Effects of combined application of organic fertilizer and microbial agents on tobacco soil and tobacco agronomic traits

Z G Zhai 1, Q L Hu 2, J R Chen 2, C X Liu 1, S Guo 1, S Q Huang 3, W A Zeng 1, 4

1 Changsha Tobacco Company of Hunan Province, Changsha, Hunan 410011, China
2 Hunan Agricultural University, Changsha, Hunan 410128, China
3 Liuyang branch of Changsha tobacco company, Hunan Liuyang 410300, China
4 E-mail: zwa10537@163.com

Abstract. Using the tobacco variety Yunyan87 as an example, we investigated the effects of the combined application of organic fertilizer and microbial agents on the physicochemical properties and enzyme activities of tobacco soil and on tobacco agronomic parameters. Compared with the control group, the contents of alkali-hydrolyzed nitrogen, available potassium, and available phosphorus in the soil treated with organic fertilizer and microbial agents increased by 27.7, 12.3, and 133.7%, respectively. The activities of soil urease, phosphatase, sucrase, and catalase increased by 86.3, 85.1, 46.6, and 33.5%, respectively, while the numbers of bacteria and actinobacteria increased by 89.9 and 183.4% respectively. In contrast, the numbers of fungi decreased by 64.8%. Plant height, effective leaf number, stem circumference, stalk width, cutting length, and cutting width increased by 8.1, 7.8, 12.1, 15.7, 20.5, and 11.5%, respectively. Based on our results, the application of organic fertilizer combined with microbial agents significantly improved soil properties, enzyme activities, microbial numbers, and tobacco agronomic parameters.

1. Introduction
Tobacco is one of the most important crops as income contributor in China, where its yield and quality are influenced by various factors such as ecological environment, climatic conditions, flue-cured tobacco varieties, soil fertility, and agronomic parameters [1]. Among agricultural measures, precision fertilization is an important means to improve the quality of tobacco leaves in flue-cured tobacco production [2]. In flue-cured tobacco production, long-term application of single type fertilizer can cause numerous problems, including soil acidification and hardening, imbalance of the soil nutrient supply, imbalance of the soil microbial community structure and aggravation of soil-borne diseases, leading to the decrease of tobacco leaf quality. In this context, it is of great practical significance to investigate the substitute technology to reduce the application of chemical fertilizers [3].

Organic fertilizers are rich in organic matter and various nutritious elements [4]. Adequate application of organic fertilizers can improve the soil quality, enhance tillage performance, increase the contents of organic matter and available nutrients, and improve the micro-ecological environment of the rhizosphere soil. At the same time, the application of organic fertilizers can improve the appearance of tobacco leaves and adjust the chemical composition of the soil [4]. Microbial agent had several beneficial live bacteria and a variety of natural active substances. Relevant studies have shown that organic fertilizer combined with a microbial agent can more effectively improve the physical and chemical soil,
regulate the soil microecological environment, improve crop stress resistance, promote healthy crop growth and improve crop yield quality [5, 6].

The effects of combined application of organic fertilizer and a microbial agent on the physicochemical properties, enzyme activities and microbial quantity of tobacco rhizosphere soil against the background of a reduced application of chemical fertilizers are still largely unclear. In this context, the objective of this study was to investigate the effects of individually applying organic fertilizer and microbial agents, as well as a combination of organic fertilizer and microbial agents on the physicochemical properties, enzyme activities and microbial quantity of tobacco rhizosphere soil. This work provides a data reference and a theoretical foundation for the reduction of chemical fertilizers and the reasonable use of microbial agents in tobacco cultivation.

2. Material and methods

2.1. Materials

The experimental field was located at the Hunan Agricultural University base of the South-Central Tobacco Test Station, China National Tobacco Corporation (28°11’ N, 113°04’ E). The tested variety was Yunyan87 in tobacco-paddy rotating field. The soil texture were loam soils. The soil physical and chemical properties were: pH= 5.61; organic matter content= 1.85% (% w/w); alkali-hydrolyzed nitrogen= 87.64 mg.kg⁻¹; rapidly available phosphorus= 9.83 mg.kg⁻¹ and rapidly available potassium= 95.64 mg.kg⁻¹. The organic fertilizer was produced by the Tianda Biological Company, China, containing an organic matter content of 75% and a total nutrient content ≥ 6%. The compound microbial agent was plant growth promoting rhizobacteria (PGRG) from Shandong Lvlong Technology Co., LTD of China, including Bacillus subtilis, Bacillus polymyxis, Trichoderma, and Actinomyces. The total living bacteria count was more than or equal to 4 x 10¹¹ CFU.g⁻¹.

2.2. Experimental design

In the field experiment, we established fertilization treatments:

(1) CK: Conventional fertilization. 750 kg/ha of compound fertilizer for tobacco (total nutrient content ≥29%, and N/P/K ratio = 8:10:11) and 750 kg/ha of a special topdressing of compound fertilizer for tobacco (total nutrient content ≥ 42%, and N/P/K ratio = 10:0:32).

(2) T1: 80% Conventional fertilization + microbial agent. The microbial agent was applied once in the small holes near the plants 7 days prior to transplanting and once by the root-irrigation method about 20 days after transplanting. Each dosage was 3.75 kg/ha.

(3) T2: 80% Conventional fertilization + organic fertilizer. 600 kg/ha of compound fertilizer for tobacco, 1500 kg/ha of organic fertilizer, 600 kg/ha of special topdressing for tobacco.

(4) T3: 80% Conventional fertilization + organic fertilizer + microbial agent. 600 kg/ha of compound fertilizer for tobacco, 1500 kg/ha of organic fertilizer, 600 kg/ha of special topdressing for tobacco. The microbial agent was applied once in the small holes near the plants 7 days prior to transplanting and once by the root-irrigation method about 20 days after transplanting. Each dosage consisted of 3.75 kg/ha.

Three repeated plots were set for each treatment, with a total of 12 plots. Each plot had an area of 30 m².

2.3. Main agronomic parameters

Prior to harvesting, five representative tobacco plants were selected from each treatment plot after 80 days of growth, according to the YC/T 142-1998 survey method for agronomic parameters of tobacco [7], to measure their agronomic parameters, including leaf number, leaf length, leaf width, plant height, stem circumference, and cutting width.

2.4. Sample collection and determination
After harvesting, three samples of 1 kg surrounding rhizosphere soil were collected from the plough layer (0-20 cm) from each plot by the five-point method. After natural air drying, impurities, such as large stones and root debris, were removed, and subsequently, the samples were screened. The physical and chemical soil properties were determined using previously described methods [8]. Soil enzyme activity was determined according to Guan’s method [9]. Soil urease was determined by phenol-sodium hypochlorite colorimetry, sucrase was determined by 3, 5-dinitrosalicylic acid colorimetry, polyphenol oxidase was determined by catechol colorimetry, catalase was determined by KMnO₄ titration, dehydrogenase was determined by TTC reduction colorimetry, and phosphatase was determined by benzene disodium phosphate colorimetry [10]. The number of soil microorganisms was determined using the solid dilution-plate method. Bacteria, fungi, and actinobacteria were determined by the surface spread plate method on beef extract-peptone medium, PDA medium, and modified go1 medium, respectively, and the results were expressed as the quantity of colony-forming units per gram of dry soil [11].

2.5. Data processing
The experimental data were entered into Excel 2013, and significance test was carried out by using the SPSS 20.0 software.

3. Results

3.1. Effects of combined application of organic fertilizers and microbial agents on the physical and chemical properties of tobacco soil
Table 1 shows the effects of the combined application of organic fertilizers and microbial agents on the soil physical and chemical properties. For the fertilization treatments of T1 and CK, there were no significant differences in pH value, organic matter content, total nitrogen content, and rapidly available potassium content, but we observed significant differences in available phosphorus content and alkali-hydrolyzed nitrogen content. For the fertilization treatments of T2 and T3, the pH value, the organic matter content, the total nitrogen, the alkali-hydrolyzed nitrogen, the available potassium, and the available phosphorus values were significantly increased.

Compared with the application of organic fertilizer, the combined application of organic fertilizer and microbial agent significantly increased the contents of alkali-hydrolyzed nitrogen, available potassium, and available phosphorus in the soil. The contents of alkali-hydrolyzed nitrogen, available potassium, and available phosphorus in soil treated with organic fertilizer and microbial agent, increased by 27.7, 12.3, and 133.7% in comparison to those in the control treatment. Based on our results, the combined application of organic fertilizer and microbial agent significantly promotes soil fertility and improves soil quality.

| Treatment | pH              | Organic matter (%) | Total nitrogen (g/kg) | Alkaline hydrolysis nitrogen (mg/kg) | Available potassium (mg/kg) | Available phosphorus (mg/kg) |
|-----------|-----------------|--------------------|-----------------------|--------------------------------------|-----------------------------|-------------------------------|
| CK        | 5.45 ± 0.05b    | 2.12 ± 0.06b       | 1.56 ± 0.07b          | 105.31 ± 1.28d                       | 115.81 ± 1.10c              | 11.42 ± 0.49d                 |
| T1        | 5.53 ± 0.03b    | 2.19 ± 0.12b       | 1.61 ± 0.11b          | 115.69 ± 1.17c                       | 119.37 ± 2.07bc             | 16.77 ± 0.53c                 |
| T2        | 5.85 ± 0.05a    | 2.42 ± 0.08a       | 1.85 ± 0.10a          | 125.64 ± 1.55b                       | 124.0 ± 2.05b               | 20.66 ± 0.74b                 |
| T3        | 5.90 ± 0.04a    | 2.45 ± 0.20a       | 1.88 ± 0.13a          | 134.44 ± 1.77a                       | 130.1 ± 1.13a               | 26.69 ± 0.72a                 |
Results are reported as the mean value plus or minus standard deviation. Different letters in the same column show significant differences \((P<0.05)\), similarly hereinafter.

**Figure 1.** Effects of different treatments on the soil enzyme activities. Error bars, mean ± standard deviation of three replicates.

### 3.2. Effects of combined application of organic fertilizers and microbial agents on the enzymatic activity of tobacco soil
Soil enzyme activity is a potential indicator of soil biological activity and productivity and largely influences soil fertility [10]. When compared to conventional fertilization (CK), application of microbial agent (T1), organic manure (T2), and organic fertilizer combined with microbial agent (T3) significantly increased urease and phosphatase activities (Figure 1), and the combined application of organic fertilizer and microbial agent resulted in the highest levels of soil urease, phosphatase, invertase, and catalase activity. In addition, there were significant differences in soil urease and phosphatase activity among the four fertilization treatments, but there were no significant differences in sucrase and catalase activity between the fertilization treatments T1 and CK. Compared with CK, for the treatment with organic manure and a microbial agent, the soil urease, phosphatase, sucrase, and catalase activities increased by 86.3, 85.1, 46.6, and 33.5%, respectively. This suggests that organic fertilizer combined with a microbial agent can significantly improve the activities of soil urease, phosphatase, sucrase, and catalase.

**Figure 2.** Effects of different treatments on soil microbial numbers. Error bars, mean ± standard deviation of three replicates.
3.3. Effects of combined application of organic fertilizers and microbial agents on microbial numbers in tobacco soil

Soil microorganisms represent an important part of the soil microecosystem and play an important role in the transformation and recycling of soil organic matter and soil nutrients, soil microbial quantity is an important reference index for soil health and fertility [12]. As shown in Figure 2, the applications of the microbial agent, organic fertilizer, and organic fertilizer combined with the microbial agent significantly increased the quantity of bacteria compared with CK, with the following order: T3 > T2 > T1 > CK. In T3, the microbial number was 89.9% higher than in CK. The quantity of fungi showed a significant decline with the different treatments, following the order CK > T1, T2 > T3. The levels for T2 and T1 were similar, while that for T3 was 64.8% lower compared to CK. The quantity of actinobacteria also increased significantly, following the order T3 > T2, T1 > CK; the levels of T2 and T1 were similar, while that of T3 increased by 183.4% compared with that for CK. The combined application of organic fertilizer and microbial agents resulted in the highest levels of bacteria and actinobacteria and in the lowest levels of fungi compared with other fertilization treatments. Bacteria and actinobacteria are the largest and second-largest microbial groups, respectively, in the soil environment, and our results indicate that the combined application of organic fertilizer and microbial agents promotes the improvement of the tobacco soil environment.

3.4. Effects of combined application of organic fertilizers and microbial agents on agronomic parameters of tobacco

Based on Table 2, the application of organic fertilizer and the microbial agent and the combined application of organic fertilizer and microbial agent could effectively improve tobacco agronomic parameters. We observed increased plant height, higher effective leaf number, higher stem circumference, higher stalk width, increased cutting length, and greater cutting width. Among the four treatments, the combined application of organic fertilizer and microbial agent showed the most significant effects; plant height, effective leaf number, stem circumference, stalk width, cutting length, and cutting width increased by 8.1, 7.8, 12.1, 15.7, 20.5, and 11.5%, respectively, compared to the control treatment.

| Treatment | Plant height (cm) | Effective leaf number | Stem circumference (cm) | Stalk width (cm) | Cutting length (cm) | Cutting width (cm) |
|-----------|-------------------|-----------------------|-------------------------|-----------------|---------------------|-------------------|
| CK        | 85.6b             | 15.3a                 | 9.1ab                   | 5.17b           | 62.5c               | 28.8b             |
| T2        | 87.4b             | 15.8a                 | 8.9b                    | 5.24b           | 66.5b               | 29.2b             |
| T3        | 91.23a            | 16.4a                 | 9.8a                    | 5.83a           | 72.4a               | 30.5b             |
| T4        | 92.54a            | 16.5a                 | 10.2a                   | 5.98a           | 75.3a               | 32.1a             |

4. Discussion

Organic fertilizer is rich in organic matter and can effectively increase soil nutrient levels. At the same time, the porous nature of organic fertilizer can provide a habitat for microorganisms, which is conducive to microbial growth. In addition, organic fertilizer can activate soil nutrients and increase soil fertility [13, 14]. Microbial agents have been widely used in the production of different crops; in combination with organic fertilizers, they have more significant positive effects on soil quality, the maintenance of soil ecological functions, soil stability, and fertilizer use [15]. In the present study, we demonstrated that the combined application of organic fertilizer and microbial agents can significantly increase the pH value as well as the contents of organic matter, total nitrogen, alkali-hydrolyzed nitrogen, available potassium, and available phosphorus. These results are in agreement with previous findings [16, 17].
Animal and plant residues as well as microbial metabolism are the main sources of soil enzymes, and the activities of soil enzymes are closely related to soil health and soil nutrient transformation [18]. Soil enzymes are an important driving force of soil ecosystem metabolism, and all biological and biochemical processes in soil require catalysis by soil enzymes [19]. The final product of enzymatic reaction of urease is Ammonium (NH₄⁺) and CO₂, where ammonium is source of nitrogen for plant, and urease activity can be used to indicate the ability of a soil to supply nitrogen. Phosphatase catalyzes the mineralization of soil organophosphorus compounds and plays an important role in improving the availability of phosphorus in the soil. Sucrase can directly participate in the organic matter catabolism, and its level can reflect the conversion efficiency of carbon sources in soil. Soil catalase can reduce the accumulation of hydrogen peroxide in soil [20, 21]. Organic fertilizers and microbial agents carry rich and diverse enzymes. Their porous physical nature and their high amount of organic matter facilitate the growth of enzyme-producing microorganisms, in this sense, the combined application of organic fertilizers and microbial agents can significantly improve the activities of soil enzymes [22]. Our study demonstrates that the activities of soil urease, phosphatase, sucrase, and catalase in the soil treated with the combined application of organic fertilizer and microbial agents increased by 86.3, 85.1, 46.6, and 33.5%, respectively, relative to those in the control treatment. This leads us to infer that this approach can significantly improve soil enzyme activities and facilitate soil nutrient conversion, thereby improving the nutrient supply of tobacco plants. These results are in agreement with the findings of Liu et al. [16] and Li et al. [17].

The soil microbial community largely governs soil fertility and plays an important role in soil quality maintenance, soil remediation, and material circulation, while fertilization is one of the most important factors affecting the microbial community composition [23]. The combined application of organic fertilizer and microbial agents resulted in the highest levels of bacteria and actinobacteria, similar to the findings of Zhang et al. [24] and Huang et al. [25]. On the other hand, the combined application of organic fertilizer and microbial agents significantly reduced the quantity of fungi. Wang et al. [26] found that fungi were abundant in low-fertility soil and appeared in lower numbers in highly fertile soil, suggesting that the combined application of organic fertilizer and microbial agents could improve soil biological fertility.

Our study also shows that the combined application of organic fertilizer and microbial agent can significantly increase plant height, effective leaf number, stem circumference, stalk width, cutting length, and cutting width, which respectively increased by 8.1, 7.8, 12.1, 15.7, 20.5, and 11.5% compared to the values in the control treatment. On the one hand, application of organic fertilizer and microbial agent may lead to a large number of beneficial microorganisms colonizing into the soil and promoting the absorption and utilization of nutrients by tobacco root [14]. On the other hand, the increase of soil enzyme activity can further enhance the root activity and promote the absorption of nutrients and growth of tobacco roots [27]. This approach can significantly promote tobacco growth and increase tobacco yield, which agrees with the findings of Zhang et al. [27].

5. Conclusions
The combined application of organic fertilizer and microbial agents can significantly increase the pH value and the levels of organic matter, total nitrogen, alkali-hydrolyzed nitrogen, available potassium, and available phosphorus in tobacco soil, promote the activities of soil urease, phosphatase, sucrase, and catalase, and facilitate the growth of soil bacteria and actinobacteria, thus improving the agronomic parameters of tobacco. This study provides a theoretical basis and technical support for improving tobacco soil and promoting tobacco growth and development.

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References

[1] Tang Y J and Zhang J P 2006 Preliminary partition on ecological quality styles of major flue-cured tobacco production bases for Shanghai tobacco (group) Corp. Chin. Tob. Sci. 27(3) 1-5.

[2] Xie J, Yan M L, Chen J J, Lu Y H, Deng S G, Cai Y X, Chen Z P, Qiu M W, Chen Y M and Wang W 2014 Effect of nitrogen forms on yield, quality and main chemical components of flue-cured tobacco. J. Plant Nutr. Fert. 20(4)1030-7.

[3] Jin H Y, Li J, Zhu Y, Qi S W, Liang Z Z and Dan J H 2019 Effect of soil conditioner on root vigor and carbon metabolism characteristics of rhizosphere soil microorganisms in flue-cured tobacco. J. Nucl. Agric. Sci. 33(1) 0158-65.

[4] Ning C C, Wang J W and Cai K Z 2016 The effects of organic fertilizers on soil fertility and soil environmental quality: A Review. Ecol. Environ. Sci. 25(1) 175-81.

[5] Liu H J, Xi X Y, Liu C K, Xiong S P, Luo W, Xie X B and Zhang C Y 2011 Effects of microbial agents on enzyme activities in soil after continuous cropping of flue-cured tobacco. Tob. Sci. Technol. 5 68-72.

[6] Mao K J, Li S T, Li X N, Wu W, Li B S, Zhang L X, Wei C C, Zhang Y F, Chen M S and Mu Y H 2018 Effects of mixed fertilization of earthworm feces and humic acid on quality of tobacco-planting soil and flue-cured tobacco leaves. Chin. J. Soil Sci. 49 911-8.

[7] State Tobacco Monopoly Bureau 1998 Investigation methods of tobacco agronomic parameters. YC/T 142-1998. Beijing: China Standards Press.

[8] Hu Q L, Zeng W A, Li F, Huang Y N, Gu S S, Cai H L, Zeng M, Li Q and Tan L 2018 Effect of nano zeolite on the transformation of cadmium speciation and its uptake by tobacco in cadmium-contaminated soil. Open Chem. 16 667–73.

[9] Guan S Y 1986 Soil enzyme and its research methods. Beijing: Agriculture Press.

[10] Wu W T, Xue M J, Zhang J, Xu S S, Lv D F, Hu Y F, Nie Y F and Wang Y 2018 Effects of multiple plantations of marigold tobacco on flora and soil enzyme activity related to soil fertility. Mol. Plant Breed. 16 7907-12.

[11] Shen P and Chen X D 2007 Microbiology Experiment. 4th ed. Beijing: Higher Education Press.

[12] Guo P, Wen T C, Dong L L, Wei C X, Shi J X and Li B 2011 Effect of fertilizer to content of soil nutrient, amount of soil microorganism and soil enzyme activities. Res Agric. Modern. 32 362-6.

[13] Wang L G, Li W J, Qiu J J, Ma Y L and Wang Y C 2004 Effect of biological organic fertilizer on crops growth, soil fertility and yield. Soil. Fertil. 5 12-6.

[14] Shi H L, Tan J, Qin X C and Wang D 2014 Effects of different bio-organic fertilizers on growth and development, yield and quality of flue-cured tobacco. Chin. Tob. Sci. 2 74-8.

[15] Liang L B, Xu J M and Zhang X H 2014 Effect of microbial fertilizer and organic-chemical fertilizer on physical properties of calcareous cinnamon soil in North of China. J. Irrig. Draine. 33 105 - 8.

[16] Liu F, Han D, Zhao M Q, Li X Y and Guan C W 2017 Effects of application of microbial agents along with humic acid potassium on tobacco-planting soil and economic benefit of flue-cured tobacco. Acta Agric. Zhejiangensis 29 1064-9.

[17] Li L, Han Z, Zhang Y, Yan X M, Zhang G C, Gao X D, Zhang Y N, Ye C and Li S B 2019 Effects of reducing nitrogen fertilizer combined with microbial agents on rice root growth and soil enzyme activities. Chin. J. Soil Sci. 50(4) 932-9

[18] Liu L, Huang B J, Sun J, Guo S R, Li L Q and Guo H W 2013 Relationship between soil microbial quantity, enzyme activity and soil fertility in hot pepper greenhouse soils of different continuous cropping years. Chin. Soil Fertil. 2 5-10.

[19] Pajares S, Gallardo J F, Masciandaro G, Ceccanti B and Etchevers J D 2011 Enzyme activity as an indicator of soil quality changes in degraded cultivated Acrisols in the Mexican Trans-volcanic Belt. Land Degrad. Dev. 22 373–81.

[20] Dick R P, Sandor J A and Eash N S 1994 Soil enzyme activities after 1500 years of terrace agriculture in the Colca Valley, Peru. Agric. Ecosyst. Environ. 50 123-31.
[21] Balota E L, Kanashiro M, Filho A C, Andrade D S and Dick R P 2004 Soil enzyme activities under long-term tillage and crop rotation systems in subtropical agro-ecosystems. *Braz. J. Microbiol.* **35** 300-6.

[22] Nayak D R, Jagadeesh Babu Y and Adhya T K 2007 Long-term application of compost influences microbial biomass and enzyme activities in a tropical Aeric Endoaquept planted to rice under flooded condition. *Soil Biol. Biochem.* **39** 1897-1906.

[23] Witter E, Martensson A M and Garcia F V 1993 Size of the soil microbial biomass in a long-term field experiment as affected by different N-fertilizers and organic manures. *Soil Biol. Biochem.* **25**(6) 659-69.

[24] Zhang X D, Cao H, Xu D Q, Jin Y F and Chen Y K 2008 Effects of photosynthetic bacteria and organic fertilizer on soil microorganisms and soil enzyme activities. *Soil* **40**(3) 443-7.

[25] Huang W, Zhang J H, Liu Q N, Zhang Z Z, Wand WZ, Li F R and Li G 2019 Effects of the microbial fertilizers on enzymic activity and microorganism in lettuce soil. *Hubei Agric Sci.* **22**(58) 54-64.

[26] Wang C, Wu F, Liu X L and Liu B 2005 Tobacco rhizosphere microorganism in different fertility of soil. *Chin. Tob. Sci.* **2** 12-4.

[27] Zhang L, Liu H B, Gu J G, Lei Q, Yin Y, Qu J K, Wang Y and Song Y F 2013 Co-effect of compound microbial inoculum and organic-inorganic fertilizer on growth, yield and quality of flue-cured tobacco. *Tob. Sci. Technol.* **12** 67 - 73.