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# The Dynamic Effects of High-carbon Biochar-based Fertilizer on Microbial Communities in Tobacco-planting Soil

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**Abstract:** Soil microorganism is an important component of the soil, it plays an important role in the formation of soil fertility and plant nutrition transformation. Soil microbial species and quantity can be used as evaluation index of soil fertility. In order to solve soil quality degradation caused by long-term single planting patterns, ZhongYan 100 was planted in the experiment of high-carbon biochar-based restoration fertilizer, and its effect on soil microbial community was evaluated. It was found that high-carbon biochar-based restoration fertilizer could increase the number of rhizosphere bacteria and reduce the number of rhizosphere fungi, improving soil microbial community composition. Therefore, the appropriate application of high-carbon biochar-based restoration fertilizer can improve continuous cropping soil through adjusting soil microbial community.
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**Keywords:** Soil; Tobacco; High-carbon Biochar; Microorganism; Restoration fertilizer

## 1. Introduction

In recent years, with the annual decrease of the cultivated land area in China, a large amount of fertilizer has been applied to the soil to improve the overall agricultural productivity, which not only leads to soil acidification, hardening and nutrient loss, but also reduces the utilization rate of fertilizer, seriously affecting the quality of agricultural products. Biomass carbon can not only reduce soil bulk density, improve soil pH value, aggregates and water holding capacity, but also act as a natural nitrogen fertilizer release agent to delay the release of fertilizer in the soil and reduce nutrient leaching. High-carbon biochar-based fertilizer is a kind of high carbon and low nitrogen fertilizer, mainly including biochar, organic carrier, natural mineral fertilizer, humic acid, trace elements and other components. A large number of studies have shown that the mixed application of biomass carbon and fertilizer has a positive effect on the growth and yield of crops [1-4].

There are close relationships among soil, plant and fertilizer, and they are mutually influenced and restricted. These relationships should be given full consideration for scientific fertilization, and rationally fertilize should be applied in different soil and crops. Different soil nutrients have a great impact on soil microbial community, which in turn affects the utilization of soil nutrients and soil improvement [5]. Therefore, it is still necessary to study the rational application of high-carbon biochar-based fertilizer in specific soil.

Zhumadian city is one of the main areas of flue-cured tobacco production in Henan Province. Long-term single tobacco planting mode is easy to cause soil quality degradation, the destruction of microbial community balance, and crop diseases and insect pests. So, the yield and quality will be decreased through affecting the normal growth and development of tobacco. Therefore, the construction of a healthy and harmonious soil environment in tobacco fields, which can improve soil physical and chemical properties, is the final approach for sustainable development of tobacco leaf production in Zhumadian City. Here, the changes of soil microbial community are analyzed through evaluating the effect of high-carbon biochar-based fertilizer improving the soil, which will provide scientific basis for sustainable development of tobacco fields and rational fertilization technology.

## 2. Material and Methods

### 2.1 Experiment materials

The tested tobacco variety is ZhongYan 100, and the high-carbon biochar-based fertilizer is from Henan Huinong Soil Conservation and Development Co.,

LTD., including the total nutrient ($N+P_2O_5+K_2O$) ⩾ 5%, organic matter (dry base) ⩾ 45%, biochar ⩾ 20%, crude fat ⩾ 1%, water content ⩽ 20%. The tobacco specific fertilizer contains N 10%, $P_2O_5$ 12% and $K_2O$ 18%.

2.2 Experiment design

In zhumadian city, low- and medium-yield fields (pH 4.98, organic matter 11.9 g/Kg, hydrolytic nitrogen 77.4 mg/Kg, available phosphorus 11.6 mg/Kg, and rapidly available potassium 175 mg/Kg) where tobacco has been continuously planted for more than 3 years were selected. A completely random zone group design was adopted. There were 4 treatments. In T0, conventional fertilization (including tobacco-special fertilizer 750 Kg/hm$^2$, cake fertilizer 600 Kg/hm$^2$) was used. In T1, conventional fertilization (the reduction of 74.96 kg tobacco-special fertilizer/hm$^2$) + 375 Kg/hm$^2$ high-carbon soil remediation fertilizer were used; In T2, conventional fertilization (the reduction of 149.92 kg of tobacco-specific fertilizer/hm$^2$) + high-carbon based soil remediation fertilizer 750 kg/hm$^2$ were applied; In T3, conventional fertilization (the reduction of 224.88 kg of tobacco-specific fertilizer/hm$^2$) + high-carbon soil remediation fertilizer 1125kg/hm$^2$ were used. Each treatment was 0.013 hm$^2$, and each treatment was repeated three times. At 30 days, 60 days and 90 days after transplanting, 6 healthy tobacco plants were randomly selected from each treatment. The roots of the tobacco plants were completely dug out, and the peripheral soil around the rhizosphere was removed. The soil attached to the roots (within 4 mm) was gently rubbed and shaken into the sealing bag, which was then transported back to the laboratory in a refrigerator, and the soil DNA was extracted after removing the stray roots. Residual rhizosphere soil was used for the determination of physiological indexes.

2.3 Soil microbial analysis

The total DNA of soil samples was extracted and a sequencing library was formed by PCR. After the library was built and checked, the qualified library was sequenced with Illumina HiSeq 2500. The original data were jointed (FLASH, Version 1.2.11) and filtered (Trimmomatic, version 0.33), and high-quality Tags sequences were obtained through removing chimera (UCHIME, version 8.1). UCLUST in QIIME (Version 1.8.0) software was used to cluster Tags and obtain OTU at 97% similarity level. Taxonomic annotation was executed according to bacteria Silva database (Release128, http://www.arb-silva.de) and fungi Unite database (Release 7.2, http://unite.ut.ee/index.php). Mothur (version V.1.30, http://www.mothur.org/) software was used to analyze the diversity of soil microorganisms, including Chao1 richness index, Ace richness index, Shannon index and Simpson index.

2.4 Statistics and analysis of data

SPSS 20.0 was used for collating and analyzing the collected data, Duncan's multiple comparison was used for the difference analysis among different processes, and Excel 2013 was used for chart drawing.

**3. Results**

3.1 Effects of high-carbon biochar-based remediation fertilizer on soil microbial diversity

As an important part of soil, soil microorganisms play an active role in the formation of soil fertility and the transformation of plant nutrients. The species and quantity of soil microorganisms can be used as indicators for the evaluation of soil fertility [10-12]. The OTU numbers of tobacco rhizosphere and non-rhizosphere soil bacteria were showed in Fig.1, and the OUT numbers of the three treatments were significantly higher than that of the control group, indicating that the application of high-carbon-based soil remediation fertilizer could increase the number and species of soil bacteria. The OTU numbers of tobacco rhizosphere and non-rhizosphere soil fungi were showed in Fig.1, and the OTU numbers of each fungus treatment were significantly lower than that of the control treatment, indicating that the application of high-carbon-based soil remediation fertilizer can reduce the number and species of soil fungi.

3.2 Effects of High-carbon Biochar-based remediation fertilizer on soil bacterial community

As can be seen from Fig.2, the bacteria phyla in tobacco soil with relative abundance of more than 1% included Proteobacteria, Acidobacteria, Actinobacteria, Gemmatimonadetes, Bacteroidetes, Chloroflexi, Firmicutes, Planctomycetes, Saccharibacterias, Cyanobacteria, Nitrospirae, Armatimonadetes and Verrucomicrobia, which account for more than 95% of the total bacterial community in the soil. Proteobacteria, Acidobacteria and Actinomycetes were the three main bacteriums, with relative abundance accounting for more than 70%. Proteobacteria was the largest phylum of soil bacteria and contained some pathogenic bacteria as well as nitrogen-fixing bacteria. The relative abundance of the rhizosphere Proteobacteria was 35-45%, while that of the non-rhizosphere was 30-35%, showing a rhizosphere effect. In different periods after transplanting, the changes in relative abundance of the same treatment were 30d>90d>60d. In the same period after transplanting, the relative abundance of both rhizosphere and non-rhizosphere Proteobacteria

decreased in high-carbon-based fertilizer treatment, and the relative abundance in T2 treatment was the lowest except for non-rhizosphere soil at 90d. The relative abundance of both rhizosphere and non-rhizosphere of Acidobacteria was 15-25%, and there was no rhizosphere effect. After transplanting, the relative abundance of Acid-bacilli in rhizosphere and non-rhizosphere soil under the same treatment showed an increasing trend. In the same period after transplantation, the relative abundance of rhizomatous Bacillariophyta increased in high-carbon fertilizer treatment, and non-rhizomatous Bacillariophyta also showed an increasing trend except for 30 days, and the relative abundance of T2 was relatively large. The relative abundance of soil Actinomycetes was 8-20%. At 30 days, its relative abundance in non-rhizosphere soil was greater than rhizosphere soil, with no significant difference between 60 and 90 days. The relative abundance of Actinomycetes in both rhizosphere and non-rhizosphere soil increased first and then decreased at different stages after transplanting. In the same period after transplanting, the relative abundance of Actinobacteria in the treatments with high-carbon-based soil remediation fertilizer was greater than that in the control, and the relative abundance in T2 treatment was the largest.

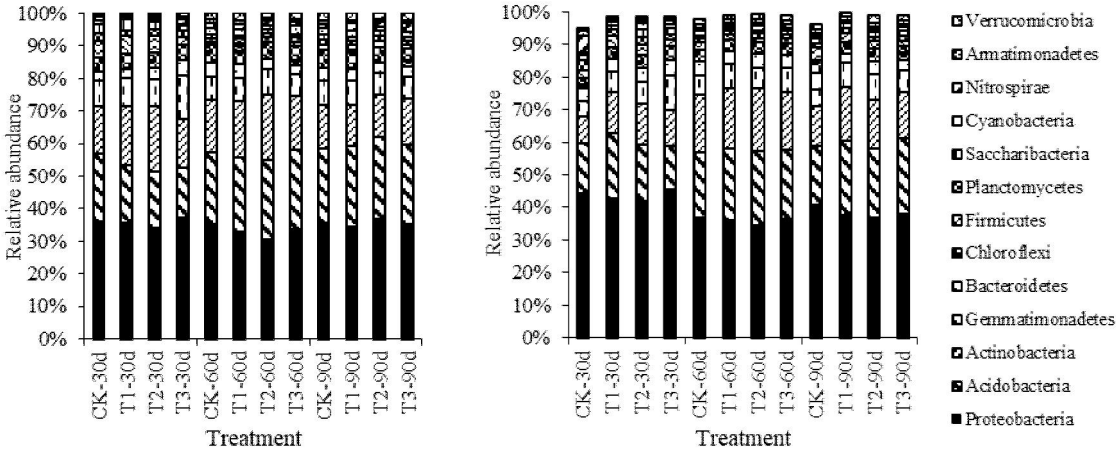


**Figure 2 The effect of high-carbon soil remediation fertilizer on the relative abundance of soil bacteria phyla. (Left) non-rhizosphere soil, (Right) rhizosphere soil**

As can be seen from Fig.3, the bacterial genus having relatively high abundance included uncultured_bacterium_f_Acidobacteriaceae_[Subgroup_1], Gemma-Timonas, undetermined Actinomycetes Gaiellales (uncultured_bacterium_o_Gaiellales), Sphingomonas, ncultured_bacterium_f_Gemmatimonadaceae, Candidatus_-Solibacter. In rhizosphere and non-rhizosphere soils, high-carbon-based fertilizer could increase the relative abundance of uncultured_bacterium_o_Gaiellales, Gemmatimonas and Candidatus_Solibacter, and reduce the relative abundance of Uncultured_bacterium_f_Acidobacteriaceae_[Subgroup_1] and Sphingomonas. In addition, high-carbon-based fertilizer could decrease the relative abundance of Uncultured_Bacterium_f_odp1230B8.23, Burkholderia-Paraburkholo-deria and Phenylobacterium, and could increase the relative abundance of Nitrospira in the rhizosphere soil. In the non-rhizosphere soil, high-carbon-based fertilizer could reduce the relative abundance of uncultured_bacterium_f_Nitrosomonadaceae and increase the relative abundance of uncultured_bacterium_f_DA111.

3.3 Effects of high-carbon biochar-based remediation fertilizers on soil fungal communities

As can be seen in Fig.4, the fungi categories having relative abundance of more than 1% included Ascomycota, Basidiomycota, Glomeromycota, Chytridiomycota, Mucoromycota and Mortierellomycota, of which Ascomycota and Basidiomycota was dominant fungi (Fig.4). At 30 d after transplanting, the relative abundance of fungi in rhizosphere and non-rhizosphere soil had no obvious difference. At 60 and 90 d after transplanting, the relative abundance among treatments was bigger, showing that high-carbon-based fertilizer had bigger influence on the soil fungi at 60 d after transplanting. The relative abundance of Ascomyceta in rhizosphere and non-rhizosphere soil in all treatments was lower than the control, and the relative abundance in T2 was the lowest, showing high-carbon fertilizer can obviously reduce the relative abundance of Ascomyceta. At 60d after transplanting, the relative abundance of Basidiomycta in rhizosphere soils in the

three treatments was lower than the control, and its relative abundance was the lowest in T2, while its relative abundance in non-rhizosphere presented an opposite trend.

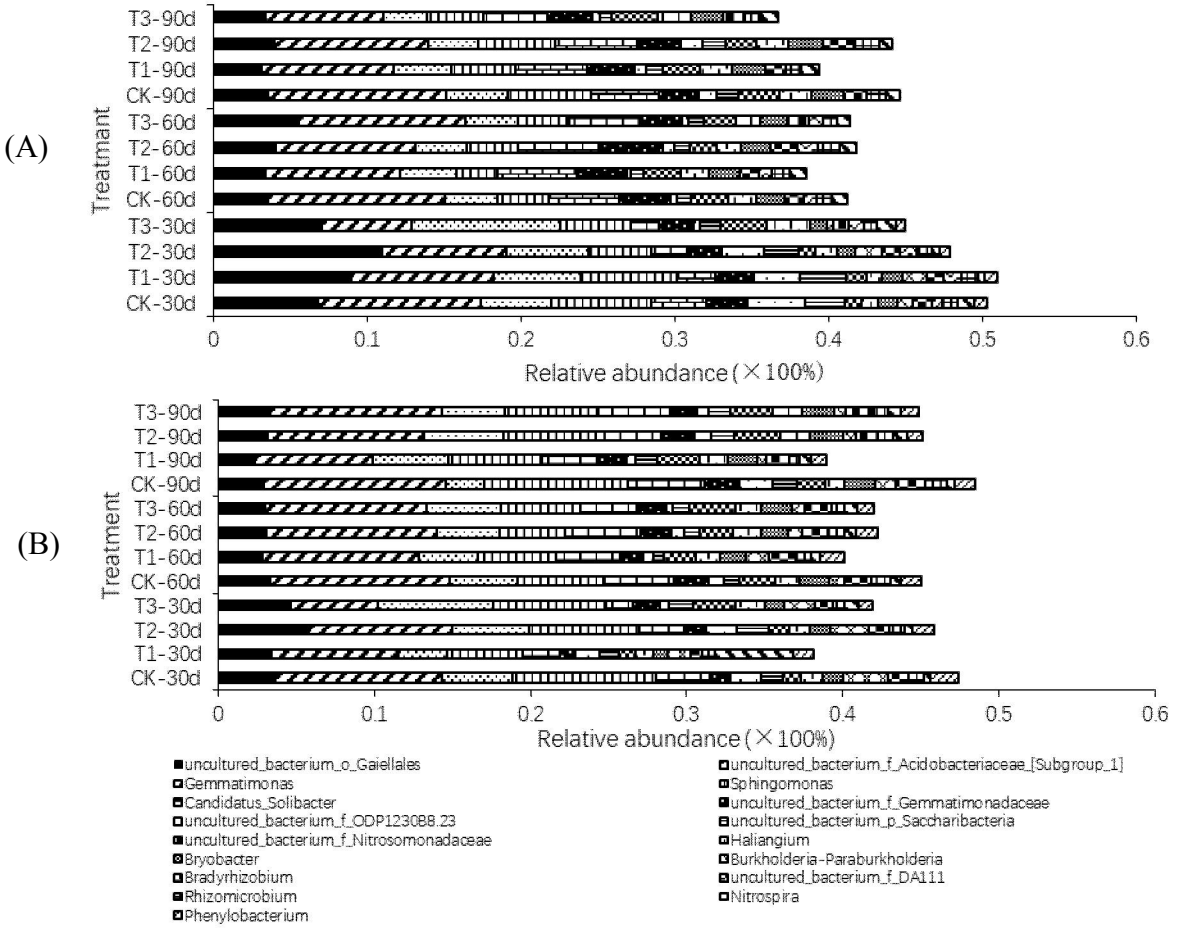


**Figure 3 The effect of high-carbon soil remediation fertilizer on the relative abundance of soil bacteria genus. (A) non-rhizosphere soil, (B) rhizosphere soil.**

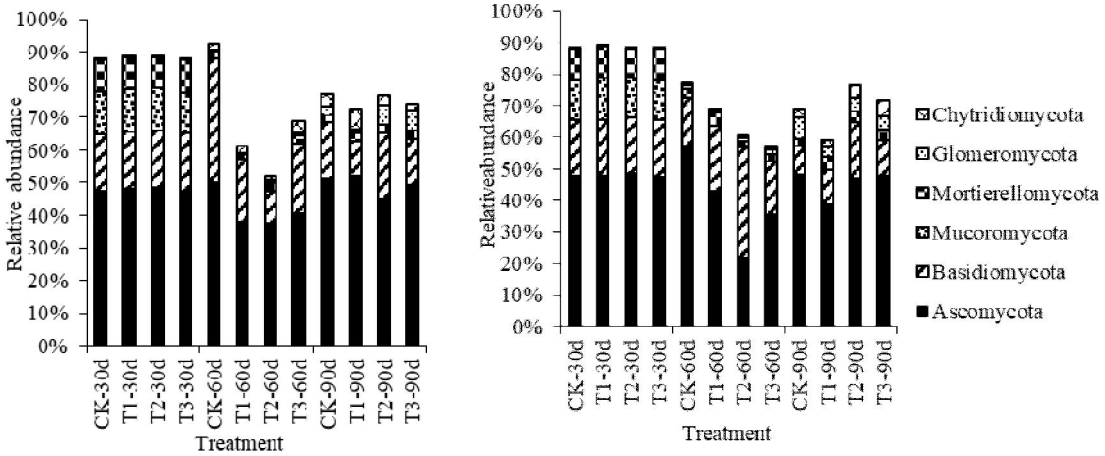


**Figure 4 The effect of high-carbon soil remediation fertilizer on the relative abundance of soil fungi phyla. (Left) non-rhizosphere soil, (Right) rhizosphere soil.**

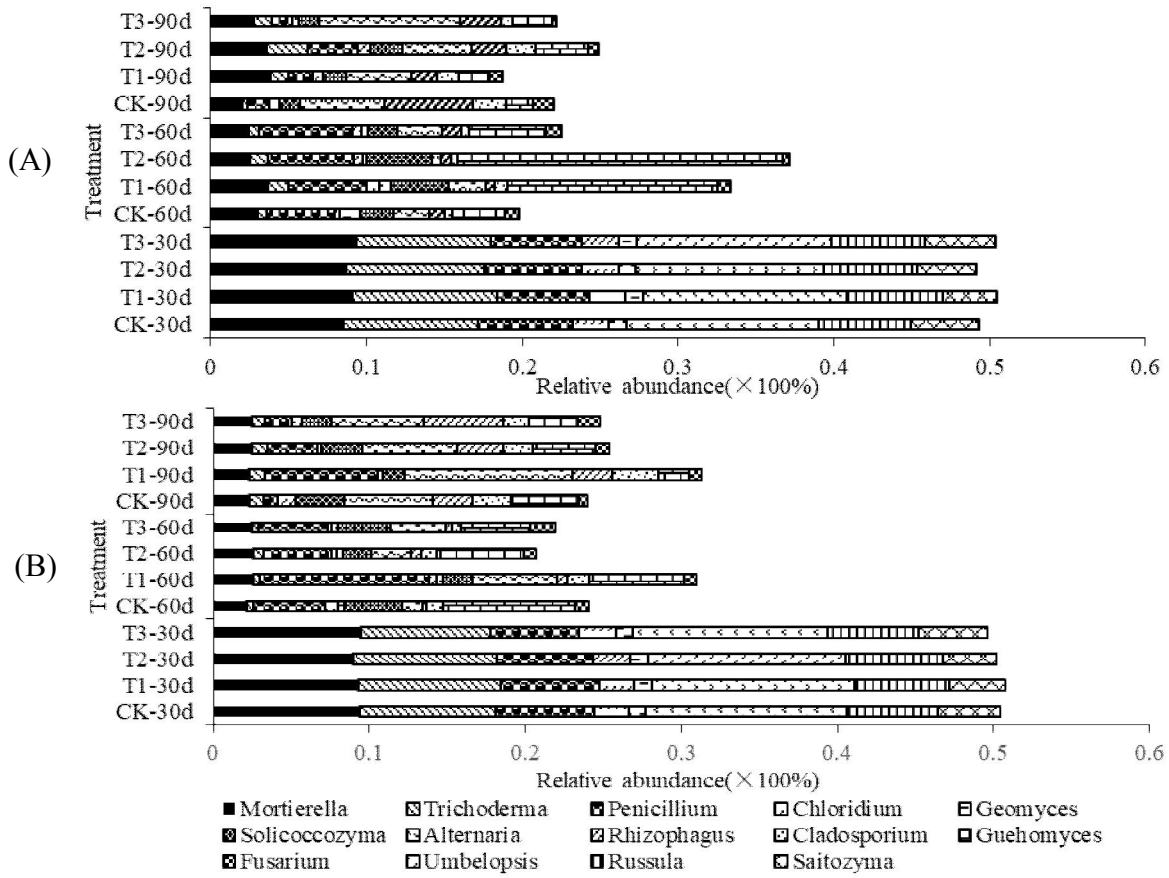


**Figure 5 The effect of high-carbon soil remediation fertilizer on the relative abundance of soil fungi genus. (A) non-rhizosphere soil, (B) rhizosphere soil.**

As shown in Fig.5, the fungi genus with relatively high relative abundance in rhizosphere and non-rhizosphere included Mortierella, Trichoderma, Penicillium, Umbelopsis, Russula and Saitozyma at 30 days after transplanting, but the differences among treatments were not obvious. At 60d after transplanting, the relative abundance of Penicillium, Solicoccozyma, Alternaria and Guehomyces in rhizosphere and non-rhizosphere soil was high. The relative abundance of Alternaria and Penicillium could be increased, and the relative abundance of Guehomyces, Chloridium and Solicoccozyma could be significantly reduced in rhizosphere by high-carbon fertilizer. The relative abundance of Penicillium, Solicoccozyma, Guehomyces and Trichoderma could be increased, and the relative abundance of Geomyces, Alternaria and Rhizophagus can be significantly reduced in non-rhizosomes. At 90d after transplanting, the high relative abundance of fungi in rhizosphere and non-rhizosphere soil included Guehomyces, Cladosporium, Rhizophagus, Alternaria, Solicoccozyma, Penicillium and Mortierella. In rhizosphere, the relative abundance of Penicillium, Alternaria and Fusarium could be obviously increased, and the relative abundance of Solicoccozyma and Chloridium could be significantly decreased. In non-rhizosphere soils, high-carbon-based fertilizers could significantly increase the relative abundance of Mortierella, Trichoderma and Guehomyces, and reduce the relative abundance of Fusarium, Cladosporium and Rhizophagus.

## 4. Discussion

The deterioration of soil microbial communities is an important cause of tobacco continuous cropping disorder. Biochar can improve soil bacterial activity and quantity through various approaches. In this study, it was found that high-carbon-based soil remediation fertilizer can significantly increase the number of soil bacteria and reduce the number of fungi in tobacco fields, which is similar to the results in cucumber and rice cultivation [6-7]. Biochar can not only provide more C sources for soil bacteria, but also provide good habitat for soil bacteria growth. These beneficial bacteria have direct antagonism to some soil pathogenic fungi and reduce the number of fungi [8-9].

The recent studies have shown that biomass carbon can improve soil pH, soil nutrients and water, and promote crop growth[10] by changing soil physicochemical properties and rhizosphere soil microbial community. It has also been reported that biomass carbon can not only promote the expression of protein metabolism-related genes in rice leaves, but also increase the expression of tomato resistance genes [11-12]. So, high-carbon-based soil remediation can possibly improve the expression of tobacco nutrient absorption-related and resistance-related genes, and then promote tobacco growth and development.

In a word, high-carbon-based soil remediation can increase the number of bacteria in the rhizosphere soil, reduce the number of rhizosphere fungi, and improve the soil microbial population structure, promoting the growth and development of tobacco plants.

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