EFFECTS OF DIFFERENT APPLICATION PERIODS AND RATES OF MICROBIAL AGENTS ON SOIL MICROBIAL DIVERSITY AND DISEASES OF TOBACCO

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Abstract. Tobacco (*Nicotiana tabacum* L.) is a significant cash crop in China. In this study, we investigated the effect of microbial agent application with different rates and application periods on tobacco diseases and soil microorganisms. During the tobacco growing process, the doses of 0, 30 and 60 kg hm^{-1} were applied at the time of ridging, transplanting and resettling, respectively. 16S rRNA and ITS gene sequencing techniques were utilized to analyze the microbial communities in tobacco fields. The results indicated that the application of microbials significantly decreased the incidence rate and disease index of *Ralstonia solanacearum* and *Phytophthora nicotianae*. Moreover, the application of microbial agents resulted in an increase in the abundance of specific fungi *(Basidiomycota*) and bacteria (*Nitrospirae*), while reducing the abundance of other fungi (*Blastocladiomycota*) and bacteria (*Spirochaetes*). The optimum application dose of microbial agents at the budding stage was recommended as 60 kg.hm^{-2} for improving tobacco production and soil health, as well as for increasing the economic value of the tobacco leaves of roasted cigarettes significantly. This study emphasizes soil organic matter and pH as key factors influencing tobacco root microbiota. After microbial fertilizer treatment, the relative abundance of various microorganisms in tobacco rhizosphere soil positively correlated with soil enzyme activity and negatively correlated with soil nutrients, which reflected that tobacco rhizosphere microorganisms were regulated by the combination of soil nutrients and enzyme activity.
Keywords: *tobacco, microbial agent, disease, soil microorganism, economic return*

Introduction

Tobacco is one of the important economic crops in China and plays a key role in the national economy (Chen et al., 2022). However, over recent years, there has been a continual reduction in the tobacco planting area. This reduced area represents a significant problem for tobacco farmers in China who generally lack scientific fertilization concepts and adopt poor fertilizer application habits, thus resulting in the excessive application of chemical fertilizers (Zhang et al., 2007). The excessive application of chemical fertilizers will lead to soil compaction and a decline in soil fertility, thus resulting in an insufficient supply of soil nutrients, the formation of a vicious circle, and a decline in tobacco yield. According to data from the National Bureau of Statistics, China's roasted tobacco planting area declined from 133 million hectares to 0.97 billion hectares between 2014 and 2020. During the same period, the production of roasted tobacco also fell from 2,696,800 quintals in 2014 to 2,020,700 quintals in 2021 (Li et al., 2013; Qin et al., 2014). The main production areas are

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concentrated in Yunnan, Guizhou and other provinces (Si et al., 2008). Yunnan produces Yunnan produces the most tobacco, accounting for approximately 40% of the national total. During the same time period, there was a general improvement in the income of residents and changes in the cigarette market. The overall demand for cigarettes in China has shown a decreasing trend over recent years, with a slight rebound in 2021.

High-quality tobacco production requires good soil conditions. For sustainable tobacco production, soil quality must be improved (Liao et al., 2003). The inter-root microbiota plays an important role in improving crop yield (Li et al., 2007). However, due to the heavy use of chemical fertilizers, and a series of poor tillage practices in tobacco growing areas, soil microbial abundance, and diversity have been reduced, thus leading to significant problems, including soil acidification, hardening, and reduced fertility, which is not only severely affect crop yields, but also negatively impact sustainability and greening within the ecosystem (Yang et al., 2022).

Microbial fungicide control of soil-borne diseases is the use of soil beneficial microorganisms to improve soil physicochemical properties, promote crop growth, reduce harmful microbial infestation, so as to inhibit the occurrence of crop diseases, is the current crop disease biological control of the main methods and important ways. The use of microbial agents to control soil-borne diseases of crops has become a hot spot in current research (Chang et al., 2017). The study proved that Bacillus subtilis wettable powder mixed with seed has a good effect on the prevention and control of wheat root rot and total erosion disease. Yang et al. (2023a) showed that microbial agents utilize soil beneficial microorganisms to maintain soil micro-ecological balance to achieve the effect of disease prevention, yield increase and crop quality improvement. Huang et al. (2023a) and He et al. (2021) proved that the application of microbial agents can improve the disease resistance of tobacco plants, and has a certain effect on the prevention and control of tobacco ‘two blacks and one green. An earlier study showed that the application of microbial fertilizers improved soil fertility and the quality of kiwi fruits by balancing the structure of the soil microbial community, regulating soil enzyme activities, and increasing the efficiency of nutrient use. Moreover, another study indicated that the application of microbial fertilizers mixed with organic fertilizers could increase cotton yield and quality by reducing soil bulk density, increasing cation exchange capacity, and improving soil physicochemical properties, which is because organic fertilizers provide abundant nutrients for soil microorganisms to exert fertilizer effects.

Soil microbial communities have been recognized to play a crucial role in crop growth and soil fertility, as highlighted by Gou et al. (2023) and Schweitzer et al. (2008). These communities influence a range of important factors such as nutrient cycling, plant symbiosis, soil structure, and nutrient availability, thereby positively impacting crop productivity and soil health (Fabra et al., 2010; Duan et al., 2012a). It is important to identify key microbes or microbial communities that influence plant productivity as indicators to predict soil health and crop productivity, rather than solely assessing overall microbial diversity. Microbial biomass and diversity have been identified as important indicators for evaluating soil quality due to their high sensitivity to changes in soil nutrients, pH, and organic matter content. Microbes possess the ability to adapt to different soil conditions by adjusting their diversity and community composition to rapidly respond to changing environmental factors. This adaptability allows them to effectively respond to changes in their surrounding environment and play a critical role in maintaining soil health and promoting the growth of plants.

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In addition, previous studies have shown that the activities of soil enzymes can be influenced by microbial communities and play a crucial role in nutrient cycling and transformation processes. Tan et al. (2021) identified a strong positive correlation between soil enzyme activity and microbial abundance. Mao et al. (2014) identified a significant correlation between urease activity and the abundance of bacteria and fungi following the application of microbial agents. Deshoux et al. (2023) found that the application of microbial agents led to significant changes in the microbial community, there was a significant increase in the bacterial abundance in the soil, however, the abundance of fungi decreased significantly and the soil shifted from a fungal to a bacterial type. Wang et al. (2020) reported that the application of microbial fertilizers to roasted tobacco led to an increase in bacterial abundance, however, there was then a reduction in abundance with the prolongation of the growth period, with a gradual decrease in the number of fungi. The main objective of this study was to determine the effectiveness of different doses and times of application of microbial agents in mitigating tobacco diseases and enhancing soil microbial health, thereby improving tobacco yield and soil quality.

Materials and methods

Location and climate conditions

The experiment was conducted in 2021 in Jingxi County, Baise City, Guangxi, China (105°56′-106°48′E, 22°51′-23°34′N, elevation: 800-1400 m). The average annual temperature in 2021 was approximately 19.1°C, with an annual rainfall of 1604 mm. The soil at the experimental site is classified as brown calcareous soil. The soil pH was approximately 6.47, and the concentrations of potassium, total nitrogen, total phosphorus, organic matter, and available potassium were 11.49 $mg.kg^{-1}$, 2.58 mg.kg-1, 1.65 $mg.kg^{-1}$, 19.11 $g.kg^{-1}$, and 227.09 $mg.kg^{-1}$, respectively.

Experimental design

The experiment was conducted in a split-plot design with two factors: the time of microbial inoculant application (A) and the dose applied (B). The main plots were assigned to different application times: A1 (microbial inoculant applied at the ridging stage): A2 (microbial inoculant applied at the transplanting stage) and A3 (microbial inoculant applied at the resettling stage). The application rates of microbial inoculant were used as the subplots: B1 (0 $kg.hm^{-2}$): B2 (30 $kg.hm^{-2}$): and B3 (60 $kg.hm^{-2}$). The length of each subplot was 57.68 m and the width was 1.1 m. So, the area of each subplot was about 63.45 m^2. Each subplot was replicated three times and a total of 70 plants were grown in each subplot. The control (CK) group received microbial 0 $kg.hm^{-2}$ of inoculant.

Experimental materials

Experimental variety: tobacco variety K326.

Microbial agents: Purchased from Beijing Engelan Environmental Technology Co., Ltd., this tobacco-specific microbial agent has an effective live bacterial count of $\geqslant$ 30.0 billion $CFU.g^{-1}$, including Bacillus amyloliquefaciens $\geqslant$ 15.0 billion $CFU.g^{-1}$, Bacillus subtilis $\geqslant$ 5.0 billion $CFU.g^{-1}$, Bacillus megaterium $\geqslant$ 2.0 billion $CFU.g^{-1}$, and other beneficial anti-disease bacteria $\geqslant$ 8.0 billion $CFU.g^{-1}$.

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Classification of tobacco grades and determination of economic traits

After harvest and curing, the tobacco leaves were sorted and weighed to determine the yield for each plot according to the national tobacco grading standards (GB2635-92). Subsequently, the average price and production value were calculated based on the tobacco purchase prices, as well as the year and production area. The comparison of tobacco leaf purchase prices for the years 2022 and 2021 conducted by the Tobacco Science department is grounded in the "Notice on the Tobacco Leaf Purchase Price Policy for 2022" issued by the China National Tobacco Corporation (Zhongyan Office, 2021, No. 133).

Investigation of soil-borne diseases in the field

The incidence of Ralstonia solanacearum and Phytophthora nicotianae in each plot was investigated at 95 days after transplanting, and the incidence rate, disease index. According to Tobacco Pest and Disease Grading and Survey Methods (GB/T 23222-2008), the severity of tobacco Ralstonia solanacearum was graded:

Grade 0: no disease on the whole plant.

Grade 1: occasional greenish spots on the stem, or a few leaves withered on the side with spots.

Grade 2: black spots on the stem, but not yet reached the top, or more than half of the leaves on the diseased side withered.

Grade 3: black spots on the stem reach the top of the plant, or more than two thirds of the leaves on the diseased side wither.

Grade 4: the plant is basically dead.

$$\text{Incidence rate (\%)} = (\text{number of diseased plants} / \text{total number of plants surveyed}) * 100 \quad \text{(Eq.1)}$$

$$\text{Disease index} = 100 \times \frac{\sum(\text{Number of Strains at Each Level} * \text{Representative Value for Each Level})}{\text{Total Number of Samples} * \text{Highest Representative Value}} \quad \text{(Eq.2)}$$

Soil sampling

Before the first tobacco harvest June 2022: soil samples were collected from the 15-20 cm soil layer of the tobacco field using a spiral auger. A total of 27 soil samples were collected. The collected soil samples were divided into three portions for further processing. The first portion of the sample was passed through a 2.0 mm sieve to remove residual roots and gravel. This portion was then used to determine the physical and chemical properties of the soil after natural air drying. The second portion was stored in a refrigerator at 30°C for the determination of soil enzyme activity. The third portion was stored in a freezer at -80°C for high-throughput sequencing analysis of soil bacteria and fungi.

Soil physical and chemical properties

The physicochemical properties of the soil we analyzed are as follows. The air-dried soil samples were ground and sieved through a 2.0 mm mesh to obtain a homogeneous texture. The chemical properties of the soil were then assessed using a variety of methods. Soil pH was measured using a pH meter (FE-20, Mettler-Toledo) with a soil-to-water ratio of 1:2.5. The soil organic matter (SOM) content was determined by potassium dichromate titration according to the Chinese national standard. The total

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nitrogen (TN) content was quantified using the Kjeldahl method. For total phosphorus (TP) content was quantified by the sodium hydroxide melting method. The total potassium (TK) content was determined using the sulfuric acid-potassium permanganate digestion method. The alkaline diffusion method was employed to determine the available nitrogen (AN) content, and the available phosphorus (AP) content was measured using the ammonium molybdate antimony anti-segmented flow spectrophotometry method.

Determination of soil enzyme activity

To determine soil enzyme activities, specifically urease (S-UE): sucrase (S-SC): catalase (S-CAT): and polyphenol oxidase (S-PPO): we used Solarbio Soil Enzyme Assay Kits. These enzyme assay kits were provided by Solarbio (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). The specific methods and measurement procedures were conducted in accordance with the manufacturer's instructions.

Determination of soi microbial community

Total soil DNA was extracted from 0.5 g of each soil sample using the MagPure Soil DNA LQ Kit in accordance with the manufacturer's instructions. For bacterial community analysis, the V3-V4 region of the 16S rRNA gene was amplified by PCR. For fungal community analysis, the ITS1 region of the ITS gene was amplified. Tks Gflex DNA Polymerase (Takara, catalog number: R060B) was used for two rounds of PCR amplification. The PCR products were then subjected to gel electrophoresis, followed by magnetic bead purification and a second round of PCR amplification. PCR reaction conditions and cycling parameters were as follows. For the first round of PCR, the reaction mixture featured 15 μl of 2× Gflex PCR buffer, 1 μl of each primer (5 pmol/μl): ≥ 1 μl of DNA template ≥ 1 μl, and 0.6 μl of Tks Gflex DNA polymerase, water was added to a total volume of 0.6 μl. The cycling conditions were as follows: initial denaturation at 94°C for 5 min followed by 30 cycles of denaturation (94°C for 30 s): annealing (56°C for 30 s): and extension (72°C for 20 s). The reaction was completed by final extension at 72°C for 5 min. For the second round of PCR, the reaction mixture contained 15 μl of 2× Gflex PCR buffer, 0.6 μl of Tks Gflex DNA polymerase, 1 μl of each aptamer, 50 ng of first-round PCR product, and water was added to a total volume of 30 μl. The cycling conditions were as follows: initial denaturation at 94°C for 5 min, followed by 7 cycles of denaturation (94°C for 30 s): annealing (56°C for 30 s): and extension (72°C for 20 s): this was followed by a final extension at 72°C for 5 min. Finally, paired-end sequencing of bacteria and fungi was performed on an Illumina MiSeq sequencer at OEBiotech Co., Ltd. (Shanghai, China).

Data analysis

Microsoft Excel 2010 was used for statistics and calculations (Microsoft Corporation, Redmond, WA, USA): SPSS version 26.0 was used for significance and correlation analysis (IBM, Armonk, NY, USA): and Origin 2021 software was used to plot graps (OriginLab, Northampton, MA, USA). The T-test was used for significance analysis, and the LSD test was applied after analysis of variance （ANOVA） to clarify differences between groups. The R package and LEfSe software were used for charting and microbial data analysis. The "mantel" function from the "vegan" and "ecodist" packages in RStudio

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(RStudio, Boston, MA, USA) was used to compute and visualize correlations between species composition, environmental factors, and ecological distances (Crabot et al., 2019; Goslee et al., 2007). Redundancy analysis (RDA) was performed using Canoco 5 (Microcomputer Power, Ithaca, NY, USA) to assess the correlation between soil microbial composition and soil properties. Data were tested for normality using the Shapiro-Wilk test in SPSS (IBM, Armonk, NY, USA) prior to RDA analysis. Non-normal data were transformed using log10 or sqrt transformation.

Results

Disease occurrence of tobacco plants

The application of microbial agent significantly decreased the occurrence of Ralstonia solanacearum and Phytophthora nicotianae in tobacco plants (*Table 1*). Compared with CK, all microbial agent treatments except A2B3 significantly decreased the disease index of Ralstonia solanacearum. Compared with CK, all microbial agent treatments except A1B2 significantly decreased the disease index of Phytophthora nicotianae.

For Ralstonia solanacearum, the A3B3 treatment exhibited the lowest morbidity and disease index, with significantly higher values of incidence and disease index observed only in CK and A1B2 for morbidity, and in CK and A2B2 for disease index. Similarly, for Phytophthora nicotianae, the A3B2 treatment achieved the lowest morbidity and disease index, surpassing the efficacy of all other treatments relative to the control, except for A1B2. the application times of microbial agent significantly reduces Phytophthora nicotianae incidence and disease index, while the application rates of which obviously decreases Ralstonia solanacearum incidence and disease index.

Table 1. *Effects of microbial agent application with different rates and applied periods on the occurrence of Ralstonia solanacearum and Phytophthora nicotianae in tobacco plants*

Treatment	Incidence rate of *Ralstonia solanacearum*	Disease index of *Ralstonia solanacearum*	Incidence rate of *Phytophthora nicotianae*	Disease index of *Phytophthora nicotianae*
CK	3.27 ± 0.05a	3.66 ± 1.03a	5.44 ± 0.62a	3.98 ± 0.47a
A1B2	1.84 ± 0.37b	1.65 ± 0.32bc	4.01 ± 0.39b	3.11 ± 0.43ab
A1B3	1.42 ± 0.33bc	1.61 ± 0.32bc	3.25 ± 0.06bc	1.98 ± 0.17c
A2B2	1.45 ± 0.35bc	1.45 ± 0.35bc	2.93 ± 0.35bc	2.2 ± 0.01c
A2B3	2.12 ± 0.59b	2.49 ± 0.71ab	3.57 ± 0.3bc	2.68 ± 0.27bc
A3B2	1.47 ± 0.37bc	1.47 ± 0.37bc	2.54 ± 0.40c	2.00 ± 0.39c
A3B3	1.36 ± 0.36c	1.36 ± 0.36c	2.89 ± 0.39bc	2.35 ± 0.39c
FA	1.12	1.23	7.33**	10.41**
FB	2.21**	8.25***	16.02**	7.34
FA*B	3.14	5.19**	0.92	8.64**

The same letters after the values indicate no significant differences between treatments ($p < 0.05$)

Yield and economic traits of tobacco

The application of microbial agent significantly enhanced the economic return of tobacco production (*Table 2*). Compared with CK, all microbial agent treatments had no

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significant effect on yield while all microbial agent treatments significantly increased the proportion of top-grade tobacco leaves, average price, and economic return. The proportion of top-grade tobacco leaves and medium-quality tobacco leaves for the treatments of A1B3, A2B2, A3B2, and A3B3 were higher than that of CK. However, the treatments of A1B2, A1B3, A3B2, and A3B3 showed higher average price than CK. Therefore, compared with CK, the treatments of A1B2, A1B3, A2B2 achieved much more economic return.

Table 2. *Effect of application of microbial preparations at different rates and application times on yield and economic traits of post-roasted tobacco leaves*

Treatment	Yield ($kg.hm^{-2}$)	Proportion of top-grade tobacco leaves (%)	Proportion of medium-quality tobacco leaves (%)	Purchase price ($kg.RMB^{-1}$)	Economic return ($RMB.hm^{-2}$)
CK	3138.01 ± 26.41ab	70486.66 ± 849.42c	22.46 ± 0.09d	0.44 ± 0b	0.2 ± 0.01a
A1B2	3112.53 ± 35.67ab	75357.88 ± 1122.96a	24.21 ± 0.19b	0.49 ± 0a	0.15 ± 0c
A1B3	3101.31 ± 15.77ab	74185.28 ± 772.62ab	23.92 ± 0.14bc	0.49 ± 0.01a	0.12 ± 0.01d
A2B2	3020.68 ± 64.66b	71270.46 ± 1813.53bc	23.59 ± 0.1c	0.49 ± 0a	0.17 ± 0.01bc
A2B3	3154.07 ± 77.16ab	76555.65 ± 1156.45a	24.28 ± 0.23b	0.5 ± 0.01a	0.16 ± 0c
A3B2	3090.82 ± 12.52ab	76567.01 ± 163.53a	24.77 ± 0.05a	0.5 ± 0a	0.19 ± 0.01ab
A3B3	3218.77 ± 25.19a	75537.08 ± 978.86a	23.47 ± 0.14c	0.48 ± 0a	0.17 ± 0abc
A	1.28	1.98	0.86	0.08	15.89***
B	2.95	5.91*	44.99***	38.43***	20.86***
A* B	1.79	5.81*	24.28***	3.55	0.89

The same letters after the values indicate no significant differences between treatments ($p < 0.05$)

Fungal community in soil

The application of microbial agent at different times and different rates exerted significant effects on fungi in paddy soil (*Fig. 1*). For fungi, 638 Operational taxonomic units (OTUs) were detected in the samples of all treatments. The number of special OTUs detected in A1B2, A1B3, A2B2, A2B3, A3B2, A3B3, and CK were 990, 942, 809, 537, 911, 1153, and 1035, respectively. Compared with CK, the A3B2, A1B3, and A2B2 treatments reduced the abundance of *Blastocladiomycota*, *Glomeromycot*a, and *Ascomycota*. A higher abundance of *Basidiomycot*a was recorded for the A3B2 and A1B3 treatments than for the CK treatment. Compared with CK, A1B3 treatment increased the abundance of *Chytridiomycot*a. A higher abundance of *Cercozoa* was recorded for the A2B2 and A2B3 treatments than CK.

Bacteria community in soil

For bacteria, there were 3023 OTUs detected in the samples across all treatments. The number of OTUs detected in A1B2, A1B3, A2B2, A2B3, A3B2, A3B3, and CK were 2755, 2455, 2491, 1697, 2540, 3253, and 2670, respectively. Compared with CK, the A1B3, A3B3, and A2B3 treatments increased the abundance of *Gemmatimonadetes* and *Actinobacteria*. A higher abundance of *Nitrospirae*, *Patescibacteria*, *Chloroflexi*, *Elusimicrobia*, *Acidobacteria*, and *Verrucomicrobia* was recorded in A1B2 and A2B3 treatments than CK. A lower abundance of *Spriochaetes*, *Fusobacteria*, *Firmicutes*, and *Epsilonbacteraeota* were recorded in A2B2, A1B3, and A3B3 treatments than CK. Compared with CK, the A2B2 treatment increased the abundance of Bacteroidetes (*Fig. 2*).

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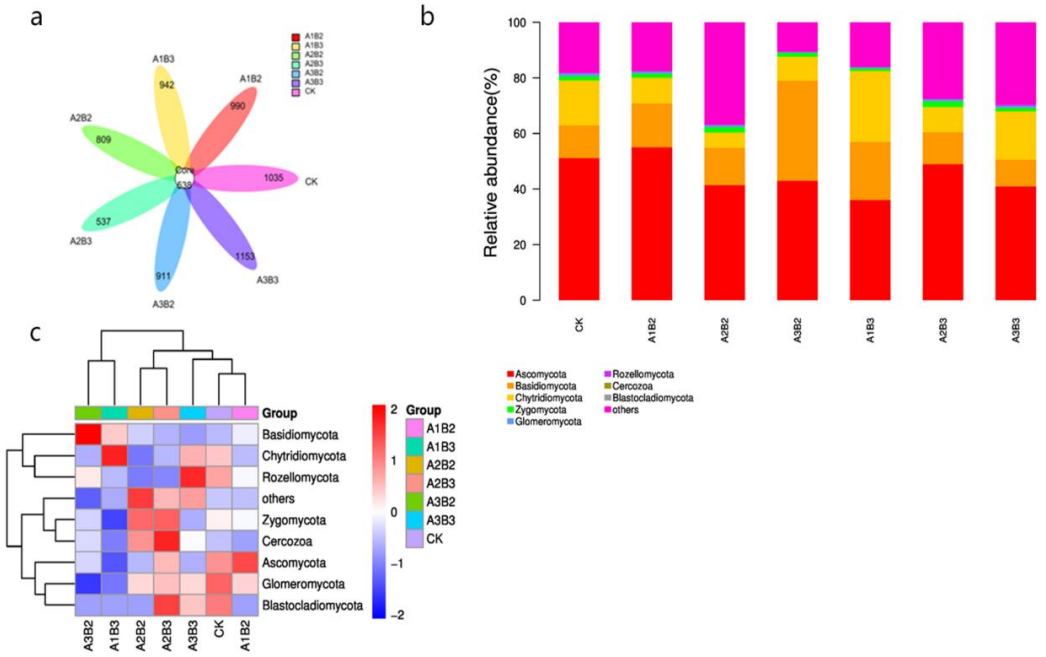

Figure 1. *The effects of microbial agent application on fungi in soil. (a) The number of fungal OTUs in different treatments. (b) Bar chart presenting the relative abundance of the top 15 fungal phyla. (c) Clustered heatmap displaying the relative abundance of the top 15 fungal taxonomic groups*

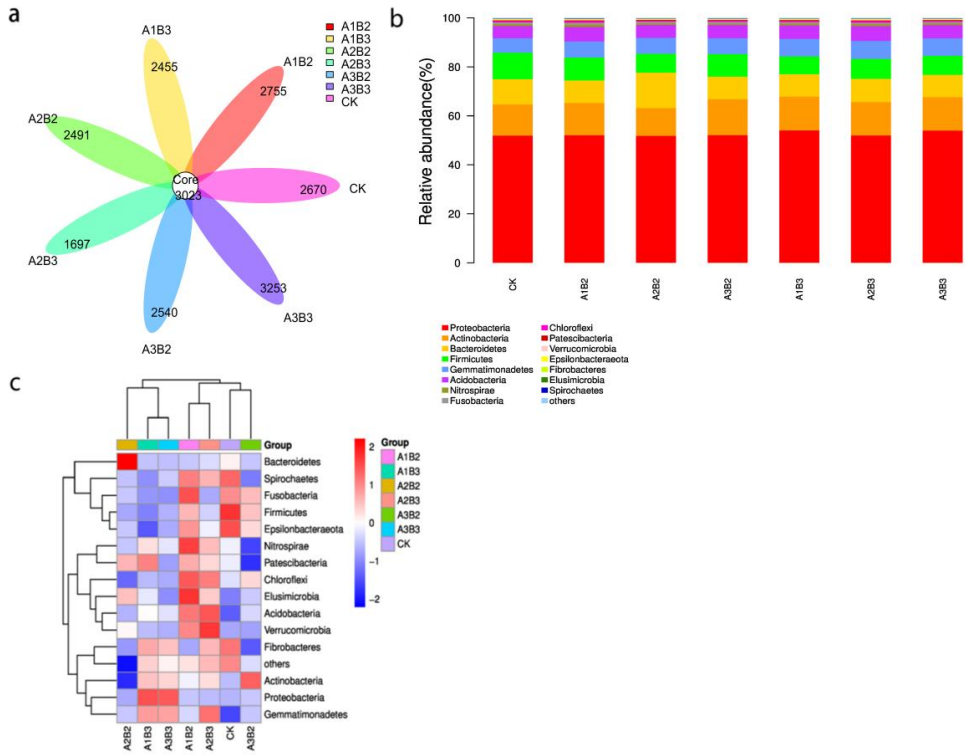

Figure 2. *The effects of microbial agent application on bacteria in soil. (a) The number of OTUs of bacteria in different treatments. (b) Bar chart presenting the relative abundance of the top 15 bacterial phyla in the rhizosphere soil samples. (c) Clustered heatmap showing the relative abundance of the top 15 bacterial taxonomic groups in the rhizosphere soil samples*

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Soil enzyme activities

The application of microbial agent at different times and different rates significantly affected the activities of soil enzymes, including S-PPO, and S-UE (*Fig. 3*). All microbial treatments produced significant or highly significant increases in the activities of two soil enzymes S-UE, while, except the A1B2 and A2B3, other treatments also reduced the activities of S-PPO markedly when compared to CK.

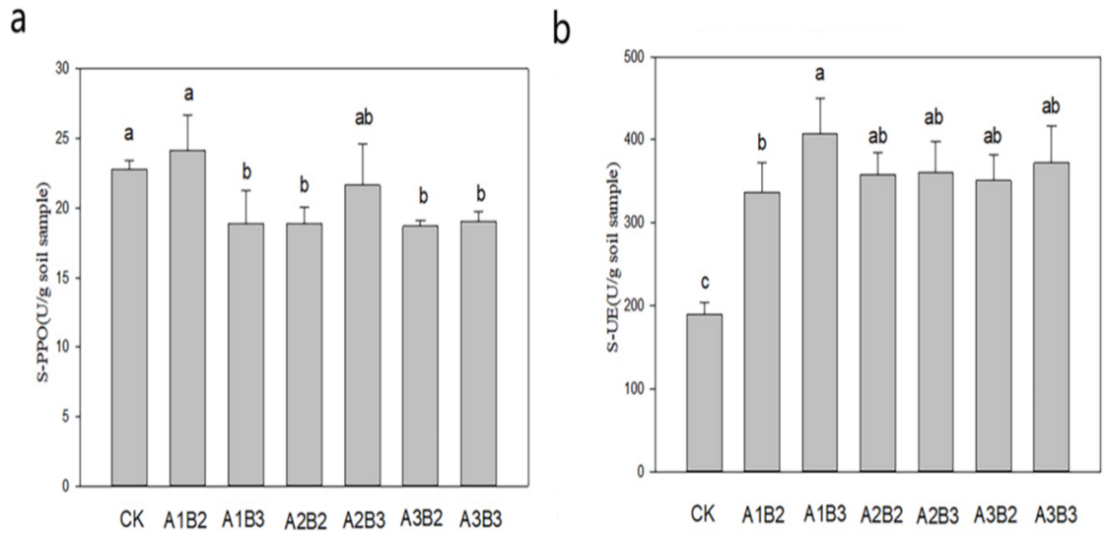

Figure 3. *The effects of microbial agent application on soil enzyme activity. S-PPO, soil polyphenol oxidase, S-UE, soil urease. Values are reported as repeated mean ± standard error. The average of the different letters (such as a, b, and c) in each column was significantly different at* $p < 0.05$

Comparison of physico-chemical properties of inter-root soils with different application periods and application rates of microbial agents

The application period (A) and application amount (B) of microbial agents had significant effects ($p < 0.01$) on soil organic matter, nutrients, enzyme activities and other indexes, and there was a significant interaction between the two. There were significant differences in the data of various indexes under different treatment combinations: The most significant differences between the soil organic matter (SOM) content of treatment A3B2 and the other treatment groups were observed. These two values were very close to each other and the differences were not significant. the significance ratings of FA (91.95**) and FB (45.78**) indicated that there were significant differences in the SOM content among the treatments. total nitrogen (TN): total potassium (TK): and the lowest pH value, A3B3 treatment had the highest content of total phosphorus (TP) and available potassium (AK). The results indicated that microbial agents could regulate soil nutrient conditions, enzyme activities and microbial activities, and ultimately affect the inter-root microbial composition of tobacco. The next step needs to clarify the mechanism of different treatments on specific microbial communities and correlation studies with tobacco disease occurrence to provide theoretical basis for the development of environmentally friendly microbial fertilizers (*Table 3*).

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Table 3. *Comparison of physico-chemical properties of inter-root soils with different application periods and application rates of microbial agents*

Treatment	SOM g.kg^{-1}	TN g.kg^{-1}	TP g.kg^{-1}	TK g.kg^{-1}	AN mg.kg^{-1}	AP mg.kg^{-1}	AK mg.kg^{-1}	pH
CK	27.57 ± 0.27c	7.62 ± 0.11d	2.65 ± 0.02a	1.17 ± 0.01c	225.23 ± 0.59d	73.77 ± 0.27d	11.18 ± 0.09e	7.63 ± 0.03b
A1B2	32.47 ± 0.74a	15.6 ± 0.06a	2.54 ± 0.02a	1.23 ± 0.01bc	252.98 ± 1.24b	60.35 ± 0.51e	21.86 ± 0.48a	6.3 ± 0.06e
A1B3	26.18 ± 0.51d	9.15 ± 0.22c	2.76 ± 0.09a	1.33 ± 0.04bc	212.26 ± 2.63e	79.62 ± 0.51cd	18.83 ± 0.17b	7.73 ± 0.03b
A2B2	32.38 ± 0.25a	11.39 ± 1.11b	2.12 ± 0.22b	1.13 ± 0.06a	274.2 ± 3.48a	80.63 ± 1.02cd	18.56 ± 0.24b	6.83 ± 0.03d
A3B2	22.5 ± 0.41e	6.86 ± 0.1d	2.9 ± 0.11a	1.25 ± 0.03c	220.2 ± 2.94d	91.68 ± 1.53bc	15.03 ± 0.37c	7.87 ± 0.03a
A3B3	27.89 ± 0.42c	16.14 ± 0.01a	2.9 ± 0.04a	1.56 ± 0.01a	233.18 ± 0.11c	96.85 ± 6.73b	12.67 ± 0.32d	5.77 ± 0.03f
FA	91.95**	18.34**	9.2**	10.57**	147.5**	38.87**	56.57**	39.02**
FB	45.78**	81.43**	22.63**	31.04**	60.53**	8.97**	38.99**	594.48**
FA*B	51.01**	143.3**	52.44**	10.44**	84.07**	24.2**	391.72**	695.72**

FA and FB marked with * indicated significant difference within A and B factors at the 0.05 level, respectively; and that with ** indicated significant difference within A and B factors at the 0.01 level, respectively. SOM: organic matter, TN: total nitrogen, TP: total phosphorus, TK: total potassium, AN: available nitrogen, AP: available phosphorus, AK: available potassium, pH: soil pH value

Effects of microbial inoculants application with different times and different rate on α-diversity of soil microbial communities

The period of microbial application and the amount of microbial application had some effects on the abundance (Chao1) and diversity index (Shannon, Simpson) of soil fungi and bacteria. The differences in Chao1 indices of fungi and bacteria were also obvious, indicating that the application of microbial agents caused changes in the abundance of soil fungi and bacteria. The fungal abundance and diversity indices varied greatly among different treatments, such as the Shannon and Simpson indices of the A3B2 group, while the differences among the bacterial indices were small (*Table 4*).

Table 4. *Alpha diversity of fungi and bacteria in soil*

Microorganisms	Treatment	Chao1	Shannon	Simpson
Fungi	CK	915.26 ± 44.03ab	5.93 ± 0.07a	0.94 ± 0.002a
	A1B2	849.44 ± 48.15ab	5.57 ± 0.17a	0.93 ± 0.012a
	A2B2	770.04 ± 54.57b	5.15 ± 0.55a	0.91 ± 0.026a
	A3B2	851.02 ± 114.09ab	3.88 ± 1.24b	0.66 ± 0.185a
	A1B3	899.31 ± 10.56ab	5.31 ± 0.56ab	0.89 ± 0.044a
	A2B3	875.15 ± 28.88ab	5.40 ± 0.46ab	0.90 ± 0.046a
	A3B3	989.10 ± 31.04a	5.77 ± 0.45a	0.93 ± 0.025 a
Bacteria	CK	4188.51 ± 42.31c	9.28 ± 0.06a	0.99 ± 0.001b
	A1B2	4290.85 ± 94.94bc	9.26 ± 0.10a	0.99 ± 0.001ab
	A2B2	4415.59 ± 66.06bc	9.29 ± 0.10a	0.99 ± 0.001a
	A3B2	4374.5 ± 134.07bc	9.32 ± 0.13a	0.99 ± 0.001ab
	A1B3	4516.52 ± 15.17ab	9.42 ± 0.05a	0.99 ± 0.000a
	A2B3	4558.81 ± 72.27a	9.51 ± 0.04a	0.99 ± 0.000a
	A3B3	4529.07 ± 52.73ab	9.48 ± 0.09a	0.99 ± 0.000a

Means followed by the same letter are not significantly different at the 0.05 probability level according to the least significant difference test (LSD 0.05)

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Influence of community composition, diversity and soil physico-chemical properties of inter-root soil microbial communities of tobacco plants on the incidence of Ralstonia solanacearum and Phytophthora nicotianae

RDA analysis of soil enzyme activities and chemical properties at the bacterial community gate level showed (*Fig. 4*) that RDA1 and RDA2 explained 15.2% and 9.23% of the variance, respectively, with a cumulative contribution of 24.43%, while RDA1 and RDA2 explained 18.62% and 13.29% of the variance, respectively, with a cumulative contribution of 31.91%. Soil polyphenol oxidase (S-PPO) was positively correlated with *Fusarium*, *Sclerotinia*, *Bacteroidetes*, N*itrifying*, Bacteria and negatively correlated with C*hlorobacterium*, *Acidobacterium*, *Graminobacterium*, and *Proteus* in the inter-root soil, Soil urease (S-UE) was positively correlated with *Acidobacteria* and *Proteobacteria* and negatively correlated with *Fusobacteria* and *Firmicutes* (*Fig. 4a*).

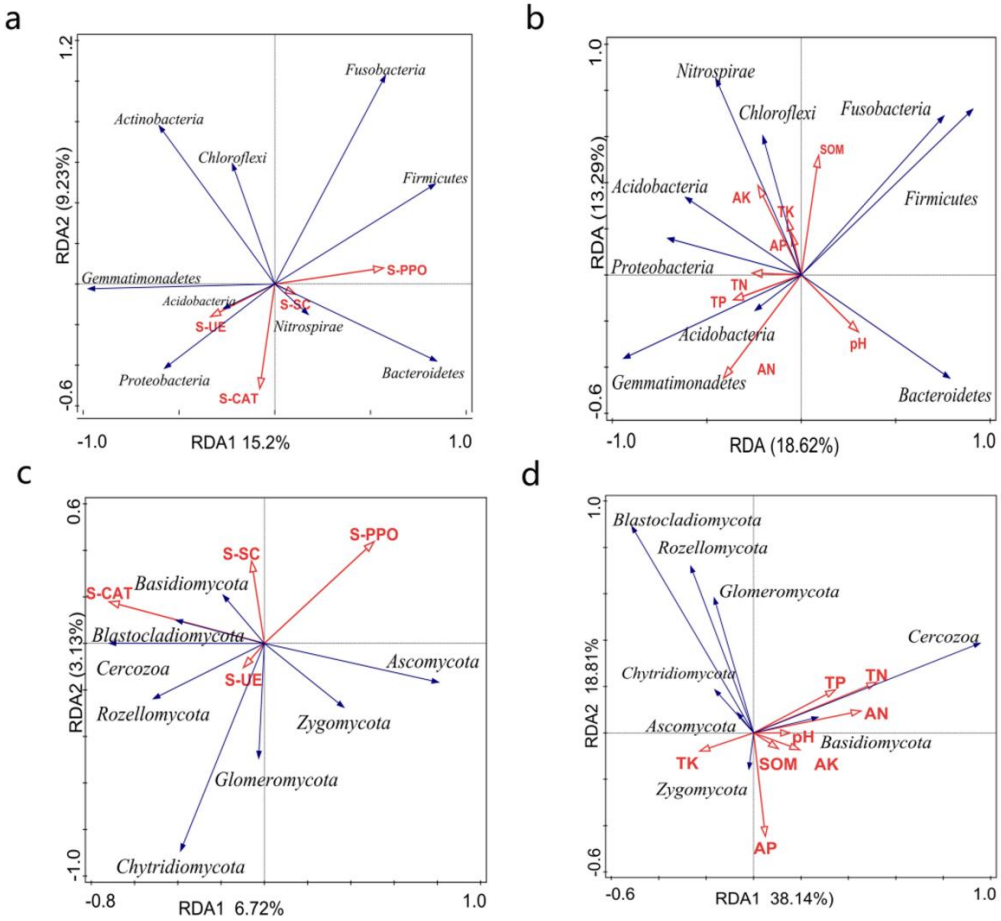

Figure 4. *Typical correspondence analysis (RDA) of key species composition of tobacco inter-root soil bacterial communities at phylum (a) and genus (b) level and fungal phylum (c) and genus (d) with environmental factors. Blue arrows represent the top 10 phylum and genus species in terms of abundance, red arrows represent soil physicochemical indexes, and the length of arrows represents the degree of influence*

Soil organic matter (SOM) and pH were positively correlated with *Fusobacteria*, *Firmicutes* and *Bacteroidota* in the inter-root soil, and negatively correlated with

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Chloroflexi, *Nitrospirae*, *Acidobacteria*, *Gemmatimonadetes*, *Acidobacteria* and *Proteobacteria*, *Acidobacteria*, *Gemmatimonadetes* were negatively correlated with their distributions, whereas the total potassium (TK): effective potassium (AK): effective phosphorus (AP): total nitrogen (TN): total phosphorus (TP): and quick-acting nitrogen (AN) contents were negatively correlated with the distribution of *Fusobacteria*, *Firmicutes*, and *Bacteroidota* (*Fig. 4b*).

The effects of community composition, diversity and soil physicochemical properties of inter-root soil microbial communities of tobacco plants on the incidence of Ralstonia solanacearum and Phytophthora nicotianae were analyzed by Mantel's test (*Fig. 5*). The incidence of tobacco plant blight was strongly influenced by soil microbial communities and diversity, and to a lesser extent by the incidence of Phytophthora nicotianae, where the incidence of blight was mainly related to the soil bacterial community, and the incidence of Phytophthora nicotianae was related to the soil fungal community. Cyanosis incidence was correlated with bacterial Simpson's index and fungal Chao1 index, and at the phylum level, cyanosis incidence was correlated with bacterial *Neisseeria* and *Streptomonas* phylum (*Fig. 5a*): Phytophthora nicotianae incidence was correlated with fungal *Ambiguous_taxa* phylum (*Fig. 5b)*.

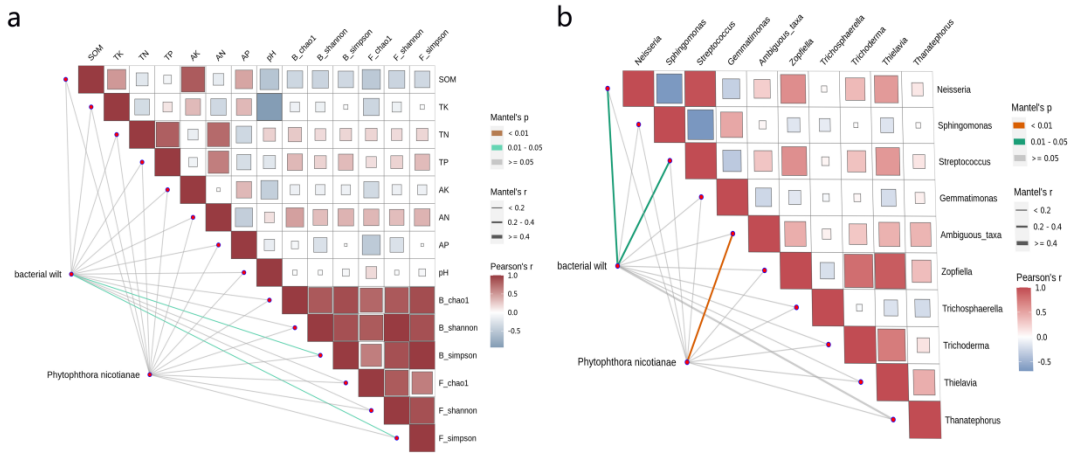

Figure 5. *Mantel test for correlation between the key species composition of bacterial (a) and fungal (b) communities in the inter-root soil of tobacco plants in the occurrence of downy blight and the incidence of blight and Phytophthora nicotianae in diseased tobacco plants. The color gradient of the Mantel test represents Spearman's correlation coefficient, with more positive values (red) indicating stronger positive correlation and more negative values (blue) indicating stronger negative correlation, and the edge width corresponds to the Mantel's r statistic for correlation, and the color gradient of the Pearson's correlation coefficient, r, represents pairwise correlation of the variables, with * indicating 0.01 < P < 0.05, ** indicating P < 0.01, and *** indicating P < 0.001*

Discussion

Tobacco production plays an important role in the national economy of China because of its high economic value. In the present study, the effect of microbial agent application with different rates application periods performances and soil microorganisms. Our results showed that the application of microbial agent increased Significantly increase tobacco yields and economic returns. The improvement in economic return was attributed to the higher proportion of top-grade tobacco leaves which benefited from the decline in

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tobacco disease including Ralstonia solanacearum and Phytophthora nicotianae. Our results agreed with the previous study which indicated that the microbial agent input is an effective way in improving crop quality and soil health.

In this study, it was found that the moderate use of microbial agents was effective in controlling Ralstonia solanacearum and Phytophthora nicotianae, A3B3 (60 $kg.hm^{-2}$ microbial inoculant applied at ridging stage) was the most effective in controlling Ralstonia solanacearum, with an incidence of 1.36%, a disease index of 1.35%, and a preventive effect of up to 90.22%, the Phytophthora nicotianae of A3B3 (30 $kg.hm^{-2}$ microbial inoculant applied at the transplanting stage) treatment had the lowest incidence rate was 2.54%, reducing Ralstonia solanacearum incidence by 80.50%-20.63% and increasing disease reduction by 19.5%-79.37%

In a previous study (Liu et al., 2017), microbial fertilizer treatment was shown to significantly improve the yield, quality and economic benefits of tobacco, all indices were better than in the control group. Of these indices, the yield increased by 11.69%, the proportion of top-grade tobacco increased by 7.66%, the production value increased by 18.97%, and the net profit increased by 18.29%.

A previous study reported by Shi et al. (2019) showed that microbial agents containing different concentrations of Bacillus subtilis and Pseudomonas fluorescens led to a significant increase in the yield and production value of tobacco, this finding was consistent with our present findings. In this study, we found that the application of microbial agents significantly improved the yield of tobacco plants compared to CK, and the yield, production value, average price, and proportion of top-grade tobacco in the A3B3 treatment increased by 4801.28 $CNY.hm^{-2}$, 1.64 $CNY.kg^{-1}$, and 3.68%, respectively, the best performance was exhibited by economic traits when compared to the control group. This also confirmed that microbial agents can promote the growth of roasted tobacco and improve both yield and quality, Previous studies by Huang et al. (2023b). He et al. (2021) showed that when compared with chemical fertilizer application alone, Engerland stubble-resistant treatments increased the proportion of top-grade cigarettes by 4.39–15.48%, the average price by 3.89–9.64%, the yield by 1.96–2.55%, and yield by 3.47–7.91%. Furthermore, the application of 60 $kg.hm^{-2}$ of microbial fungi during the ridging stage increased the unit price, yield and proportion of medium to top quality tobacco and increased the economic value of roasted tobacco as compared to controls. This study provides preliminary evidence for the feasibility and potential of microbial fertilization for tobacco, although there are limitations that need to be considered. We only evaluated a single strain and dose, thus limiting the scope of our experiments. Furthermore, we did not identify the mechanism of action involved. Follow-up studies can remedy these shortcomings by optimizing the protocol, expanding the experimental scope, and by identifying the specific mechanism involved, thus increasing the application value of this technology.

It is well known that high-quality tobacco production requires good soil condition. In the present study, we observed that microbial agent application had substantial effects on soil microorganisms. For fungi, we found that the application of microbial inoculants (A3B2 and A1B3 treatments) increased the abundance of Basidiomycota which is able to break down organic matter and enhance the decomposition and recycling of soil organic matter (Baldrian, 2008). This result indicated that the application of microbial inoculants might regulate fungi to improve the organic matter of soil and thus benefit soil health. For bacteria, A2B2 treatment increased the abundance of Bacteroidetes while A1B3 and A3B3 treatments increased the abundance of Gemmatimonadetes,

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these bacteria have been reported as beneficial taxa for the immobilization of cadmium in soil. A previous study by Wu et al. (2022) indicated that Bacteroidales dominated paddy soil and promoted nitrogen cycling. We found that the application of microbial inoculants (A1B2 and A2B3 treatments) increased the abundance of Patescibacteria and Actinobacteria. According to a previous study, these bacteria are related to the metabolism of soil nitrogen. Moreover, we found that the abundance of Proteobacteria increased in response to certain microbial inoculant treatments (A1B3 and A3B3): an earlier study indicated that Proteobacteria was beneficial to the bacteria in the soil.

The activity of enzymes in the soil is also used as a sensor in studies investigating the effects of soil treatments on microbial activity and the fertility of soil. Soil catalase is a key enzyme that catalyzes the decomposition of urea into ammonia and carbon dioxide. Furthermore, this enzyme plays an important role in nitrogen cycling in the soil and is commonly used to evaluate the nitrogen transformation capacity of soil. Soil catalase is also a crucial antioxidant enzyme that plays a key role in the breakdown of hydrogen peroxide (a type of oxygen-free radical) into oxygen and water, furthermore, this enzyme is a critical component in the activity of soil microbes and redox reactions. Soil polyphenol oxidase is known to participate in the degradation and decomposition of polyphenolic compounds in the soil and oxidizes polyphenolic substances, converting them into high molecular weight polymers. Soil sucrase is involved in the degradation and conversion of sucrose in the soil catalyzes the hydrolysis of sucrose and reflects sugar metabolism and carbon cycling processes in the soil.

On the other hand, and consistent with the findings of Shen et al. (2023): the application of organic fertilizers significantly increased the activity of soil urease (S-UE): thus leading to improved nitrogen efficiency. Similarly, we observed a significant increase in the activity of soil urease with the application of microbial inoculants. This could be due to the presence of microorganisms in the inoculants that are capable of producing or activating urease-related enzyme systems, thereby promoting nitrogen cycling and enhancing nitrogen utilization efficiency. However, we also observed that microbial inoculants had an inhibitory effect on the activity of soil polyphenol oxidase (S-PPO): these results are consistent with the findings of Zeng et al. (2016). Ku et al. (2018) reported higher activity of soil polyphenol oxidase activity in forest ecosystems. The use of microbial inoculants may lead to changes in the structure and function of the soil microbial community, thus resulting in a reduction in the activity of soil polyphenol oxidase. This inhibition could be attributed to certain microorganisms present in the inoculants that competitively utilize the substrates required for polyphenol oxidase, thereby reducing its activity. Despite the observed inhibitory effect of microbial inoculants on soil polyphenol oxidase activity, we believe that this inhibition is not necessarily unfavorable. Excessive polyphenol oxidase activity could lead to the over-decomposition of soil organic matter and the loss of nutrients. Thus, the inhibition of polyphenol oxidase by microbial inoculants may help maintain the balance of soil organic matter and nutrient cycling, thus contributing to a healthier soil ecosystem.

In this study, it was found that microbial agents could cause changes in the number and diversity of soil microorganisms, especially on fungal populations (*Table 4*). Kwak et al. (2018) also showed that microbial agents are effective means to regulate soil microorganism. Jack et al. (2021) also suggested that the composition and structure of inter-root microorganisms were regulated by soil conditions, and that different microbial populations were closely related to tobacco health and disease. Microbial agents contain complex organic compounds, enzymes, peptides and other components,

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which may affect soil microorganisms through different pathways (Zhao et al., 2023). They may affect soil microorganisms in different ways: by directly inhibiting or stimulating the growth and reproduction of specific microorganisms. Different agents have different toxicity and growth-promoting effects on specific genera/species (Brözel, 2022; Duan et al., 2012b). Influence the signaling molecular pathways between microorganisms and between microorganisms and plants, altering the dynamics of microbial communities. Indirectly affects microbial growth by altering soil nutrient and environmental conditions (Alori et al., 2017). Different microorganisms have different sensitivities to these effects. It is necessary to study the interaction mechanism between the fungicide components and the gene expression, protein activity and metabolic network of different microorganisms at the molecular level, so as to clarify the targets and effects of different agents on specific microorganisms.

The present study showed that soil pH and organic matter (SOM) were the key factors affecting tobacco inter-root microorganisms, which was in agreement with the findings of Wang et al. (2023), that soil pH, temperature and humidity, and organic matter (SOM) significantly affected the survival and colonization of pathogenic bacteria. Significant changes in the microbial composition of tobacco inter-roots were observed after the application of microbial fertilizers. We observed that the relative abundance of Phytophthora thicketi, Phytophthora bacilliformis and Phytophthora anisopliae was positively correlated with soil pH, phenol oxidase activity and sucrase activity, whereas, it was negatively correlated with soil quick nitrogen, catalase activity and urease activity. This reflects a complex regulatory mechanism, in which soil nutrient conditions and enzyme activities jointly affected the inter-root microorganisms. In addition, the roles of inter-root microorganisms in plant nutrient cycling and disease suppression are closely related (Xu et al., 2018). Appropriate regulation of soil nutrients, enzyme activities and microbial composition can effectively affect the structure of tobacco inter-root microorganisms and the health of the host, and the present study verified the conclusion of Yang et al. (2023b), that soil microbial composition and diversity have an important impact on tobacco health.

Conclusions

The application of microbial agent had no significant effect on yield and growth parameters of tobacco plants but significantly decreased the disease index and morbidity of Ralstonia solanacearum and Phytophthora nicotianae. Microbial agent application also increased the proportion of top-grade tobacco leaves and thus increased the economic return of tobacco production. Our analyses demonstrated the significant impact of microbial agent application on soil fungi and bacteria. Moreover, compared with controls, the application of microbial inoculations significantly enhanced the activity of soil enzymes including CAT, SSC, and UE. Organic matter (SOM) and pH can affect the inter-root microbial composition of tobacco. After the application of microbial fertilizer treatment, the inter-root microbial composition was co-regulated by soil nutrients and enzyme activities, and the application of microbial fungi can change the community structure and diversity of soil microorganisms, which in turn affects the inter-root microbial composition of tobacco and the health status of the plant.

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DOI: http://dx.doi.org/10.15666/aeer/2204_28852902

REFERENCES

[1] Alori, E. T., Dare, M. O., Babalola, O. O. (2017): Microbial Inoculants for Soil Quality and Plant Health. – In: Lichtfouse, E. (ed) Sustainable Agriculture Reviews, vol. 22. Springer, Cham., pp. 281–307. https://doi.org/10.1007/978-3-319-48006-0_9

[2] Baldrian, P. (2008): Wood-inhabiting ligninolytic basidiomycetes in soils: Ecology and constraints for applicability in bioremediation. – Fungal Ecology 1: 4-12.

[3] Brözel, V. S. (2022): Microbial interactions in soil. – Microorganisms 10 (10): 1939. doi: 10.3390/microorganisms10101939

[4] Chang, N., Zhang, X. J., Ma, L. L., Shi, J. J. (2017): Effects of microbial agents on wheat growth and soil-borne disease prevention. – Crop Journal 1: 155-160. doi: 10.16035/j.issn.1001-7283.2017.01.028.

[5] Chen, S., Yao, F., Mi, G., Wang, L., Wu, H., Wang, Y. (2022): Crop rotation increases root biomass and promotes the correlation of soil dissolved carbon with the microbial community in the rhizosphere. – Frontiers in Bioengineering and Biotechnology 10: 1081647. doi: 10.3389/fbioe.2022.1081647.

[6] Crabot, J., Clappe, S., Dray, S., Datry, T. (2019): Testing the Mantel statistic with a spatially-constrained permutation procedure. – Methods in Ecology and Evolution 10: 532-540. doi: 10.1111/2041-210x.13141.

[7] Deshoux, M., Sadet-Bourgeteau, S., Gentil, S., Prévost-Bouré, N. C. (2023): Effects of biochar on soil microbial communities: a meta-analysis. – Science of the Total Environment 902: 166079. doi: 10.1016/j.scitotenv.2023.166079.

[8] Duan, Y. Q., Chen, D. M., Jin, Y., Wang, H. B., Yang, Y. H., You, C. Y. (2012a): Effects of different fertilizers on microbial and enzyme activities in inter-root soils of continuous tobacco. – Chinese Agricultural Science and Technology Bulletin 3: 122-126.

[9] Duan, Y., Yang, J., Yang, J., Cao, N., Li, X., Zhou, X., Li, Y. (2012b): Screening and identification of Bacillus subtilis SH7 resistant to Bacillus solanacear. – Journal of Jilin Agricultural University 1: 52-57. doi: 10.13327/j. jjlau. 2012.01.011.

[10] Fabra, A., Castro, S., Taurian, T., Angelini, J., Ibañez, F., Dardanelli, M., Tonelli, M., Bianucci, E., Valetti, L. (2010): Interaction among Arachis hypogaea L. (peanut) and beneficial soil microorganisms: how much is it known? – Critical Reviews in Microbiology 36: 179-194. doi: 10.3109/10408410903584863.

[11] Goslee, S. C., Urban, D. L. (2007): The ecodist package for dissimilarity-based analysis of ecological data. – Journal of Statistical Software 22(7): 1-19. doi: 10.18637/jss.v022.i07.

[12] Gou, Z., Zheng, H., He, Z., Su, Y., Chen, S., Chen, H., Chen, G., Ma, N. L., Sun, Y. (2023): The combined action of biochar and nitrogen-fixing bacteria on microbial and enzymatic activities of soil N cycling. – Environmental Pollution 317: 120790. doi: 10.1016/j.envpol.2022.120790.

[13] He, Z., Jiang, N., Luo, G., Zhu, Y. (2021): Effect of application of Engeran anti-residue fungicide on yield and appearance quality of roasted tobacco. – Agriculture and Technology 41: 29-32. doi: 10.19754/j.nyyjs.20210228008.

[14] Huang, G., Dong, L., Ma, B., Bin, W., Zi, Z., He, X., Bai, X., Zhang, J. (2023a): Application effects of three microbial agents on Yunyun 99. – Hunan Agricultural Science 4: 45-49+54. doi: 10.16498/j.cnki.hnnykx.2023.004.009.

[15] Huang, X. J., Fu, L. M., Li, S. Y., Li, C., Zhao, X. T., Zhang, Z. L., Kong, G. C., Li, X. H., Chen. H. R., Dao, J. H., Zhang, Z. H. (2023b): Investigation on the effect of different microbial agents on the growth of tobacco plants and the prevention and control of “two blacks and one green”. – Chinese Agronomy Bulletin 39(27): 133-139.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_28852902

[16] Jack, C. N., Petipas, R. H., Cheeke, T. E., Rowland, J. L., Friesen, M. L. (2021): Microbial inoculants: silver bullet or microbial Jurassic park? – Trends in Microbiology 29(4): 299-308.
[17] Kwak, M. J., Kong, H. G., Choi, K., Kwon. S. K., Song, J. Y., Lee, J., Lee, P. A., Choi, S. R., Seo, M., Lee, H. J., Jung, E. J., Park, H., Roy, N., Kim, H., Lee, M. M., Rubin, E. M., Lee, S.-W., Kim, J. F. (2018): Rhizosphere microbiome structure alters to enable Ralstonia solanacearum resistance in tomato. – Nature Biotechnology 36 (11): 1100-1109. doi: 10.1038/nbt.4232.
[18] Ku, Y. L., Xu, G. Y., Zhao, H., Dong, T. W., Cao, C. L. (2018): Effects of microbial fertilizer on soil improvement and fruit quality of kiwifruit in old orchard. – Journal of Applied Ecology 29: 2532-2540. doi: 10.13287/j.1001-9332.201808.025.
[19] Li, W., Li, H. (2007): Effects of nutrients on tobacco yield and quality. – Gansu Science and Technology 1: 210-212.
[20] Liao, Y., Zhou, X. (2003): The impact of climate change on tobacco production and habitat selection in China. – Chinese Journal of Eco-Agriculture 04: 142-143.
[21] Mao, G. J., Meng, N., Liu, H. S., Pan, G. W., Han, J. F., Yuan, S. H., Pu, W. X. (2014): Effects of different microbial agents on biological activity of tobacco rhizosphere soil under dichloroquinoline acid stress. – Jiangxi Journal of Agricultural Sciences 26(5): 25-28. doi: 10.19386/j.cnki.jxnyxb.2014.05.006.
[22] Qin, H., He, B., Zhao, X., Li, Y., Mao, W., Zeng, Q. (2014): Influence of different slope position and profile in Disporopsis pernyi forest land on soil microbial biomass and enzyme activity in southwest Karst mountain of China. – Environmental Sciences 35: 3580-3586. doi: 10.13227/j.hjkx.2014.09.046.
[23] Schweitzer, J. A., Bailey, J. K., Fischer, D. G., LeRoy, C. J., Lonsdorf, E. V., Whitham, T. G., Hart, S. C. (2008): Plant – soil – microorganism interactions: heritable relationship between plant genotype and associated soil microorganisms. – Ecology 89: 773-781. doi: 10.1890/07-0337.1
[24] Shen, Y., Zhao, J., Zou, X., Shi, Z., Liao, Y., He, Y., Wang, H., Chen, Q., Yang, P., Li, M. (2023): Differential responses of bacterial and fungal communities to siderophore supplementation in soil affected by tobacco bacterial wilt (Ralstonia solanacearum). – Microorganisms 11 (6): 1535. doi: 10.3390/microorganisms11061535.
[25] Shi, C., Yu, F. M., Shang, G. L., Zhu, B. Y., Zeng, L. (2019): Effect of microbial agents on the prevention and control of root and stem diseases of roasted tobacco and yield. – Modern Agricultural Science and Technology 21: 107-109.
[26] Standardization Administration of the People's Republic of China (1992): Roasted tobacco. – GB/T 2635-1992.
[27] Tan, X., Nie, Y., Ma, X., Guo, Z., Liu, Y., Tian, H., Megharaj, M., Shen, W., He, W. (2021): Soil chemical properties rather than the abundance of active and potentially active microorganisms control soil enzyme kinetics. – Science of the Total Environment 770: 144-500. . doi: 10.1016/j.scitotenv.2020.144500.
[28] Wang, J. D., Zheng, J. Y., Gong, Z. L., Liu, H. M., Diao, Y. H., Li, X. Y., Yan, W. L., Jiang, X. J., Liang, Y. J. (2023): Effects of microbial agents on soil physical and chemical properties and cotton growth. – Xinjiang Agricultural Reclamation Science and Technology 46(5): 65-68.
[29] Wang, P., Li, Y., Ling, G., Sun, S., Bao, J., Wu, D., Zheng, F., Zhang, Z. (2020): Effects of microbial fertilizer on the growth and development of Yunyan 87 roasted tobacco and the chemical composition of tobacco leaves. – Anhui Agricultural Science 48(1): 157-159.
[30] Wu, Z., Sun, L., Dong, Y., Xu, X., Xiong, Z. (2022): Contrasting effects of different field-aged biochars on potential methane oxidation between acidic and saline paddy soils. – Science of the Total Environment 853: 158-643. doi: 10.1016/j.scitotenv.2022.158643.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_28852902

[31] Xu, Z. S., Yang, Y. M., Chen, X. J., Zhang, B. W., Zhang, X. L., Liu, J. H. (2018): Effects of bacterial fertilizer on soil enzyme activity and microorganisms in mixed pasture. – Soil and Fertilizer 6: 77-81.
[32] Yang, J. F., Tian, T., Chen, J. L., Geng, Y. G. (2023a): Application test of two microbial agents in the control of soil-borne diseases of crops. – Tianjin Agricultural and Forestry Science and Technology 2: 23-25 + 29. doi: 10.16013/j.cnki.1002-0659.2023.0016.
[33] Yang, X. F., Guo, R., Yao, Y. L., Zhu, W. J., Hong, L. D., Hong, C. L., Zhu, F. X., Wang, W. P. (2023b): Effects of microbial agents on growth, soil nutrients, and microbial communities of strawberry in continuous cropping plots. – Journal of Nuclear Agriculture 37(6): 1253-1262.
[34] Yang, Y., Wang, T., Li, Y., Wang, M., Chen, W., Dai, Y. (2022): Mitigating cadmium contamination of rice soils supporting tobacco–rice rotation in southern China: win–win or lose–lose? – Journal of Hazardous Materials 425: 128052. doi: 10.1016/j.jhazmat.2021.128052.
[35] Zeng, Q., Li, T., Wang, C., Cai, Y., Yang, J., Zhang, R., Li, B. (2016): Effects of microbial agents on urease and catalase activities in inter-root soil of roasted tobacco. – Chinese Agronomy Bulletin 32: 46-50.
[36] Zhao, X. Y., Zhu, D., Tan, J., Wang R., Qi, G. F. (2023): Cooperative action of fulvic acid and Bacillus paralicheniformis ferment in regulating soil microbiota and improving soil fertility and plant resistance to bacterial wilt disease. – Microbiol Spectr. 11(2):e0407922. doi: 10.1128/spectrum.04079-22.

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