EFFECTS OF BIO-ORGANIC FERTILIZER ON YIELD AND QUALITY OF FLUE-CURED TOBACCO AND SOIL MICROECOLOGY

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Abstract. This study aims to promote the development and application of organic fertilizer, improve soil quality, enhance the yield of flue-cured tobacco, provide a scientific basis for large-scale organic fertilizer production and application, and support the growth of the tobacco industry. The field randomized block design experiment included no-fertilizer and conventional fertilizer controls, as well as various bio-organic fertilizer treatments. The study analyzed soil quality indices, flue-cured tobacco growth, disease incidence, leaf quality, and economic traits under different treatments to evaluate the functional restoration effect of bio-organic fertilizer on tobacco-planting soil. The application of organic fertilizer can improve soil nutrients, promote the growth of flue-cured tobacco and improve soil microbial structure. Comprehensive analysis showed that organic fertilizer could improve the indexes of tobacco leaves and soil, and the soil function was restored. Among the tested treatments, formula product 1 of the functional bio-organic fertilizer demonstrated the most significant effects.
Keywords: *bio-organic fertilizer, flue-cured tobacco, soil, physicochemical property, microorganism*

Introduction

Yunnan Province has the largest tobacco cultivation area in China. The tobacco industry represents a significant source of taxation and serves as a vital pillar of the national economy. Over time, extensive chemical fertilizer use and continuous cropping have been employed to maximize tobacco leaf yield and quality. These practices have significantly degraded the soil environment in tobacco fields, leading to compaction, acidification, reduced fertility, imbalanced nutrient ratios in tobacco leaves, destruction of soil aggregates, and diminished biological activity and diversity (Lv, 2022). Optimal soil conditions are essential for high-quality tobacco leaf production. Therefore, repairing soil functions, balancing the nutrient supply of soil to tobacco plants, and creating a good soil environment to achieve the purpose of promoting tobacco growth

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and development and achieving sustainable development of tobacco (Zhang, 2016). Actively developing tobacco soil remediation technology is the top priority of flue-cured tobacco planting. Applying organic fertilizer in tobacco planting can not only provide nutrients for tobacco, but also improve soil properties and regulate soil microbial status (Xiao et al., 2021). Studies have shown that within a certain range, the content of organic matter, available phosphorus and available potassium in soil will increase with the increase of organic fertilizer application (Ke et al., 2022). Organic fertilizer can increase the nutrient content in soil and improve the living environment of soil microorganisms (Liu et al., 2000; Huang et al., 2003). Organic fertilizer can promote the increase of edible cassava plants, effectively improve the quality and yield of edible cassava (Lu et al., 2023). At present, the soil environment of tobacco planting is mainly improved by applying organic fertilizer, returning straw to the field, implementing rotation system and using soil structure modifier. The results showed that organic fertilizer could promote the growth of tobacco plants and increase the number of leaves (Xing et al., 2020; Dan et al., 2022). Studies have shown that the application of organic fertilizer can improve the disease resistance of flue-cured tobacco in the field (Wang et al., 2016). Organic fertilizer can change the groups and functions of crop rhizosphere microorganisms (Wang, 2022; Li et al., 2024; Huang et al., 2024). Organic fertilizer can enhance soil microbial metabolism, thereby promoting soil health (Huang, 2023; Kong et al., 2024). Organic fertilizer can promote the increase of soil microbial community activity and the remodeling of community structure (Liu et al., 2024). In conclusion, bio-organic fertilizer can increase soil nutrients, improve crop yield and quality, and promote soil microbial community activity, which has a good application prospect. The effects of different organic fertilizers on tobacco planting were clarified, which provided suggestions for the development of bio-organic fertilizers (Guo et al., 2023). In this study, the effects of different organic fertilizers on the yield traits of tobacco and the physical and chemical properties of soil were compared through field random experiments, and the types of organic fertilizers suitable for flue-cured tobacco planting were screened out, in order to provide a theoretical basis for the selection of bio-organic fertilizers in tobacco production, and provide a theoretical basis for local soil improvement and improvement of tobacco leaf quality, so as to achieve the purpose of protecting the environment and realizing the sustainable development of tobacco industry (Huo et al., 2013; Li et al., 2019).

Materials and methods

Testing material

The flue-cured tobacco variety was Yunyan 121, a local main cultivar, which was hybridized with Yunyan 97 as the female parent and PY5 as the male parent. The tobacco seedlings are uniform and grow normally. Fertilizer: inactivated bio-organic fertilizer, flue-cured tobacco special functional bio-organic fertilizer formula products, flue-cured tobacco special functional bio-organic fertilizer mature products, compound fertilizer, potassium nitrate, potassium sulfate, calcium superphosphate, etc. The fertilizer information of each treatment is shown in *Table 1*.

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***Table 1.** Fertilizer information of each treatment*

Sample ID	Fertilizer Information	Manufacture sources
C2	no fertilization. Treatment	-
C3	conventional fertilizer: compound fertilizer (15: 8: 25), potassium nitrate (k20≥44.5%), potassium sulfate (k≥52 %), calcium superphosphate (P2O5≥12%)	Yunye Fertilizer Co., Ltd.
C4	Inactivated bio-organic fertilizer (substrate) ; product 1 / 2 / 3 of the matrix, adding 0.5‰V-aminobutyric acid, adding inactivated functional strains, net content of 40 kg / bag.	Yunye Fertilizer Co., Ltd.
C5	Flue-cured tobacco special functional bio-organic fertilizer formula product 1: add 0.5‰V-aminobutyric acid, add functional strainYWF-17-0001(growth-promoting function), effective viable count≥2x107cuf/g, net content 40kg/bag.	Yunye Fertilizer Co., Ltd.
C6	Flue-cured tobacco special functional bio-organic fertilizer formula product 2: add 0.5‰V-aminobutyric acid, add functional strain YWF-21-2122 (growth promoting function), effective viable count≥2x107cuf/g, net content 40kg / bag.	Yunye Fertilizer Co., Ltd.
C7	Flue-cured tobacco special functional bio-organic fertilizer formula product 3: add 0.5‰V-aminobutyric acid, add functional strain YWF-20-2069 (growth promoting function), effective viable count≥2x107cuf/g, net content 40kg/bag.	Yunye Fertilizer Co., Ltd.
C8	Flue-cured tobacco special functional bio-organic fertilizer mature products: marigold flower dead: 30%, peat 30%, molasses solution10%, bacteria package: 15%, distiller 's grains: 15%, microbial bacteria: 5 kg, net content 40 kg/bag.	Yunye Fertilizer Co., Ltd.

Test methods

The test was located in the main tobacco producing area of Malong District, Qujing City, Yunnan Province, China, with an altitude of 1979.3 m, 25 ° 16 ′ 39 ″ north latitude and 103 ° 21 ′ 52 ″ east longitude. A total of 7 treatments were set up, each treatment had 3 replicates, a total of 21 plots, 20 m^2 per plot, 36 plants in each plot, and the row spacing was 1.2 * 0.7 meters. The locations of each plot were divided by irregular random sorting. Each treatment was recorded as C2, C3, C4, C5, C6, C7, C8, and C1 was the soil number before planting. Cultivation techniques: seedling: 300 ~ 400 hole seedling trays were used for seedling raising by hydroponics. Ridging and fertilization: ridging height is not less than 25 cm, ditch straight, fine soil, flat soil moisture. The diameter of the pond is 35 ~ 40 cm, the depth is 15 ~ 18 cm, and the distance between the tobacco seedlings and the film is 5 ~ 10 cm. Fertilizer and organic fertilizer were applied. Transplanting: The transplanter was used to open the hole at a fixed distance on the film-covered tobacco border, and the strong seedlings with no disease and uniform size were selected for transplanting. Avoid direct contact with fertilizer when transplanting roots, in order to avoid burning seedlings. Mulching and management: Cover the film immediately after transplanting to ensure that the top of the tobacco seedlings is more than 5cm away from the film. After ensuring the survival of tobacco seedlings, the membrane was broken for ventilation. The plots of each treatment are shown in *Fig. 1*.

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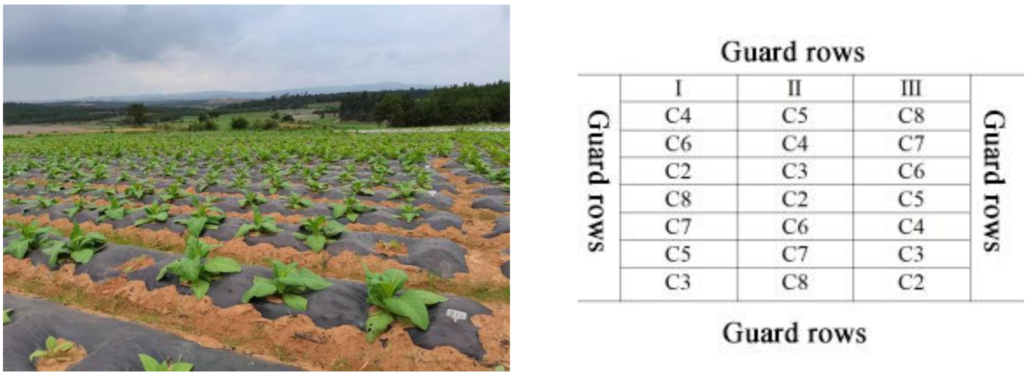

Figure 1. *Field map of each treatment*

Taking the control without fertilization as the control 1 (C2), the application of conventional chemical fertilizer as the control 2 (C3), five organic fertilizer treatments were set up, which were inactivated bio-organic fertilizer (matrix) (C4), flue-cured tobacco special functional bio-organic fertilizer formula product 1 (C5), flue-cured tobacco special functional bio-organic fertilizer formula product 2 (C6), flue-cured tobacco special functional bio-organic fertilizer formula product 3 (C7), flue-cured tobacco special functional bio-organic fertilizer mature product (C8). The above fertilizers were applied as base fertilizer before transplanting of tobacco seedlings, and potassium nitrate was used as topdressing for 30 days. The fertilizer application of each treatment is detailed in *Table 2*.

Table 2. *Fertilization per mu (kg / hm^2)*

Sample ID	N: P: K	bio-organic fertilizer	compound fertilizer	calcium superphosphate	potassium sulfate	potassium nitrate
C2	-	-	-	-	-	-
C3	1:1:3	0	514.5	352.5	190.5	150
C4	1:1:3	1500	411.0	357.0	226.5	150
C5	1:1:3	1500	411.0	357.0	226.5	150
C6	1:1:3	1500	411.0	357.0	226.5	150
C7	1:1:3	1500	411.0	357.0	226.5	150
C8	1:1:3	1500	411.0	288.0	181.5	150

Measuring indexes

Collection of soil samples

Soil C1 was taken before flue-cured tobacco planting in spring (March), and soil C2-C8 was taken after flue-cured tobacco harvest in autumn (September). 1.0 kg of soil mixed samples were collected from 0-20 cm soil layer by soil drilling in each plot, and 5 points of mixed samples were collected in each plot. Gravel and plant residues in fresh soil samples were removed and put into self-sealing plastic bags. Some soil samples were stored in refrigerator at 4 °C for the determination of soil microorganisms and soil enzyme activities. The remaining soil samples were mixed and air-dried for the determination of soil physical and chemical properties.

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Agronomic traits investigation

According to the standard YC / T142-1998, five tobacco plants were selected from each plot to investigate the agronomic traits of flue-cured tobacco. Stem circumference: The circumference of the stem was measured at 1 / 3 of the plant height during the squaring period. Maximum leaf area: measure the product of the maximum leaf length and width and the leaf area coefficient of 0.6345, length: the straight-line length from the stem-leaf junction to the leaf tip, width: the vertical length of the widest leaf surface and the main vein.

Investigation on appearance quality of cured tobacco leaves

The maturity, color, identity, structure, oil content and chromaticity of B2F and C3F tobacco leaves were scored according to the plot. Leaf appearance quality rating index: oil: more, more, more, slightly, identity: moderate, slightly thinner, thinner, slightly thicker, thicker, leaf structure: loose, loose, slightly dense, tight, raw tobacco color: lemon yellow, orange, reddish brown, micro-belt green, green yellow, miscellaneous color, chroma: strong, strong, medium, weak, weak, single leaf weight: 10 dry tobacco leaves with the same grade in the middle were weighed, expressed in grams, repeated 2-4 times to take the average.

Economic character survey

The yield, yield value, average price and proportion of first-class tobacco were calculated respectively according to the individual harvest, pole making, baking, grading and yield calculation. Economic traits: yield: 1 hm^2 area yield, yield value: 1 hm^2 area output value, average price = yield value / yield, grade index = average price / C1F price × 100 (grade index, the higher the grade index, the higher the commodity value, the better the quality of tobacco leaves), yield index = yield value / C1F price (the higher the yield index, the better the tobacco varieties), the proportion of superior tobacco: the percentage of superior tobacco weight in the total weight of treated tobacco leaves.

Soil index detection

Soil organic matter, bulk density, available phosphorus, alkali-hydrolyzable nitrogen, available potassium, pH value and other indicators were measured according to the plot.

Determination of soil microbial characteristics

High-throughput sequencing technology was used for analysis. C1 was before planting, and treatments 1 to 7 were numbered C2 to C8, respectively. PCR amplification of bacterial 16Sd: primers were used 16S: F: (‘-ACTCCTACGGGAGGCAGCA-3 ') and R: (5 ' -GGACTACHVGGGTWTCTAAT-3 '). PCR amplification of fungal ITS: primers ITS F: (5'-CTTGGTCATTTAGAGGAAGTAA-3') and R: (5 ' -GCTGCGTTCTTCATCGATGC-3 ‘) (Tong et al., 2019).

Determination of tobacco leaf index

The contents of nitrogen, phosphorus and reducing sugar in tobacco leaves were determined according to the upper, middle and lower leaves. Total nitrogen was

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determined by continuous flow analyzer, total plant alkali was determined by gas chromatography, and the content of chlorine was determined by potentiometric titration with silver nitrate standard solution.

Disease investigation

The investigation of root rot (*Fusarium*) and black shank (*Phytophthora nicotianae var. nicotianae*) was carried out by 5-point sampling method. According to GB / 23222-2008 ' tobacco pest classification and investigation methods ' standard survey, and calculate the incidence and disease index.

Data analysis

All data were statistically analyzed using WPS Office and IBM SPSS Statistics 27 software. The data in the table were average and the significance level was 0.05. OTUs were classified and annotated using RDP software. MOTHUR software was used to calculate the microbial diversity of each soil sample, including OTU richness, Chao1 index, Shannon index and phylogenetic diversity index (Mao et al., 2015).

Results

Comparison of soil physical and chemical properties under different treatments

As shown in *Table 3*, compared with the application of chemical fertilizer (C3), the application of organic fertilizer increased soil pH, organic matter, hydrolyzable nitrogen and available potassium, and reduced soil conductivity and bulk density. Among them, the pH value, organic matter, hydrolyzable nitrogen and available potassium of C5 treatment increased most significantly, and the conductivity decreased most significantly, which increased by 20.46%, 32.82%, 16.86% and 9.34% respectively compared with chemical fertilizer (C3), and the conductivity decreased by 18.12%.

Table 3. *Comparison of soil physical and chemical properties under different treatments*

Sample ID	pH	Organic matter (g/kg)	Hydrolysable N(mg/kg)	Available phosphorus (mg/kg)	Rapidly available potassium (mg/kg)	Electric conductivity (us/cm)	Volumetric weight (g/cm3)
C2	5.15±0.13bcd	26.1±2.3b	107±11c	49.8±5.3b	316±18d	863.0±24.5a	1.08ab
C3	4.79±0.26d	26.2±3.1b	166±8b	122.9±6.1a	899±21c	632.0±22.2b	1.11a
C4	4.89±0.31cd	34.6±2.2a	183±7ab	127.6±4.4a	970±28a	536.0±16.0cd	1.02b
C5	5.77±0.24a	34.8±1.6a	194±9a	126.9±6.9a	983±16a	517.5±19.2d	1.02b
C6	5.60±0.37ab	34.5±3.1a	188±9b	120.9±4.9a	954±23ab	553.5±13.8c	1.02b
C7	5.23±0.21bcd	32.2±2.4a	185±16b	122.8±7.1a	920±32bc	544.5±20.3cd	1.03b
C8	5.33±0.30abc	32.9±4.2a	183±12ab	121.6±6.4a	906±25c	548.5±27.1cd	1.04b

Note: The lowercase English letters indicate that there is a significant difference at the 0.05 level

Comparison of agronomic traits of different treatments

The comparison results of agronomic traits of different bio-organic fertilizer treatments are shown in *Figure 2*. Among them, the leaf number, plant height, stem circumference and maximum leaf area of C5 treatment were the most significant, which

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were 93.45 %, 238.75 %, 104.16 % and 324.74 % higher than those of C2. However, there was no significant difference between organic fertilizer and chemical fertilizer treatments. It can be seen from the figure that the best growth in the field is the flue-cured tobacco-specific functional bio-organic fertilizer formula product 1 (C5), and the worst is the non-fertilization control group.

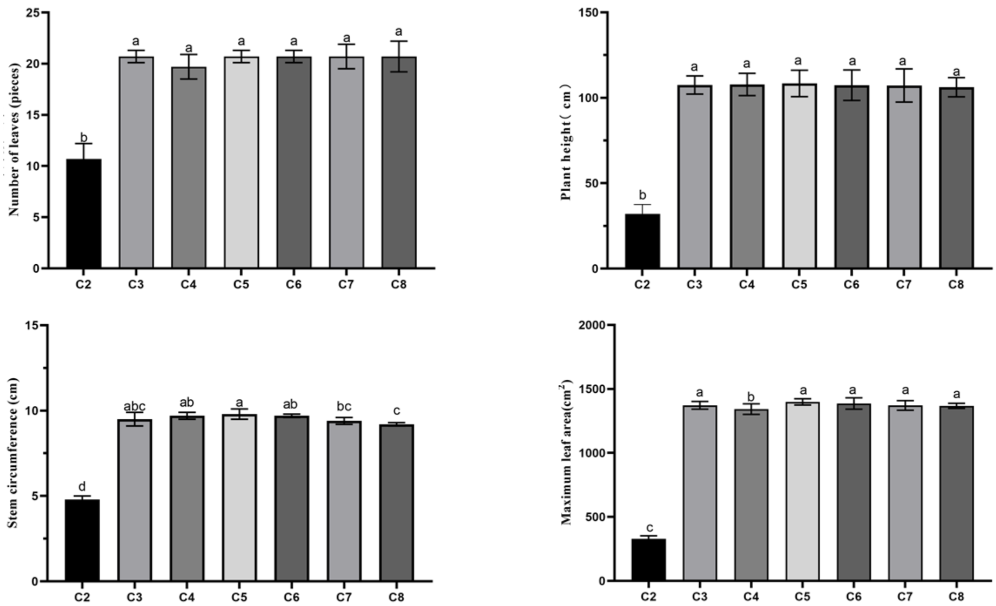

Figure 2. *Comparison of agronomic traits of different treatments. Note: The lowercase English letters indicate that there is a significant difference at the 0.05 level*

Comparison of incidence of flue-cured tobacco in different treatments

The growth of non-fertilized treatment (C2) was too poor to investigate. From *Table 4*, it can be seen that compared with C3, organic fertilizer significantly reduced the occurrence and harm of tobacco black shank and root rot. Among them, the C5 treatment reduced the harm of black shank and root rot most significantly, and the control effect was the best compared with the application of chemical fertilizer.

Table 4. *Comparison of incidence of flue-cured tobacco in different treatments*

Sample ID	Black shank disease incidence rate (%)	Black shank disease index	Control effect (%)	Root rot incidence (%)	Root rot disease index	Control effect (%)
C2	-	-	-	-	-	-
C3	8.33±2.80a	3.60±1.06ab		1.69±0.57a	0.83±0.45ab	
C4	7.41±3.30ab	4.11±0.78a	-14.17	0.93±0.48ab	0.93±0.29a	-12.05
C5	2.78±1.40b	1.54±0.42b	57.22	0.30±0.52b	0.10±0.17c	87.95
C6	5.56±2.80ab	2.26±0.70ab	37.22	0.59±0.51b	0.59±0.56abc	28.92
C7	3.70±1.20ab	2.06±0.63ab	42.77	0.37±0.32b	0.17±0.15bc	79.52
C8	5.56±3.00ab	3.50±1.50ab	2.77	0.86±0.11ab	0.79±0.35abc	4.82

Note: The lowercase English letters indicate that there is a significant difference at the 0.05 level

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Comparison of chemical components and appearance quality of flue-cured tobacco under different treatments

There was no economic value in the treatment of no fertilizer (C2). It can be seen from *Table 5* that compared with the application of conventional chemical fertilizer (C3), the total nitrogen, total plant alkali, reducing sugar and chlorine in the upper leaves of flue-cured tobacco increased after the application of organic fertilizer. The content of potassium increased except C8, and the content of phosphorus decreased except C4. Among them, C5 increased total nitrogen, total alkaloid and reducing sugar by 34.23%, 22.14% and 49.02%, respectively.

Table 5. *Comparison of main chemical components in different parts of flue-cured tobacco under different treatments (%)*

Site	Sample ID	Total nitrogen	Total alkaloid	Reducing sugar	Potassium	Phosphorus	Chlorine
upper leaves	C2	-	-	-	-	-	-
	C3	2.22±0.15b	2.80±0.36b	14.30±0.85c	2.17±0.14a	0.17±0.02a	0.28±0.03b
	C4	2.87±0.21a	3.28±0.28ab	16.90±1.17b	2.34±0.09a	0.17±0.01a	0.33±0.04b
	C5	2.98±0.23a	3.42±0.22a	21.31±0.97a	2.24±0.21a	0.15±0.03a	0.30±0.03b
	C6	2.97±0.17a	2.84±0.16ab	17.38±0.76b	2.22±0.19a	0.15±0.02a	0.32±0.04b
	C7	2.92±0.18a	3.11±0.31ab	16.96±0.96b	2.19±0.11a	0.16±0.03a	0.44±0.05a
	C8	2.87±0.13a	2.98±0.27ab	16.98±0.90b	2.08±0.12a	0.14±0.01a	0.34±0.02b
middle leaves	C2	-	-	-	-	-	-
	C3	1.81±0.11d	1.51±0.21d	16.72±0.71d	2.30±0.11b	0.21±0.03a	0.38±0.06b
	C4	2.43±0.13bc	2.54±0.23bc	17.80±0.69cd	2.61±0.17a	0.17±0.02abc	0.36±0.06b
	C5	2.78±0.19a	2.52±0.14bc	22.29±1.06a	2.42±0.09ab	0.18±0.03ab	0.36±0.05b
	C6	2.64±0.21ab	2.36±0.11c	20.72±0.74b	2.41±0.13ab	0.15±0.01bc	0.47±0.08b
	C7	2.35±0.15c	2.86±0.13a	18.84±0.84c	2.36±0.08ab	0.15±0.03bc	0.49±0.09b
	C8	2.26±0.12c	2.65±0.16ab	18.31±0.67c	2.40±0.18ab	0.14±0.01c	0.36±0.07b
lower leaves	C2	-	-	-	-	-	-
	C3	1.27±0.13c	0.55±0.09c	14.13±0.37d	2.43±0.10c	0.22±0.02a	0.45±0.06a
	C4	2.18±0.18ab	2.46±0.12a	16.73±0.72b	2.85±0.16ab	0.18±0.03bc	0.57±0.05a
	C5	2.26±0.23a	2.48±0.19a	19.47±0.84a	3.08±0.21a	0.20±0.02ab	0.58±0.06a
	C6	1.98±0.26ab	2.15±0.15b	16.26±0.51bc	2.87±0.14ab	0.19±0.01abc	0.52±0.07a
	C7	2.06±0.15ab	2.33±0.22ab	15.43±0.45c	2.59±0.18bc	0.18±0.02bc	0.56±0.06a
	C8	1.87±0.11b	2.47±0.14a	15.64±0.64c	2.65±0.17bc	0.16±0.01c	0.47±0.07a
quality tobacco		1.5%~3.5%	1.5%~3.5%	16%~18%	<2%	0.15%~0.5%	<1%

Note: The lowercase English letters indicate that there is a significant difference at the 0.05 level

Compared with the conventional chemical fertilizer (C3), the contents of total nitrogen, total plant alkali, reducing sugar and potassium in the middle leaves of flue-cured tobacco were increased and the phosphorus content was decreased after the application of organic fertilizer. Among them, C5 increased the total nitrogen, reducing sugar and potassium content of middle leaves by 53.59%, 33.31% and 4.96%, respectively, and C7 increased the total alkaloid content by 89.40%.

Compared with the conventional chemical fertilizer (C3), the contents of total nitrogen, reducing sugar, potassium and chlorine in the lower leaves of flue-cured

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tobacco were increased after the application of organic fertilizer, and the contents of total plant alkali and phosphorus were decreased. Among them, C5 increased the content of total nitrogen, reducing sugar, potassium and chlorine in the middle leaves by 77.95%, 37.79%, 26.75% and 28.89%, respectively.

From different parts, the contents of total nitrogen and total alkaloid in the upper leaves were higher than those in the middle and lower leaves, the content of reducing sugar in the middle leaves was higher than that in the upper and lower leaves, and the contents of potassium, phosphorus and chlorine in the lower leaves were higher than those in the upper and middle leaves.

Comparison of economic characters of flue-cured tobacco leaves under different treatments

It can be seen from *Figure 3* that compared with C3, after the application of organic fertilizer, the yield, average price, grade index, yield index and proportion of superior tobacco all increased. Among them, C5 increased the yield, yield, average price, grade index, yield index and proportion of superior tobacco most significantly, increasing by 17.40%, 27.11%, 8.27%, 8.28%, 27.11% and 18.95%, respectively.

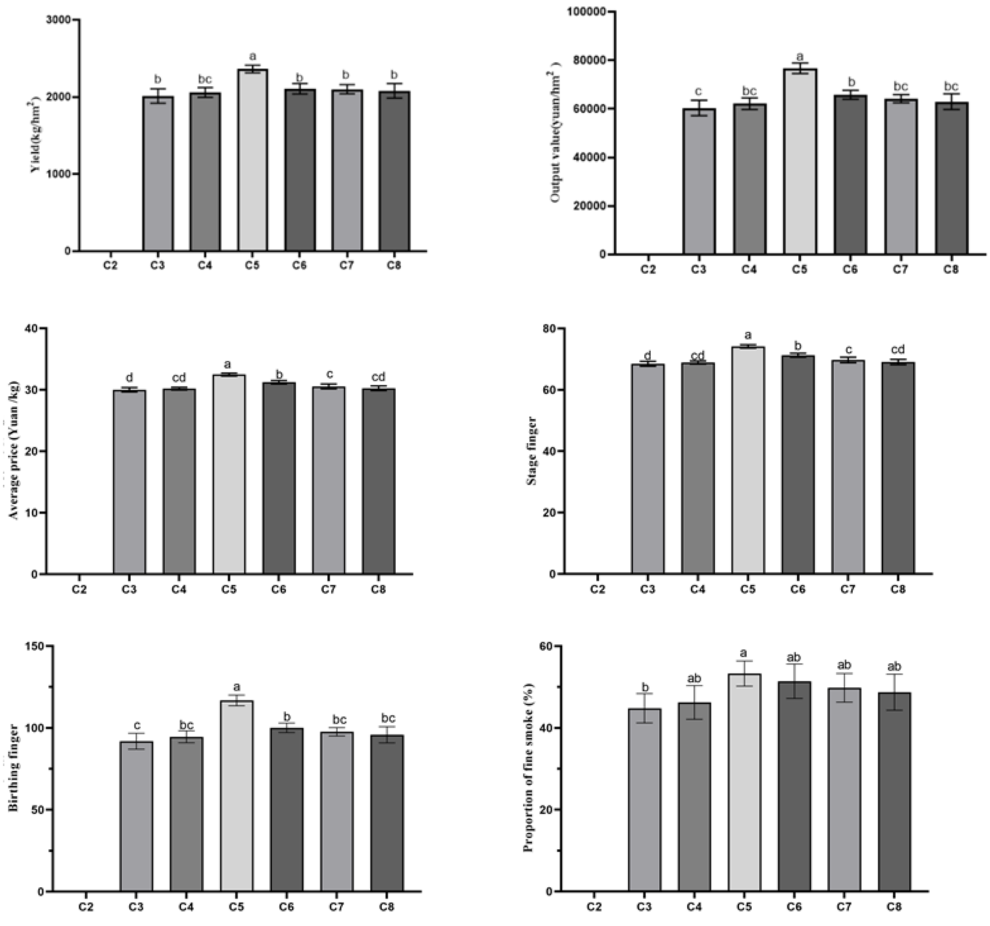

Figure 3. *Comparison of economic traits of flue-cured tobacco under different treatments. Note: The lowercase English letters indicate that there is a significant difference at the 0.05 level*

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Effects of different treatments on biological characteristics of tobacco field soil

Analysis of soil microbial community diversity

The number of bacterial Features in C1 was the highest before planting, and the number of bacterial Features decreased significantly after planting flue-cured tobacco. Compared with no fertilization (C2), except for C8, the species abundance of other treatments decreased. Compared with conventional chemical fertilizer (C3), the species richness of C5 and C8 with organic fertilizer increased, and the species richness of C8 bacteria increased most significantly (*Fig. 4A*).

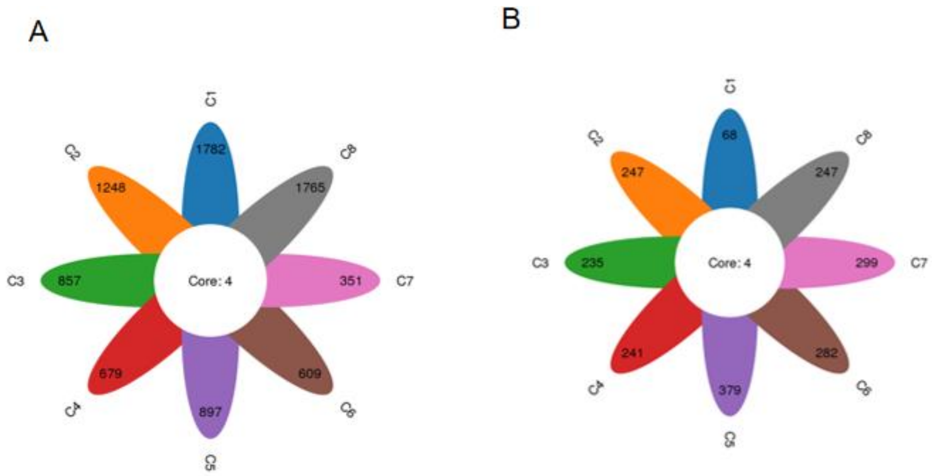

Figure 4. *Venn diagram of microbial OTUs in tobacco planting soil under different treatments: (A)the Wayne diagram of OTUs of bacteria with different treatments; (B) the Wayne diagram of fungal OTUs*

The number of fungal OUTs in C1 was the least before planting, and the number of fungal OUTs increased significantly after planting flue-cured tobacco. Compared with no fertilization (C2), the abundance of fungal species in all treatments except C3 and C4 increased. Compared with the treatment (C3), the fungal species richness of the treatment with organic fertilizer increased, and the fungal species richness of C5 increased most significantly (*Fig. 4B*).

It can be seen from *Table 6* that in the 8 groups of soil, the Feature number, ACE index and Chao1 index are distributed between 589 and 2339, with a certain span. The Shannon index is distributed between 6.85 and 9.76, and the minimum values of these indexes are from C7. On the whole, each sample has rich bacterial diversity, and the bacterial flora has reached a high level. The uniformity and diversity of bacterial flora in C5 were the most significant. The Feature number, ACE index and Chao1 index of C1 and C2 were higher. It may be that these two groups were closer to the natural state, and there were more natural bacteria.

Compared with the soil before planting (C1), the diversity of soil fungi increased significantly after planting flue-cured tobacco. Compared with C3, the diversity of soil fungi increased after organic fertilizer. Among them, C5 increased the Feature, ACE, Chao1, PD _ whole _ tree index of soil fungi most significantly, and C8 increased Simpson and Shannon index most significantly.

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Table 6. *Alpha diversity index of soil microorganisms in different treatments*

Microorganism	Sample ID	Feature	ACE	Chao1	Simpson	Shannon	PD_whole_tree	Coverage
Bacteria	C1	2,339.00	2,339.16	2,339.00	0.9875	9.5314	176.67	1.0000
	C2	1,866.00	1,866.18	1,866.00	0.9975	9.6724	144.28	1.0000
	C3	1,561.00	1,561.43	1,561.01	0.9969	9.3885	115.91	1.0000
	C4	1,417.00	1,419.46	1,417.21	0.9961	9.2629	98.57	0.9998
	C5	1,690.00	1,691.32	1,690.09	0.9979	9.7605	114.38	0.9999
	C6	865.00	865.17	865.00	0.9845	7.5434	92.20	1.0000
	C7	589.00	589.00	589.00	0.9802	6.8545	62.22	1.0000
	C8	2,315.00	2,315.00	2,315.00	0.9885	9.5459	191.32	1.0000
Fungus	C1	97.00	97.00	97.00	0.9843	6.2554	38.85	1.0000
	C2	412.00	412.00	412.00	0.9898	7.6444	91.63	1.0000
	C3	412.00	412.61	412.00	0.9902	7.7424	93.74	1.0000
	C4	421.00	421.48	421.00	0.9909	7.7983	90.69	1.0000
	C5	629.00	629.00	629.00	0.9895	7.7526	139.95	1.0000
	C6	545.00	545.00	545.00	0.9463	6.4479	114.17	1.0000
	C7	535.00	535.00	535.00	0.9544	6.7922	131.09	1.0000
	C8	424.00	424.00	424.00	0.9903	7.8258	91.79	1.0000

Analysis of soil microbial community phylum level

It can be seen from *Figure 5(A)* that the dominant bacterial groups in the soil are Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria, Acidobacteria, Chloroflexi, and Cyanobacteria, accounting for more than 80% of the total. Compared with before planting flue-cured tobacco (C1), after planting flue-cured tobacco, except for C6, C7 and C8, the other treatments increased the contents of Proteobacteria, Actinobacteria, Acidobacteria and Chloroflexi, and decreased the contents of Bacteroidetes and Firmicutes. Compared with C3, Organic fertilizer increased the content of Firmicutes and decreased the content of Proteobacteria, Actinobacteria and Anhydrideomycetes. C6 and C7 reduced Proteobacteria and increased Bacteroidetes and Firmicutes most significantly, and the content of C5 bacteria was most evenly distributed.

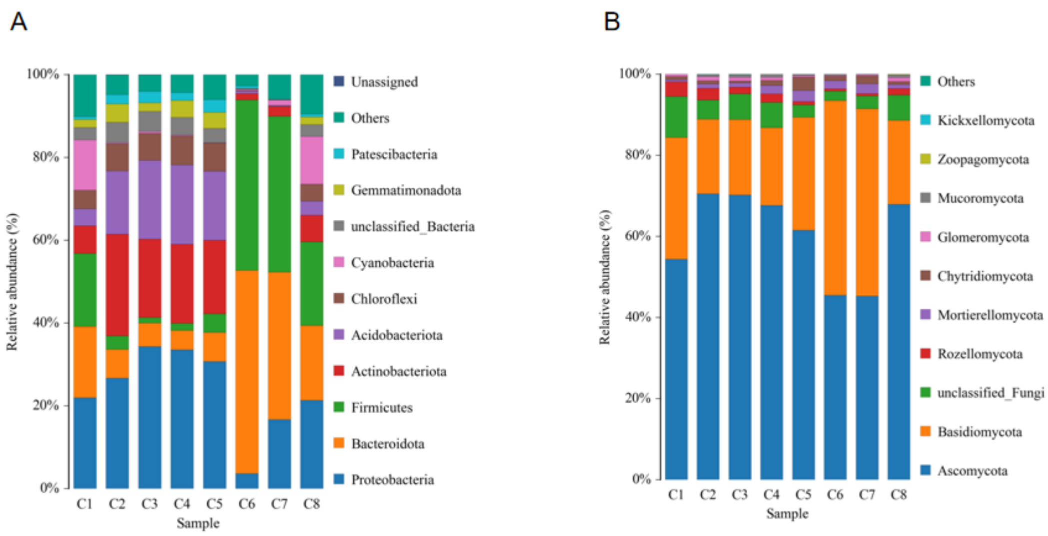

Figure 5. *Relative abundance of soil microbial phylum level in different treatments: (A) the phylum of bacteria in different treatments; (B) the phylum of fungi*

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It can be seen from *Figure 5(B)* that the dominant bacterial groups in the soil are Ascomycota and Basidiomycota, accounting for more than 80% of the total. On the whole, compared with before planting (C1), except for C6 and C7, after planting flue-cured tobacco, the Ascomycota increased and the Basidiomycota decreased. Compared with C3, organic fertilizer decreased the number of Ascomycota. Except for C4, the number of Basidiomycota, Mycomycota and Chytridiomycota increased, and the number of Mycobacterium decreased.

Correlation analysis

In this paper, Pearson was used to analyze the relationship between chemical indexes of middle leaves of flue-cured tobacco and soil nutrient elements, bacterial community diversity index and fungal community diversity index. The results are shown in *Figure 6*. It can be seen from *Fig. 6(A)* that the reducing sugar content in the middle leaves was positively correlated with soil pH and significantly positively correlated with hydrolyzed nitrogen content (HN). The potassium content of middle leaves was positively correlated with soil organic matter and available potassium content, and negatively correlated with soil bulk density. Chlorine in the middle leaves was negatively correlated with Chao1 and Shannon index of bacterial community. From *Figure 2-5C*, it can be seen that chlorine in the middle leaves is negatively correlated with the Shannon index of the fungal community; the reducing sugar content in the middle leaves was significantly positively correlated with the Chao1 index of the fungal community.

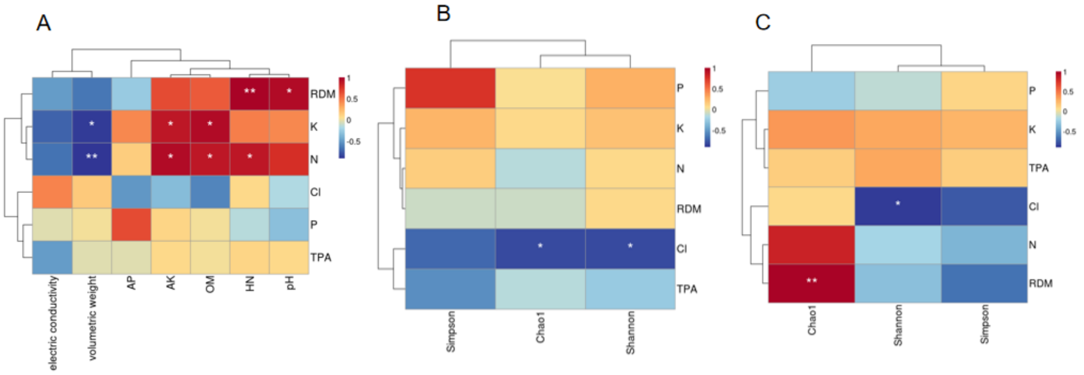

Figure 6. *Related factors of chemical indexes of middle flue-cured tobacco leaves. * Indicates significant difference (p < 0.05), * * indicates extremely significant difference (p < 0.01)*

Discussion

Bio-organic fertilizer has a good application prospect in agricultural production. It is of great significance to the realization of an environment-friendly society (Fu et al., 2017). Bio-organic fertilizer has a good application effect in field crops, vegetables and fruit trees, and can also improve the soil ecological environment (Tian et al., 2023). Organic fertilizer contains a lot of nutrients. Organic fertilizer can improve soil water retention, aeration, fertilizer retention and drought resistance. It can also improve the soil structure and make the soil looser. In this experiment, the p H value, organic matter, phosphorus and potassium of tobacco soil treated with organic fertilizer were significantly increased, which confirmed that organic fertilizer had the effect of

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improving soil nutrients. Organic fertilizer can increase the content and exchange capacity of soil exchangeable base ions, thus alleviating soil acidity (Hai et al., 2024).

Compared with the single application of chemical fertilizer, the leaf area and effective leaf number of flue-cured tobacco treated with organic fertilizer increased to varying degrees (Zhang et al., 2024). In this study, except for the control group without fertilization, there was no significant difference in the growth of flue-cured tobacco among different fertilization treatments, which may be related to the single-year planting of flue-cured tobacco. Whether organic fertilizer can promote the growth of flue-cured tobacco needs to be further verified by later experiments. However, it also confirms the previous research conclusion that bio-organic fertilizer has no obvious promoting effect on these indicators in single-year tobacco planting (Feng et al., 2022). Black shank and root rot have become the main diseases that hinder local tobacco cultivation. The occurrence and development of the two are closely related to the soil rhizosphere microecology. Organic fertilizer can improve soil microecology and control the occurrence of some tobacco diseases. Therefore, it can be used as an effective measure to control tobacco diseases (Shi et al., 2018). Organic fertilizer can reduce the severity of tobacco black stem disease and root rot, and increase tobacco yield (Luo et al., 2023). Organic fertilizer treatment significantly reduced the occurrence of tobacco black shank and root rot. The application of organic fertilizer improved the soil environment and promoted root growth. Therefore, the application of organic fertilizer had a good effect on improving the quality of tobacco leaves. Compared with the application of special chemical fertilizer for tobacco, Functional organic fertilizer increased the agronomic traits and yield of tobacco plants (Liu et al., 2019). In this experiment, the indexes of flue-cured tobacco leaves treated with organic fertilizer were improved to varying degrees, and the economic traits were improved to varying degrees. In this study, under the same maturity conditions, the potassium content of the middle leaves was the highest (Li et al., 2024).

The improvement of soil physical and chemical properties can better adapt to the survival of soil microorganisms. In addition to enriching soil nutrients, bio-organic fertilizer can also improve plant adaptability and soil remediation efficiency by regulating soil microbial communities (Wang, 2023). Commercial organic fertilizer and cow dung can increase the richness and diversity of bacteria in tobacco-planting soil and recruit more potential beneficial bacteria (Meng et al., 2023). Organic fertilizer increased soil microbial diversity. The application of commercial organic fertilizer can change the rhizosphere bacterial community structure: the abundance of beneficial microorganisms Actinobacteria and Firmicutes increased, and the abundance of harmful microorganisms Acidobacteria and Flammulina decreased (Dan et al., 2022). In this experiment, organic fertilizer increased the content of Firmicutes, Basidiomycota, Mould and Chytridiomycota, and decreased the content of Proteobacteria, Actinobacteria, Acidobacteria and Ascomycota. The mechanism of the decrease in the abundance of Proteobacteria and Actinobacteria needs to be further verified.

In the experiment, different organic fertilizers have obvious differences in promoting growth and improving quality of flue-cured tobacco. However, the experimental data are the results of single-year planting, and there may be some accidental conditions, which also need to be verified by field planting in the later period. From the perspective of various indicators, especially the impact on soil microecology, it shows that bio-organic fertilizer has played a very good role in the restoration of soil function in tobacco fields.

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Conclusions

The results showed that the control group without fertilization completely lost its economic benefits. Compared with the control group applying ordinary chemical fertilizer, the treatment group applying bio-organic fertilizer significantly increased soil nutrients, flue-cured tobacco yield, increased the diversity of soil bacteria and fungi, increased the content of Firmicutes, and decreased the content of Acidobacteria and Ascomycota. In different bio-organic fertilizer treatments, the formula product 1 of flue-cured tobacco special functional bio-organic fertilizer showed good fertilizer effect in the process of tobacco production. However, this study also has some limitations, and there is a lack of research on the mechanism of the effects of different organic fertilizers on microorganisms, which needs further study in the future. However, this study clarified that different organic fertilizers that are beneficial to dryland flue-cured tobacco cultivation are effective technologies to improve the soil environment and have broad application prospects.

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REFERENCES

[1] Chen, D., Wang, M., Wang, G., Zhou, Y., Yang, X., Li, J., Zhang, C., Dai, K. (2022): Functional organic fertilizers can alleviate tobacco (*Nicotiana tabacum* L.) continuous cropping obstacle via ameliorating soil physicochemical properties and bacterial community structure. – Frontiers in Bioengineering and Biotechnology 10: 1023693.

[2] Feng, Y., Chen, W., Huang, Y., Fu, X., Zhang, Y., Zhang, H., Liu, Z. (2022): Study on the Role of Bio-organic Fertilizer in Tobacco Production. – Seed Science & Technology 40: 121-123.

[3] Guo, N., Lv, G., Zhao, Y., Fan, T., Zhao, D., Chen, W. (2023): Effects of Different Bio-organic Fertilizers on Tomato Growth Yield and Soil Nutrients in Solar Greenhouse. – Vegetables, pp. 17-25.

[4] Huang, X., Fan, Z., Cai, J., Yang, Z., Zhao, L., Yang, Z., He, X., Zhang, L., Zhang, C. (2003): Effects of Organic Fertilizer and Micro-fertilizer on the Quality of Sun-cured Tobacco. – Journal of Yunnan Agricultural University, pp. 10-13.

[5] Huang, B., Chen, Y., Pei, Z., Jiang, L., Zhang, Y., Wang, J., Wang, J. (2023): Application of microbial organic fertilizers promotes the utilization of nutrients and restoration of microbial community structure and function in rhizosphere soils after dazomet fumigation. – Frontiers in Microbiology 13: 1122611.

[6] Huang, B., Wang, P., Wang, Y., Zhang, L., Sheng, H., Yu, X., Ge, J. (2024): Functional endophytic organic fertilizer alleviates phthalates burden and promotes vegetable growth with the assistance of indigenous Sphingomonas. – Case Studies in Chemical and Environmental Engineering 9: 100671.

[7] Huo, G., Meng, G., Wang, Z., Li, Z., Jiang, P., Hua, D. (2013): Effects of Biological Organic Fertilizer on Flue-cured Tobacco Chemical Composition. – Journal of Anhui Agricultural Sciences 41: 4815-4818+4833.
[8] Kong, C., Zhang, S., Yuan, S., Wang, W., Song, X., Guo, D., Lawi, A. S. (2024): Soil bacterial community characteristics and its effect on organic carbon under different fertilization treatments. – Frontiers in Microbiology 15: 1356171.
[9] Li, J., Li, L., Yang, S.-N., He, P.-P., Zhang, J.-S. (2019): Effects of bio-organic fertilizer on soil microorganism and quality of roasted tobacco. – Jiangxi Academy of Agricultural Sciences, Nanchang, China.
[10] Li, Z., Qin, N. (2024): Comparative analysis of tobacco leaf quality of different flue-cured tobacco varieties. – Modern Agricultural Science and Technology, pp. 136-139.
[11] Li, D., Duan, W., Guo, H., Zong, J., Chen, J., Wang, J. (2024): High-nitrogen organic fertilizer promotes the growth of bermudagrass (*Cynodon dactylon*), zoysiagrass (*Zoysia japonica*) and paspalum grass (*Paspalum vaginatum*) by enhancing nitrogen use efficiency associated with Bacillus-stimulated bacterial community. – Scientia Horticulturae 329: 113027.
[12] Liu, T., Li, C., Xiong, D., Zeng, W., Xie, F. (2000): Effect of Combined Application of Organic Fertilizer and Chemical Fertilizer on Flue-cured Tobacco. – Chinese Tobacco Science, pp. 27-30.
[13] Liu, L., Yang, P., Tian, J., Tan, Z., Peng, G. (2019): Effects of Bio-organic Fertilizer on Yield and Quality of Tobacco. – Guangdong Agricultural Sciences 46: 69-77.
[14] Liu, T., Wang, Q., Li, Y., Chen, Y., Jia, B., Zhang, J., Guo, W., Li, F. Y. (2024): Bio-organic fertilizer facilitated phytoremediation of heavy metal (loid) s-contaminated saline soil by mediating the plant-soil-rhizomicrobiota interactions. – Science of The Total Environment 922: 171278.
[15] Lu, S., Song, E., Lei, K., Xu, C., Chen, R., Ma, C. (2023): Effects of Bio-organic Fertilizer on Quality and Yield of Edible Cassava. – Southern Agricultural Machinery 54: 5-8.
[16] Luo, C., Ding, H., Li, Y., Liu, T., Dong, Y. (2023): Effect of Enhanced Organic Material Addition during Reductive Soil Disinfestation on Disease Resistance, Yield Increase, and Microbial Community in Flue-Cured Tobacco. – Agronomy 13: 2458.
[17] Lv, W. (2022): Effects of Biological Organic Fertilizer on Tobacco Soil and Tobacco Quality. – Master's thesis.
[18] Mao, W., Wu, S., Zhang, X. (2015): Construction and application of Ion Torrent PGM high-throughput detection method for 16S rDNA of soil microorganisms. – Acta Agriculturae Zhejiangensis 27: 2165.
[19] Shi, H., Sun, L., Tan, J., Zhao, X., Wang, R. (2018): Effects of Bio-organic Fertilizer on Tobacco Bacterial wilt and Soil Bacterial Community. Chinese Tobacco Science 39: 54-62.
[20] Tang, H., Shi, L., Sun, G., Wen, L., Long, Z., Li, C., Cheng, K., Sun, M., Li, W., Luo, Z. (2024): Long-Term Organic Manure Retarding Soil Acidity in the Double-Cropping Rice Field of Southern China. – Communications in Soil Science and Plant Analysis 55: 1420-1429.
[21] Tian, M., Li, P., Zhao, S., Zhao, T., He, D., Jiang, H., Liu, W. (2023): Bio-organic Fertilizer and Its Application Effect. – Modernizing Agriculture, pp. 27-30.
[22] Tong, L., Gao, J., Jin, Y. (2019): Dynamic Changes of Rhizosphere Microorganisms in Strawberry at Different Growth Stages. – Journal of Beijing University of Agriculture 34: 10-15.
[23] Wang, L., Liu, B., Jiang, Y., Yan, C., Zheng, Z., Liu, J. (2016): Effects of Different Organic Fertilizers on the Growth Yield and Quality of Yunyan 99. – Journal of Anhui Agricultural Sciences 44: 17-19.

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DOI: http://dx.doi.org/10.15666/aeer/2302_29412956

[24] Wang, Z., Yang, T., Mei, X., Wang, N., Li, X., Yang, Q., Dong, C., Jiang, G., Lin, J., Xu, Y. (2022): Bio-organic fertilizer promotes pear yield by shaping the rhizosphere microbiome composition and functions. – Microbiology Spectrum 10: e03572-22.
[25] Wang, R., Liu, T., Lu, C., Zhang, Z., Guo, P., Jia, B., Hao, B., Wang, Y., Guo, W. (2023): Bioorganic fertilizers improve the adaptability and remediation efficiency of *Puccinellia distans* in multiple heavy metals-contaminated saline soil by regulating the soil microbial community. – Journal of Hazardous Materials 448: 130982.
[26] Xiao, B., Zhang, S., Li, H. (2021): Application Status and Development Trend of Bio-organic Fertilizer in Tobacco Production. – Seed Science & Technology 39: 72-73.
[27] Xing, S., Jiang, H., Deng, G., Xu, B., Wang, G., Liu, D. (2020): Effects of Different Organic Fertilizer and Chemical Fertilizer Ratio on Growth Yield and Quality of Flue-cured Tobacco. – Bulletin of Agricultural Science and Technology, pp. 85-88.
[28] Zhang, T. (2016): Effects of Soil Organic Improvement Measures on Growth Development and Quality of Flue-cured Tobacco. – Master's thesis.
[29] Zhang, L., Guo, Y., Ke, Y., Ji, Y., Zhang, Y., Zhang, Z. (2023): Effects of Bio-organic Fertilizer Combined with Subsoiling on Farmland Soil Fertility and Crop Yield. – Journal of Agricultural Science and Technology 25: 157-166.
[30] Zhang, S., Wang, Y., Huang, P., Ma, Y., Wang, Y., Wen, X., Shen, H., Wang, H., Wang, Y., Liu, L. (2024): Effects of chemical fertilizer reduction combined with sheep manure organic fertilizer on growth, yield and quality of flue-cured tobacco and soil nutrients. – Shandong Agricultural Sciences 56: 72-80.
[31] Zhu, M., Xu, D., Si, G., Peng, C., Yuan, J., Zhao, S. (2023): Effects of different organic fertilisers on the microbial functional diversity and bacterial communities in a tobacco soil. – Archives of Agronomy and Soil Science 69: 1566-1578.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_29412956