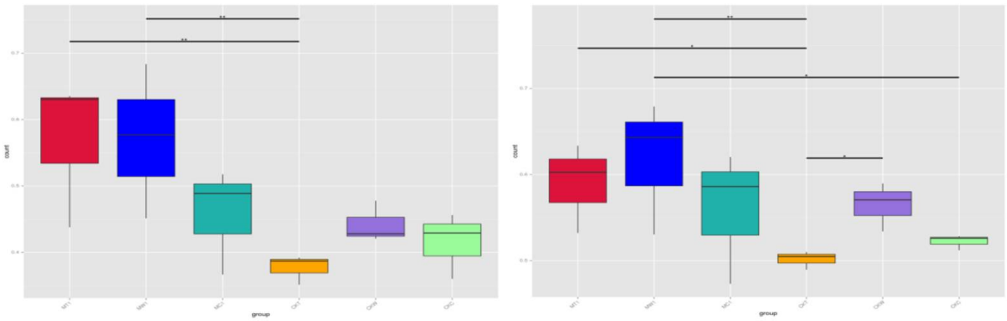
Fig. 4. The NMDS plots of rhizosphere bacterial (a) and fungal (b) communities in the tobacco field

Using unweighted unifrac, the distance between any two communities can be calculated. The 18 samples in this study were divided into 6 groups each containing 3 samplesrepresenting three communities, so that each group had 3 unweighted unifrac values. The Wilcox test can analyse whether the difference of the unweighted unifrac values of each group pair is significant. If the unweighted uniFrac value of a certain group is significantly greater than that of another group, it indicates that the difference between different communities in the first group is greater than that of the latter. Our unweighted uniFrac values and Wilcox test results indicate that for both bacterial and fungal communities, differences among communities in the group of intercropping aromatic plants were greater than those in the control group (Fig. 5).

In root soil, in addition to the substances secreted by the root during its growth, there are also active microbial populations. The direct or indirect, beneficial or unfavourable, effects of microorganisms or plants on other microorganisms or plants in the same system is termed allelopathy. Most of the allelopathic effects between plants and between plants and micro-organisms involves detoxification or enhanced toxification through soil microorganisms. It is necessary to study the allelopathic effects of soil microorganisms on plants. Previous studies have shown that the effect of rhizosphere microorganisms on tobacco plants is significant (Zhu *et al*. 2019, Zhang *et al*. 2018). When the number of microbes in the tobacco rhizosphere soil increases, the corresponding tobacco leaf yield is higher and the proportion of top and medium quality tobacco leaves is increased, which means improved agronomic quality of tobacco leaves. There is a correlation between the number of rhizosphere bacteria, actinomycetes and fungi of tobacco plants and the chemical composition of tobacco leaves (Ma *et al*. 2012). Studies have shown that the content of nicotine and water-soluble sugars in tobacco leaves are related to the quantity of bacteria and fungi in the rhizosphere soil.

Actinomycetes were certainly dominant in the intercropping soil, indicating that the influence of intercropping Lamiaceae plants may change the rhizosphere microenvironment of the intercropping flue-cured tobacco soil, resulting in a significant increase in the number of actinomycetes. It is likely caused by a class of small allelopathic molecules secreted by Lamiaceae plants that can stimulate the growth of actinomycetes in the soil. It is widely known that actinomycetes are the group that produces the largest amount of antibiotics. Antibiotics are widely

used in medicine and agriculture to inhibit pathogenic bacteria of many plants. The rhizosphere soil of flue-cured tobacco contains a considerable amount of actinomycetes, which can enhance the disease resistance of plants to a certain extent and reduce the use of pesticides. This is important in improving the quality of flue-cured tobacco. Our research found that the number of tobacco bacteria and actinomycetes in the intercropping mode was superior to other treatments. Therefore, the intercropping of Lamiaceae and flue-cured tobacco can significantly improve the soil micro-ecological environment and improve the quality of tobacco leaves to a certain extent.



(a) (b)

Fig.5. Differences in the soil rhizosphere communities of bacteria (a) and fungi (b) in the tobacco field.

The vertical axis value (Count) is the distance between the communities calculated by using the unweighted unifrac method. The horizontal line and asterisk above the box plot indicate the significant level of the Count difference between groups (* P< 0.05; ** P< 0.001).

The comparison between the soil microbial flora of the intercropping tobacco field and the control field showed that at the resettling stage, the diversity of bacterial communities was higher than that of the control, and the dominant bacteria belonged to *Proteobacteria*, *Firmicutes*, and *Nitrospirae*.The phylum *Proteobacteria* contains many bacteria that are and rhizobia. These bacteria can promote the growth of plants, and therefore these may be bacteria that are beneficial to plant growth and crop quality (Liu *et al*. 2018). *Proteobacteria*, the absolute dominant flora in a normal environment, also accounted for a relatively high proportion in both groups of soils, and especially in intercropping group soils, indicating that intercropping can indeed increase microbial diversity.

Previous studies have shown that the positive effects of tobacco rhizosphere soil microbes are significant, and that tobacco rhizosphere microbes are related to the soil nitrogen mineralization and nitrification processes of tobacco field. It is generally considered that *Chloroflexi* can generate energy through photosynthesis and degrade the contamination in soil environment. *Actinobacteria* prefer alkaline environment and soil with a high N/C ratio and low molecular organic matter content can better promote their growth (Chang *et al*.2017). Amides and rhizosphere soil bacteria such as *Gemmatimonas* play an important role in soil's nitrogen cycle and nitrogen supply, and the quantity of nitrification and nitrosation bacteria (e.g., *Nitrospirae*) affects tobacco's nicotine synthesis and accumulation (Zhang2018). *Bacillaceae* (e.g., bacillus) can be utilised to increase available phosphorus, nitrogen and potassium contents in soil, enhancing disease resistance in tobacco plants and effectively inhibiting the growth of pathogens.

Xu *et al*. (2019) have shown that the long-term continuous cropping of soil fungal flora structure changed, pathogens increased, and tobacco leaf spot pathogens, *Pythium*, *Mortierella*, *Alternaria*, the genus Sclerotia, Scenedesmophilus, Fusarium and Xylariomycetidae were closely related to soil nutrient metabolism.

It is worth noting that in all the samples, Fusarium's ITS fragment was detected, and the average abundance was around 10%. The most abundant species of this genus was Fusariumoxysporum. Wu *et al*. (2021) found the garlic root exudates and its main components had significant inhibitory effects against *F. oxysporum* from the intercropping garlic to control tobacco Fusarium root rot.

Based on molecular analysis and pathogenicity assay results, Qiu *et al*. (2018) concluded that among the three causative agents of the root rot disease, namely, *F. oxysporum*, *F. proliferatum*, and *F. equiseti*, *F. oxysporum* is the major one. In our study, no root rot symptom was observed, indicating that the k326 of tobacco varieties we planted have resistance in root rot, and the intercropping cultivation mode also controlled the occurrence of diseases to a certain extent.

In tobacco field intercropped with aromatic Lamiaceae plants, the total amounts of soil microbes in the resettling, flourishing, and harvesting stages were all higher than those of the control, indicating that the intercropping may change the microenvironment of the tobacco rhizosphere soil, thereby causing the quantity of the microbial flora in it to alter greatly. The biodiversity of each soil sample was analysed by using the PCR amplification method. Our results showed that in comparison to the control, the amount, species and Alpha diversity index of rhizosphere soil microorganisms were all higher in the intercropping (between Lamiaceae aromatic raw material plants and tobacco plants) group samples at the resettling and flourishing stages. The dominant flora mainly belonged to *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Nitrospira*, all of which can promote the proliferation of beneficial microorganisms, such as bacillus, actinomycetes, and nitrifying and nitrosating bacteria in the tobacco field in intercropping mode. Actinomycetes displayed significant dominance. Tobacco rhizosphere soil contains a considerable portion of actinomycetes, which can inhibit a variety of plant pathogenic bacteria, and to a certain extent enhance the disease resistance of tobacco plants. Nitrifying and nitrosating bacteria play important roles in the nitrogen cycle and supply in soil, and can affect the synthesis and accumulation of nicotine in tobacco. Bacillus can increase the content of available nitrogen, phosphorus, and potassium in soil, enhance the disease resistance of tobacco plants, and effectively inhibit the growth of pathogenic bacteria. Therefore, the use of Lamiaceae and tobacco intercropping can significantly improve the soil micro-ecological environment in tobacco-growing areas, reduce the use of pesticides, and be conductive in improving the quality of flue-cured tobacco.

By and large, the intercropping model of Lamiaceae aromatic raw material plants and tobacco can significantly increase the number and diversity of soil microorganisms in tobacco fields.

Acknowledgements

We thank project of the China Tobacco (Yunnan) project "Technologies on Improving Tobacco Leaf Quality Based on the Application of Biodiversity Theories" (2016YL01) for funding.

References

Chang A, Li J, Zhang T, Zhang JW, Wei DH, Wang PW, Yu JJ 2017. Analysis of bacterial community composition in tobacco rhizosphere soil based on metagenomics 16S rDNA sequencing. China Agricul. Sci. Tech. Review **2017**(02): 49-56.

Edgar RC 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics, **26**(19): 2460-2461.

Fang S, Tang L, Chen S, Gu G and Chen Y 2011. Influence of crop rotation on tobacco bacterial wilt number and pothogenesy. Chin. J. Eco-Agricul. **19**(02): 377-382.

Gao F, Qi W, Deng X and Yang S 2014. Effect of flue-cured tobacco intercropping with aromatic plant on soil nutrient contents. J. Anhui Agri. **42**(06): 1612 -1613, 1658.

Li L, Li SM, Sun JH, Zhou LL, Bao XG, Zhang HG and Zhang FS 2007. Diversity enhances agricultural productivity via rhizosphere phosphorus facilitation on phosphorus-deficient soils. Proceedings of the National Academy of Sciences of the United States of America, **104** (27): 1119211196.

Li L, Yang SC, Li XL. Zhang FS and Christie P 1999. Interspecific complementary and competitive interactions between intercropped maize and faba bean. Plant Soil, **212**(02):105-114.

Li Z, Wang C, Wang S, Yang Y, Li M and Zhu H 2014.Intercropped with different aromatic plants affect the aroma constituents of tobacco. Molecul. Plant Breeding, **12**(05): 982-991.

Liu J, Gao H, Xie Y, et al 2018.Analysis of bacterial community structure in tobacco- growing soil based on 16SrDNA gene high-throughput sequencing. Molecul. Plant Breeding, **16**(24): 120125.

Liu G, Wang S, Sha F, Ren W, Yang Y, Zhang Q and Du R 2013. Effects of long-term green manure return to field on the yield, quality of flue-cured tobacco and soil improvement. Chin. Agricul. Sci. Bull., **29**(04):173-177.

Ma J, Li X and Jia 2012. Degradation of polycyclic aromatic hydrocarbons by Pseudomonas sp.JM2 isolated from active sewage sludge of chemical plant. J. Environ. Sci. **2012**(12): 2141-2148.

Peng S, Chen X, Yang Y, Li S, Chen W and Zhao M 2014. The preliminary study on effect of intercropping tobacco with aromatic plants on agronomic and economic characters. J. Yunnan Agricul. University.**29**(01):144-148.

Qiu R, Bai J, Li C, Li S and Liu D 2018. Molecular identification, and pathogenicity analysis of Henan tobacco Fusarium. Acta Tobacco Sin. **24**(02):133138.

Sun M, Li P, Lv JH and Zhang QX 2007. Function of aromatic plants and garden application. Forestry Practical Tech. **2007** (05): 46-47.

Wang C, Kuang C and Tang W 2015. Effect of rice herbicides on tobacco leaf and its pedorelict in the tobacco-paddy rotation. Crop Res. **29**(08): 864-867.

Wu X, Lai R, Mi Y, Gu G, Zhang B and Wang Q 2021. Control effects of garlic root exudates on fusariumoxysporum root rot of tobacco. Chin. J. Biolog. Control. **37**(03): 555-563.

Xie J 2009.Tobacco fragrant raw materials. Beijing: Chemical Industry Press, China.

Xu Z, Wang F, Jin Y, Wang M, Xiong Y, Hao H and Xu X 2019. Analysis of fungal communities in rhizosphere soil of flue-cured tobacco under different cropping years using 18SrDNA-PCR-DGGE. Soils Fertilizers China. **2019**(04): 39-46.

Yang X, Li T, Zang X, Chen D, Liang Y and Zhang C 2016. Effects of tobacco garlic crop rotation and tobacco garlic crop intercropping on soil microbial groups in tobacco fields. Soils, **48**(04): 698–704.

Zhang DY, Wang J, Yang SP, Zhang X, Liu J, Zhao J, He DM, Yang HJ, Mo JJ, Gou JY, Zhao XM, Jiang W, Ding W and Chen DX 2017. Influence of *Scrophularian in gpoensis*-tobacco intercropping on bacterial community structure in soil. Acta Prata Cultur. Sin. **26**(06):120-130.

Zhang D 2018.Effects of rotation (intercropping) between flue-cured tobacco and Chinese herbal medicine on soil microbe and yield and quality of flue-cured tobacco. Southwest University. Chongqing, China.

Zhang YJ, Shao HF, Zhang K, Jia HF, Huang WX, Han D 2018. Research on the effects of fertilization on soil environment and microorganisms in continuous cropping tobacco based on high-throughput sequencing. China Agricul. Sci. Tech. Review. **20**(05): 22-31.

Zhu J, Fan Z, Chen Q, Wang J, Chen C, Lu A, Li H and Wang Y 2019. Effects of continuous cropping years on soil microbial flora in the tobacco rhizosphere. J. Northern Agricul. **47**(01): 42-48.

Zou X, Yi B, Zhang Q and Di H 2020.Effects of long-term fertilization on the microbial community structure and the population of N cycle-related functional microorganism in paddy soil. J. Plant Nutr. Fertil. **26**(12): 2158-2167.

(Manuscript received on 8 October, 2022; revised on 21 December, 2022)