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# Effect of Enhanced Organic Material Addition during Reductive Soil Disinfestation on Disease Resistance, Yield Increase, and Microbial Community in Flue-Cured Tobacco

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**Abstract:** The addition of organic materials is pivotal for the efficacy of reductive soil disinfestation (RSD). However, data on the influence of varying amounts of organic matter during RSD on soil-borne disease mitigation, yield increase, and rhizosphere microecological health in the current flue-cured tobacco season remain limited. This study analyzed various organic material addition rates (CK, G0.8, G1.0, and G1.2) at two experimental sites (K and Y). The results indicated that increasing the application of organic material improved the soil physicochemical properties (pH, AN, AP, AK, OM, and C/N), mitigated the severity of black shank and Fusarium root rot, and amplified the tobacco yield. The K/YG1.2 treatment significantly reduced the Shannon and Sobs fungal indices across both sites, and enhanced the relative abundance of the bacteria Actinobacteria, Chloroflexi, Firmicutes, and Acidobacteriota, while decreasing the relative abundance of Ascomycota. The bacterial genera were predominantly represented by *Sphingomonas* and *Bacillus*, whereas the fungal genera were represented by *Saitozyma*, *Mortierella*, and *Fusarium*. The addition of organic materials during RSD substantially decreased the relative abundance of *Mortierella* and *Fusarium*. Using FUNGuild and Tax4Fun to evaluate the application of adding organic matter during the RSD process, we identified that rhizosphere fungi in high application rates of flue-cured tobacco were primarily saprophytic or pathogenic saprophytes, which were mainly involved in the metabolism, environmental information processing, genetic information processing, and cellular processes. The results of the two experimental sites indicate that applying 15 t·ha$^{-1}$ (K/YG1.2) of solid residues such as vegetables during RSD emerges as the optimal choice. This strategy is highly effective in guaranteeing the sterilization and pest control effect of the RSD process, facilitating the reconstruction of microbial community diversity, lowering pathogen abundance, managing soil-borne diseases that are prevalent in the current flue-cured tobacco season, and leading to an increase in tobacco yield.

**Keywords:** reductive soil disinfestation; organic materials; pathogenic fungi; disease control; microbial community



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## 1. Introduction

Flue-cured tobacco (*Nicotiana tobacum* L.), the primary raw material for cigarette products, is a globally prevalent cash crop with a significant cultivation in China [1]. The robustness and sustainability of the tobacco industry are closely related to the quality of tobacco planting soil and its micro-ecological environment. Motivated by economic interests, the continuous cropping of flue-cured tobacco has led to pressing issues, such as soil-borne diseases, suboptimal plant growth, and declines in both yield and quality [2]. Continuous soil cropping obstacle issues are primarily attributed to a single soil microbial composition and the proliferation of pathogenic fungi, bacteria, nematodes, and other pathogens [3,4]. The composition and functionality of soil microbial communities determine the incidence of plant diseases [5] and growth patterns [6]. Maintaining a healthy microecosystem is crucial

for optimal crop growth. Previous methods, such as crop rotation, chemical pesticides, and biocontrol bacteria, have been employed to counteract diseases and soil microbial imbalances caused by the continuous cropping of flue-cured tobacco. However, the limited efficiency of crop rotation, the safety concerns associated with chemical pesticide residues, and the unstable antagonistic effect of biocontrol bacteria have led growers to view these measures unfavorably [7].

Reductive soil disinfection (RSD) technology, employed to mitigate soil-borne pathogens and improve continuous cropping resilience, has been successfully implemented in several countries, including Japan, the Netherlands, and the United States [8–10]. Previous studies have indicated that RSD can effectively inhibit a variety of soil-borne pathogens, including *Ralstonia solanacearum* [9], *Phytophthora cacto rum* [11], *Fusarium oxysporum* f. sp. *cubense* (FOC) [12], and *Phytophthora cassici* Leonian [13]. To eliminate soil-borne pathogens and reshape soil microbial communities, RSD relies on introducing easily degradable organic matter to the soil, irrigating to maximum field capacity, mulching, and rapidly inducing anaerobic and high-temperature conditions [14]. The judicious selection and application of organic materials are crucial to the efficacy of this method as a substitute for chemical fumigation [15].

As a pre-planting soil remediation technology, RSD has been applied to diverse economic crops, including watermelon, eggplant, okra, and chili pepper, resulting in enhanced yield and disease mitigation [13,16,17]. Khadka et al. [16] demonstrated that adding different organic materials during RSD reduced Fusarium wilt and root knot nematode incidences while increasing okra and eggplant yields. Zhu et al. [15] found through pot experiments that the application of organic materials during RSD significantly improved the microbial community ecology of chili peppers and suppressed *Fusarium oxysporum*. Strauss et al. [18] discovered that incorporating local crop straw into the soil, particularly at 20.2 $t \cdot ha^{-1}$, created an environment that was detrimental to pathogenic bacteria. Wen et al. [19] have reported that both 1.5 and 3 $t \cdot ha^{-1}$ straw additions can effectively inhibit *Fusarium oxysporum* growth. It is evident that the organic material type and quantity added during RSD are pivotal for microbial community alterations. However, these studies, generally greenhouse- or pot-based, primarily only focused on the control effects of specific pathogenic microorganisms. The literature on the application of RSD to the rhizosphere soil microenvironment and flue-cured tobacco field disease management remains limited.

Yunnan Province, which contributes to over 35% of the annual tobacco production in China, is a significant hub for flue-cured tobacco cultivation [20]. The incorporation of organic materials and the induction of an anaerobic environment during RSD treatment can modify soil physicochemical properties, mitigating the issues associated with continuous cropping [14]. However, there are currently insufficient data regarding the influence of RSD on the rhizosphere soil microbiota of crops in the current season, especially the effects of varying organic material inputs during RSD on microbial traits and disease prevalence in tobacco rhizosphere soil under distinct field conditions. We hypothesized that adding organic materials during RSD can promote microbial diversity and the relative abundance of beneficial bacteria in the rhizosphere soil of flue-cured tobacco, thereby inhibiting soil-borne diseases. Therefore, field experiments were established based on long-term flue-cured tobacco continuous cropping with different rates of organic material application across two sites. We aimed to explore the impacts of increasing the application of organic material on (1) the physicochemical properties of the rhizosphere soil of flue-cured tobacco; (2) the prevalence of soil-borne diseases in flue-cured tobacco and their influence on yield; and (3) the alterations in microbial communities in the rhizosphere soil of flue-cured tobacco, and the relationship between the communities and environmental factors. This study was conducted to elucidate the relevant mechanisms through which RSD modulated the rhizosphere soil microecology of flue-cured tobacco. Hence, this study can provide the theoretical foundation for RSD technology in controlling soil-borne diseases in flue-cured tobacco and enhancing crop yields.

## 2. Materials and Methods

### *2.1. Overview of the Experimental Site and Materials*

This study focused on two primary flue-cured tobacco planting regions in Chuxiong City, Yunnan Province: the Donghua town modern tobacco agricultural base (24°55′20″ N, 101°30′37″ E, 3657 m ASL) and the Nanhua county tobacco-scale planting base (25°19′22″ N, 101°27′35″ E, 3092 m ASL). Both are situated in the northern subtropical monsoon climate zone and are approximately 28 km apart on a straight line. The annual average temperatures for the Donghua (K) and Nanhua (Y) sites are 23.2 °C and 20.2 °C, respectively, with closely matched annual rainfalls of 864.1 mm and 864.8 mm. The temperature and precipitation patterns during the planting season are shown in Figure 1. Each site has witnessed the continuous cultivation of flue-cured tobacco for 7–8 years. The soil properties at the K site included a pH of 6.54, AN (alkali-hydrolyzable nitrogen) of 17.29 mg·kg$^{-1}$, AP (Olsen-P) of 8.90 mg·kg$^{-1}$, AK ($NH_4OAc$-exchangeable K) of 86.5 mg·kg$^{-1}$, and OM (organic matter) of 19.27 g·kg$^{-1}$. Correspondingly, the Y site has values of pH 6.75, AN 19.29 mg·kg$^{-1}$, AP 8.78 mg·kg$^{-1}$, AK 83.25 mg·kg$^{-1}$, and OM 19.01g·kg$^{-1}$. The flue-cured tobacco variety selected for testing was "Yunyan 87". Seedlings were transplanted after approximately 30 d of cultivation at the base. The organic materials utilized in the experiment were derived from the fermented biogas residues of cabbage roots and stem waste from a vegetable-planting base. These materials had a TOC content of 34.85%, a TN content of 1.63%, and a C/N ratio of 21.4. Before soil incorporation, the organic materials were dried, ground, and sieved through a 50-mesh sieve.

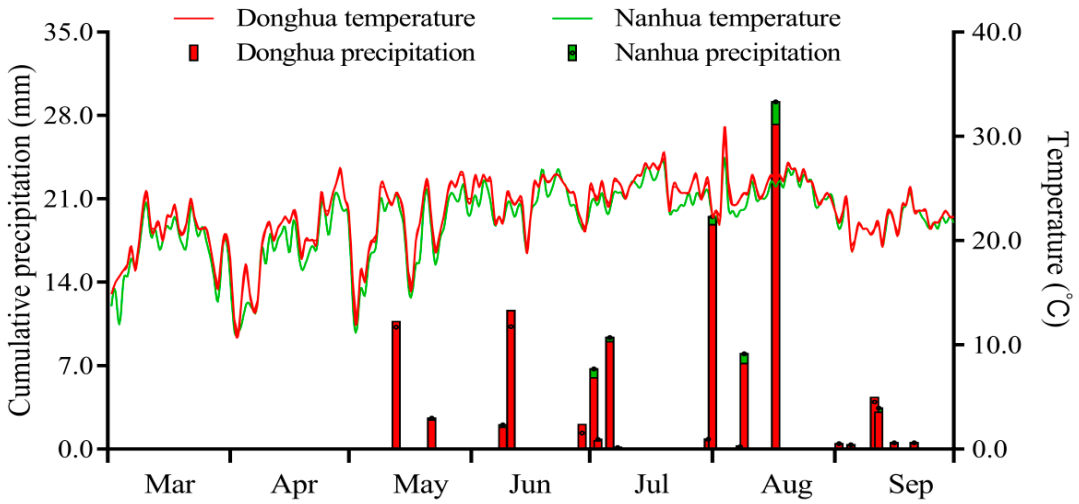


**Figure 1.** Daily average temperature and monthly cumulative precipitation during the planting season at two experimental sites.

### *2.2. Experimental Design*

This experiment adopted a complete randomized block design with four organic material application rates at each experimental site. These rates were defined as K site or Y site (i.e., K/YCK 0.8, 1.0, and 1.2 represent the application rates per 667 m$^2$, respectively, and the same applies for the other treatments) without organic material added; K/YG0.8 with 12 t·ha$^{-1}$ of organic materials; K/YG1.0 with 15 t·ha$^{-1}$ of organic materials; and K/YG1.2 with 18 t·ha$^{-1}$ of organic materials. Each treatment was repeated three times, with 12 cells at each experimental site, each measuring 38.7 m$^2$ (6.45 m × 6.00 m). The flue-cured tobacco was planted in single rows, spaced 1.2 m apart. Each row housed 11 plants with a spacing of 0.5 m between plants. The rows were demarcated by ditches, 0.4 m deep and 0.2 m wide. All other chemical management practices for flue-cured tobacco production, not specified here, adhered to the local technical specifications for high-quality tobacco cultivation [21].

The experiment commenced 30 d before the transplantation of flue-cured tobacco. After field leveling, organic materials were uniformly distributed across each plot's soil surface, followed by tilling at a depth of 10–20 cm. Upon thorough mixing and irrigation to maximum field capacity, the plots were sealed with plastic film for a 30 d anaerobic treatment. Subsequently, the film was removed, and the soil was aerated for 10 d. During the anaerobic treatment with film covering, the soil temperature ranged between 20 and 30 °C.

### *2.3. Indicator Measurement and Methods*

#### 2.3.1. Evaluation of Black Shank Disease and Fusarium Root Rot in Flue-Cured Tobacco

Disease assessment was conducted when the flue-cured tobacco approached its harvest period, approximately 60 d post-transplantation. The diagonal survey method was employed for disease investigation, with five points selected per plot and two plants sampled per point, totaling 10 plants. Disease prevalence for black shank and Fusarium root rot was enumerated in accordance with the GB/T23222-2008 standard for "Classification and Investigation Methods for Tobacco Diseases and Pests" [21]. The severity of the tobacco diseases was then categorized, and the disease severity index was calculated using the following formula.

$$\text{Disease index} = \frac{\Sigma(\text{number of diseased plants at each level} \times \text{corresponding disease level value})}{\text{total number of surveyed plants} \times \text{highest level value}} \times 100$$

#### 2.3.2. Evaluation of Flue-Cured Tobacco Leaf Yield

During the tobacco harvest period, leaves from each plot were collected and bundled before being sent to the curing chamber. They were classified and processed in accordance with the three-tier tobacco leaf curing technique, including the upper, middle, and lower parts. Subsequent to product grading, both the total yield for each plot and the yield for each grade were calculated and then normalized to a per-unit-area basis.

#### 2.3.3. Soil Sample Collection

Upon entering the harvesting phase for flue-cured tobacco (approximately 60 d post-transplantation of seedlings), we collected rhizosphere soil samples (0–20 cm depth) from the plants. Employing the five-point sampling method, soil closely adhering to the roots was collected as rhizosphere soil from each plot. The soil from five plants was combined and homogenized, then sieved through a 20-mesh sieve, ensuring a minimum collection of 1.5 kg of rhizosphere soil from each community. The sieved samples were categorized into two parts: one portion was dried for the assessment of the primary soil physicochemical properties, and the other was stored at −80 °C for microbial analysis.

#### 2.3.4. Detection of Basic Physicochemical Properties

The indicators included pH, organic matter (OM), total nitrogen (TN), alkaline nitrogen (AN), available phosphorus (AP), and available potassium (AK). The specific methods and steps can be found in Bao's [22] "Soil agrochemical analysis".

#### 2.3.5. Soil DNA Extraction and Microbial Detection

The soil microbial genomic DNA was determined using an E.Z-N.A® Soil DNA kit (Omega Biotek, Norcross, GA, USA). The DNA concentration and purity were assessed using 2% agarose gel electrophoresis. Genomic DNA samples were submitted to GENEWIZ for bacterial community sequencing, targeting the high-variation regions of the 16S rRNA gene. The selected primers were 338F (5′-ACTCCTACGGGGGGCAGG-3′) and 806R (5′-GACTACHVGGGTWTCTAAT-3′), focusing on the V3-V4 regions [23]. For fungal sequencing, primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGTGTTCATCGATGC-3′) were used, targeting the ITS1 region [24]. The PCR conditions followed the methods outlined by Xiong et al. [25]. The post-PCR procedures, including sequencing, operational taxonomic unit (OTU) clustering, and species annotation,

were facilitated via the cloud platform of Shanghai Meiji Biopharmaceutical Technology Co., Ltd. (Shanghai, China) (https://cloud.majorbio.com accessed on 10 March 2023) [26]. The initial sequence data were archived in the SRA (sequence reading archive) of the NCBI (National Biotechnology Information Center), with subsequent analysis conducted using the Quantitative Insight Microbial Ecology (QIIME) software package version 1.8.0 [27]. The accession numbers for the sequences were PRJNA1011446 (Bacteria) and PRJNA1011012 (Fungi).

### *2.4. Data Analysis and Statistical Methods*

The data were analyzed using Microsoft Excel 2010. Significant differences ($p < 0.05$) among treatments were determined via a one-way analysis of variance (ANOVA) and least significant difference (LSD) multiple comparison tests using SPSS 20.0. Sequence quality control was performed using Fastp 0.19.6, and the remaining sequences were clustered into OTUs based on 97% similarity. After clustering, the sequences were aligned to the Silva and Unite databases using QIIME, enabling community composition analyses across taxonomic levels. Community richness and diversity, represented by the Chao and Shannon indices, were calculated using the mothur software (version 1.30.2). Principal coordinate analysis (PCoA) and correlation heat maps were generated using R packages, specifically Vegan and Pheatmap. The pathogen data, derived from the OTU table statistics, were visualized using GraphPad Prism 7.0. Variations in the community structures across the samples were assessed to determine the correlation between the microorganisms and the environmental factors. In addition, following a linear discriminant analysis (LDA) based on group distinctions, key species with significant sample partitioning differences were identified using Lefse multi-level species difference analysis (http://huttenhower.sph.harvard.edu accessed on 10 March 2023). Functional predictions for the bacterial and fungal communities were facilitated via Tax4Fun and FUNGuild, with subsequent data analysis conducted on the ShengxinCloud Platform provided by Shanghai Meiji Biomedical Technology Co., Ltd. (Shanghai, China) (https://cloud.majorbio.com accessed on 10 March 2023).

## 3. Results

### *3.1. Physicochemical Properties of Tobacco Rhizosphere Soil*

The effect of the addition of organic material during the RSD process on the physicochemical properties of tobacco rhizosphere soil is shown in Table 1. For both experimental sites, K: Donghua and Y: Nanhua, an increase in the quantity of organic materials during the RSD process enhanced the soil physicochemical properties to varying degrees (pH, AN, AP, AK, OM, and C/N). Specifically, compared to the CK treatment, both the YG1.2 and KG1.2 treatments significantly increased the pH, AN, AP, AK, OM, and C/N values ($p < 0.05$). The increments in pH, AN, AP, and C/N at the Y site exceeded those at the K site, recording increases of 2.8%, 0.8%, 4.1%, and 1.2%, respectively. However, AK and OM peaks were observed at the K site, achieving values of 93.45 mg·kg$^{-1}$ and 27.34 g·kg$^{-1}$, respectively.

**Table 1.** Effect of adding organic materials on soil physicochemical properties.

| Treatments | pH | AN (mg·kg$^{-1}$) | AP (mg·kg$^{-1}$) | AK (mg·kg$^{-1}$) | OM (g·kg$^{-1}$) | C/N |
|---|---|---|---|---|---|---|
| KCK | 6.54 ± 0.22 c | 17.29 ± 0.55 c | 8.90 ± 1.21 b | 86.75 ± 3.21 c | 19.27 ± 1.25 d | 23.02 ± 0.12 b |
| KG0.8 | 6.62 ± 0.21 bc | 19.24 ± 1.25 b | 9.21 ± 0.75 b | 89.25 ± 1.21 b | 21.15 ± 2.12 c | 23.91 ± 0.12 b |
| KG1.0 | 7.04 ± 0.31 ab | 21.33 ± 0.85 b | 10.01 ± 0.95 ab | 90.12 ± 2.10 ab | 23.85 ± 2.41 b | 24.01 ± 0.20 ab |
| KG1.2 | 7.17 ± 0.30 a | 23.79 ± 0.45 a | 12.25 ± 1.29 a | 93.45 ± 1.89 a | 27.34 ± 1.58 a | 24.75 ± 0.15 a |
| YCK | 6.75 ± 0.22 c | 19.29 ± 0.75 b | 8.78 ± 1.01 b | 83.25 ± 1.21 c | 19.01 ± 0.75 d | 22.82 ± 0.12 b |
| YG0.8 | 6.90 ± 0.24 bc | 19.95 ± 1.23 ab | 9.07 ± 0.85 b | 83.75 ± 2.11 b | 20.19 ± 2.02 c | 23.78 ± 0.12 b |
| YG1.0 | 7.14 ± 0.23 ab | 22.13 ± 0.63 a | 10.21 ± 0.75 ab | 86.12 ± 2.00 ab | 22.95 ± 2.01 b | 24.77 ± 0.20 ab |
| YG1.2 | 7.37 ± 0.43 a | 23.99 ± 0.48 a | 12.75 ± 1.04 a | 90.45 ± 1.59 a | 25.84 ± 1.88 a | 25.05 ± 0.15 a |

Note: different letters in the same column for the same experimental site indicate significant differences among treatments at $p < 0.05$.

### *3.2. Occurrence of Flue-Cured Tobacco Diseases and Tobacco Leaf Yield*

The influence of the addition of organic material during the RSD process on the occurrence of soil-borne disease in flue-cured tobacco and tobacco leaf yield is shown in Figure 2. At both experimental sites, there was a significant decline in black shank disease and Fusarium root rot in the flue-cured tobacco with an increase in the application of organic material. Compared to KCK and YCK, the KG1.2 and YG1.2 treatments notably decreased the black shank disease index by 26.8% and 70.0% and lowered the Fusarium root rot disease index by 207.6% and 104.9%, respectively ($p < 0.05$). Employing organic materials during the RSD process not only diminished the soil-borne disease indices, but also enhanced the tobacco yields. Compared to the CK treatment, the KG1.2 and YG1.2 treatments increased the tobacco yields by 6.7% and 12.2%, respectively ($p < 0.05$). Although the G0.8 treatment significantly reduced the prevalence of both diseases at the K and Y sites, it did not notably enhance the tobacco yield. The overall efficacy of the G1.2 treatment was better at the K and Y sites, demonstrating its superior performance in disease mitigation and yield augmentation.

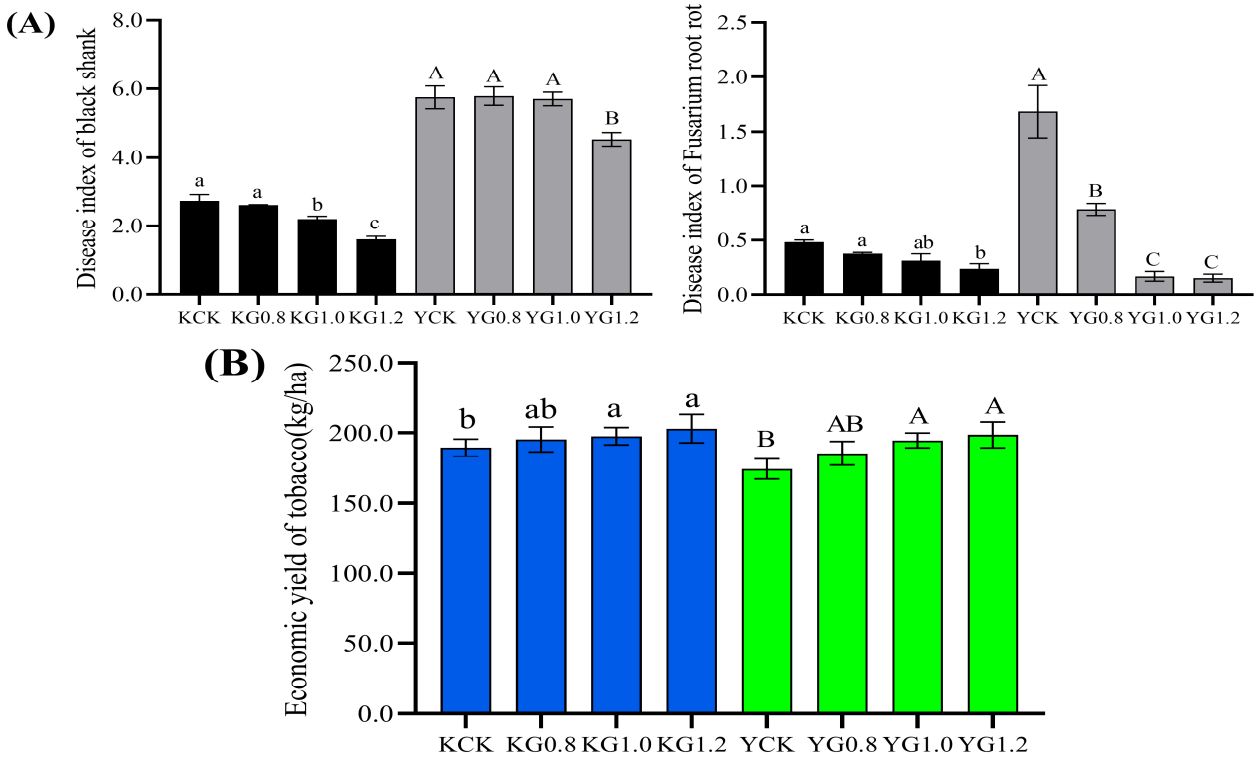


**Figure 2.** Effect of adding organic materials on the occurrence of soil-borne diseases in flue−cured tobacco and the yield of tobacco leaves. (**A**) Disease index of two diseases and (**B**) tobacco leaf yield of in the K and Y experimental sites. Different uppercase (lowercase) letters at the same experimental site represent significant differences among treatments at $p < 0.05$.

### *3.3. Characteristics of Microbial Community in the Rhizosphere Soil of Flue-Cured Tobacco*

#### 3.3.1. α Diversity

The influence of the application of organic material during the RSD process on the α-diversity of the fungi and bacteria in the rhizosphere soil of flue-cured tobacco is shown in Figure 3. The results from both experimental sites indicated that compared to the CK treatment, the application of organic material increased the Chao, Shannon, and Sobs indices for bacteria. Notably, the KG1.2 and YG1.2 treatments exhibited peak values for the Chao, Shannon, and Sobs indices. Furthermore, the enhancement of the bacterial diversity index was more pronounced at the K site than at the Y site. In terms of the fungal α-diversity, the application of organic material led to a significant decline in both

the Shannon and Sobs indices ($p < 0.05$). However, the Chao index demonstrated an initial increase followed by a decrease as the application of organic material increased.

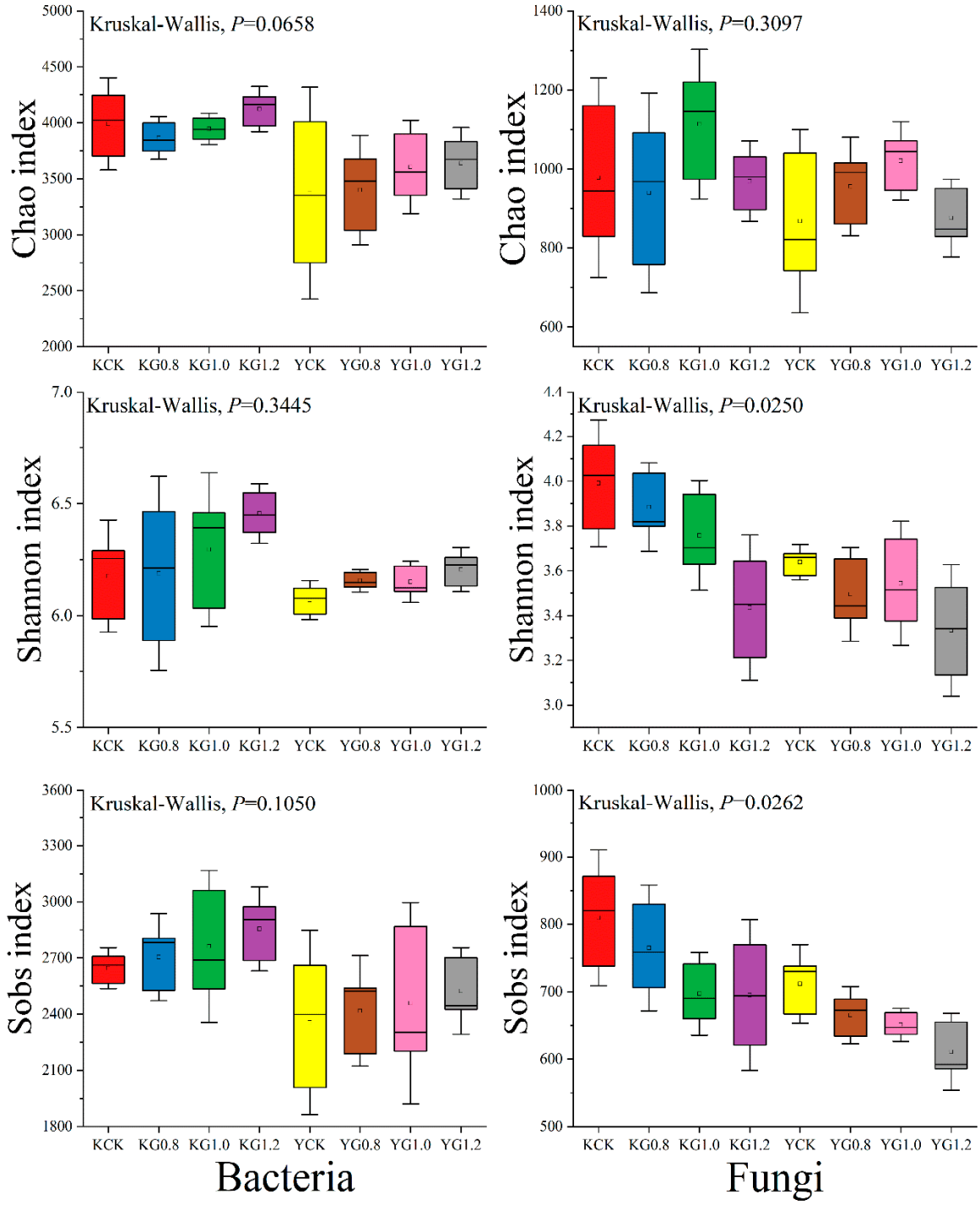


**Figure 3.** Microbial diversity and richness indices of rhizosphere soil in flue-cured tobacco.

### 3.3.2. Comparison of OTUs

The impact of adding organic materials on the OTU abundance distribution of microorganisms (fungi and bacteria) in the rhizosphere soil of flue-cured tobacco is illustrated in Figure 4. For fungi, adding organic materials during the RSD process reduced the total OTU count. Compared to the KCK and YCK, the G1.2 treatment at both sites recorded 352 and 305 shared OTUs, and 30 and 21 unique OTUs, respectively. The KG12 and YG12

treatments displayed the lowest counts for both total and unique OTUs compared to KCK and YCK. In the bacterial domain, although there was a decline in shared OTUs with the addition of organic materials, the effects on the unique OTU counts across the two sites varied. In contrast to the CK treatment, the KG1.2 treatment resulted in a reduction of 517, whereas the YG1.2 treatment led to an increment of 477 unique OTUs.

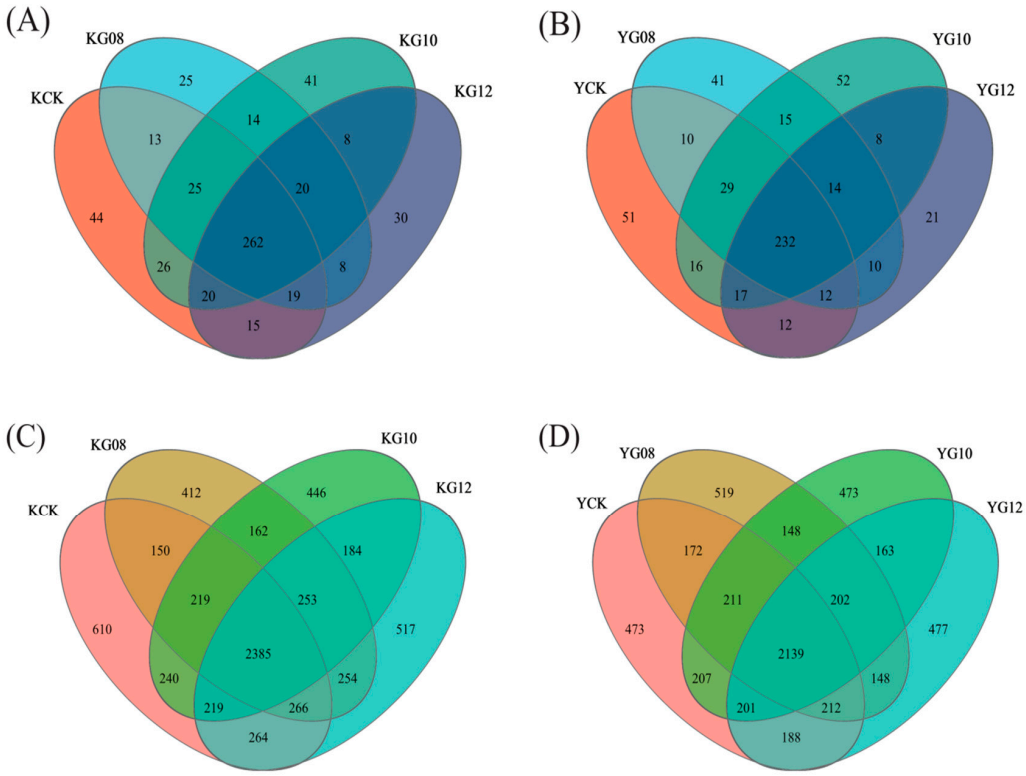


**Figure 4.** Distribution of OTU abundance of fungi and bacteria in the rhizosphere soil of flue-cured tobacco ((**A**): fungi at the K site; (**B**): fungi at the Y site; (**C**): bacteria at the K site; (**D**): bacteria at the Y site).

### *3.4. Changes in Microbial Community Structure in the Rhizosphere Soil of Flue-Cured Tobacco*

#### 3.4.1. Community PCoA Analysis

Based on the PCoA analysis at the OTU level, samples that were treated by adding organic materials during the RSD process exhibited evident variations, although the distribution density of the replicate soil samples remained relatively low (Figure 5). In addition, the results at both experimental sites revealed no significant disparity in the fungal community across the organic material treatments. However, the bacterial community structure at the K site showed significant alterations, a phenomenon that was absent at the Y site ($p < 0.05$, Figure 5C,D). Variations in the bacterial community structure at the Y site were predominantly affected by three principal coordinate components, accounting for 33.33%, 17.46%, and 49.21% of the variance, respectively. The difference between the coordinate components PCoA1, PCoA2, and PCoA3 reached a significant degree. This suggests that the application of organic material at the K site substantially affected the bacterial community structure in the rhizosphere soil of the flue-cured tobacco.

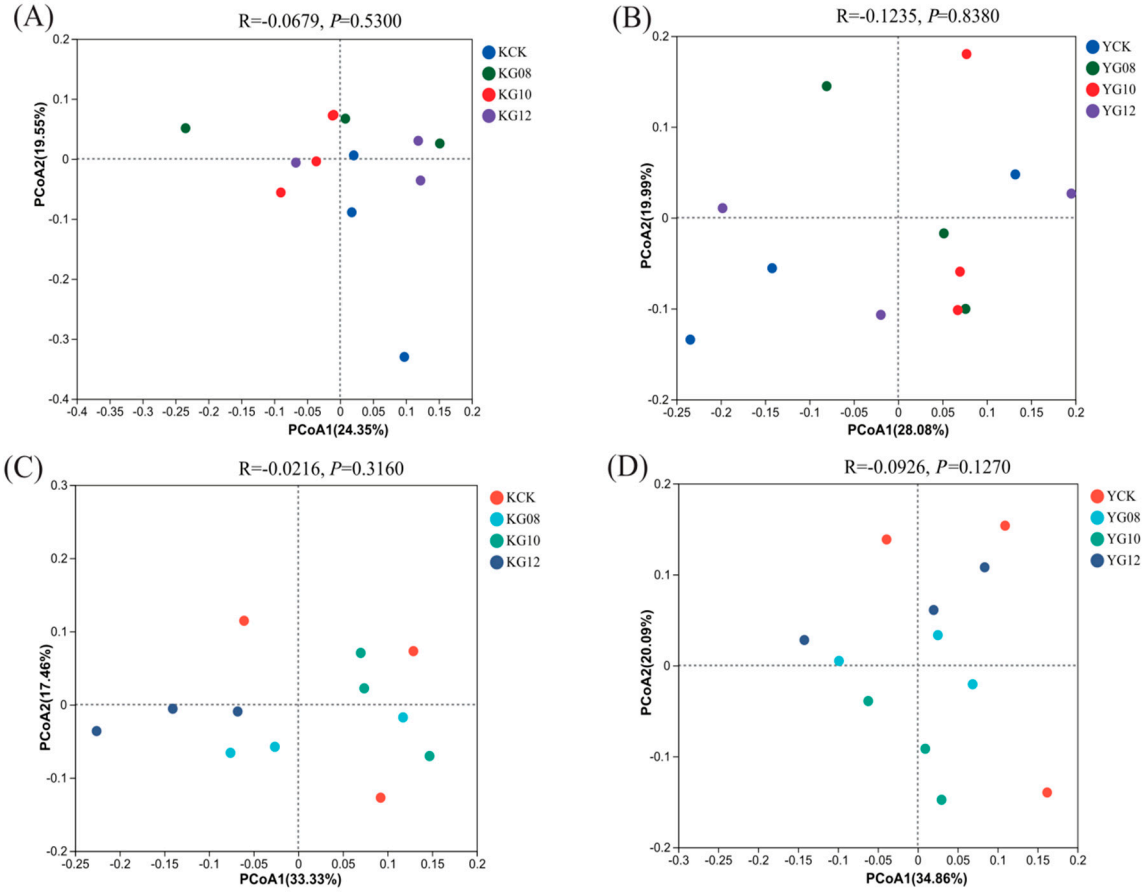


**Figure 5.** PCoA analysis of fungi and bacteria in the rhizosphere soil of flue−cured tobacco ((**A**): K site fungi; (**B**): Y site fungi; (**C**): K site bacteria; (**D**): Y site bacteria).

### 3.4.2. Abundance of Rhizosphere Soil Microbial Communities

Figure 6 illustrates the microbial community abundance in the rhizosphere soil of flue-cured tobacco at the phylum and genus levels when organic materials were added during the RSD process. In terms of the bacteria, compared to the CK treatment, the KG12 and YG12 treatments increased the relative abundance of Actinobacteria, Chloroflexi, Firmicutes, and Acidobacteriota at the phylum level, while reducing that of Proteobacteria. At the genus level, the dominant bacterial genera included unclassified_f_*Micrococcaceae*, *Sphingomonas*, *Bacillus*, norank_f_norank_o_*Gaiellales*, and *Terrabacter*. Their relative abundance under KG1.2 and YG1.2 exceeded 3.00%, constituting 20.26% and 24.19% of the entire bacterial population, respectively. Adding organic materials increased the prevalence of the genera unclassified_f_*Micrococcaceae*, *Bacillus*, and norank_f_norank_o_*Gaiellales*. Regarding the fungi, the principal phyla were Ascomycota, Basidiomycota, Mortierellomycota, and unclassified_K_Fungi. Compared with the KCK and YCK treatment, the addition of organic material significantly reduced the relative abundance of *Ascomycota* and unclassified_k_Fungi ($p < 0.05$) and increased that of *Basidiomycota* and *Mortierellomycota*. At the genus level, the dominant fungi spanned 28 genera, including *Saitozyma*, *Mortierella*, *Fusarium*, *Cephalotrichum*, and *Chaetomium*. Compared to the CK treatment, the KG1.2 and YG1.2 treatments significantly promoted the relative abundance of *Saitozyma* while reducing that of *Mortierella* and *Fusarium*, with the latter two reaching statistical significance ($p < 0.05$).

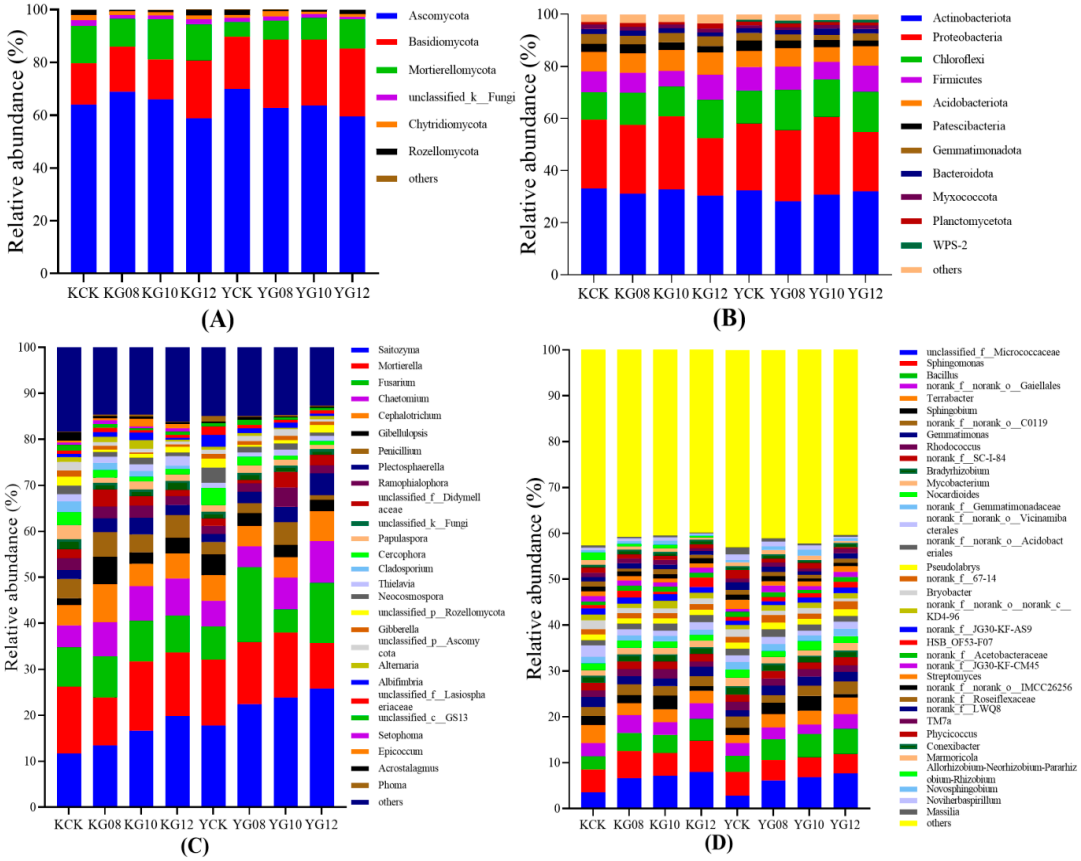


**Figure 6.** Community abundance profiling of rhizosphere soil in flue-cured tobacco at the phylum and genus levels ((**A**): fungi phylum; (**B**): bacteria phylum; (**C**): fungi genus; (**D**): bacteria genus).

### 3.4.3. Relative Abundance of Pathogenic *Phytophthora nicotianae* and *Fusarium* spp.

Figure 7 depicts an in-depth analysis of the relative abundance of the primary pathogenic genera associated with tobacco black shank disease (*Phytophthora nicotianae*) and Fusarium root rot (*Fusarium* spp.). The application of organic materials during the RSD process appeared to reduce the relative abundance of these pathogenic microbes. Compared to the CK treatment, the KG1.2 and YG1.2 treatments at the two experimental sites significantly reduced the relative abundance of these pathogens ($p < 0.05$). Specifically, the reductions in *Phytophthora nicotianae* were 31.4% and 43.3%, and those for *Fusarium* spp. were 22.9% and 55.7%, respectively.

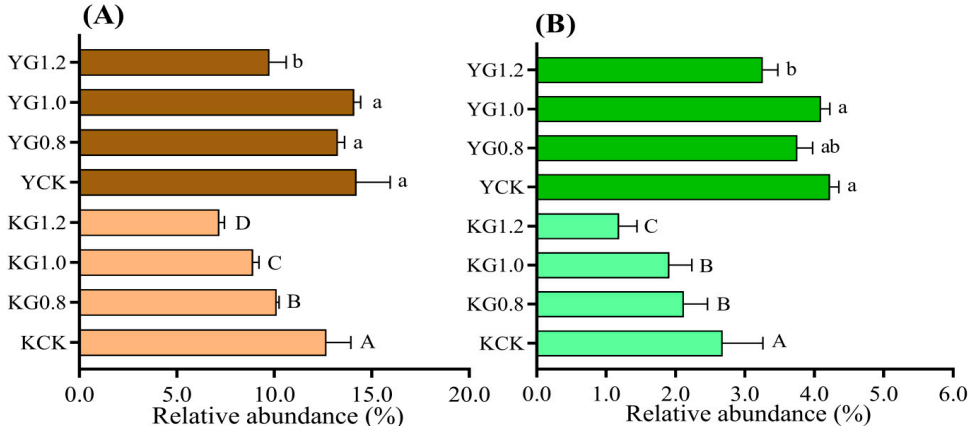


**Figure 7.** Relative abundance of pathogenic bacteria in the rhizosphere soil of flue-cured tobacco ((**A**): *Phytophthora nicotianae*; (**B**): *Fusarium* spp.). Different uppercase (lowercase) letters at the same experimental site represent significant differences among treatments at $p < 0.05$.

### 3.4.4. LEfSe Multi-Level Species Difference Discriminant Analysis

Based on a previous study, Figure 8 illustrates the LEfSe multi-level species difference discriminant analysis, highlighting significant microbial species differences from the phylum to genus level (including unclear classification at the genus level). In Figure 8, the distinct colored nodes represent microbial groups that are significantly enriched in the corresponding groups, exerting a pronounced impact on intergroup variations. In contrast, the light-yellow nodes represent microbial groups without substantial influence across the groups. Sequentially, from the innermost to the outermost circle, the species levels represented are phylum, class, order, family, and genus. For the fungi, distinctions at the class level between the KCK and KG12 treatments were evident, with o__Agaricales, g __Agaricomycetes, o__Agaricomycete, f __Agaricomycetes, and g__Lecanicillium indicating significant variances ($p < 0.05$). However, YCK and YG1.2 did not exhibit notable differences. Regarding the bacteria, a substantial discrepancy ($p < 0.05$) between the CK and YG1.2 treatments was observed at the phylum level, where f__JG30-KF-CM66, c__JG30-KF-CM66, o__JG30-KF-CM66, and g__JG30-KF-CM66 were significantly different ($p < 0.05$).

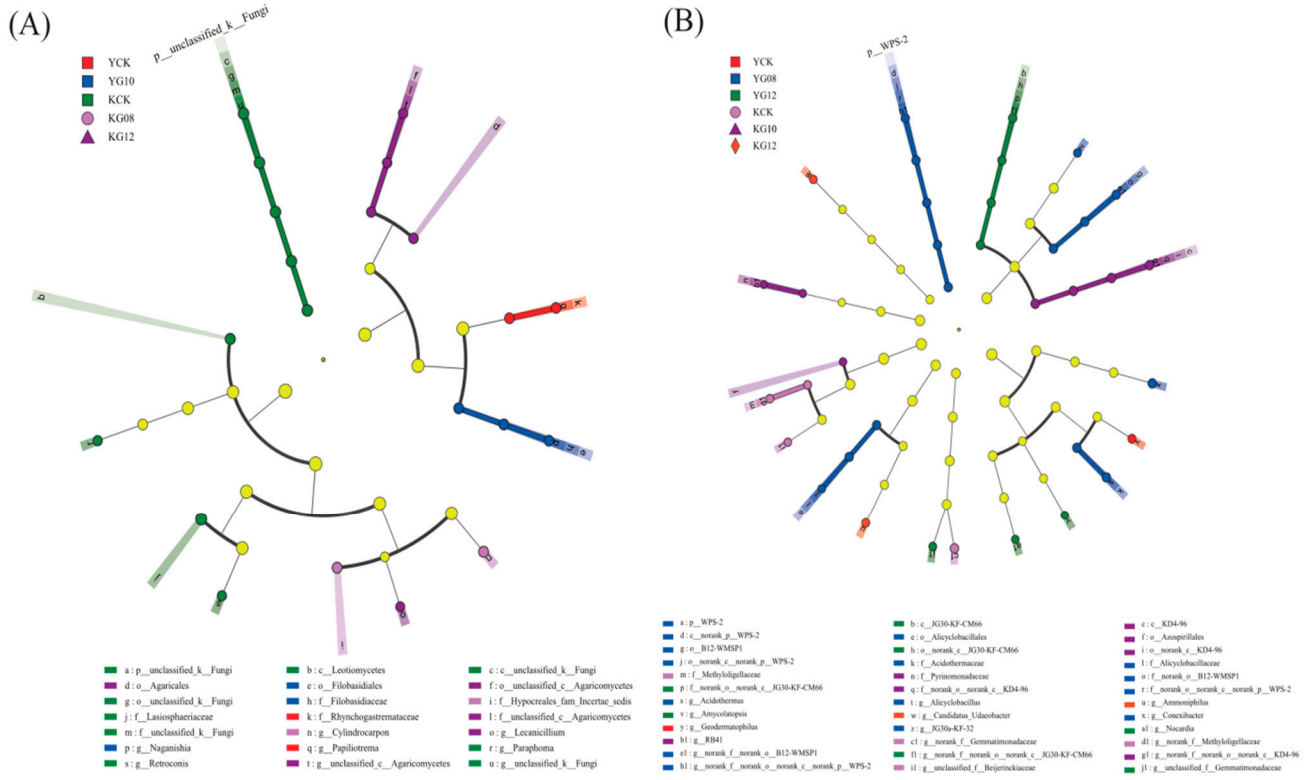


**Figure 8.** LEfSe multi-level species discriminant analysis of microbial differences in the rhizosphere soil of flue-cured tobacco ((**A**): fungi; (**B**): bacteria).

### 3.4.5. Correlation between Microbial Communities and Environmental Factors

Figure 9 illustrates the correlation statistics between the predominant 50 fungal and bacterial genera and the environmental factors following the identification of distinct microbial species. The fungi were notably influenced by the AK (one genus), pH (five genera), OM (one genus), and C/N (one genus). In particular, five genera significantly affected the yield and eleven affected the occurrence of black shank, though the occurrence of Fusarium root rot remained unaffected. For the bacteria, the key influencing factors included the AN (five genera), OM (two genera), pH (two genera), and C/N (one genus). Specifically, 16 genera substantially affected the yield, 20 affected the occurrence of black shank, and five affected the occurrence of Fusarium root rot. Notably, the fungal genera *Cercophora*, *Cylindrocarpon*, and unclassified_C__*Agaricomycetes* demonstrated a substantial positive correlation with black shank disease occurrence. Among the bacteria, *Marmoricola*

exhibited a pronounced negative correlation with the soil physicochemical properties, a highly significant negative correlation with the yield ($p < 0.01$), and a positive relationship with both the occurrences of black shank disease and Fusarium root rot. In addition, genera such as norank_f__norank_o__*B12-WMSP1*, *Bryobacter*, *Conexibacter*, and *Acidothermus* demonstrated a positive correlation with diseases but a significant inhibitory effect on the yield ($p < 0.01$) (Figure 9B).

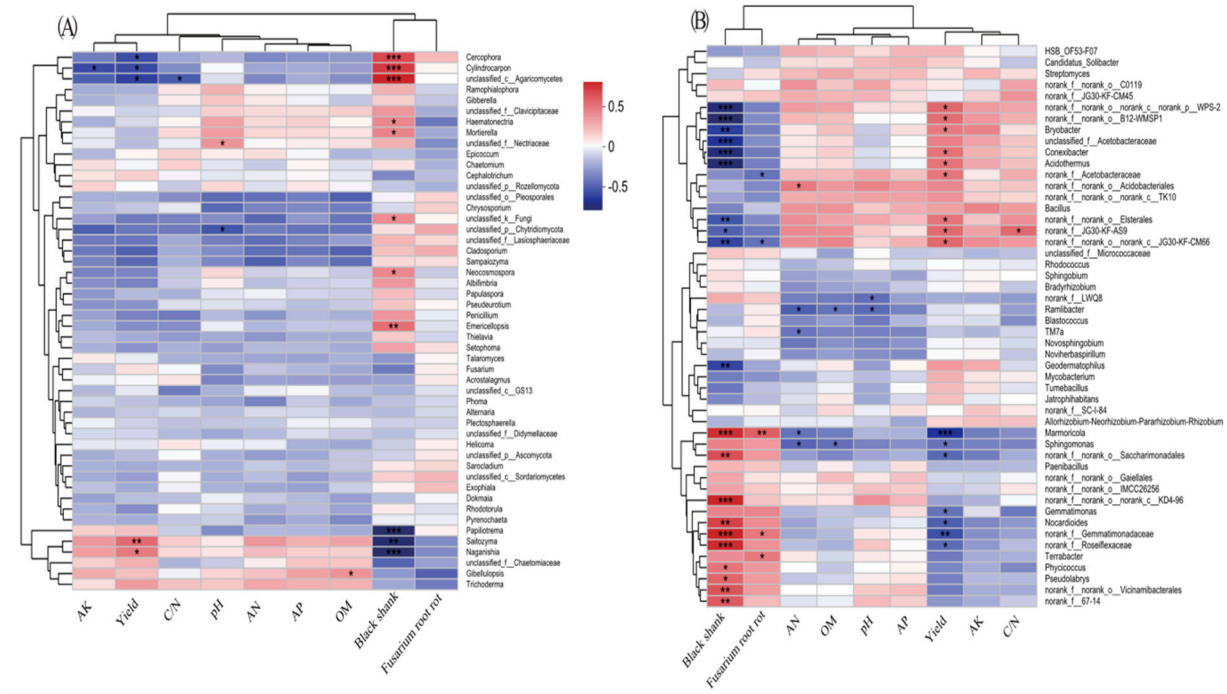


**Figure 9.** Correlation analysis between dominant microbial genera and environmental factors in the rhizosphere soil of flue−cured tobacco ((**A**): fungi; (**B**): bacteria). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

### 3.4.6. Function Prediction

Figure 10A illustrates the composition of the fungal functional groups derived from the FUNGuild database. The predominant functional groups included Fungal Parasite—Undefined Saprotroph, Undefined Saprotroph, and Endophyte—Litter Saprotroph–Soil Saprotroph–Undefined Saprotroph. These groups, primarily of the saprophytic trophic or pathological saprotroph types, constituted over 50% of the total abundance. Compared to CK, increasing the application of organic material notably enhanced the abundance of fungal parasite—undefined saprotroph. This increase was the maximum in the KG1.2 and YG1.2 treatments, achieving statistical significance ($p < 0.05$). The predominant contributors to this trend include Trimorphomyceae, Mortierellomycetes, and Sortariomycetes.

Based on the bacterial communities, KEGG function prediction using Tax4Fun (Figure 10B) revealed that the primary functions of each sample community were distributed among four categories: metabolism (62.30%, 11 pathways), environmental information processing (18.45%, three pathways), genetic information processing (11.54%, four pathways), and cellular processes (4.79%, five pathways). The application of various organic materials induced distinct functional abundance differences within the bacterial communities across the treatments. When compared to the CK treatment, the KG1.2 and YG1.2 treatments exhibited no notable differential abundance across most functions. However, there was a substantial increase in pathways related to carbohydrate metabolism, glycan biosynthesis and metabolism, and nucleotide metabolism. Moreover, the amino acid metabolism, energy metabolism, and xenobiotic biodegradation and metabolism pathways demonstrated significant reductions in the KG1.2 and YG1.2 treatments compared to the CK treatment.

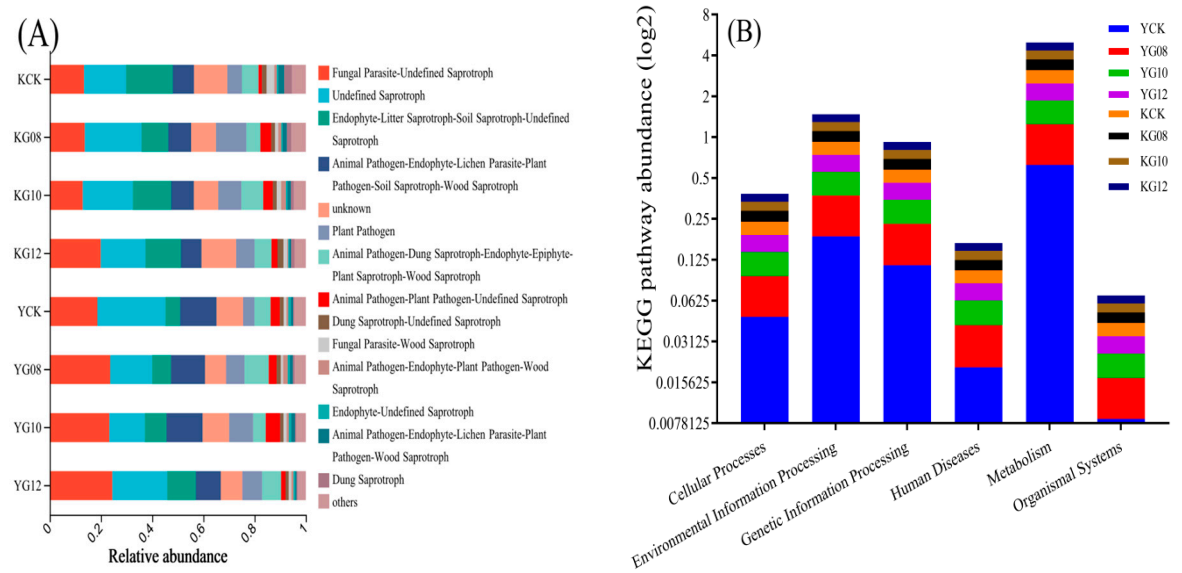


**Figure 10.** Prediction and analysis of microbial community functions in the rhizosphere soil of flue-cured tobacco ((**A**): fungi; (**B**): bacteria).

### *3.5. RDA Analysis*

Figure 11 reflects the contribution of the soil physicochemical properties (pH, AN, AP, AK, OM, and C/N), soil-borne diseases (black shank and Fusarium root rot), and tobacco yield to the structure of the fungal and bacterial communities. RDA analyses suggested that after the application of organic materials, the physicochemical properties of the soil had assorted positive contributions to the fungal and bacterial communities, mainly driven by the AN, AP, AK, and OM. There was a noticeable positive association between the yield of flue-cured tobacco and its physicochemical properties. However, within the fungal communities, the yield exhibited a negative relationship with the pH. Notably, a pronounced negative correlation between the yield and soil-borne diseases adversely affected the community structure, with this effect being more pronounced in the bacterial communities.

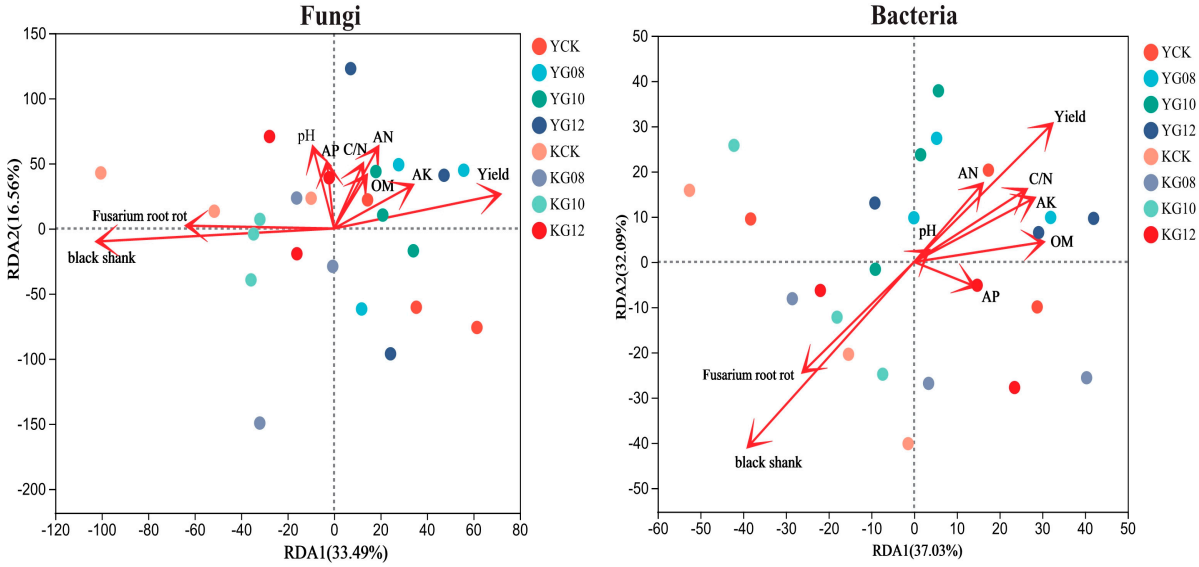


**Figure 11.** RDA analysis of microorganisms and environmental factors in the rhizosphere soil of flue−cured tobacco.

## 4. Discussions

### *4.1. Physicochemical Properties, Disease Occurrence, and Yield Characteristics of Tobacco Rhizosphere Soil under RSD and Application of Organic Materials*

Continuous soil cropping obstacles caused by monoculture and excessive agricultural chemical utilization currently impede sustainable agricultural development [28]. To avoid this in tobacco cultivation, strategies have primarily focused on plant growth and fertilization management to sustain yield without directly addressing the persistent issues of continuous cropping [29]. Soil RSD technology, which can improve soil fertility and reshape microbial communities under soil culture and greenhouse conditions, has emerged as a potential solution to these continuous cropping challenges [15,18]. However, there is a noticeable shortage of studies on RSD treatment for the improvement of field crop disease control and yield. This study at two experimental sites suggested that adding organic materials during the RSD process increased the pH, AN, AP, AK, OM, and C/N levels in the rhizosphere soil of flue-cured tobacco. Notably, when compared to the CK treatment, higher applications of organic material (KG1.2 and YG1.2) significantly enhanced the soil physicochemical properties (Table 1) ($p < 0.05$), aligning with previous findings [30,31]. The introduction of small-molecule organic matter, resulting from organic material decomposition during the RSD process not only increased the organic matter content in the soil but also modified the $NH_4^+$, the $NO_3^-$-N levels, the $NH_4^+$ microbial fixation, and the $NH_4^+$ to $NO_3^-$-N transition, consequently boosting the soil's pH, AN, AP, and AK levels [17,32]. In addition, as the application of organic material increased, the abundant carbon sources elucidated the observed increase in C/N in the rhizosphere soil of the flue-cured tobacco. Despite applying equivalent quantities of organic material, the effectiveness of enhancing the physicochemical properties varied due to inherent differences in the initial soil characteristics and types. However, higher application rates (KG1.2 and YG1.2) proved more beneficial in ameliorating soil physicochemical properties post-harvest; this may be related to the fact that the organic materials selected in this study are easily decomposable and can be quickly decomposed.

RSD technology, with the advantages of short-term consumption, ease of material procurement, and pronounced control over soil-borne diseases, is a potent intervention for controlling soil-borne pathogens and reconstructing soil health prior to planting [33–35]. The addition of organic materials is pivotal for RSD efficacy and serves as the nutrient foundation, facilitating an enhanced nutrient uptake and yield improvement in crops [33,36]. However, few studies have focused on the performance of using this technology in the field of seasonal crops. Our findings suggest that adding organic materials during the RSD process mitigated the severity of common soil-borne pathogens, such as *Phytophthora nicotianae* and *Fusarium* spp., in flue-cured tobacco. Notably, the YG1.2 and KG1.2 treatments markedly reduced the disease severity by 26.8% to 1007.6% ($p < 0.05$). Moreover, the addition of these organic materials increased the leaf yield of the flue-cured tobacco, thereby achieving the dual benefits of disease suppression and yield enhancement (Figure 1). The superior performance of the YG1.2 and KG1.2 treatments suggested that promoting the amounts of organic material in RSD was effective for controlling soil-borne diseases in flue-cured tobacco and amplifying the yield, especially when applied at higher rates. Enhanced levels of organic material application facilitated the decomposition of organic matter by anaerobic microbes, thereby releasing diverse bactericidal substances, such as acetic acid, butyric acid, $H_2S$, and $NH_3$, as well as reductive agents, such as $Fe^{2+}$ and $Mn^{2+}$. These agents act deleteriously on pathogenic bacteria, curtailing disease incidence [9,31]. Interestingly, while site Y (Nanhua) displayed a pronounced edge in disease mitigation via the organic material enhancement, site K (Donghua) shone in terms of its tobacco yield. This distinction likely resulted from the superior physicochemical properties of the K site, where ample nutrient resources contributed to a substantial yield. Figure 11 highlights a significant positive contribution between the tobacco leaf yield and the physicochemical properties, such as AK, OM, and AN. Conversely, a pronounced inverse relationship be-

tween the occurrences of Fusarium root rot and black shank disease with the tobacco leaf yield was observed, aligning with the findings of [33].

### *4.2. Effect of RSD Treatment and Application of Organic Materials on Changes in Microbial Community Structure*

The balance of the microbial community structure in rhizosphere soil is a critical indicator of soil ecosystem health. Therefore, devising efficacious crop management strategies to optimize this microbial structure, suppress soil diseases, and increase crop yields is imperative [28,37]. Adding organic materials during the RSD process not only utilizes a soil environment abundant in high-temperature anaerobic conditions and small-molecule organic acids, as well as reducing metal ions to combat soil-borne pathogens, but also refines the soil microbial community structure [23,31]. In this study, the application of organic material in the RSD process amplified the unique OTU count of the bacteria and the Chao, Shannon, and Sobs indices in the soil, while substantially decreasing the total OTU count, and the Shannon and Sobs indices for the fungi ($p < 0.05$). This indicated the modulatory influence of organic materials in RSD on the diversity of rhizospheric fungi and bacteria during the crop's current season, aligning with the findings of Huang et al. [35]. Compared with the YCK and KCK treatments, an increased application of organic material (YG1.2 and KG1.2) led to a pronounced increase in the relative abundance of Actinobacteria, Chloroflexi, Firmicutes, and Acidobacillus at the phylum level, mirroring Huang et al. [35] and Rao et al. [33]'s observations. These bacterial phyla are pivotal for augmenting soil nutrient levels via plant residue degradation, lignin and protein decomposition, and humus formation [38–40]. Hence, the addition of organic material in the RSD process notably promoted nutrient levels in the rhizosphere soil of the continuous cropping of flue-cured tobacco. Moreover, this was further corroborated by the RDA results, which suggested that the organic material amplification enhanced the abundance of Actinobacteria, Chloroflexi, Firmicutes, and Acidobacteriota in the rhizosphere soil of flue-cured tobacco during the current season. In addition, there was a decline in the relative abundance of *Ascomycota*, countered by the increased representation of *Basidiomycota* and *Mortierellomycota*, diverging from prior studies [29]. This shift could be attributed to the diminishing easily decomposable organic matter and robust anaerobic reducing conditions that occurred with the escalated organic material input. This reduced *Ascomycota* thrived in oxidizing environments that were rich in easily decomposable organic matter [31], whereas *Basidiomycota* and *Mortierellomycota*, via a symbiotic relationship with plant roots, enhanced their nutrient acquisition, leading to their increased relative abundance [41].

In the RSD process, adding organic materials significantly increased the relative abundance of *Sphingomonas*, *Bacillus*, and *Terrabacter* at the bacterial level, with YG1.2 and KG1.2 demonstrating superior outcomes. The relative abundance of these beneficial bacterial genera increased, which not only antagonized the killing of *Fusarium* spp. and *Phytophthora nicotianae*, but also compressed the living space of the pathogenic bacteria, thereby reducing their number. This was attributed to the organic materials providing more substrates and energy, promoting the proliferation of these bacterial genera, and subsequently enhancing the organic matter degradation during the RSD process [5,35,36]. After RSD, these genera became dominant beneficial entities in the rhizosphere soil of extant crops, defending against pathogens and detrimental microbes via the production of antibiotics, antifungal agents, and enzymes [42]. In previous studies, *Bacillus*, which reduced $Fe^{3+}$ and $Mn^{4+}$ during RSD, produced metabolites ($H_2S$, $NH_3$, $Fe^{2+}$, and $Mn^{2+}$) that counteract pathogenic bacteria [35]. As the application of organic material increased, there was a notable upward trend in the relative abundance of the genera *Saitozyma* and *Mortierella*, whereas *Fusarium*'s relative abundance decreased (Figure 5). This was potentially due to *Saitozyma* and *Mortierella*'s robust competitive niches as beneficial bacteria, and RSD's capability to neutralize a majority of Fusarium pathogens. Although *Saitozyma*, a member of the *Saccharomyces* genus, is ubiquitously present, its influence on plant growth remains limited [43]. On the other hand, *Mortierella* significantly facilitates soil nutrient

transformation and availability, profoundly impacting soil fertility and its ecological environment [44]. *Fusarium oxysporum* and *Phytophthora nicotianae*, the principal pathogenic culprits of soil-borne diseases in flue-cured tobacco, demonstrated a significant positive correlation between their soil quantity and abundance, and the occurrence of these diseases [32]. The findings across both experimental sites in this study demonstrated that adding organic materials during the RSD process effectively reduced pathogenic bacterial abundance, enhanced the diversity and community structure of specific advantageous bacteria, and restored the soil's microecological balance and health.

### *4.3. Effect of RSD Treatment on Microbial Community Function in Tobacco Planting Soil*

Differential microbial community structures arise from the adaptive survival strategies of microorganisms to environmental shifts, manifesting prominently in community functions [17,35]. In this study, as the addition of organic materials increased, the primary nutritional profiles discerned for fungi were saprophytic and pathogenic saprophytic types. These fungal categories promoted the degradation and recycling of organic materials, and there was an increase in the relative abundance of fungi with disease-suppressing capabilities, enhancing the microbial community's stability [37]. Concurrently, the application of organic material increased the functional abundance of the bacterial carbohydrate metabolism, the degradation and metabolism of exogenous substances, and the metabolism of terpenoids and polyketones. Previous studies have indicated that an increased carbohydrate metabolism as well as an increased degradation and metabolism of exogenous substances facilitate the bacterial community decomposition of added materials and organic matter accumulation. The enhanced microbial metabolism of terpenoids and polyketones contributes to the disease resistance and stress tolerance of subsequent crops [45,46].

Higher application rates of easily degradable organic materials (K/YG1.2) markedly influenced the physicochemical properties and fungal community dynamics of the soil, diminishing the abundance of soil-borne pathogens. Liu [46]'s research indicated that environmental factors determine the recombination of bacterial communities and the performance of beneficial bacterial functionalities. Genera such as *Cercophora*, *Cylindrocarpon*, *Marmoricola*, *Bryobater*, *Conexibacter*, and *Acidothermus* are positively correlated with soil-borne diseases, while being inversely associated with certain physicochemical factors and the yield. Notably, *Cercophora*, *Marmoricola*, *Conexiactor*, and *Acidothermus* undergo the decomposition of cellulose, lignin, and other organic substrates, enriching the soil's organic matter profile [46,47]. *Cylindrocarpon* and *Acidothermus* participate in nitrogen and nutrient cycling processes [48]. *Mortierella* has the capacity to transform organic matter into arachidonic acid during fermentation [49], while *Bacillus* is a pivotal decomposer of hemicellulose and polycyclic aromatic hydrocarbons, releasing cellulase to decompose organic matter into low-molecular-weight compounds [50]. These findings demonstrate the efficacy of the RSD process in controlling diseases and elevating yields in flue-cured tobacco. This is achieved by promoting the application of organic materials in the RSD process, reconstructing microbial community diversity, reducing pathogen abundance, increasing antagonistic taxa, and amplifying the roles of various functional microorganisms [45,46].

## 5. Conclusions

Field tests from two experimental sites demonstrated that adding organic materials during the RSD process effectively sterilized the soil and controlled pests. Promoting the application of organic materials enhanced the physicochemical properties of the rhizosphere soil for flue-cured tobacco during the growth season. This not only reduced the relative abundance of soil-borne pathogens, such as *Fusarium oxysporum* and *Phytophthora nicotianae*, mitigating the severity of soil-borne diseases, but also increased the tobacco leaf yield. Concurrently, this procedure modified the structure and composition of the microbial community. It enhanced the antagonistic effect of beneficial bacteria such as *Sphingomonas* and *Bacillus*, influenced the relative abundance of Actinobacteria and Chloroflexi, improved

the organic material decomposition by *Cercophora*, *Marmoricola*, *Conexiber*, and *Acidothermus*, and ultimately refined the rhizosphere microbial community and its functionality.

**Author Contributions:** C.L. and H.D. conceived the original screening and research plans. C.L. finished writing this thesis. Y.D. supervised the experiments, agreed to serve as the author responsible for contact, and ensures communication. Y.L. provided technical assistance to C.L. and Y.D. designed the experiments. Y.L. and T.L. analyzed the data. All authors have read and agreed to the published version of the manuscript.

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## References

1. Li, C.; Ahmed, W.; Li, D.; Yu, L.; Xu, L.; Xu, T.; Zhao, Z. Biochar suppresses bacterial wilt disease of flue-cured tobacco by improving soil health and functional diversity of rhizosphere microorganisms. *Appl. Soil Ecol.* **2022**, *171*, 104314. [CrossRef]
2. Jiang, J.L.; Lu, Y.; Su, Y.; Pan, L.; Tang, X.D.; Chen, X.S.; Niu, F.F.; Zhao, L.C.; Lu, M.H.; Zhu, S.X. Chemical characteristics and usability of flue-cured tobacco growing in main tobacco producing areas in China. *Acta Tabacaria Sin.* **2017**, *23*, 13–27. [CrossRef]
3. Huang, X.Q.; Zhao, J.; Zhou, X.; Zhang, J.B.; Cai, Z.C. Differential responses of soil bacterial community and functional diversity to reductive soil disinfestation and chemical soil disinfestation. *Geoderma* **2019**, *348*, 124–134. [CrossRef]
4. Pervaiz, Z.H.; Iqbal, J.; Zhang, Q.; Chen, D.; Wei, H.; Saleem, M. Continuous cropping alters multiple biotic and abiotic indicators of soil health. *Soil Syst.* **2020**, *4*, 59. [CrossRef]
5. Wei, Z.; Gu, Y.A.; Friman, V.P.; Kowalchuk, G.A.; Xu, Y.C.; Shen, Q.R.; Jousset, A. Initial soil microbiome composition and functioning predetermine future plant health. *Sci. Adv.* **2019**, *5*, eaaw0759. [CrossRef] [PubMed]
6. Wang, W.X.; Shen, B.; Jia, H.B.; Qiao, J.Q.; Niu, B. Application of rhizospheric biocontrol consortia and the potential mechanisms of their enhancing efficacy on disease-suppressive effect. *Biotechnol. Bull.* **2020**, *36*, 31–41. [CrossRef]
7. Geng, S.J.; Pan, X.P.; Zhao, F.; Zhou, G.S.; Zha, H.B.; Tao, Y.P.; Chen, X.; Zhao, Z.X. Effect of different anaerobic soil disinfestation by organic materials on the occurrence of root-knot nematode disease, yield and quality of flue-cured tobacco. *J. Yunnan Agric. Univ. Nat. Sci.* **2019**, *34*, 785–792. [CrossRef]
8. Blok, W.J.; Lamers, J.G.; Termorshuizen, A.J.; Bollen, G.J. Control of soilborne plant pathogens by incorporating fresh organic amendments followed by tarping. *Phytopathology* **2000**, *90*, 253–259. [CrossRef] [PubMed]
9. Momma, N.; Yamamoto, K.; Simandi, P.; Shishido, M. Role of organic acids in the mechanisms of biological soil disinfestation (BSD). *J. Gen. Plant Pathol.* **2006**, *72*, 247–252. [CrossRef]
10. Shrestha, U.; Wszelaki, A.L.; Butler, D.M. Implementing Anaerobic Soil Disinfestation in Tennessee. 2014. Available online: https://extension.Tennessee.edu/publicatios/Documents/SP765-B (accessed on 1 May 2023).
11. Samaniego-Gaxiola, J.A.; Balagurusamy, N. Survival of soil-borne fungus *Phymatotrichopsis omnivora* after exposure to volatile fatty acids. *J. Gen. Plant Pathol.* **2013**, *79*, 105–109. [CrossRef]
12. Huang, X.Q.; Wen, T.; Meng, L.; Zhang, J.B.; Zhu, T.B.; Cai, Z.C. The inhibitory effect of quickly and intensively reductive soil on *Fusarium oxysporum*. *Acta Ecol. Sin.* **2014**, *34*, 4526–4534. [CrossRef]
13. Wang, G.F.; Ma, Y.; An, X.; Zhao, J.F.; Luo, D.X.; Sun, Y.D. Effect of different intensively reductive treatments by organic materials on soil character and disease control of *phytophthorablight* of chilli pepper. *Soil Fertil. Sci. China* **2016**, *5*, 124–129. [CrossRef]
14. Runia, W.T.; Thoden, T.C.; Molendijk, L.P.G.; Van Den Berg, W.; Termorshuizen, A.J.; Streminska, M.A.; Van Der Wurff, A.W.G.; Feil, H.; Meints, H. Unravelling the mechanism of pathogen inactivation during anaerobic soil disinfestation. *Acta Hortic.* **2014**, *1044*, 177–193. [CrossRef]
15. Zhu, W.J.; Wang, W.P.; Hong, C.L.; Ding, J.; Zhu, F.X.; Hong, L.D.; Yao, Y.L. Influence of reductive soil disinfestation on the chemical and microbial characteristics of a greenhouse soil infested with *Fusarium oxysporum*. *Physiol. Mol. Plant Pathol.* **2022**, *118*, 101805. [CrossRef]
16. Khadka, R.B.; Marasini, M.; Rawal, R.; Testen, A.L.; Miller, S.A. Effects of anaerobic soil disinfestation carbon sources on soilborne diseases and weeds of okra and eggplant in Nepal. *Crop Prot.* **2019**, *135*, 104846. [CrossRef]
17. Liu, L.; Chen, S.; Zhao, J.; Zhou, X.; Wang, B.; Li, Y.; Zheng, G.; Zhang, J.; Cai, Z.; Huang, X. Watermelon planting is capable to restructure the soil microbiome that regulated by reductive soil disinfestation. *Appl. Soil Ecol.* **2018**, *129*, 52–60. [CrossRef]
18. Strauss, S.L.; Greenhut, R.F.; Mcclean, A.E.; Kluepfel, D.A. Effect of anaerobic soil disinfestation on the bacterial community and key soilborne phytopathogenic agents under walnut tree-crop nursery conditions. *Plant Soil* **2017**, *415*, 493–506. [CrossRef]

19. Wen, T.; Huang, X.; Zhang, J.; Zhu, T.; Meng, L.; Cai, Z. Effects of water regime, crop residues, and application rates on control of *Fusarium oxysporum* f. sp. *cubense*. *J. Environ. Sci.* **2015**, *31*, 30–37. [CrossRef]
20. Chen, J.; He, X.; Zhang, X.; Chen, Y.; Zhao, L.; Su, J.; Qu, S.; Ji, X.; Wang, T.; Li, Z.; et al. The applicability of different tobacco types to heated tobacco products. *Ind. Crops Prod.* **2021**, *168*, 113579. [CrossRef]
21. *GB/T 23222-2008*; Grade and Investigation Method of Tobacco Diseases and Insect Pests. China Standards Press: Beijing, China, 2008.
22. Bao, S.D. *Methods of Agrochemical Analysis of Soils*; China Agricultural Science and Technology Press: Beijing, China, 2000.
23. Liu, C.; Zhao, D.; Ma, W.; Guo, Y.; Wang, A.; Wang, Q.; Lee, D.-J. Denitrifying sulfide removal process on high-salinity wastewaters in the presence of *Halomonas* sp. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 1421–1426. [CrossRef]
24. Adams, R.I.; Miletto, M.; Taylor, J.W.; Bruns, T.D. Dispersal in microbes: Fungi in indoor air are dominated by outdoor air and show dispersal limitation at short distances. *ISME J.* **2013**, *7*, 1262–1273. [CrossRef] [PubMed]
25. Xiong, W.; Zhao, Q.; Zhao, J.; Xun, W.; Li, R.; Zhang, R.; Wu, H.; Shen, Q. Different continuous cropping spans significantly affect microbial community membership and structure in a vanilla-grown soil as revealed by deep pyrosequencing. *Microb. Ecol.* **2015**, *70*, 209–218. [CrossRef] [PubMed]
26. Zhang, Y.; Yang, Y.; Yu, L.; Wang, A.; Xue, C.; Zhang, J.; Duan, A.; Zhao, M. Composition and characteristics of soil microbial communities in cotton fields with different incidences of Verticillium wilt. *Plant Signal. Behav.* **2022**, *17*, e2034271. [CrossRef] [PubMed]
27. Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Fierer, N.; Gonzalez Peña, A.; Goodrich, J.K.; Gordon, J.I.; et al. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **2010**, *7*, 335–336. [CrossRef]
28. Zhao, J.; Zhou, X.; Jiang, A.Q.; Fan, J.Z.; Lan, T.; Zhang, J.B.; Cai, Z. Distinct impacts of reductive soil disinfestation and chemical soil disinfestation on soil fungal communities and memberships. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 7623–7634. [CrossRef] [PubMed]
29. Wei, G.Y.; Hu, Y.; Wu, Y.Q.; Zhang, Q.Z.; Li, X. Effects of reductive soil disinfestation on fungal community structure and tobacco yield and quality. *Hunan Agric. Sci.* **2021**, *2021*, 34–39. [CrossRef]
30. McCarty, D.G. Anaerobic Soil Disinfestation: Evaluation of Anaerobic Soil Disinfestation (ASD) for Warm-Season Vegetable Production in Tennessee. Master's Thesis, University of Tennessee, Knoxville, TN, USA, 2012. Volume 15, pp. 1–156. Available online: http://trace.tennessee.edu/utk_gradthes/1393 (accessed on 1 May 2023).
31. Huang, X.Q.; Wen, T.; Zhang, J.B.; Meng, L.; Zhu, T.B.; Cai, Z.C. Toxic organic acids produced in biological soil disinfestation mainly caused the suppression of *Fusarium oxysporum* f. sp. *cubense*. *Biol. Control* **2015**, *60*, 113–124. [CrossRef]
32. Zhu, T.B.; Dang, Q.; Zhang, J.B.; Müller, C.; Cai, Z.C. Reductive soil disinfestation (RSD) alters gross N transformation rates and reduces NO and $N_2O$ emissions in degraded vegetable soils. *Plant Soil* **2014**, *382*, 269–280. [CrossRef]
33. Rao, D.E.; Liu, P.Y.; Zou, L.Y.; Teng, Y.; Yu, H.Y.; Yin, G.T. Effects of long-term continuous cropping and reductive soil disinfestation on fungal community in flue-cured tobacco rhizosphere. *Soil Fertil. Sci. China* **2022**, *300*, 47–56. [CrossRef]
34. Liu, L.L.; Kong, J.J.; Cui, H.L.; Zhang, J.B.; Wang, F.H.; Cai, Z.C.; Huang, X.Q. Relationships of decomposability and C/N ratio in different types of organic matter with suppression of *Fusarium oxysporum* and microbial communities during reductive soil disinfestation. *Biol. Control* **2016**, *101*, 103–113. [CrossRef]
35. Huang, X.Q.; Liu, L.L.; Wen, T.; Zhang, J.B.; Wang, F.H.; Cai, Z.C. Changes in the soil microbial community after reductive soil disinfestation and cucumber seedling cultivation. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 5581–5593. [CrossRef] [PubMed]
36. Huang, X.Q.; Cui, H.L.; Yang, L.; Lan, T.; Zhang, J.B.; Cai, Z.C. The microbial changes during the biological control of cucumber damping-off disease using biocontrol agents and reductive soil disinfestation. *Biocontrol* **2017**, *62*, 97–109. [CrossRef]
37. Rokunuzzaman, M.; Hayakawa, A.; Yamane, S.; Tanaka, S.; Ohnishi, K. Effect of soil disinfection with chemical and biological methods on bacterial communities. *Egypt. J. Basic Appl. Sci.* **2016**, *3*, 141–148. [CrossRef]
38. Singh, B. *Myceliophthora thermophila* syn. *Sporotrichum thermophile*: A thermophilic mould of biotechnological potential. *Crit. Rev. Biotechnol.* **2016**, *36*, 59–69. [CrossRef] [PubMed]
39. Vinale, F.; Sivasithamparam, K.; Ghisalberti, E.L.; Marra, R.; Lorito, M. Trichoderma–plant–pathogen interactions. *Soil Biol. Biochem.* **2008**, *40*, 1–10. [CrossRef]
40. Silveira, M.H.L.; de Siqueira, F.G.; Rau, M.; da Silva, L.; Moreira, L.R.d.S.; Ferreira-Filho, E.X.; Andreaus, J. Hydrolysis of sugarcane bagasse with enzyme preparations from *Acrophialophora nainiana* grown on different carbon sources. *Biocatal. Biotransformation* **2014**, *32*, 53–63. [CrossRef]
41. Zhang, S.N.; Wang, Y.; Sun, L.T.; Qiu, C.; Ding, Y.Q.; Gu, H.L.; Wang, L.J.; Wang, Z.S.; Ding, Z.T. Organic mulching positively regulates the soil microbial communities and ecosystem functions in tea plantation. *BMC Microbiol.* **2020**, *20*, 103. [CrossRef]
42. Mowlick, S.; Hirota, K.; Takehara, T.; Kaku, N.; Ueki, K.; Ueki, A. Development of anaerobic bacterial community consisted of diverse clostridial species during biological soil disinfestation amended with plant biomass. *Soil Sci. Plant Nutr.* **2012**, *58*, 273–287. [CrossRef]
43. Tedersoo, L.; Bahram, M.; Põlme, S.; Kõljalg, U.; Yorou, N.S.; Wijesundera, R.; Ruiz, L.V.; Vasco-Palacios, A.M.; Thu, P.Q.; Suija, A.; et al. Global diversity and geography of soil fungi. *Science* **2014**, *346*, 1256688. [CrossRef]
44. Osorio, N.W.; Habte, M. Soil phosphate desorption induced by a phosphate-solubilizing fungus. *Commun. Soil Sci. Plant Anal.* **2014**, *45*, 451–460. [CrossRef]

45. Atsuko, U.; Nobuo, K.; Katsuji, U. Role of anaerobic bacteria in biological soil disinfestation for elimination of soil-borne plant pathogens in agriculture. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 6309–6318. [CrossRef]
46. Liu, L.L.; Huang, X.Q.; Zhao, J.; Zhang, J.B.; Cai, Z.C. Characterizing the key agents in a disease-suppressed soil managed by reductive soil disinfestation. *Appl. Environ. Microbiol.* **2019**, *85*, e02992–e03018. [CrossRef]
47. Tayyab, M.; Islam, W.; Lee, C.G.; Pang, Z.; Khalil, F.; Lin, S.; Lin, W.; Zhang, H. Short-term effects of different organic amendments on soil fungal composition. *Sustainability* **2019**, *11*, 198. [CrossRef]
48. Jiang, X.; Zhou, X.; Liu, Q.; Zheng, L.; Li, W. Expression of acidothermus cellulolyticus thermostable cellulases in tobacco and rice plants. *Biotechnol. Biotechnol. Equip.* **2016**, *31*, 23–28. [CrossRef]
49. Lu, J.M.; Peng, C.; Ji, X.J.; You, J.Y.; Cong, L.L.; Ouyang, P.; Huang, H. Fermentation characteristics of mortierella alpina in response to different nitrogen sources. *Appl. Biochem. Biotechnol.* **2011**, *164*, 979–990. [CrossRef] [PubMed]
50. Qian, D.; Du, G.; Chen, J. Isolation and culture characterization of a new polyvinyl alcohol-degrading strain: *Penicillium* sp. WSH02-21. *World J. Microbiol. Biotechnol.* **2004**, *20*, 587–591. [CrossRef]