


Article

The Effect of Flame Sterilization on the Microorganisms in Continuously Cultivated Soil and the Yield and Quality of Tobacco Leaves

Xueying Han ¹ , Liang Wang ², Ruyan Li ¹ and Qingli Han ^{1,*}¹ College of Forestry, Southwest Forestry University, Kunming 650224, China; h18314350214@outlook.com (X.H.)² China RongTong Agricultural Development Group Corporation Limited, Beijing 100020, China

* Correspondence: hanqingli@swfu.edu.cn

Abstract: Flame disinfection is a new technology that uses high temperatures to kill pathogens and control soil-borne diseases. In order to determine the feasibility of applying flame disinfection technology to flue-cured tobacco, a field experiment was conducted in Pianpo Village (test site I) and Lühuai Village (test site II), Luquan County, Yunnan Province. The effects of flame disinfection on soil-borne disease control, flue-cured tobacco growth agronomic traits, the tobacco yield and quality, and the soil microbial community in the flue-cured tobacco field were investigated. The results were as follows. (1) After flame disinfection, the control rates of the four main soil-borne diseases—black root rot, root rot, wilt, and root knot nematodes—were all over 70%. (2) Samples were taken from the experimental site of Pianpo Village at 0 and 114 days after disinfection (tobacco boom period) to study the effects of soil microbial communities by high-throughput sequencing. Compared with the control group, after 0 days of flame disinfection, the abundance of bacterial actinobacteria, *Nocardia*, *Streptomyces*, and fungal ascomycetes decreased, while the abundance of bacterial Proteobacteria, Bacteroidetes, *Arthrobacter*, and mycospora increased. After 114 days of disinfection, the abundance of bacterial actinobacteria, Proteobacteria, chloromycetes, and fungal ascomycetes decreased. The abundance of *Mortierella* was recovered, the abundance of *Gibberella* and *Fusarium* increased, and the abundance of *Trichospora* and Basidiomycetes decreased in both periods. (3) After flame disinfection treatment, the tobacco yield in the two experimental areas was increased by 50.80% and 54.70%, respectively, and the proportion of high-quality tobacco was also increased. In conclusion, flame disinfection before tobacco planting can improve the soil conditions, effectively control soil-borne tobacco diseases, and improve the quality and yield of tobacco leaves.

Keywords: tobacco-growing soil; flame disinfection; soil microbial community; tobacco yield and quality



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1. Introduction

Yunnan is the main producing area of high-quality tobacco in China. Over the years, the planting area of flue-cured tobacco has been relatively fixed, and continuous cropping is very common. However, continuous cropping has degraded the soil environment of flue-cured tobacco and caused soil-borne pathogens to be continuously accumulated in the soil, resulting in frequent soil-borne pests and diseases in flue-cured tobacco and leading to cropping obstacles that have seriously affected the development of the flue-cured tobacco planting industry [1].

Even with good field management systems, crops under long-term continuous cropping systems may experience slow growth, reduced yields and quality, and an increased incidence of soil-borne diseases [2,3]. Soil-borne diseases are the most important factor in terms of continuous cropping obstacles. Studies have shown that when the same crop is continuously planted on a piece of land for more than three crop rounds, soil-borne

diseases will cause a 30%~40% crop yield reduction and even lead to no harvest in serious cases [4,5].

The soil-borne diseases that seriously affect the growth of flue-cured tobacco include black shin disease, root black rot, bacterial wilt disease, root knot nematode disease, etc. [6,7], which are caused by invasion and infection by *Phytophthora nicotianae*, *Thielaviopsis basicola*, *Ralstonia solanacearum*, and root knot nematodes, respectively [8–10]. At present, regarding the prevention and control of soil-borne diseases among crops and obstacles to continuous cropping, researchers have proposed various prevention and control measures. Among these, the regulation of the soil micro-environment is one of the research hotspots, mainly including the selection of different planting systems and the selection of crop-resistant varieties, the application of organic fertilizers, soil-intensive reduction treatment and biological fumigation, and the application of biochar, etc. [11]. Soil disinfection is a measure to kill or reduce harmful organisms such as weeds [12], phytopathogenic fungi, bacteria [13,14], and nematodes [15,16] in the soil before planting crops.

Soil disinfection mainly includes chemical fumigation, biological material fumigation, and physical disinfection [17].

Flame disinfection technology is a type of physical disinfection that uses high-temperature flames to kill insects. The flame disinfection machine consists of three parts: one is the power traction device that enables the machine to drive forward, one is the high-temperature fuel-burning device for the combustion of natural gas or liquefied gas, and the other is the soil rotary tillage equipment. When the machine rolled along, it produces a series of flames and rotates the soil. The machine rotary tillage device can extract soil from a depth of 30~50 cm to the equipment body for crushing and send it into the high-temperature box for instant high-temperature sterilization and insect killing via software for temperature control. The soil created by rotary tillage passes through the 400~600 °C high-temperature flame box for 2~3 s treatment, so as to achieve high-temperature disinfection.

The flame sterilizer refurbishes the soil after high-temperature sterilization and effectively removes the residual organic compounds in the soil [18–20]. It does not destroy the nutrients, such as organic matter and inorganic salts, in the soil [21]. The flame disinfection technique has many advantages, such as low time consumption, no secondary pollution, good prevention efficiency, and labor and time savings [22–24]. In recent years, flame soil disinfection technology has mostly been used in facility agriculture, and it can effectively prevent and control the damage caused by diseases, insects, and grasses in combination with high solar temperatures and the increased application of microbial agents [25,26]. Cao and his research team applied the soil flame disinfection technique to cucumber and pepper greenhouses; the yields of the two crops increased significantly and the number of pathogenic nematodes, such as *Fusarium* and *Phytophthora infestans*, in the soil, as well as the number of grass weeds and broad-leaved weeds, decreased, reducing the crop root knot index. Moreover, the effect of two flame disinfection treatments was better than that of one treatment [20]. Yue et al. showed that flame disinfection was suitable for the control of sweet potato stem nematodes, root rot disease, and weeds; specifically, the control effect for sweet potato stem nematodes reached up to 100% [27].

At present, there are few reports about flame disinfection used in flue-cured tobacco field trials at home and abroad. In this study, flame disinfection experiments were carried out in a tobacco field in Luquan County, Kunming City, Yunnan Province of China, to study the effect of flame disinfection on the prevention and control of four soil-borne tobacco diseases and its influence on tobacco growth and development and the production quality. Through high-throughput sequencing, the differences in the microbial community structure, such as bacteria and fungi, in the soil after flame disinfection were examined to determine whether flame disinfection could be used as an effective soil disinfection method in flue-cured tobacco cultivation.

2. Materials and Methods

2.1. Test Materials

The two experimental fields were located on Pingshan Street, Luquan County, Kunming City, Yunnan Province, China (102°32' E, 25°35' N, 1,950 m above sea level) and Lühuai Village (102°54' E, 25°58' N), where tobacco has been cultivated for many years. The distance between the two test sites was 2 km. The physical and chemical properties of the soil in the two experimental plots of Pianpo (field I) and Lühuai (field II) are shown in Table 1.

Table 1. Soil physical and chemical properties at different experimental sites.

Physical or Chemical Property	Field I	Field II
pH (water-to-soil ratio = 2.5:1)	7.46	7.32
Organic material (g/kg)	22.7	21.56
Moisture (%)	5.01	6.28
Ammonium nitrogen (mg/kg)	2.88	2.95
Total nitrogen (g/kg)	1.13	1.03

Flame disinfection machine: self-propelled precision rotary soil flame disinfection machine (3SHJG-135) produced by Anhui Chunhui Ecological Environment Technology Co., Ltd. (Wuhu, China) Transplanting flue-cured tobacco variety: K326. The transplanting date was 5 April 2023.

2.2. Test Design and Flame Disinfection Operation

The flame disinfection operation time was 8 March 2023. According to a completely randomized block design, flame disinfection treatment F and control CK were set up at site I and site II, respectively. There were 3 replicates in both F and CK. The area of each replicate at test site I was 200 m² (20 m long and 10 m wide), and that at site II was 300 m² (30 m long and 10 m wide).

Flame disinfection operation: The flame disinfection rotary tiller was operated by the company's professionals, with a 1 m/min moving forward speed, a 30 cm rotary tillage depth in the soil, and a 1200 °C flame temperature. The soil temperature dropped to 55~60 °C after landing.

Day of seedling transplanting: 5 April 2023.

The day of flame disinfection was 8 March, which was set as 0d_F in the flame disinfection treatment and as 0d_CK in the control. During the rapid growth of the tobacco, the second soil samples were taken on July 1, marked as 114d_F and 114d_CK. The soil was sampled according to the five-point sampling method, placed into a dry-ice box, and brought back to the laboratory. All samples were divided into two parts: one was stored in a −80 °C refrigerator for the subsequent extraction of the soil microbial DNA, and the other was placed in a 4 °C refrigerator for soil physicochemical property and soil-borne pathogen determination.

2.3. Determination of Soil Physical and Chemical Properties

The physical and chemical properties of the soil were determined according to soil agrochemical analysis [28]. The soil total nitrogen and ammonium nitrogen were detected using the continuous flow method; organic matter was measured using the potassium dichromate volumetric method; the moisture content was detected by the oven method; and the pH value was detected using the glass electrode method.

2.4. Evaluation and Detection Method for the Effect of Flame Disinfection on Soil-Borne Pathogens

There are many types of pathogens that cause soil-borne diseases. In this study, the effects of flame disinfection on soil-borne pathogens were evaluated by measuring the numbers of *Fusarium* spp., *Phytophthora* spp., and *Meloidogyne* spp. in the soil before and after flame disinfection at trial site I. The colonies of *Fusarium* and *Phytophthora* in

the soil were detected according to the methods of Komada [29] and Masago et al. [30], respectively. The heavy sugar separation method was used for the isolation and counting of root knot nematodes [31]. Pathogen inhibition rate (%) = (number of colonies in control treatment – number of colonies in flame disinfection treatment)/number of colonies in control treatment × 100%, root knot nematode control effect (%) = (number of nematodes in control area – number of nematodes in treatment area)/number of nematodes in control area × 100% [32].

2.5. Incidence of Four Soil-Borne Diseases of Flue-Cured Tobacco

The incidence of the soil-borne diseases in flue-cured tobacco was investigated according to the National Standard for Grade and Investigation Methods of Tobacco Diseases and Insect Pests (GB/T 23222-2008) [33]. Black shank disease and bacterial wilt disease were investigated in the two experimental plots during the rapid growth period of flue-cured tobacco (1 July). After all tobacco leaves were harvested on 30 August, the tobacco roots were dug up to investigate root knot nematode disease and root black rot on 5 September, and the rate of diseased plants and the control effectiveness were calculated.

The rate of the diseased plants (%) = (number of diseased plants/total number of plants) × 100%; disease index (%) = Σ (disease progression × number of diseased plants)/(maximum disease progression × total number of plants) × 100%; control effect (%) = (control group disease index – treatment group disease index)/control disease index × 100%.

2.6. Agronomic Characteristics and Yield Quality of Flue-Cured Tobacco

Ten days after the top of the tobacco plant was knocked off (26 July), 10 tobacco plants were randomly selected from each treatment plot in the two experimental plots to measure the agronomic traits of flue-cured tobacco, such as the plant height, stem diameter, leaf length and leaf width of maximum effective leaf, effective leaf number, and chlorophyll, according to the YC/T 142-2010 Survey Method for Tobacco Agronomic Traits [34]. Maximum single leaf area = leaf area coefficient 0.6345 × maximum leaf length × maximum leaf width. Chlorophyll was detected with an OPTI-Science CCM-200 plus portable chlorophyll analyzer.

Measurement of yield: Before tobacco harvesting and baking, the effective tobacco leaves that could be harvested from the tobacco plants under the flame disinfection treatment and the control were labeled. A 5-point method was used to select 4 consecutive tobacco plants at each point, i.e., 60 tobacco plants were listed for both flame disinfection and the control.

After picking and baking, the yields of the cured tobacco leaves were measured and graded by professional tobacco leaf grading personnel, and the proportions of first-class tobacco rated as medium orange—1, medium orange—2, medium orange—3, medium lime—1, medium lime—2, upper orange—1, upper orange—2, upper lime—1, and lower orange were calculated. Tobacco leaf samples of middle tangerine three—C3F and upper tangerine two—B2F were selected for chemical composition analysis.

2.7. Effects of Flame Disinfection on Soil Microbial Community

The diversity of the bacterial and fungal communities in the soil samples from test point 1's flame disinfection treatment and the CK at 0 d and 114 d was analyzed. The soil samples stored in the –80 °C refrigerator were extracted using a Powersoil® DNA extraction kit (Mo Bio, Carlsbad, CA, USA).

The DNA quality and purity were tested and it was sent to Shanghai Meiji Biomedical Technology Co., LTD (Shanghai, China). The bacterial universal primer 338F (5'-ACTCCTACGGGAGCAGCAG-3')-1146R (5'-GGACTACHGGGTWTCTAAT-3') [35] and the fungal universal primer ITS1F were used (5'-CTTGGTCATTAGGAAGTAA-3')-ITS2R (5'-GCTGCGTTCTTCATCATGATGC-3') [36] to amplify the V3-V4 region of the 16S rRNA gene in the soil bacteria and the ITS region in the fungi, respectively. After quality filtering

and merging the raw sequencing data using the Illumina MiSeq platform, the OTU was obtained by clustering at a 97% similarity level.

2.8. Data Analysis

The sequencing data were analyzed and a cloud diagram was drawn with the cloud platform (<https://cloud.majorbio.com/page/project/overview.html> accessed on 24 March 2024) provided by Meiji biological Medicine Science and Technology Co., LTD., in Shanghai. Mothur was used to analyze the alpha diversity; the R language tool was used to encode the soil microbial relative abundance percentage data and draw a soil microbial community histogram. The OTU sequence data were used for UPGMA clustering and principal coordinate analysis (PCoA) based on the weighted Bray–Curtis distance, in order to evaluate the microbial community structure under the different treatments [37]. The IBM SPSS Statistics 20 software and the Kruskal–Wallis H test were used to test the significance between the different treatments.

3. Results and Analysis

3.1. Prevention and Control of Soil Pathogens and Four Diseases

The killing and control effects of flame disinfection regarding the genera *Fusarium* and *Phytophthora* and nematodes in the soil of experimental site I are shown in Table 2.

Table 2. The preventive and therapeutic effects of flame disinfection on three pathogens in experimental site I.

Treatment	Phytophthora		Fusarium		Root Knot Nematode	
	Colony Number	Control Effect	Colony Number	Control Effect	Number	Control Effect
	(cfu·g ^{−1})	%	(cfu·g ^{−1})	%	(per ·100 g ^{−1})	%
0d_CK	760.0 ± 72.1 b	-	3693.3 ± 424.0 b	-	223.3 ± 51.3 a	-
0d_F	60.0 ± 20.0 d	92.11	360.0 ± 120.0 c	90.25	13.3 ± 5.8 b	94.01
114d_CK	1253.3 ± 140.5 a	-	5680.0 ± 1180.5 a	-	256.7 ± 55.1 a	-
114d_F	366.7 ± 122.2 c	70.74	1093.3 ± 260.3 c	80.75	76.7 ± 15.3 b	70.13

Note: The data in the table are the mean ± standard deviation; different lowercase letters indicate significant differences between treatments ($p \leq 0.05$) according to the Kruskal–Wallis H test.

The occurrence of harmful pathogens in the tobacco field was serious, and the number of pathogens increased rapidly with the growth of the tobacco plant. In the control group, at 0 d and 114 d, the number of *Phytophthora* colonies in the soil was 760 cfu·g^{−1} and 1253.3 cfu·g^{−1}, respectively; the number of *Fusarium* colonies was 360 cfu·g^{−1} and 5320 cfu·g^{−1}, respectively; and the number of root knot nematodes was 223 per 100 g^{−1} and 256 per 100 g^{−1}, respectively. Compared with the control group, the number of pathogens in the soil decreased significantly after the flame disinfection treatment. At 0 d and 114 d, the number of *Phytophthora* colonies was 60 cfu·g^{−1} and 366 cfu·g^{−1}, respectively, with the corresponding control effects reaching 92.11% and 70.74%; the number of *Fusarium* colonies was 360 cfu·g^{−1} and 1093 cfu·g^{−1}, with the corresponding control effects reaching 90.25% and 80.75%, respectively; and there were 223 and 256 root knot nematodes per 100 g soil, respectively, with corresponding control effects of 94.01% and 70.13%. The results showed that flame disinfection had a good control effect on the three soil pathogens ($p < 0.05$).

The incidence rates of the two experimental sites are shown in Table 3 with the incidence indices abbreviated as follows: disease plant rate—DPR; disease index—DI; control effect—CE. The incidence indices of the three main soil-borne diseases did not show a significant difference ($p < 0.05$), either in the control group or in the treatment group, between test points 1 and 2. However, there was a significant difference in the incidence indices of each disease between the control group and the treatment group. Taking test point 1 to illustrate, the disease indices of black shank disease, wilt disease, root rot disease, and nematode disease in the tobacco in control group were 12.48, 14.03, 16.70, and 29.33,

respectively, and those for the flame sterilization treatment were 1.60%, 3.33%, 3.33%, and 2.78%, respectively, while the corresponding control effects were 87.18%, 76.27%, 80.06%, and 90.52%. The results also indicated that flame disinfection had the strongest effect on root knot nematode disease, followed by black shank disease.

Table 3. Incidence of four soil-borne diseases of flue-cured tobacco under different treatments.

Disease	Index (%)	Field I		Field II	
		CK	F	CK	F
Black shank	DPR	19.39 ± 1.05 a	3.19 ± 3.13 b	23.46 ± 4.62 a	1.60 ± 1.41 b
	DI	12.48 ± 0.73 a	1.60 ± 1.56 b	17.09 ± 8.69 a	3.05 ± 3.28 b
	CE	-	87.18	-	82.14
Bacterial wilt	DPR	22.17 ± 3.55 a	3.33 ± 5.77 b	17.54 ± 4.95 a	2.24 ± 2.18 b
	DI	14.03 ± 4.53 a	3.33 ± 5.77 b	8.37 ± 3.86 a	2.38 ± 2.38 b
	CE	-	76.27	-	71.56
Black root rot	DPR	25.76 ± 5.17 a	1.11 ± 1.92 b	16.16 ± 4.37 a	0.72 ± 1.26 b
	DI	16.70 ± 1.28 a	3.33 ± 5.77 b	8.41 ± 2.40 a	2.17 ± 3.77 b
	CE	-	80.06	-	74.14
Root knot nematode disease	DPR	33.23 ± 3.18 a	2.78 ± 2.55 b	18.56 ± 1.01 a	2.10 ± 0.22 b
	DI	29.33 ± 15.03 a	2.78 ± 2.55 b	18.46 ± 10.46 b	2.40 ± 0.22 b
	CE	-	90.52	-	87.03

Note: The data in the table are the mean ± standard deviation; different lowercase letters indicate significant differences between treatments ($p \leq 0.05$) according to the Kruskal–Wallis H test.

3.2. Effect of Flame Disinfection on Agronomic Characteristics, Yield, and Quality of Flue-Cured Tobacco

After the leaves of the flue-cured tobacco were mature and the top of the tobacco plant was removed, the agronomic characteristics of the tobacco plant and the tobacco yield after roasting were as shown in Table 4. The flame disinfection treatment significantly increased the plant height, stem diameter, maximum leaf area, leaf number, chlorophyll, and other relevant agronomic traits of flue-cured tobacco.

Table 4. Agronomic characteristics and yields of flue-cured tobacco at maturity stage when treated by flame disinfection.

Field	Treatment	Plant Height (cm)	Stem Diameter (cm)	Maximum Leaf Area (cm ²)	Number of Blades (Slice)	Chlorophyll (%)	Yield (kg/hm ²)	Prime Tobacco Proportion (%)
Field I	CK	105.90 ± 16.29 b	3.87 ± 0.53 a	1640.50 ± 247.29 b	17.20 ± 2.15 b	19.11 ± 2.34 b	2173.8 b	51.37 b
	F	132.60 ± 9.25 a	4.07 ± 0.21 a	2072.98 ± 363.40 a	20.20 ± 1.81 a	20.63 ± 3.82 a	2776.8 a	69.71 a
Field II	CK	91.30 ± 8.62 b	3.62 ± 0.33 a	1473.25 ± 240.03 b	17.30 ± 2.16 b	18.63 ± 2.27 b	1995.15 b	48.51 b
	F	128.50 ± 10.48 a	4.18 ± 0.22 a	2047.79 ± 294.75 a	20.30 ± 1.60 a	22.21 ± 2.64 a	2531.13 a	72.06 a

Note: The data in the table are the mean ± standard deviation; different lowercase letters indicate significant differences between treatments ($p \leq 0.05$) according to the Kruskal–Wallis H test.

The yield in plot I increased by 603 kg/hm², and the proportion of high-grade tobacco was 35.70% higher than that in the control. The yield in test site II increased by 535.98 kg/hm², and the proportion of high-grade tobacco was 48.55% higher than that in the control.

The main chemical components of B2F and C3F tobacco leaves after baking were analyzed (Table 5). Compared with the control, flame disinfection had no significant effect on the content of total sugars, reducing sugars, total nitrogen, total plant alkaloids, K₂O, and Cl⁻, as well as the ratio of reducing sugars to total sugars, the ratio of sugars to plant alkaloids, and the ratio of chlorine to potassium. The ideal chemical composition for tobacco leaves is as follows: total sugars 18%~24%; reducing sugars 16%~22%; total plant alkaloids 1.5%~3.5%; total nitrogen 1.5%~3.5%; potassium content greater than 2%; chlorine content less than 1%, with a recommended potassium to chlorine ratio of 4~10; a sugar to alkaloid ratio of around 10; and a nitrogen to alkaloid ratio of around 1 [38]. After the flame

disinfection treatment, the main chemical components of the tobacco leaves fell within the appropriate range.

Table 5. Chemical composition indices of tobacco leaves in test plot I.

Tobacco Leaf	Total Sugars (%)	Reducing Sugars (%)	N (%)	Total Alkaloids (%)	K ₂ O (%)	Cl [−] (%)	Reducing Sugar to Total Sugar Ratio (%)	Sugar to Alkaloid Ratio (%)	Chlorine to Potassium Ratio (%)
CK (C3F)	26.5	18.37	2.48	3.36	2.9	0.35	0.69	7.89	0.12
CK (B2F)	27.69	19.4	2.19	2.34	2.88	0.23	0.7	11.83	0.08
F (C3F)	25.07	17.57	2.7	4.41	2.51	0.32	0.7	5.68	0.13
F (B2F)	28.52	20.09	1.86	2.06	2.99	0.22	0.7	13.84	0.07

3.3. Effects of Flame Disinfection on Soil Microbial Community Diversity

In test site I, rhizosphere soil samples of the tobacco plants were taken from the control area and the flame disinfection area at day 0 and day 114 after flame disinfection, and they were labeled as 0d_CK, 0d_F, 114d_CK, and 114d_F, respectively. The control and treatments were repeated three times and yielded 12 soil samples in total.

3.3.1. Analysis of Effectiveness of Soil Sample Sequencing

Twelve trial soil samples were subjected to high-throughput sequencing and optimized for the original sequences. A total of 6,451,140 effective sequences were obtained by bacterial 16S sequencing, with an average length of 416.5 bp, and a total of 822,943 effective sequences were obtained after ITS sequencing, with an average length of 236.6 bp.

Figure 1 shows the dilution curves of bacteria (A) and fungi (B) in the soil treated with flame disinfection. With the increase in the sample size, the dilution curves of all samples show a tendency to reach a plateau, indicating that the database obtained by the bacterial 16S rRNA and fungal ITS gene sequencing of the 12 samples in this study covered most of the samples. The results showed that the amount and depth of sequencing were reasonable, and the sequencing results could reflect the composition and diversity of the bacterial and fungal communities in the soil.

3.3.2. OTU Specificity Analysis of Microbial Communities

After optimizing the original sequences, the OTUs were divided based on a 97% sequence similarity threshold. As shown in Figure 2, the total number of bacterial OTUs and fungal OTUs in the 12 samples was 3517 and 1362, respectively. Among them, there were 2379 common bacterial OTUs and 329 common fungal OTUs. At 0 d after flame disinfection, 921 bacterial OTUs were found in the soil, amounting to 169 more than in the control; at 114 d after flame disinfection, there were six more than in the control. At 0 d after disinfection, the number of fungal OTUs in the soil was higher than that in the control, at 35, and it was lower than that in the control after 114 d, amounting to 53. The results showed that the OTUs of bacteria and fungi in the soil increased at 0 d after flame disinfection, and, with the passage of time, the OTUs of bacteria increased and the OTUs of fungi decreased during the prosperous tobacco growth period up to 114 d.

3.3.3. Alpha Diversity of Microbial Community in Soil

The alpha diversity index is used to characterize the diversity of microbial communities. Generally, biodiversity is positively correlated with the “Shannon”, “ACE”, and “Chao1” indices and negatively correlated with the “Simpson” index [39]. The α diversity index of the soil microbial community under flame disinfection is shown in Table 6 (Bacteria) and Table 7 (Fungi). The coverage of the bacterial community and fungal community in all sequenced treatments reached more than 96%, and the coverage of the fungal community reached more than 99%. The sequencing depth was sufficient to meet the requirements for subsequent analyses.

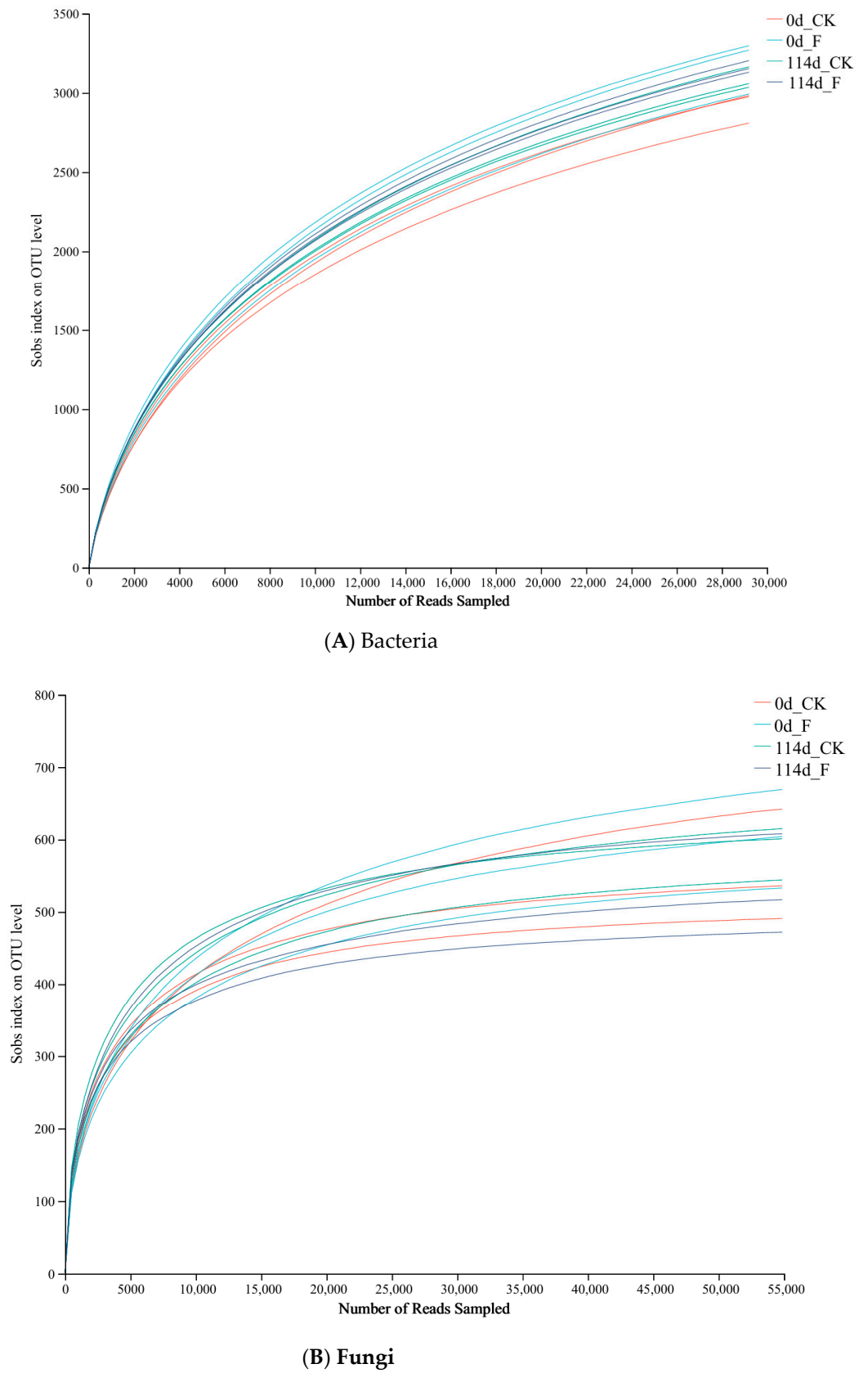


Figure 1. Rarefaction curves of bacteria (A) and fungi (B) in flame disinfection and control soil samples.

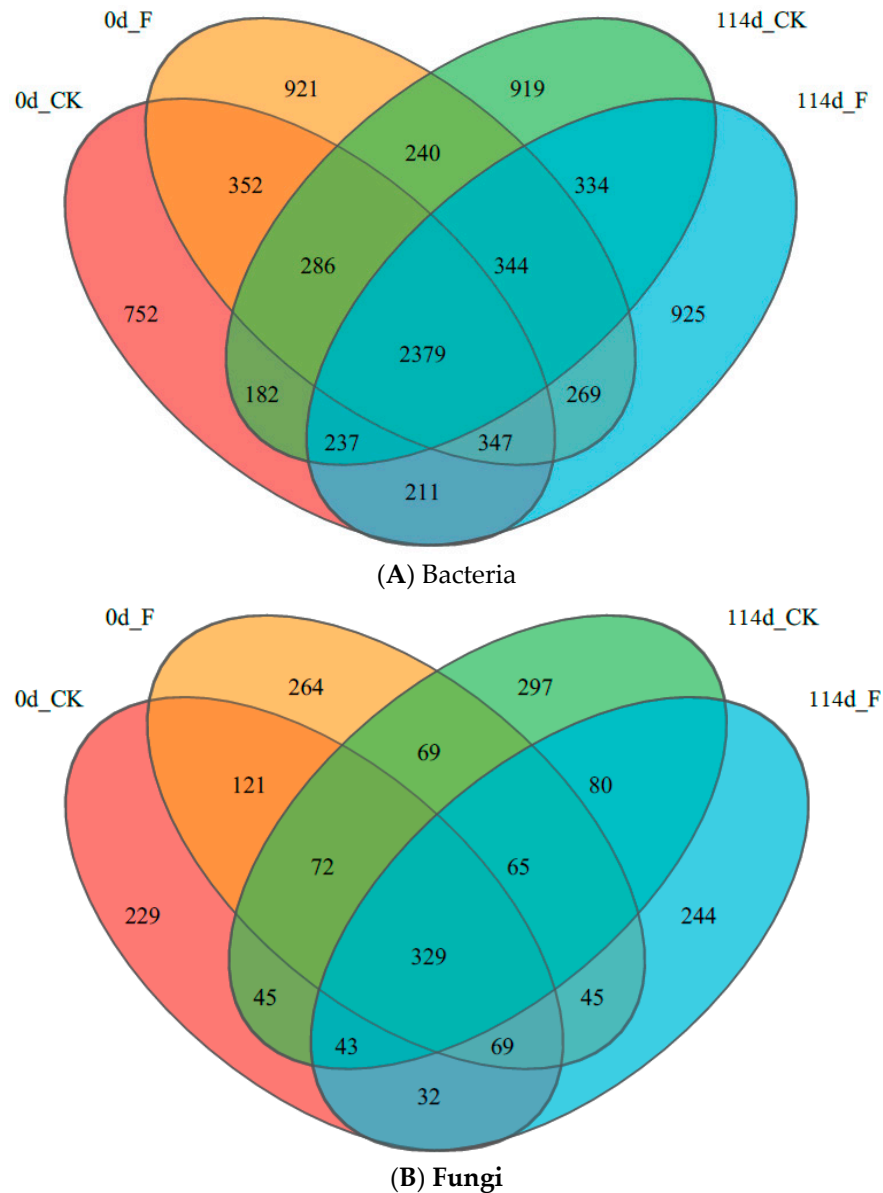


Figure 2. Numbers of specific OTUs of bacteria (A) and fungi (B) in flame disinfection treatment and control. (Note: Different colors in the figure represent different soil samples, and overlapping areas indicate the OTU shared by microorganisms among different soil samples).

Table 6. Alpha diversity indices of flame-sterilized soil bacteria.

Soil Sample	Richness Index			Diversity Index		Coverage Index
	Sobs	Chao1	Ace	Shannon	Simpson	Coverage
0d_CK	3186.33 ± 168.02 a	4085.09 ± 188.40 a	4176.44 ± 173.65 a	6.61 ± 0.24 ab	0.0092 ± 0.0060 a	0.97 ± 0.001 b
0d_F	2922.33 ± 98.23 b	3787.36 ± 112.21 b	3827.26 ± 131.84 b	6.40 ± 0.08 b	0.0091 ± 0.0012 a	0.97 ± 0.002 a
114d_CK	3161.67 ± 37.45 a	3947.77 ± 165.91 ab	4125.24 ± 175.31 a	6.72 ± 0.02 a	0.0047 ± 0.0035 a	0.97 ± 0.001 ab
114d_F	3085.33 ± 68.24 ab	3893.44 ± 122.26 ab	4028.77 ± 111.24 ab	6.70 ± 0.03 a	0.0047 ± 0.0005 a	0.97 ± 0.001 ab

Note: The data in the table are the mean ± standard deviation; different lowercase letters indicate significant differences between treatments ($p \leq 0.05$) according to the Kruskal–Wallis H test.

Effects on bacterial communities: The richness index for 0d_F was significantly lower than that of 0d_CK, and the diversity, Shannon, and Simpson indices were not significantly different. Compared with 114d_CK, the richness, Shannon, and Simpson indices were not significantly different; compared with 114d_F, the richness index and Shannon index of 114d_F were significantly increased, but there was no significant difference in the Simpson index. Compared with 114d_F, there were no significant differences in the richness,

Shannon, and Simpson indices between 114d_CK and 114d_F. The results showed that the diversity of the soil bacterial community decreased initially after flame disinfection, but the diversity was recovered with the extension of time after disinfection.

Table 7. Alpha diversity indices of fungi in flame-disinfected soil.

Soil Sample	Richness Index			Diversity Index		Coverage Index
	Sobs	Chao1	Ace	Shannon	Simpson	Coverage
0d_CK	556.33 ± 77.53 a	590.22 ± 94.85 a	591.35 ± 109.88 a	4.31 ± 0.22 ab	0.0341 ± 0.0071 ab	0.9989 ± 0.0007 a
0d_F	602.00 ± 68.02 a	661.53 ± 98.44 a	651.96 ± 92.73 a	4.13 ± 0.10 b	0.0421 ± 0.0047 a	0.9985 ± 0.00055 a
114d_CK	586.67 ± 62.10 a	613.21 ± 78.94 a	612.69 ± 42.29 a	4.56 ± 0.19 a	0.0231 ± 0.1024 b	0.9990 ± 0.00022 a
114d_F	532.33 ± 69.28 a	555.97 ± 70.70 a	553.41 ± 73.67 a	4.55 ± 0.08 a	0.0214 ± 0.0374 b	0.9992 ± 0.00019 a

Note: The data in the table are the mean ± standard deviation; different lowercase letters indicate significant differences between treatments ($p \leq 0.05$) according to the Kruskal–Wallis H test.

Effects on fungal communities: There was no significant difference between 0_F and 0d_CK, as well as between 0d_CK and 114d_CK, regarding the richness, Shannon, and Simpson indices. Compared with 114_F, the richness index of 0F had no significant difference, while the Shannon index of 114d_F was significantly increased and the Simpson index was significantly decreased. For 114d_CK compared to 114d_F, there were no significant differences in the richness, Shannon, and Simpson indices. The results showed that flame disinfection had no significant effect on the diversity of the soil fungi at the initial stage, but the diversity increased at a later stage.

The results showed that flame disinfection mainly reduced the diversity and richness of bacteria, but, after a certain period of time, the bacterial community recovered to the control level, while the fungal community diversity was higher than the control level.

3.3.4. Beta Diversity Analysis of Soil Microbial Community Structure

The β diversity is used to analyze the similarity of microbial species between different treatments. The smaller the β diversity, the more similar the species types between the populations. The results of the PCoA analysis based on the Bray–Curtis distance showed that the flame disinfection treatment and sampling period were the main factors that caused differences in the microbial community compositions of the samples. (Figure 3) The different sample points were dispersed in four quadrants with a certain distance, indicating that there were significant differences in the composition of the microbial communities in different soil samples.

Regarding the first and second principal coordinates, 31.63% and 14.24% of the differences in the bacterial community and 29.66% and 20.77% of the differences in the fungal community in the 12 soil samples were detected, respectively. Overall, compared with the CK, the compositions of the soil bacterial community and fungal community were different after the flame disinfection treatment. At 0 d and 114 d after disinfection, the difference between the flame disinfection and control treatments was large, indicating that there was still a significant difference after flame disinfection treatment over a long period of time.

UPGMA was used to construct a tree structure, and the shorter the tree branch length, the more similar the species composition among the samples. As can be seen in Figure 4, with the exception of bacterial 0d_F1 samples, all processing repeats were clustered together, indicating good repeatability among the samples. According to the bacterial clustering tree, the samples from 0 d and 114 d contained two different types and were far apart, indicating that the time had a greater impact on the bacterial community, while the samples after flame disinfection were closer together. From the fungal clustering tree, the total samples were clustered into three categories: 0d_F and 0d_CK were grouped into the same category, and 114d_F and 114d_CK were grouped into two different categories, respectively. The results showed that flame disinfection had significant effects on the soil bacterial and fungal community compositions.

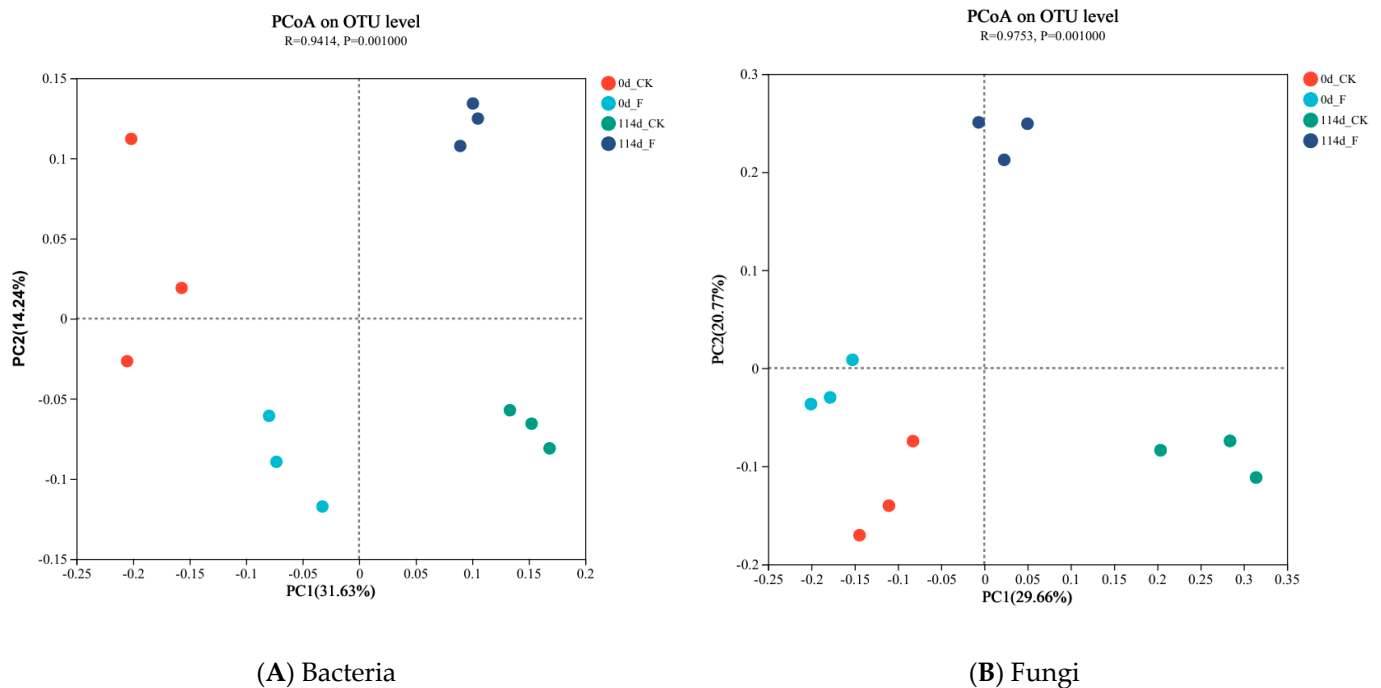


Figure 3. Principal coordinate analysis of flame disinfection and control bacterial and fungal communities.

3.3.5. Effects of Flame Disinfection on Phylum-Level Species of Bacterial and Fungal Communities

Figure 5 shows the relative abundances of the top 10 phylum levels of the bacterial (A) and fungal (B) community compositions under the flame disinfection treatment and the control. In the bacterial community, at the phylum level, *Actinobacteriota*, *Proteobacteria*, *Acidobacteria*, *Chloroflexi*, *Firmicutes*, *Bacteroidetes*, *Myxococcota*, *Methylophila*, and *Nitrospiraea* were mainly detected in all soil samples. Among them, *Actinobacteriota*, *Proteobacteria*, *Acidobacteria*, and *Chloroflexi* were the dominant bacterial groups. According to the mean values of the samples in the group, the dominant categories of soil bacteria in the different treatments were the same, but the relative abundance was different.

At 0 d after flame disinfection, the relative abundance of *Actinobacteriota* and *Acidobacteria* decreased by 8.45% and 7.78%, respectively, and that of *Proteobacteria* and *Bacteroidetes* increased by 0.5% and 1.78%, respectively. After 114 days of flame disinfection, the relative abundance of *Actinobacteriota* and *Proteobacteria* decreased by 3.95% and 0.47%, respectively, and the relative abundance of *Acidobacteria* and *Chloroflexi* increased by 1.85% and 1.66%, respectively.

Regarding the fungal communities, *Ascomycota*, *Mortierellomycota*, *Basidiomycota*, and *Chytridiomycota* were mainly detected in the soil samples at the phylum level. *Ascomycota* was the dominant bacterial group, with the relative abundance of 71.93–82.84%. Compared with the control group, the relative abundance of the *Ascomycota* community at 0 d and 114 d significantly decreased by 5.82% and 7.82%, respectively. However, the relative abundance of *Mortierellomycota* and *Basidiomycota* increased significantly, by 3.03% and 1.84% at 0 d and 0.34% and 4.73% at 114 d, respectively.

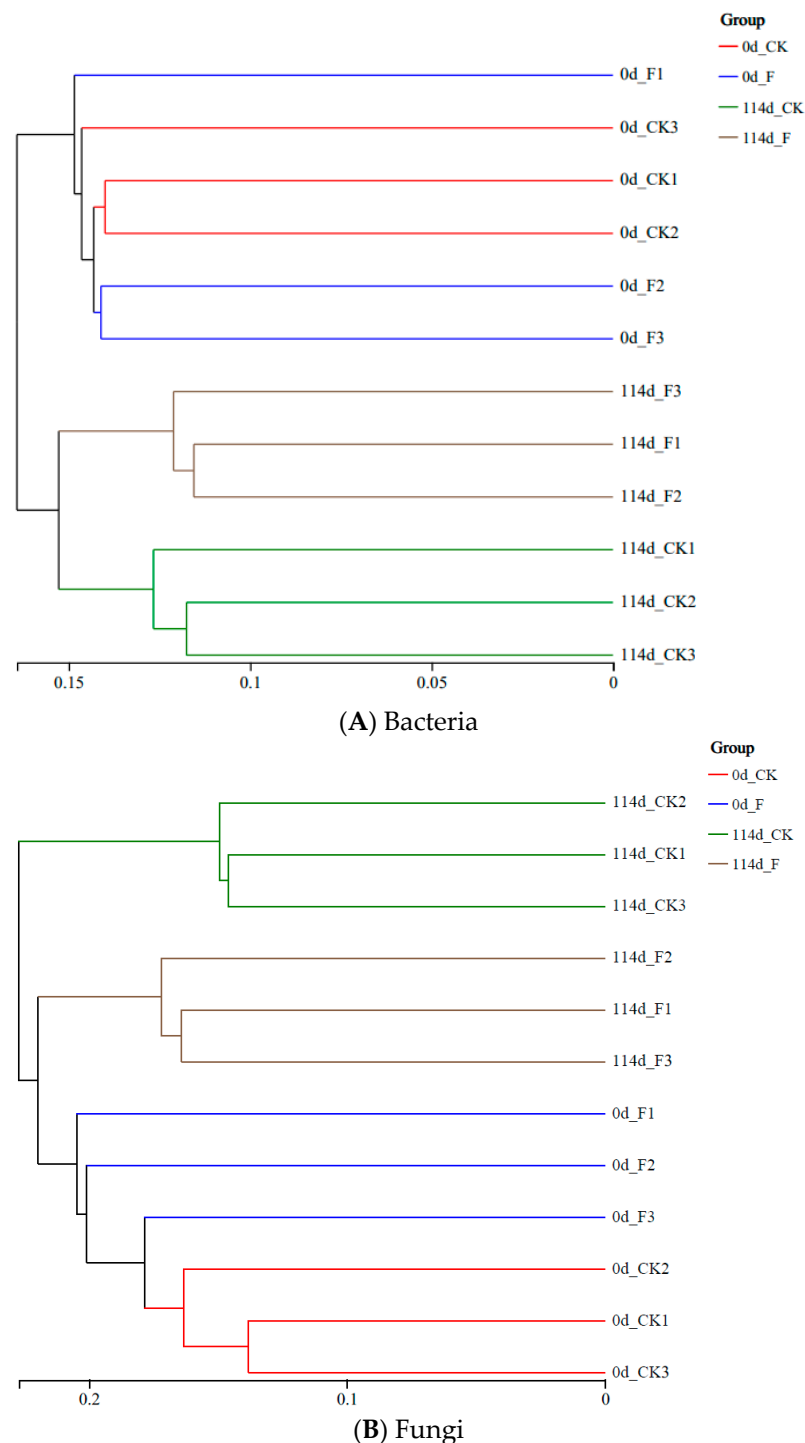


Figure 4. Hierarchical clustering analysis (based on Bray–Curtis method) of bacteria (A) and fungi (B) at the OTU level under different treatments.

3.3.6. Effect of Flame Sterilization on Bacterial and Fungal Communities

Figure 6 shows the abundances of the most represented bacterial (A) and fungal (B) taxa in the soil after the flame disinfection treatment. At the generic level, the top ten most abundant bacterial communities in all samples were *Arthrobacter*, *norank_f_norank_o_Vicinamibacteriales*, *norank_f_Vicinamibacteraceae*, *Nocardioides* and *Streptomyces*, *Bacillus*, *norank_f_Gemmatimonadaceae*, *norank_f_JG30-KF-CM45*, *norank_f_norank_o_Gaiellales*, and *norank_f_6 Wait 7-14*. At 0 d after flame disinfection, compared with the control group, the colony abundance of *Arthrobacter* increased by 0.52%, and that of *Nocardioides* and

Streptomyces decreased by 1.04% and 2.29%. At 114 d, the abundances of *Arthrobacter*, *norank_f_norank_o_vicinamiales*, and *norank_f_Gemmatimonadaceae* were significantly reduced by 0.34% and 0.93%, respectively, 0.9% and 0.78%.

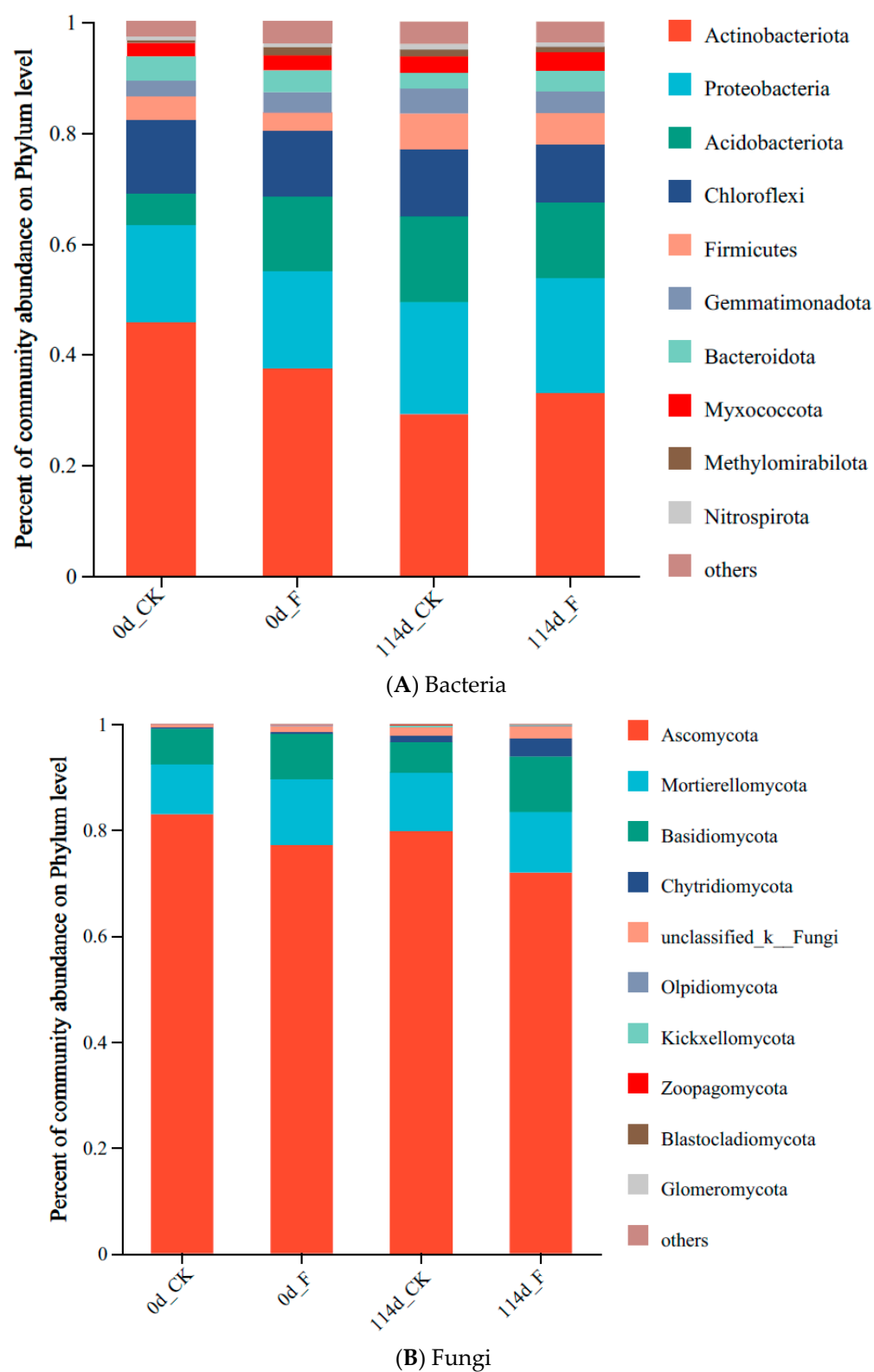


Figure 5. Taxonomic composition and relative abundance of the top 10 phyla of bacteria (A) and fungi (B).

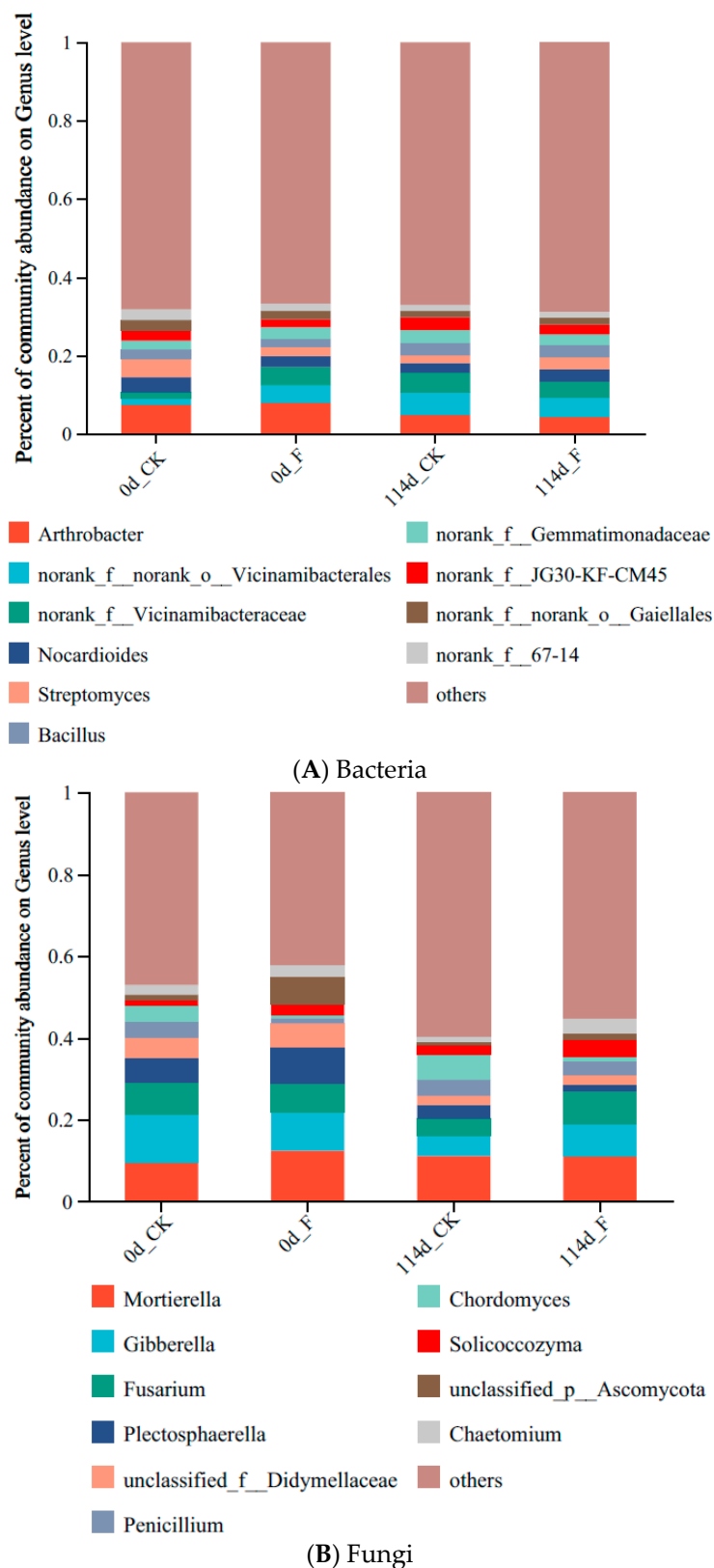


Figure 6. Taxonomic composition and relative abundance of the top 10 genera of bacteria (A) and fungi (B).

The top ten most abundant fungal communities in all samples were *Mortierella*, *Gibberella*, *Fusarium*, *Plectosphaerella*, *Penicillium*, *Chordomyces*, *Solicoccozyma*, *unclassified_p_Ascomycota*, *Chaetomium*, etc. At 0 d after flame disinfection, the colony abundance of *Mortierella* increased

by 3.02% compared with the control group, while the colony abundance of *Gibberella* and *Fusarium* decreased by 2.45% and 0.71%, respectively. At 114 d after flame disinfection, the colony abundance of *Mortierella* was only 0.01% different from that in the control group, indicating that the colony abundance of *Mortierella* returned to a normal level, while that of *Gibberella* and *Fusarium* increased by 2.96% and 4.01%, respectively.

4. Discussion

4.1. Prevention and Control of Soil-Borne Pathogens and Soil-Borne Diseases

Flame disinfection is mostly applied in facility agriculture [40]. The research results of Lou et al. [41] showed that the number of pathogenic bacteria after high-end flame treatment was significantly lower than that after mid-range flame treatment, and the possibility of fungal diseases was lower than that under mid-range flame treatment.

The key to preventing and controlling soil-borne diseases is to reduce the amount of pathogens in the soil before planting. The flame disinfection machine used in this study can quickly kill bacteria, pests, and weeds in the soil through high temperatures. The research results of Wang et al. [25] showed that, after flame disinfection treatment, the presence of three pathogens, *Aspergillus*, *Fusarium*, and nematodes, was significantly lower than in untreated plots. The results obtained in the present research are consistent with this.

The rapid and efficient killing of pathogens by flame disinfection has a significant preventive and control effect on soil-borne diseases in tobacco. Compared with untreated plants, the incidence of shank disease and bacterial wilt disease in flue-cured tobacco in the rapid growth period was reduced by 87.18% and 76.27%, respectively, and the control effect of root black rot and root knot nematode disease in the harvest period was 80.06% and 90.52%.

4.2. Agronomic Traits, Yield, and Quality of Flue-Cured Tobacco

In this study, after flame disinfection, the plant height, stem diameter, leaf number, and other agronomic characteristics of the two experimental plots were all improved; the final yield was increased; and the superior tobacco ratio was increased. The reasons are as follows: first, flame disinfection kills soil pests before the transplantation of tobacco seedlings so that the tobacco plants grow in healthy soil and grow vigorously; second, flame disinfection reduces the soil permeability, enhances the vitality of the tobacco roots, and enhances the absorption of nutrients, so the yield is high.

Sun et al. found that the application of an organic fertilizer after flame disinfection could significantly improve the growth of leaf vegetables such as amaranth and green cabbage in the second crop stage and improve the soil salinization to a certain extent [42].

4.3. Effects of Flame Disinfection on Soil Fungal and Bacterial Communities

Soil microorganisms are an important part of the soil ecosystem and can not only affect the formation and transformation of organic matter but also affect the nutrient cycle of the soil [43]. The results showed that the community structure of fungi and bacteria in the flame disinfection treatment and the control soil changed significantly before transplanting and during the flourishing period of flue-cured tobacco. The relative abundance of Acidobacteria decreased at 0 d after flame disinfection, which was consistent with the results of Liu et al. [44], and it recovered after 114 days.

Bacterial Acidobacteria occupy a relatively high percentage of the soil and belong to the eutrophic group, often gathering in soil environments with high levels of nutrients, affecting soil material circulation and ecological environment construction [45]. It was found that flame disinfection did not reduce the abundance of the Acidobacteria community in the soil for a long time, but only inhibited it for a short period of time.

After flame disinfection, the relative abundance of actinomycetes decreased in both periods, and the influence on Proteobacteria was not significant. Most actinomycetes can decompose plant and animal residues in the soil, and a small number of actinomycetes belong to pathogenic bacteria. Proteobacteria play a crucial role in nutrient cycling, carbon

and cellulose degradation, and humus formation [46]. They belong to the eutrophic group and often gather in soil environments with high levels of nutrients.

At 0 d after flame disinfection, at the bacterial genus level, the abundance of *Nocardia* and *Streptomyces* decreased, but, after 114 days, the abundance of the *Nocardia* community was higher than that in the control, and that of the *Streptomyces* community was higher than that in the control. *Nocardia* is a common animal and plant pathogen [47], and most strains of *Streptomyces* can be used as root knot nematode biocontrol resources [48,49]. The results showed that flame disinfection had a short-term killing effect on *Nocardia* and other possible pathogenic bacteria, and they could not be prevented for a long period of time. For some actinomycetes bacteria, it may have a long-term control effect. For *Acidobacteria*, *Streptomyces*, and other beneficial plant bacteria, their levels could recover at a certain period of time after killing.

In terms of the fungal communities, the abundance of ascomycetes decreased significantly in both periods of flame disinfection, while the abundance of Mortispora and basidiomycetes increased significantly. Ascomycetes can accelerate organic metabolism [50]. Certain *Mortierella* fungi contribute to soil nutrient transformation and availability [51]. Basidiomycetes mainly decompose refractory organic matter at the later stage of the decomposition process [52].

At the fungal genus level, the abundance of the *Mortierella* community increased at day 0 d after flame disinfection, while the abundance of colonies of *Gibberella* and *Fusarium* decreased. At day 114 d after flame disinfection, the *Mortierella* community returned to a normal level, while the populations of *Gibberella* and *Fusarium* increased. *Gibberella* and *Fusarium* are some of the main soil pathogens and can infect a variety of crops and cause serious crop reductions [53,54]. Flame disinfection can increase the beneficial bacteria such as mortieres and Basidiomycetes, which affect soil fertility, but it can only reduce the abundance of *Gibberella* and *Fusarium* in a short period of time, indicating that the control effect against soil-borne diseases may be relatively weakened in the later period.

5. Conclusions

The results of this study show that flame disinfection had a significant control effect on *Phytophthora* spp., *Fusarium* spp., and root knot nematodes in soil obtained from the continuous cropping of tobacco, and it had a significant control effect on the main soil-borne diseases of flue-cured tobacco, such as black shin, root black rot, bacterial wilt, and root knot nematodes. Flame disinfection significantly improved the quality and yield of flue-cured tobacco.

Flame disinfection significantly affected the composition of the soil microbial community, reducing the number of soil-borne bacteria from the source before planting and reducing the colonization of plant pathogens. At the same time, the relative abundance of some beneficial microbial communities increased, such as those of *Acidobacteria*, *Mortispora* spp., and *Streptomyces* spp., regulating the proportions of beneficial and harmful bacteria in the soil.

In summary, flame disinfection technology is technically feasible in preventing and controlling soil-borne diseases of flue-cured tobacco and increasing production, and it can provide a new means to overcome the obstacles of continuous tobacco cropping.

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