Co-Composted Spent Coffee Grounds with Rice Bran and Microbial Agents Promotes Cigar Tobacco Growth and Enhances the Smoking

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Abstract: Coffee grounds, though agricultural waste, are valuable as organic fertilizer, insect repellent, livestock feed, and biomass energy. This study evaluated four fermented coffee ground fertilizers (T1: coffee grounds, T2: coffee grounds + fermentation agent, T3: coffee grounds + rice bran + fermentation agent, T4: coffee grounds + rice bran) on cigar plants and soil microorganisms. T3 (coffee grounds + rice bran + fermentation agent) significantly enhanced cigar tobacco growth, increasing leaf length and width by 7.22% and 15.07% respectively. Soil organic matter, humic acid, available phosphorus, potassium, nitrogen, and total nitrogen all improved under T3. It also reduced harmful fungi (Fusarium spp., Burkholderia spp., Aspergillus spp., Pythium spp.) and increased beneficial Mortierella spp. Sensory evaluation ranked T3 highest in tobacco quality. This study indicates T3 fertilizer’s effectiveness in promoting cigar plant growth and soil health, offering a reference for its use in cigar cultivation.

Keywords: Coffee Grounds; Cigars; Organic Fertilizer; Soil Fertility; Soil Bacteria; Soil Fungi

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1 Introduction

The shortage of high-quality cigar raw materials has restricted the development of the cigar industry [1]. In the cultivation and production of cigar tobacco leaves, excessive reliance on chemical fertilizers remains an issue, which can lead to decreased soil organic matter (OM) content, and impacts negatively on microbial communities, all of which are detrimental to tobacco leaf quality [2, 3]. The application of organic fertilizers helps to replenish soil organic matter, enhance soil microbial activity [4], elevate total nitrogen (TN) and available nitrogen levels [5], bolster disease resistance in tobacco plants, promote tobacco growth, and enhance leaf quality [6]. Therefore, the utilization of organic fertilizers in tobacco cultivation has gained increasing attention. With the rapid growth of domestic coffee consumption in recent years [7], a significant amount of coffee grounds is generated annually [8], and they possess considerable potential benefits. Coffee grounds contain abundant sugars, dietary fibers, and nutritional components such as minerals and vitamins [9], and their porous structure amends soil structure and enhances soil nutrient content as an organic fertilizer. Thus, coffee grounds could be employed to improve soil for

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cigar tobacco planting and subsequently enhance leaf quality. However, inadequately fermented coffee grounds can exhibit toxicity to crops, thereby necessitating proper fermentation before their use in crop production. Hence, there is a need to study appropriate fermentation methods to achieve crop requirements. Nonetheless, at present, there is limited research on the application of coffee ground-based organic fertilizers in cigar tobacco cultivation, and no reports are available regarding the fermentation and preparation of coffee ground organic fertilizers for cigar tobacco cultivation. To explore fermentation formulations of coffee ground organic fertilizers suitable for cigar tobacco cultivation, this study prepared four different fermented coffee ground organic fertilizers. We analyzed their effects on soil fertility in the rhizosphere of cigar tobacco plants, bacterial and fungal communities, tobacco plant growth, and the sensory qualities of tobacco leaves. The study aimed to provide a reference for the rational utilization of fermented coffee ground organic fertilizers in cigar production.

2 Materials and Methods

2.1 Experimental Materials and Design

The experiment was conducted in Shigu Town, Shifang City, Sichuan Province, China (latitude 31°17’67" N, longitude 104°7’37" E). The experimental site had a flat terrain, soil pH of 6.05, and moderate fertility. The tobacco variety used was DeXue 3 and the tobacco-specific compound fertilizer used had a composition of N:P_2O_5:K_2O = 10%:10%:20%. The organic fertilizer materials used were Yunnan Baoshan coffee bean residues and rice bran from Shifang. The compound fermentation microbial agent used was a composite functional microbial agent provided by the Microbiology Research Group of Sichuan Agricultural University’s College of Resources. The concentration of the microbial agent was 0.2–0.4 billion mL^{-1}.

Four fermentation formulations were established for the treatments: T1, 34 kg of coffee grounds; T2, 34 kg of coffee grounds + 1.0 L of compound fermentation microbial agent; T3, 34 kg of coffee grounds + 18 kg of rice bran + 1.0 L of compound fermentation microbial agent; and T4, 34 kg of coffee grounds + 18 kg of rice bran. According to the formulations, the raw materials for each treatment were mixed thoroughly, adjusted to a moisture content of approximately 60% with added water, and then placed in insulated plastic foam boxes for proper heap fermentation. Oxygen was periodically supplied using an air pump. After 20 days of fermentation and maturation, the materials were removed and dried for later use.

A randomized block design was employed for the field experiment, with cigar tobacco planted at a row spacing of 1.2 m and a plant spacing of 0.5 m. Each plot covered an area of 30 m^2, and there were three replicates within each block. After soil plowing and turning, tobacco-specific compound fertilizer was applied at a rate of 525 kg hm^{-2}, followed by application of the four types of organic fertilizers at a rate of 1500 kg hm^{-2} as base fertilizer. Ten days later (late April), cigar seedlings were transplanted into the field. After 30 days of transplanting, an additional 225 kg hm^{-2} of tobacco-specific compound fertilizer was applied. Other cultivation management and tobacco leaf preparation measures were carried out as per previously established procedures.

2.2 Collection of Soil Samples

After 30 days and 80 days of tobacco plant transplantation (S1, rosette stage and S2, leaf harvesting stage, respectively), soil samples were collected using excavation around the main roots of the tobacco plants within a radius of 10 cm from the base and up to a depth of 20 cm. The collected soil samples were carefully shaken to remove soil particles that were not closely associated with the root system. The soil adhering to the root surfaces was meticulously collected and placed into 50 mL cryogenic tubes. These tubes were then stored at −80°C for subsequent total DNA extraction and microbial sequencing analysis. The remaining soil samples were air-dried naturally and used for soil chemical analysis.

2.3 DNA Extraction and Sequencing

Microbial DNA from the rhizosphere soil samples was extracted using a DNA extraction kit (TianGen, Beijing China). The extracted DNA was subsequently assessed for purity and concentration using agarose gel electrophoresis, followed by DNA purification. The purified genomic DNA was used as a template for polymerase chain reaction amplification of the fungal ITS2 region using the specific primers 2024F (5'-GCATCGATGAAGAACG-CAGC-3') and 2409R (5'-TCCTCCGCTTATTGATATGC-3'), and amplification of the bacterial V3~V4 region using the 16S recombinant DNA primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R

(5'-GGACTACHVGGGTWTCTAAT-3') was performed. All PCR mixtures were added with 15 μL of Phusion High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM primers, and 10 ng of genomic DNA template. The first denaturation was carried out at 98 ℃ for 1 minute, followed by 30 cycles of denaturation at 98 ℃ for 10 seconds, annealing at 50 ℃ for 30 seconds, and extension at 72 ℃ for 30 seconds, with a final extension at 72 ℃ for 5 minutes. PCR products were analyzed by electrophoresis on a 2% agarose gel. After passing the quality check, the PCR products were purified using magnetic beads and quantified with enzyme labeling. They were then mixed equally based on the concentration, thoroughly mixed, and subjected to another round of electrophoresis on a 2% agarose gel. Target bands were recovered u The qualified amplification products were subjected to sequencing on the Illumina Nova sing a universal DNA purification recovery kit (TianGen). seq 6000 platform (Novogene, Beijing, China) with three biological replicates for each treatment.

2.4 Measurement of Soil Physicochemical Properties

Dried soil samples were ground and sieved to determine OM, humic acid (HA), TN, alkaline hydrolysis nitrogen (AN), available phosphorus (AP), and available potassium content (AK). The specific measurement methods were as follows: for HA, the soil humus composition correction method (Xiaoxiao *et al*., 2012) was used; for OM content, NY/T 1121.6—2006 was used; for TN, NY/T 1121.24—2012 was used; for AN, DB51/T 1975—2014 was used; for AP, NY/T 1121.7—2014 was used; and for AK content, NY/T 889—2004, was used.

2.5 Measurement of Agronomic Indicators

Sixty days after tobacco seedling transplantation, leaf number, plant height, stem circumference, length of the waist leaf, and width of the waist leaf were investigated according to the YC/T142-2010 "Standard Methods for Investigation of Agronomic Traits in Tobacco Industry."

2.6 Statistical Analysis and Data Processing

Statistical analysis was performed using DPS software (V9.01). Bar charts for soil physicochemical properties were created in Excel 2016. Analysis and visualization of soil rhizosphere bacterial and fungal community data were conducted using the Beijing Novogene Post-analysis Platform (https://magic.novogene.com).

2.7 Evaluation of Tobacco Leaf Sensory Quality

Tobacco leaves from each treatment were collected, and sensory quality evaluation of the tobacco leaves was performed by expert evaluators from the Changcheng Cigar Factory.

3 Results

3.1 Alpha Diversity Analysis of Rhizosphere Soil Bacteria and Fungi

The sequencing results of rhizosphere soil under each treatment showed that the "coverage" indicators for bacteria and fungi were at least 97% and 99%, respectively, indicating good representativeness. The observed species count, Chao1 index, abundance-based coverage estimator (ACE) index, and phylogenetic diversity (PD) whole tree index of bacteria (Table 1) reflected the α-diversity of microbial communities in soil samples, with ranges of 1375.33–2369.33, 1768.56–3133.02, 1904.02–3113.59, and 230.48–541.29, respectively. The observed species count, Chao1 index, ACE index, and PD whole tree index of bacteria displayed the trend T1 > T2 > T4 > T3, indicating significant variations in the α-diversity of bacterial communities between the different formulations of fermented organic fertilizers. Notably, T3 exhibited the lowest α-diversity among the four treatments.

Table 1 Alpha diversity indices of bacterial communities 30 d after transplantation

Microbe	Treatment	Observed species	Chao1 index	ACE index	PD whole tree	Good Coverage %
Bacteria	T1	2369.33 ±490.75a	3133.02 ±1143.38a	3113.59 ±645.53a	541.29 ±167.80a	97.08%
	T2	2148.33 ±75.88ab	2551.07 ±59.59a	2808.03 ±12.23a	483.15 ±47.12a	97.5%

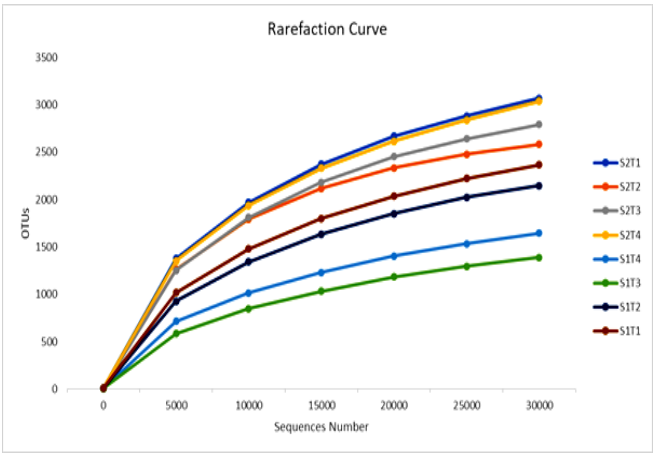
Microbe	Treatment	Observed species	Chao1 index	ACE index	PD whole tree	Good Coverage %
	T3	1375.33 ±278.21c	1768.56 ±348.78a	1904.02 ±299.38ab	230.48 ±29.84b	98.21%
	T4	1645.66 ±101.12bc	2284.45 ±247.23a	2359.52 ±232.77b	286.66 ±28.86b	97.71%
Fungi	T1	280.00 ±27.62	302.67 ±27.19a	314.47 ±28.49a	64.19 ±6.89a	99.91%
	T2	265.00 ±19.97ab	298.52 ±27.19a	316.10 ±33.16a	62.05 ±6.50a	99.89%
	T3	215.66 ±32.02b	239.61 ±35.31a	252.53 ±40.19a	56.78 ±8.97a	99.91%
	T4	217.66 ±5.85b	239.44 ±6.71a	246.04 ±4.56a	52.77 ±3.91a	99.93%

ACE, abundance-based coverage estimator; PD, phylogenetic diversity; T1, 34 kg of coffee grounds; T2, 34 kg of coffee grounds + 1.0 L of compound fermentation microbial agent; T3, 34 kg of coffee grounds + 18 kg of rice bran + 1.0 L of compound fermentation microbial agent; T4, 34 kg of coffee grounds + 18 kg of rice bran.

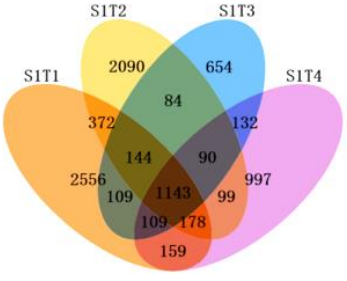
Regarding fungi, the differences in α-diversity indices among treatments were as follows: observed species count, Chao1 index, ACE index, and PD whole tree index were ranked T1, T2 > T3, T4. This pattern indicated that the α-diversity of fungi in soils treated with T3 and T4 organic fertilizers was lower than that in soils treated with T1 and T2 organic fertilizers.

3.2 Dilution Curve and Shared and Unique Operational Taxonomic Unit Analysis

The sequencing data were sufficient to characterize the characteristics and diversity of bacterial and fungal communities in the tobacco rhizosphere soil under various treatments. Figure 1b and c display the number of operational taxonomic units (OTU) in the rhizosphere soil under various treatments 30 and 80 days after transplantation, and differences in OTUs were observed among the rhizosphere soil samples treated with different organic fertilizers. Specifically, 80 days after transplantation (S2) the number of unique OTUs in soils treated with T1 and T2 decreased by approximately 80% compared with that 30 days after transplantation (S1). For T3 and T4, the number of unique OTUs decreased by 29.9% and 43.5%, respectively. However, the number of shared OTUs among treatments generally increased from 1143 to 1237. This suggests that the application of different formulations of fermented organic fertilizers led to distinct bacterial populations in the soil. The capacity of each treatment to enrich specific bacteria decreased as the growing time of tobacco in the field increased, resulting in increased convergence of bacterial community characteristics. Organic fertilizer treatments with rice bran (T3 and T4) exhibited relatively stable bacterial community characteristics compared with those of T1 and T2.



(a)



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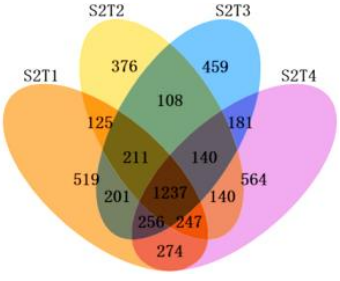

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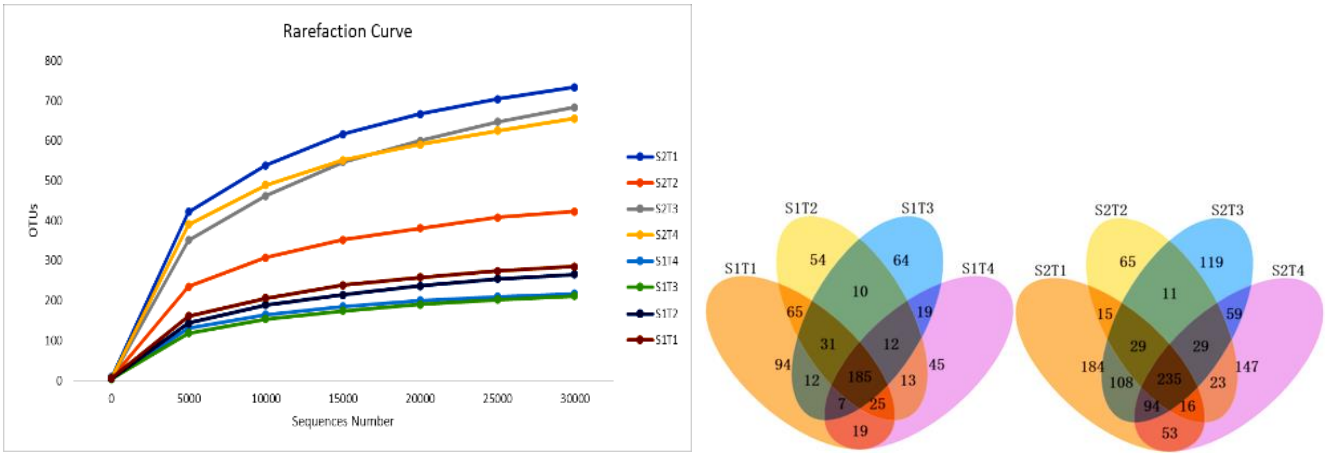


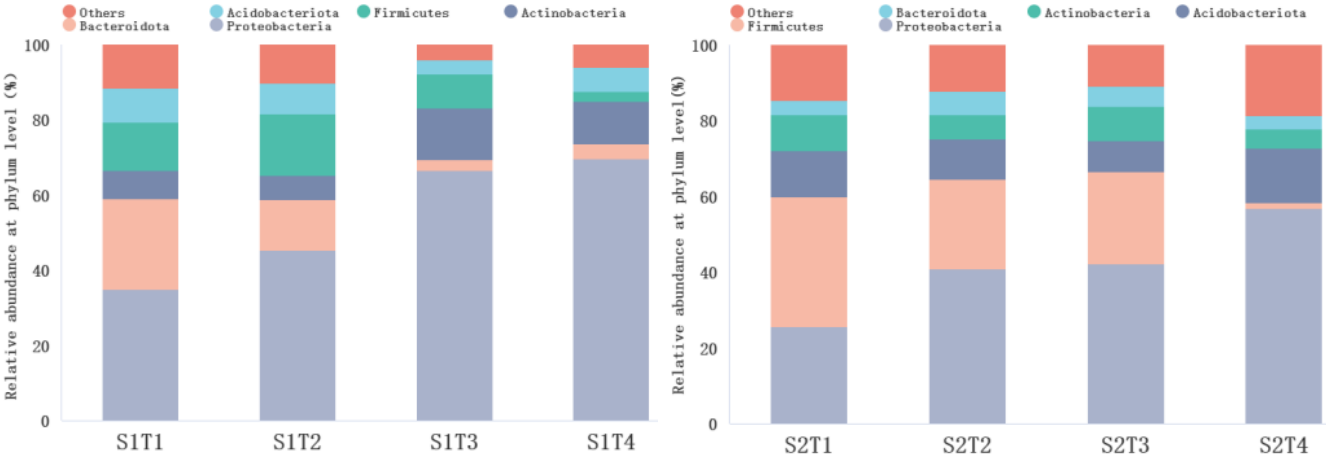
(d) (e) (f)

Figure 1 Rarefaction curve of OTUs and OTU-based Venn diagram analysis. (a) Rarefaction curve of bacterial OTUs; OTU-based Venn diagram of bacteria after (b) 30 d and (c) 80 d. (d) Rarefaction curve of fungal OTUs; OTU-based Venn diagram of fungus after (e) 30 d and (f) 80 d. OTU, operational taxonomic unit. S1, rosette stage; S2, leaf harvesting stage; T1, 34 kg of coffee grounds; T2, 34 kg of coffee grounds + 1.0 L of compound fermentation microbial agent; T3, 34 kg of coffee grounds + 18 kg of rice bran + 1.0 L of compound fermentation microbial agent; T4, 34 kg of coffee grounds + 18 kg of rice bran.

Figure 1e and f show the number of fungal OTUs in the rhizosphere soil under various treatments 30 and 80 days after transplantation. During S2, the number of unique fungal OTUs in soils under all treatments exceeded those during S1, with the increase in quantity following the pattern of T4 > T1 > T3 > T2. During S2, the number of unique fungal OTUs in soil followed the order T1 > T4 > T3 > T2. This indicated that the application of organic fertilizers containing compound fermentation microbial agents (T2 and T3) led to a more stable number of unique fungal OTUs than that of the organic fertilizers without microbial agents (T1 and T4).

3.3 Analysis of Dominant Microbial Phyla and Genera in the Rhizosphere

3.3.1 Analysis of Dominant Bacterial Phyla and Genera



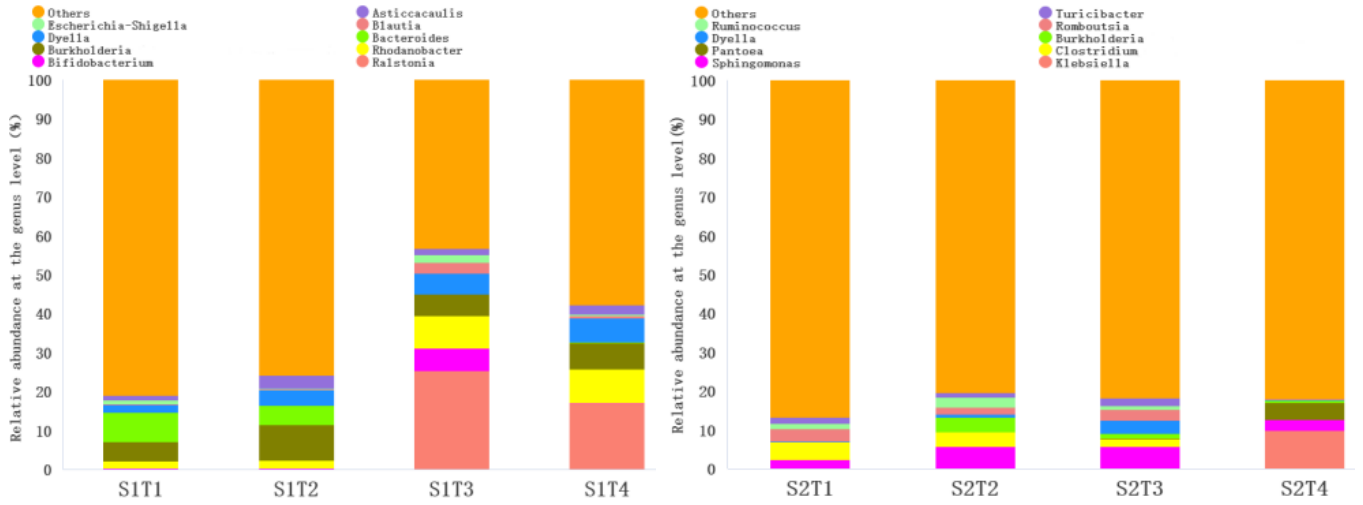
(a) (b)

Figure 2 Abundance of bacterial genera in rhizosphere soil (a) 30 d and (b) 80 d after transplantation. S1, rosette stage; S2, leaf harvesting stage; T1, 34 kg of coffee grounds; T2, 34 kg of coffee grounds + 1.0 L of compound fermentation microbial agent; T3, 34 kg of coffee grounds + 18 kg of rice bran + 1.0 L of compound fermentation microbial agent; T4, 34 kg of coffee grounds + 18 kg of rice bran

Figure 2 depicts the dominant bacteria at the phylum level (relative abundance > 1%) in the rhizosphere soil under various treatments. The dominant phyla include Proteobacteria, Bacteroidota, Actinobacteriota, Firmicutes,

and Acidobacteriota. Under all treatments, Proteobacteria had the highest relative abundance (30.6–62.8%), followed by Bacteroidota (2.6–21.6%). Eighty days after transplantation (S2), the relative abundance of Proteobacteria significantly decreased under all treatments, whereas the relative abundance of Firmicutes increased under the T1, T2, and T3 treatments. The T3 treatments, showed the most significant increase, rising from 8.3% to 19.89%.

Figure 3 displays distinct differences among the dominant bacterial genera at the genus level in the experimental soils. During S1, the dominant bacterial populations included *Ralstonia* spp., *Bifidobacterium* spp., and *Rhodanobacter* spp., accounting for 19.34–57.22% of the total abundance. During S2, the dominant bacterial genera were *Klebsiella*, *Sphingomonas*, and *Clostridium*, accounting for 13.44–21.15% of the total abundance. Notably, the genera *Ralstonia* and *Rhodanobacter* were common among treatments during S1 but were not detected during S2. Additionally, the relative abundance of *Burkholderia* during S2 was lower than that that during S1 under all treatments. The relative abundance of *Bifidobacterium* and *Dickeya* under T3 and T4 treatments was lower than that under other treatments.

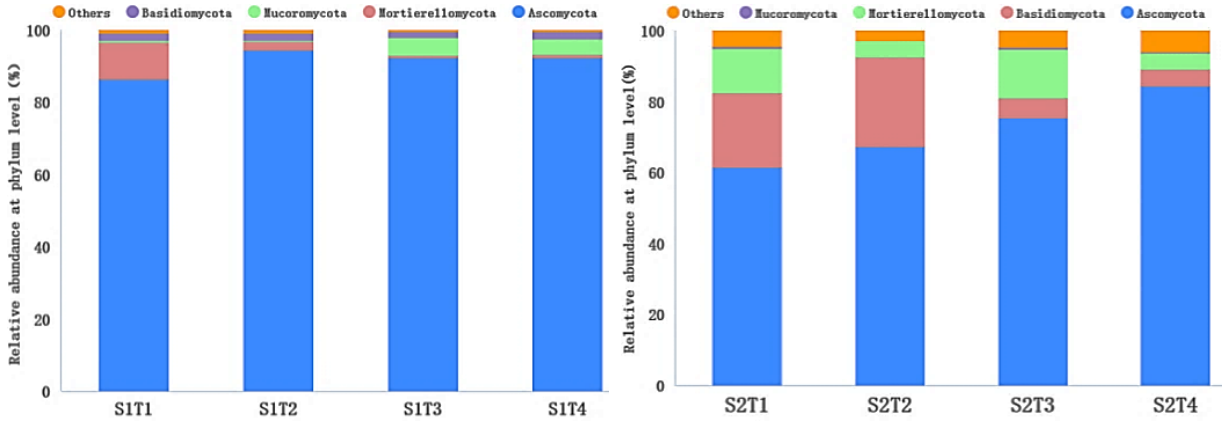


(a) (b)

Figure 3 Abundance of bacterial genera in rhizosphere soil (a) 30 days and (b) 80 days after planting. S1, rosette stage; S2, leaf harvesting stage; T1, 34 kg of coffee grounds; T2, 34 kg of coffee grounds + 1.0 L of compound fermentation microbial agent; T3, 34 kg of coffee grounds + 18 kg of rice bran + 1.0 L of compound fermentation microbial agent; T4, 34 kg of coffee grounds + 18 kg of rice bran.

3.3.2 Analysis of Dominant Fungal Phyla and Genera

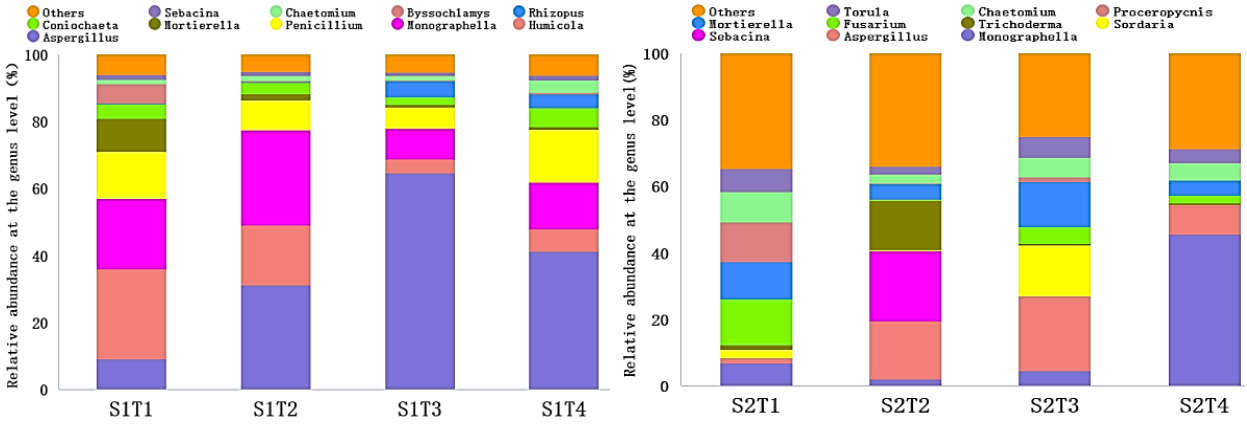
The dominant fungal species with a relative abundance greater than 1% are shown in Figure 4. Four dominant fungal phyla were identified in tobacco rhizosphere soil: Ascomycota, Mucoromycota, Mortierellomycota, and Basidiomycota. Among these, Ascomycota had the highest relative abundance at the phylum level (61.2–94.5%). The relative abundance of Ascomycota and Mucoromycota decreased between the two time periods under all soil treatments. Conversely, Mortierellomycota and Basidiomycota showed increased relative abundances under the influence of organic fertilizers. Notably, the T3 treatment, which included rice bran and microbial agents, exhibited the most significant increase in the relative abundance of Mortierellomycota from 0.62–13.69%. Thus, the four different fermentation formulas of organic fertilizers had the same effect on the dominant phyla of fungi, and all treatments reduced the relative abundance of Ascomycota and Mucoromycota phyla and increased the relative abundance of Mortierellamycota and Basidiomycota.



(a) 30 d (b) 80 d

Figure 4 Abundance of fungal genera in rhizosphere soil (a) 30 days and (b) 80 days after planting. S1, rosette stage; S2, leaf harvesting stage; T1, 34 kg of coffee grounds; T2, 34 kg of coffee grounds + 1.0 L of compound fermentation microbial agent; T3, 34 kg of coffee grounds + 18 kg of rice bran + 1.0 L of compound fermentation microbial agent; T4, 34 kg of coffee grounds + 18 kg of rice bran.

Figure 5 shows that the dominant fungal genera differed between the two time periods. During S1, the top 10 dominant fungal genera included *Aspergillus*, *Humicola*, and *Monographella*, accounting for 93.85–94.86% of the total abundance. During S2, the top 10 dominant fungal genera included *Monographella*, *Aspergillus*, *Sebacina*, *Fusarium*, and *Mortierella*, accounting for 65.34–75.04% of the total abundance. Comparing the relative abundances between S1 and S2, the dominant genera, *Aspergillus*, *Humicola*, *Penicillium*, *Coniochaeta*, *Rhizopus*, and *Trichoderma*, showed decreased relative abundances during S2 after the application of all four organic fertilizers. The relative abundance of the dominant genus *Mortierella* increased during S2 after the application of all four organic fertilizers, with the T3 treatment showing the most significant increase from 0.59% to 13.43%.



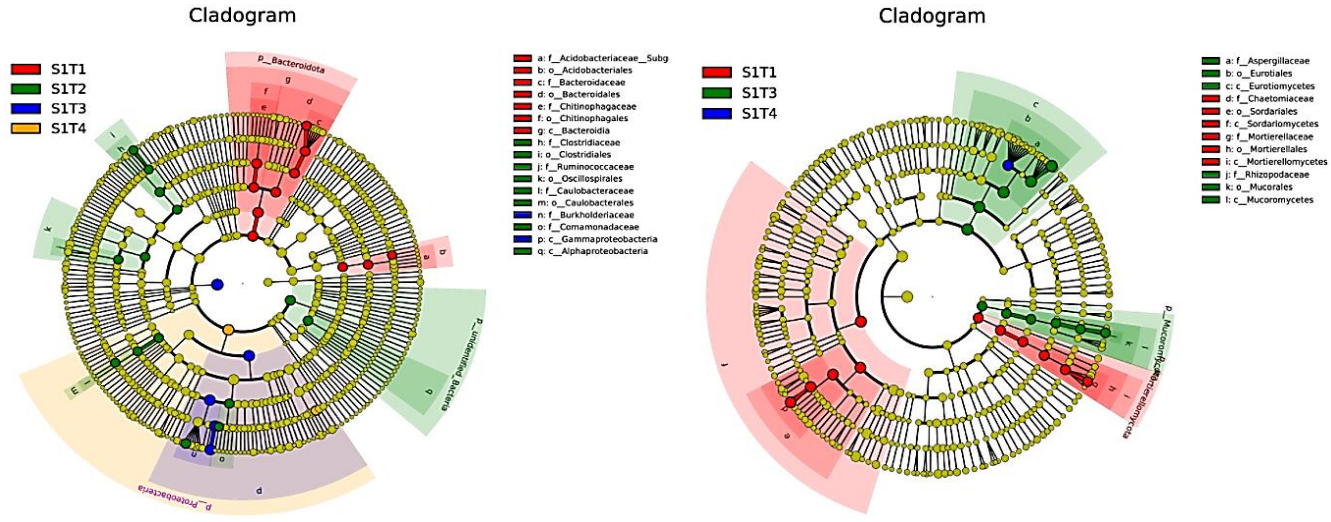
(a) 30 d (b) 80 d

Figure 5 Abundance of fungal genera in rhizosphere soil (a) 30 days and (b) 80 days after planting. S1, rosette stage; S2, leaf harvesting stage; T1, 34 kg of coffee grounds; T2, 34 kg of coffee grounds + 1.0 L of compound fermentation microbial agent; T3, 34 kg of coffee grounds + 18 kg of rice bran + 1.0 L of compound fermentation microbial agent; T4, 34 kg of coffee grounds + 18 kg of rice bran.

3.3.3 Differential Analysis of Microbial Species in Rhizosphere Soil

Using linear discriminant analysis effect size (LefSe) analysis, specific bacterial species in the tobacco rhizosphere under different fertilization treatments during S1 were identified (Figure 6a). Under the T1 treatment, specific bacterial phyla, including Bacteroidota, were identified. At the genus level, *Bacteroides*, *Faecalibacterium*, and *Terriglobus* were specific to T1. Under the T2 treatment, specific bacteria such as *Aquabacterium* and *Asticcacaulis* were identified, whereas T3 was associated with the specific genus *Ralstonia*. Under the T4 treatment the phyla Proteobacteria and genus *Rhodanobacter* were identified. This indicated that different fermented organic

fertilizers selectively enriched specific species.



(a) rosette stage (b) leaf harvesting stage

Figure 6 LEfSe analysis of rhizosphere soil microbial community of cigar tobacco plants. LEfSe, linear discriminant analysis effect size; S1, rosette stage; S2, leaf harvesting stage; T1, 34 kg of coffee grounds; T2, 34 kg of coffee grounds + 1.0 L of compound fermentation microbial agent; T3, 34 kg of coffee grounds + 18 kg of rice bran + 1.0 L of compound fermentation microbial agent; T4, 34 kg of coffee grounds + 18 kg of rice bran.

During S1, under the T1 treatment, the specific fungal phylum Mortierellomycota and the specific genera *Humicola*, *Mortierella*, and *Scytalidium* were identified. Under the T2 treatment, no specific fungal phyla or genera were identified, whereas the phylum Mucoromycota and genera *Aspergillus* and *Rhizopus* were associated with T3. Under the T4 treatment, no specific fungal phyla were identified but the specific genus *Penicillium* was identified.

3.4 Soil Nutrient Indicator Analysis

By comparing soil data after using the four different organic fertilizers with pre-tobacco planting soil data (Table 2), the soil organic matter increased by 59.87% to 85.98%, humic acid by 17.22% to 72.68%, available phosphorus by 51.79% to 130.85%, available potassium by 133.34% to 283.34%, available nitrogen by 15.92% to 33.12%, and total nitrogen by 39.65% to 58.04% under various organic fertilizer treatments. OM content showed the trend of T3 > T1 > T4 > T2. All organic fertilizer treatments led to an increase in OM content, with the T3 treatment demonstrating the highest increase. HA content followed the order of T3 > T2 > T1 > T4, with all organic fertilizer treatments resulting in increased content, and the highest in the T3 treatment. Soil TN, AN, AP, and AK contents all increased after applying the four organic fertilizers, with TN content following the order of T1 > T3 > T4> T2, AN content following the order of T4 > T3 > T1 > T2, AP content following the order of T3 > T1 > T4 > T2, and AK content following the order of T4 > T3 > T1 > T2. This indicated that soils under the T3 and T4 treatments were more conducive to plant nutrient absorption and utilization than the T1 and T2 treatments were.

Table 2 Soil physicochemical properties under each treatment

Treatment	OM (g/kg)	TN (%)	AN (mg/kg)	AP (mg/kg)	AK (mg/kg)	HA (mg/kg)
Before tobacco planting	31.4±0.73d	0.174±0.005d	157±2.64d	36.3±3.37c	150±7.54d	2.38±0.05d
T1	57.2±0.5b	0.275±0.0026a	190±2.51b	80.2±4.07a	352±6c	3.59±0.07c
T2	50.2±0.45c	0.243±0.0045c	182±1.73c	55.1±2.65b	350±8.08c	3.7±0.04c
T3	58.4±0.51a	0.264±0.002b	208±2a	83.8±2.66a	429±8b	4.11±0.14a
T4	56.3±0.62b	0.247±0.091c	209±2.64a	76.9±3.42a	575±8.73a	2.79±0.09c

OM, organic matter; TN, total nitrogen; AN, alkali hydrolyzed nitrogen; AP, available phosphorus; AK, available potassium; HA, humic acid; T1, 34 kg of coffee grounds; T2, 34 kg of coffee grounds + 1.0 L of compound fermentation microbial agent; T3, 34 kg of coffee grounds + 18 kg of rice bran + 1.0 L of compound fermentation microbial agent; T4, 34 kg of coffee grounds + 18 kg of rice bran.

3.5 Agronomic Traits of Tobacco Plants under Different Treatment at Different Stages

The agronomic traits of the rosette stage after applying the T1, T2, T3, and T4 organic fertilizers are shown in Table 3. The agronomic traits during the rosette stage of the tobacco plants treated with T4 organic fertilizer resulted in stronger growth in the field than that of tobacco plants treated with the T1, T2, and T3 organic fertilizers, with significant improvements in plant height, stem circumference, leaf length, and leaf width. As shown in Table 3, during the rosette stage, the agronomic traits of tobacco plants treated with the T1 and T4 organic fertilizers without bacterial agents were better than those treated with the T2 and T3 organic fertilizers containing bacterial agents. Thus, the T4 treatment had the optimal agronomic traits during the rosette stage.

Agronomic traits after topping, including internode length, stem girth, leaf length, and leaf width, are shown in Table 3. Tobacco plants treated with T3 organic fertilizer showed better growth in the field than that of those treated with T1, T2, and T4. The internode length, stem diameter, leaf length, and leaf width of the T3 treatment were higher than the other three organic fertilizer treatments, with the leaf length and width increasing by 7.22% and 15.07%, respectively, compared to the lowest treatment (T1). Notably, the treatments containing microbial agents (T2 and T3) exhibited better agronomic traits than that of those without microbial agents (T1 and T4), with the T3 treatment demonstrating the best agronomic traits after topping.

Table 3 Agronomic characters under each treatment

Stage	Treatment	Plant height (cm)	Pitch (cm)	Stem girth (cm)	Leaf length (cm)	Leaf width (cm)
Rossette Stage	T1	23.8 ±0.66b	/	4.13 ±0.05a	32.8 ±2.26ab	19.93 ±3.17ab
	T2	16.1 ±0.30d	/	3.46 ±0.23b	27.86 ±1c	16.53 ±0.73b
	T3	21.7 ±0.47c	/	3.7 ±0.1b	30.86 ±1.13b	17.36 ±0.64b
	T4	27.4 ±0.75a	/	4.2 ±0.1a	34.63 ±0.2a	22.4 ±0.45a
Recovery	T1	/	7.2 ±0.2a	8.5 ±0.53a	53.98 ±1.11a	26.86 ±2.03b
	T2	/	7.5 ±0.55a	8.6 ±0.4a	57.25 ±2.95a	30.81 ±0.95a
	T3	/	7.6 ±0.45a	9 ±0.32a	57.88 ±3.44a	30.91 ±0.41a
	T4	/	7.5 ±0.62a	8.5 ±0.34a	57.05 ±1.22a	29.93 ±1.47a

T1, 34 kg of coffee grounds; T2, 34 kg of coffee grounds + 1.0 L of compound fermentation microbial agent; T3, 34 kg of coffee grounds + 18 kg of rice bran + 1.0 L of compound fermentation microbial agent; T4, 34 kg of coffee grounds + 18 kg of rice bran.

3.6 Sensory Evaluation of Tobacco Leaves Under Different Treatments

The sensory evaluation results of tobacco leaves produced and processed under different treatments are shown in Figure 7. The burning characteristics and ash color scores of the tobacco leaves were consistent, with scores of 5.5 and 6, respectively. However, there were differences in other sensory attributes such as aroma intensity, richness, maturity, and irritation. When considering the composite score of all 12 sensory attributes, T3 had the highest overall score of 70.5 points, followed by T4 with 67.5 points, and T1 with 65 points. In summary, out of the four different organic fertilizers, T3 exhibited the best overall sensory quality, followed by T4, whereas T1 performed the worst. In terms of sensory evaluation total scores, the ranking was: T3 > T4 > T2 > T1.

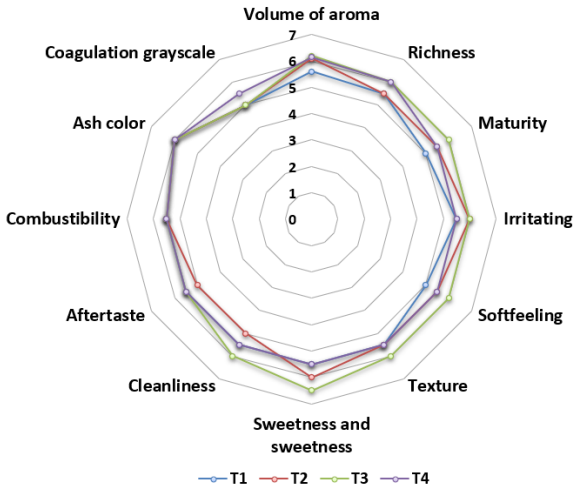


Figure 7 Sensory evaluation of tobacco leaves. T1, 34 kg of coffee grounds; T2, 34 kg of coffee grounds + 1.0 L of compound fermentation microbial agent; T3, 34 kg of coffee grounds + 18 kg of rice bran + 1.0 L of compound fermentation microbial agent; T4, 34 kg of coffee grounds + 18 kg of rice bran.

4 Discussion

Coffee is one of the most prominent beverages globally [10], and a significant amount of coffee grounds are produced annually [11]. Coffee grounds contain various chemical components such as oils, proteins, sugars, and minerals, making them valuable resources for organic fertilizer production [12]. Effective utilization of coffee grounds can reduce resource wastage, improve resource efficiency, and mitigate environmental pollution. Coffee grounds significantly increase soil organic matter through their rich content of organic carbon and nutrients such as nitrogen, potassium, phosphorus, and trace elements. This enhancement optimizes soil structure, water retention, and aeration, benefiting plant growth. Additionally, coffee grounds promote soil biodiversity and activity, accelerating the decomposition of organic matter into nutrients absorbable by plants and potentially fostering the production of substances that promote plant growth by beneficial microbes [13]. In this study, the effects of organic fertilizers derived from four fermentation formulas of coffee grounds on tobacco cultivation were analyzed. The application of coffee ground organic fertilizers enhanced soil properties such as OM, TN, AP, and AK, which aligns with the findings of Comino [14].

The variations in the number of microbial OTUs in rhizosphere soils under the four coffee ground organic fertilizer treatments revealed significant differences in microbial community structure and soil characteristics, consistent with studies by He and Calheiros [15, 16]. Beneficial rhizosphere microorganisms promote OM decomposition, humus formation, nutrient mineralization, and soil fertility improvement, and play important roles in plant growth promotion and disease prevention [17-19]. Firmicutes can decompose organic matter and release nutrients [20], All treatments increased the relative abundance of the beneficial phylum Firmicutes while reducing harmful genera such as Ralstonia and Burkholderia. Ralstonia can cause rotting of crop roots, leading to plants being unable to absorb nutrients properly and eventually wilting [21]. Burkholderia genus can cause crop diseases, harmful to tobacco plants, resulting in yellowing leaves and stunted growth [22], with the most significant effect observed under the T3 treatment. Fungi act as decomposers in soil, breaking down OM and improving soil structure, as well as promoting plant growth [23]. Basidiomycota are involved in nutrient cycling and form beneficial relationships with plant roots [24, 25], Mortierellomycota help decompose organic matter and improve soil structure [26]. This study found that all treatments increased the relative abundance of the phyla Basidiomycota and Mortierellomycota, while decreasing the relative abundance of the harmful genera Humicola and Aspergillus [27, 28], Humicola and Aspergillus fungi can disrupt soil nutrient balance and hinder plant growth by producing toxins and metabolites that harm soil microbial communities. This can lead to slow plant growth, leaf yellowing, reduced fruit production, and disease spread to other plants. Notably, the T3 treatment significantly increased the relative abundance of the beneficial genus Mortierella [29, 30]. The characteristics of bacterial and fungal communities under the T3 treatment may be related to enhanced plant growth [16]. The increase in beneficial fungi and bacteria, as well as the decrease in harmful fungi and bacteria, may be reasons for the enhanced soil nutrient levels and improved growth of tobacco plants under T3 treatment.

The α-diversity of soil microbes increases or decreases after applying organic fertilizers [15]. In this study, different treatments led to both increased and decreased α-diversity of rhizosphere bacteria and fungi. Different organic fertilizer treatments led to varied enrichment of microorganisms, with T3 favoring Mucoromycota and T4 favoring Proteobacteria. Additionally, the T3 treatment promoted the enrichment of beneficial microorganisms and suppressed harmful species. This was evident in the suppression of harmful genera, such as *Ralstonia* and *Burkholderia*, in tobacco rhizospheres while enriching beneficial genera such as *Mortierella* and Mucoromycota. These results highlight the significant impact of coffee ground organic fertilizers on soil microorganisms [16], which subsequently affecting soil nutrients and crop growth. The disadvantages and benefits of microbes in soil include the nutrient uptake from plants and conferring disease and abiotic stress resistance, respectively, making their assessment a complex endeavor [31]. Analysis of changes in microbial communities under different treatments should be combined with the final growth status of crops and product quality. In summary, among the different fertilizers tested, T3 exhibited the most favorable impact on tobacco plant agronomic traits and leaf sensory quality.

5 Conclusions

The four fermentation formulas of coffee ground organic fertilizers analyzed in this study all demonstrated

the ability to enhance nutrient content, including total nitrogen, alkali hydrolyzed nitrogen, available phosphorus, and available potassium, as well as OM and organic acids, in tobacco soil. These fertilizers also influenced the composition and diversity of rhizosphere microbial communities, ultimately promoting the growth of tobacco plants and leaves. Furthermore the coffee ground organic fertilizers were effective in suppressing harmful microorganisms in the tobacco rhizosphere, including the bacterial genera *Ralstonia* and *Burkholderia* and the fungal genera *Aspergillus* and *Humicola*. Simultaneously, they promoted the enrichment of beneficial microorganisms such as Mucoromycota and Mortierellomycota. Moreover, among the tested treatments, the T3 treatment comprising coffee grounds + rice bran + microbial agents, showed the best overall effect in improving soil fertility, promoting tobacco plant growth, and achieving good sensory characteristics in tobacco leaves, Under T3 organic fertilizer treatment, soil organic matter increased by 85.98%, humic acid increased by 72.68%, available phosphorus increased by 130.85%, available potassium increased by 186%, available nitrogen increased by 32.48%, and total nitrogen increased by 51.72%, Leaf length and width increased by 7.22% and 15.07%, respectively, compared to the worst treatment (T1). The sensory evaluation score of 70.5 points was the highest among all treatments. Overall, the comprehensive effectwas the best among all treatments. Therefore, T3 holds promising potential for application in tobacco production and soil conservation. Hence, the utilization of coffee grounds as a resource for organic fertilizer production, particularly in combination with rice bran and microbial agents, offers a promising avenue for sustainable tobacco cultivation and soil preservation.

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