

Exogenous Inoculation of Microorganisms Effect on Root Exudates and Rhizosphere Microorganism of Tobaccos

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Abstract

Studying the relationship between rhizosphere microorganisms and root exudates is of great significance for the interaction between rhizosphere microorganisms and plants, and the prevention and control of soil-borne diseases. This article analyzed the effects of different microorganisms on tobacco root exudates and rhizosphere microorganisms. It was found that the bacterial wilt pathogen can greatly increase acids and amines, while the probiotic *B. amylo-liquefaciens* ZM9 can eliminate some acids and amines. The results of the study show that the root exudates of pathogenic bacteria may contain a variety of allelochemicals that cause soil-borne diseases.

Keywords

Root Exudates, Rhizosphere Microbes, GC-MS

1. Introduction

The plant-microbe interaction maintains or dominates the ecological functions of terrestrial ecosystems. The microorganisms in the soil environment are regarded as the largest biological diversity reservoir in nature [1]; each gram of rhizosphere material can identify as many as 1011 microbial cells and more than 30,000 prokaryotes [2] [3]. Plant rhizosphere microorganisms that are regarded as the second genome of plants [4], by modulating the host immune system [5], changing the defense capabilities of plants [6] [7] or recruiting plant beneficial bacteria [8] [9], Changing plant nutrient supply, inhibiting the growth of patho-

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genic bacteria [10] and other mechanisms play a vital role in the regulation of plant growth, health, yield and other aspects [1] [11]. Root exudates are important intermediates in the interaction between rhizosphere microorganisms and plants [12] [13]. To study the interaction between rhizosphere microorganisms and root exudates is useful for exploring the interaction between rhizosphere microorganisms and plants. And the realization of comprehensive index evaluation for plant growth, soil-borne diseases and insect pests, etc. is of great significance.

The current research on the interaction between rhizosphere microorganisms and root exudates mainly focuses on the regulation of rhizosphere microbe formation, microbial metabolism, growth and diversity by root exudates [14] [15]. In fact, rhizosphere microorganisms can stimulate the abundance or quality of root exudates to affect plant growth by absorbing certain specific components in root exudates [16] [17] [18] [19]. For example, inoculation of *B. subtilis* B26 not only increased the yield of rape (*B. distachyon*), but also markedly enhanced drought resistance [20]. Even in the absence of nitrogen, *Azospirillum* and *Herbaspirillum* could promote the growth of rape [21]. These studies show that rhizosphere microorganisms play a potential role in plant root exudates. However, due to the complexity of soil microorganisms in a heterogeneous environment, it is more technically challenging to explore how soil microorganisms directly affect the changes of root exudates, so there are relatively few studies.

In this paper, tobacco is used as the research material to detect and analyze the composition of tobacco root exudates under the action of different rhizosphere microorganisms by gas chromatography-mass spectrometry. Research on signal transmission and other aspects provide effective information and provide new clues for the prevention and control of tobacco soil-borne diseases.

2. Materials and Methods

2.1. Material

R. solanacearum and *B. amyloliquefaciens* ZM9 are preserved by our laboratory, among which *B. amyloliquefaciens* ZM9 is an antagonistic bacteria of *R. solanacearum*.

2.2. Tobacco Cultivation and Root Exudates Extraction

Tobacco were transplanted to peat soils, and then 15 days later, 1 ml of *R. solanacearum* (T1), *B. amyloliquefaciens* ZM9 (T2) (1.0×10^9 CUF/mL) and ddH₂O (CK). The tobacco root exudates were collected according to the previous methods [22], and then the collected root exudates were extracted with ethyl acetate. The acidic, basic and neutral ethyl acetate extracts were concentrated under reduced pressure to 1 mL (45°C), filtered with a 0.45 µm filter membrane, and the organic phases are combined into a rotary evaporator, and the product is rotary evaporated to dryness at 50°C, and the wall is washed with 1 mL ultrapure water, and stored at low temperature (−80°C) for later use.

2.3. Derivatization of Root Exudates

Add 40 μL of 20 mg/mL methoxyamine salt pyridine and 10 μL of 5% phenethyl acetate mixture to the freeze-dried root exudates (Phenyl acetate as internal standard), and after incubation in a 60°C incubator for 60 min, Then add 50 μL of silylation reagent N,O-bis(trimethylsilyl)acetamide (BSTFA), keep it at 70°C for 60 min, and cool to room temperature for later use.

2.4. GC-MS Detection Conditions

Add 40 μL of 20 mg/mL methoxyamine salt pyridine and 10 μL of 5% phenethyl acetate/pyridine mixture (Phenethyl acetate as internal standard) to the freeze-dried root exudates sample, and place it at a constant temperature of 60°C. Keep the temperature in the box for 60 minutes. Then add 50 μL of silanization reagent N,O-bis(trimethylsilyl)acetamide (BSTFA), keep it at 70°C for 60 min, cool naturally to room temperature, GC-MS detection adopts the American Thermo Company gas chromatography/mass spectrometer (DSQII) for analysis. GC conditions: The chromatographic column is TG-5MS 30 m \times 0.25 mm \times 0.25 μm , the inlet temperature is 250°C, and the split ratio is 50:1. Column temperature rising program: increase from 50°C to 250°C at a rate of 3°C/min, continue to increase to 300°C at a rate of 25°C/min, keep for 4 minutes; carrier gas is He (99.9%), flow rate is 20 psi, the injection volume is 1 μL . MS conditions: EI source, ion energy of 70 eV, ion source temperature of 300°C, scanning range of 30 - 600 m/z, transmission line temperature of 280°C.

2.5. DNA Sequence Data Collection and Analysis

Soil DNA was extracted from 0.5 g rhizosphere soil using the FastDNA Spin Kit (MP Biomedicals, USA) following the manufacture's protocol. The integrity of DNA samples were determined by 1% agarose gel electrophoresis. Then the concentration and purity of the DNA were determined using a Nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). The extracted soil DNA was used as template to amplify 16S rRNA gene. The V4 region of 16S rRNA gene were amplified with the primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). All PCR reactions were performed on Illumina HiSeq platforms (Illumina Inc., USA) at Novogene Bioinformatics Technology Co., Ltd. (Beijing, China). The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Sequences analysis was performed by Uparse software (Version 7.0.1001, <http://drive5.com/uparse/>). Sequences with $\geq 97\%$ similarity were assigned to the same OTUs. Each representative sequence was screened for further taxonomic information annotation. FASTX Toolkit 0.0.13 software package was used to preliminarily filtrate the raw sequence data with removing the low mass base at the tail of the sequence (Q value less than 20) and the sequences with lengths less than 35 bp. The sequence quality was statistically analyzed by CASAVA1.8, and

finally, the length of the valid reads was approximately 250 bp. The operational taxonomic units (OTUs), observed-species (Sobs), Shannon, Simpson and Chao1 were calculated to evaluate richness and diversity of soil microbial community.

2.6. Statistical Analysis

The data were analyzed with Microsoft Excel 2007 and SPSS version 18.0 (IBM, USA). Differences between treatments were assessed by one-way analysis of variance (ANOVA) and least significant difference (LSD) test ($p < 0.05$). Correlation analysis was conducted by Pearson (2-tailed). Principal components analyzed (PCA) with the weighted UniFrac distance and canonical correspondence analysis (CCA) were carried out using the vegan package in R (Version 2.15.3).

3. Results and Analysis

3.1. Diversity of Tobacco Root Exudates among Different Treatments

GC-MS analyzed the composition changes of the root exudates of Yunyan 87 under different treatments. As shown in **Figure 1**, in the GC-MS total ion chromatogram after the bacterial wilt pathogen treatment, we can see 37 peaks, indicating that there are 37 types of root exudates. The composition changes of root exudates under different treatments were compared by GC-MS analysis, and it was found that different microorganisms have certain influence on the types of tobacco root exudates.

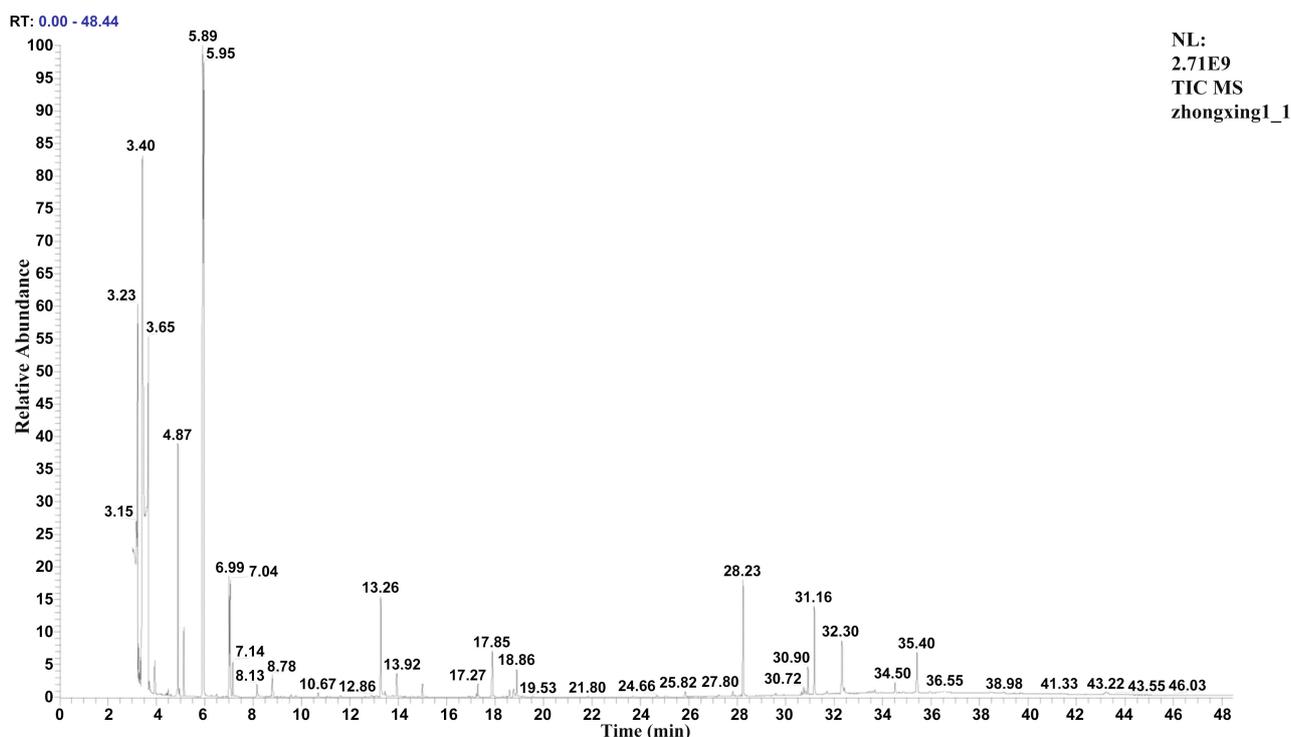
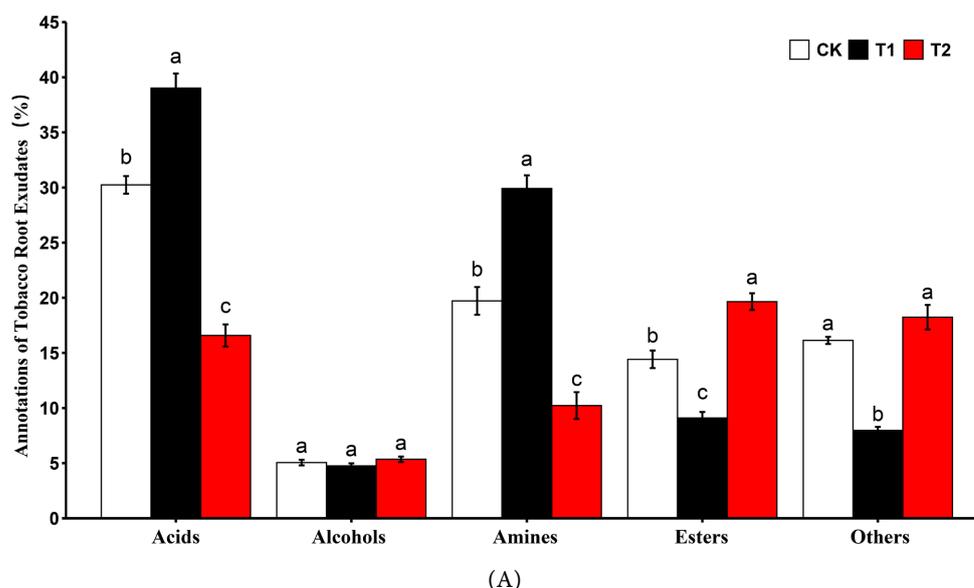
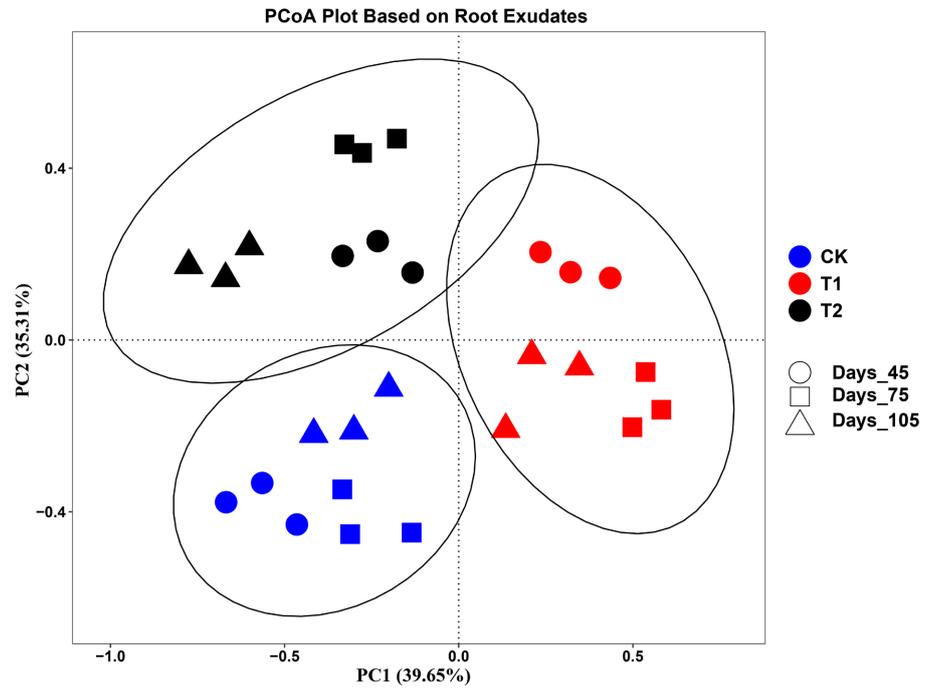


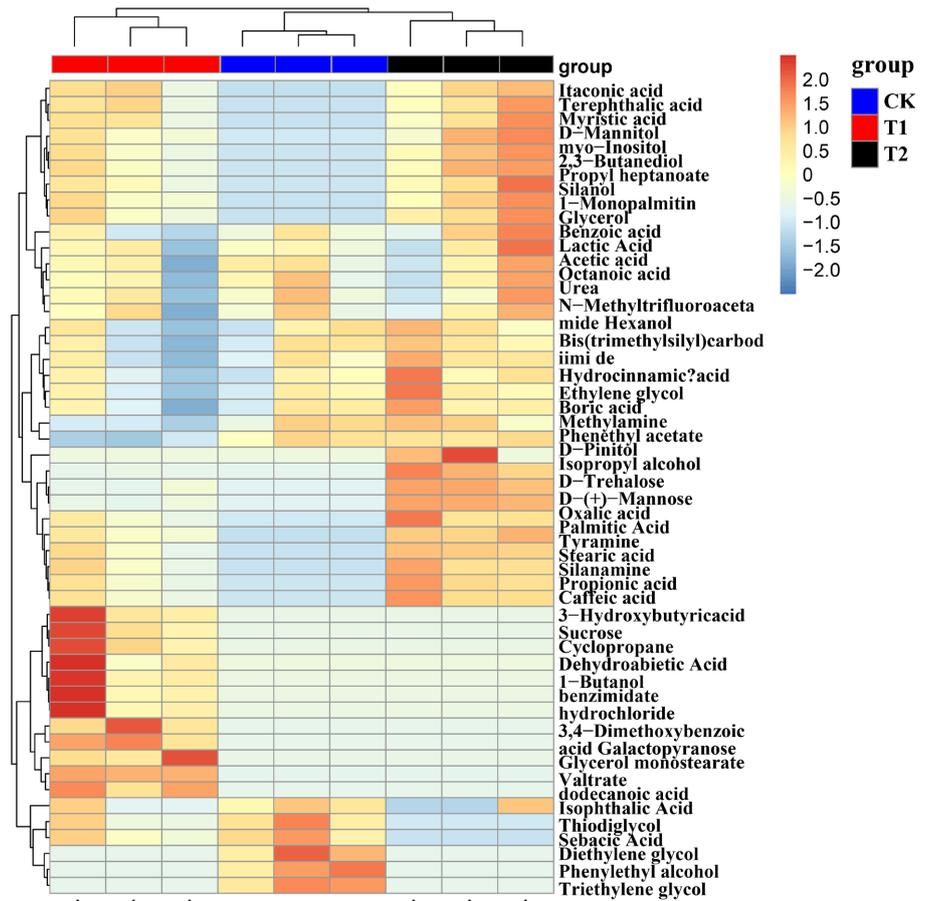
Figure 1. GC-MS total ion current after treatment with bacterial wilt pathogen.

GC-MS analyzed the composition changes of the root exudates of Yunyan 87 under different treatments. Excluding the components whose types and contents did not change under the three conditions, under the action of *R. solanacearum*, acids, alcohols, amines and esters accounted for 39.02%, 4.75%, 29.91%, and 9.10%, respectively. Under the action of its antagonistic *B. amyloliquifaciens* ZM9, acids, alcohols, amines and esters accounted for 16.58%, 5.35%, 10.23%, and 19.65%, respectively. After removing the components whose types and contents did not change under the three conditions, under the action of *R. solanacearum*, the types of root exudates of Yunyan 87 increased by 19 components and decreased by 3 components compared with the control group. The increased components mainly include 3 kinds of amines, 6 kinds of alcohols, 8 kinds of acids, 1 kind of sugars, 1 kind of esters; the reduced components are diethylene glycol, phenethyl alcohol and triethylene glycol (**Figure 2(A)**). After adding *B. amyloliquifaciens* ZM9, 24 kinds of ingredients are added, 6 of which are newly added; 6 kinds of ingredients are reduced, including 6 kinds of thiodiglycol, 1-butanol, sebacic acid, benzimidate hydrochloride, isophthalic acid, and cyclopropane Completely disappear. Compared with the types of root exudates in the T1 group and the T2 group, there were 2 more types and 6 less components. Principal coordinate analyses (PCoA) were carried out using weighted UniFrac distance in different treatments, and PC1 and PC2 explained 74.96% of root exudates. The CK groups in different periods are clustered together, and the root exudates of the corresponding T1 and T2 groups in different periods are clustered together, and the root exudates under different treatments in the same period are separated from each other. This result shows that the change of root exudates has little relationship with time, and is mainly affected by microorganism (**Figure 2(B)**). The heat map analysis of the top 53 root exudates with hierarchical clustering is used to discuss the composition changes of root exudates under different treatments. There are differences in the composition of root





(B)



(C)

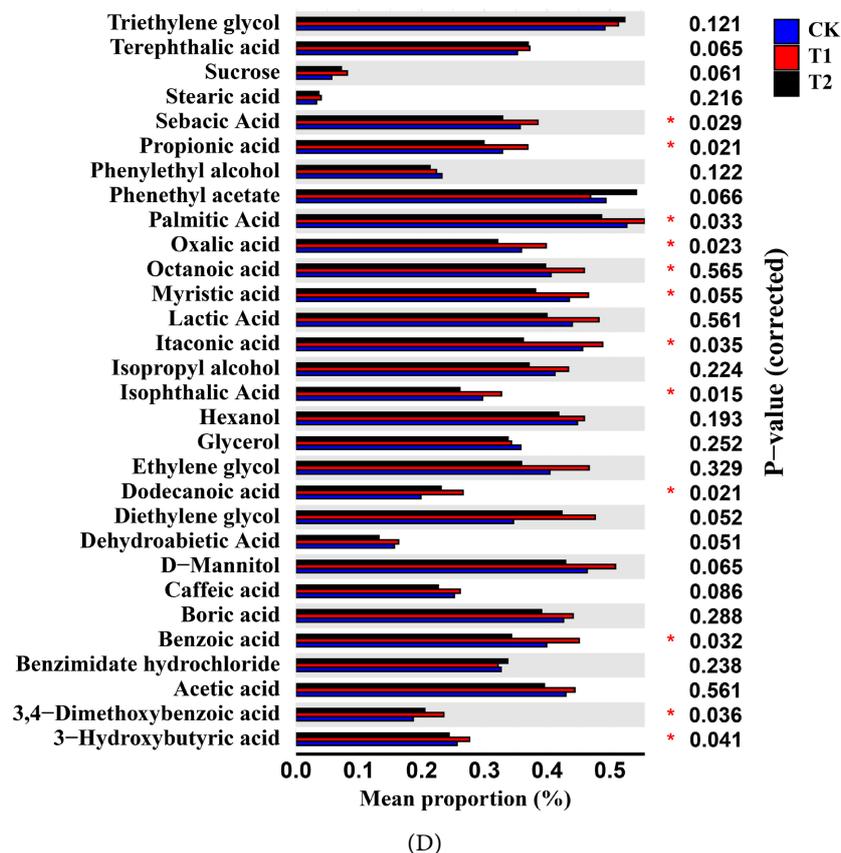


Figure 2. Types and changes of tobacco root exudates under three treatments; (A) The proportion of root exudates under different treatments; (B) Principal coordinate analysis (PCoA) of root exudates; (C) Hierarchical cluster analysis of main root exudates; (D) The relative abundance of the first 30 root exudates in different treatments. The abundance of different species was analyzed by one-way ANOVA * $p < 0.05$ and ** $p < 0.01$ (CK: ddH₂O; T1: *R. solanacearum*; T2: *B. amyloliquefaciens* ZM9).

exudates between different treatments in the heat map. The treatment of *R. solanacearum* significantly increased the abundance of 3-hydroxybutyric acid, sucrose, cyclopropane, hydroabietic acid, 1-butanol, benzimidate, hydrochloride, 3,4-dimethoxybenzoic, glycerol monostearate, and dodecanoic acid. In contrast, the treatment of *B. amyloliquefaciens* ZM9 increased terephthalic acid, d-Mannitol, myo-Inositol, 2,3-Butanediol, propyl heptanoate, Silanol, 1-monopalmitin, glycerol, benzoic acid, lactic acid, acetic acid, octanoic acid, urea, d-Pinitol, octanoic acid these abundance. Results indicate that the treatment of different microorganisms has a great influence on the composition of tobacco root exudates (Figure 2(C)). Comprehensive analysis found that stearic acid, propionic acid, oxalic acid, octanoic acid, myristic acid, itaconic acid, isophthalic acid, dodecanoic acid, benzoic acid, 3-hydroxybutyric acid and 3,4-dimethoxybenzoic acid fluctuates the most (Figure 2(D)).

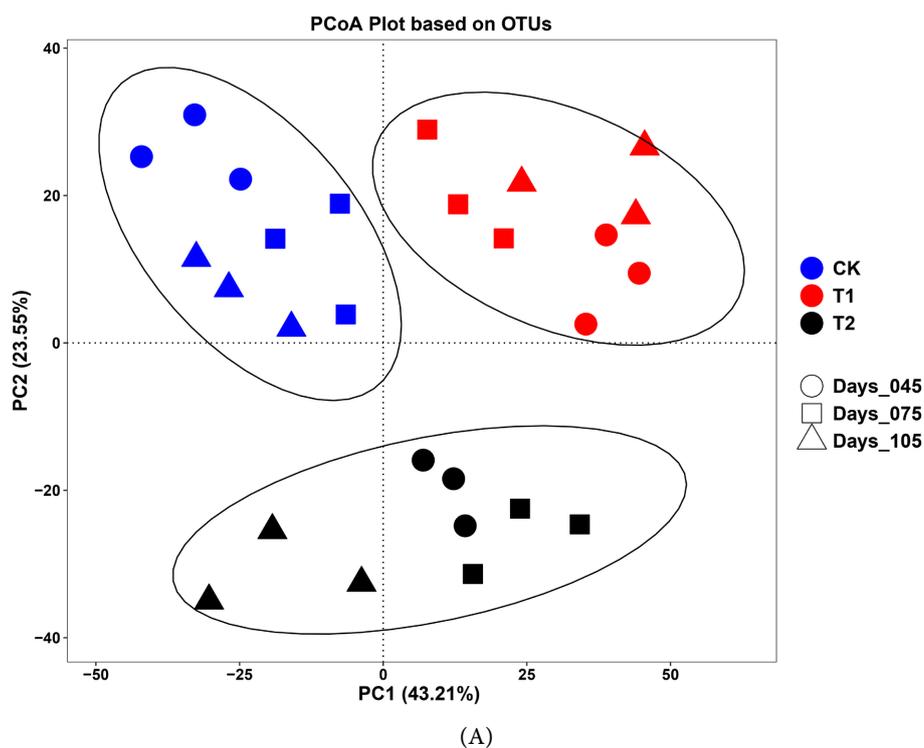
3.2. Bacterial Diversity in Soil

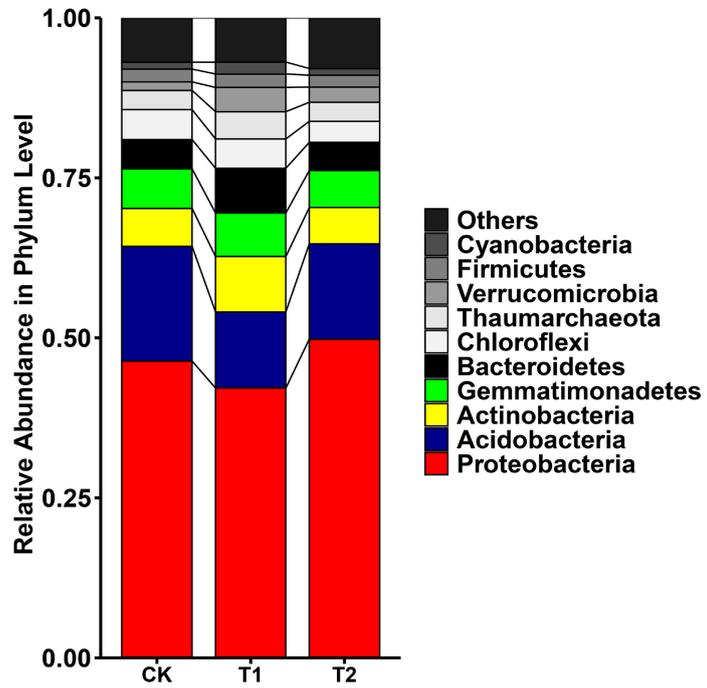
In total 679,451 high-quality raw sequences with the average length of 252 bps

for bacteria were obtained from rhizospheric soil samples after quality filtering. The OTUs, Chao1 and Shannon index were used to evaluate and compare the richness and diversity of bacterial community among different treatments (Table 1). It can be seen from the data that the OTUs, Chao1 and Shannon indexes in the rhizosphere soil treated by *R. solanacearum* were significantly lower than those in the CK and T2 groups. Compared with the CK group, the OTUs and Chao1 indexes of the *B. amyloliquefaciens* ZM9 treatment were slightly higher. The results indicate that *R. solanacearum* treatment affects the richness and diversity of soil bacterial communities to a certain extent, and its antagonistic antibacterial treatment can improve the richness and diversity of soil bacterial communities.

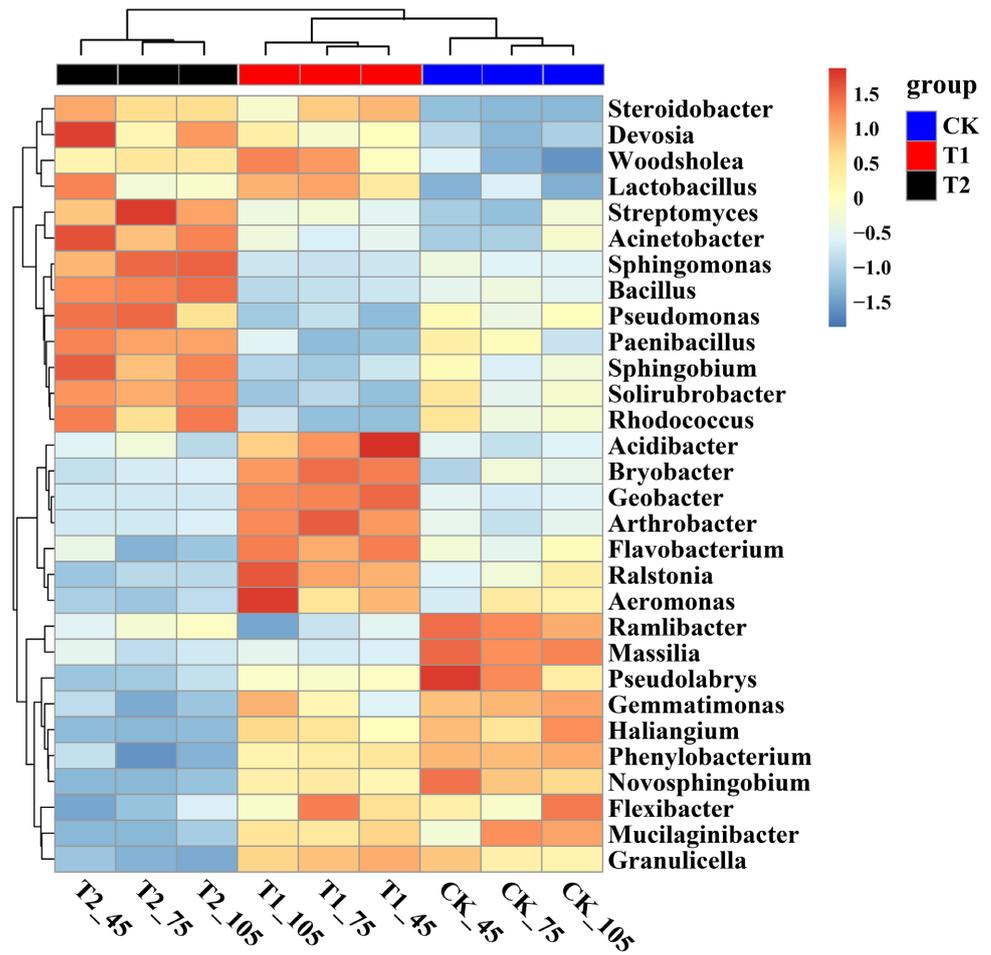
3.3. Bacterial Community Composition in Soils

In tobacco soil samples of different treatments, *Proteobacteria* accounted for 42% - 51.2% of the top 10 bacteria. The second was *Acidoobacteria* (18.35% - 23.64%), *Actinobacteria* (4.41% - 7.89%), *Gemmatimonadetes* (3.41% - 5.32%), *Bacteroidetes* (2.21% - 3.11%), *Chloroflex* (1.41% - 2.51%), *Thaumarchaeota* (1.21% - 2.01%), *Verrucomicrobia* (0.51% - 1.71%), *Firmicutes* and *Cyanobacteria* (0.71% - 1.41%). The relative abundance of *Proteobacteria*, *Acidobacteria*, *Actinomyces* and *Bacteroidetes* was more than 4% (Figure 3(A)). Principal coordinate analysis (PCoA) was performed using weighted UniFrac distance in different treatments. PC1 and PC2 accounted for 66.76% of the total bacterial community. The bacterial communities in different time periods of CK group were clustered together, the corresponding bacterial communities in different time periods of





(B)



(C)

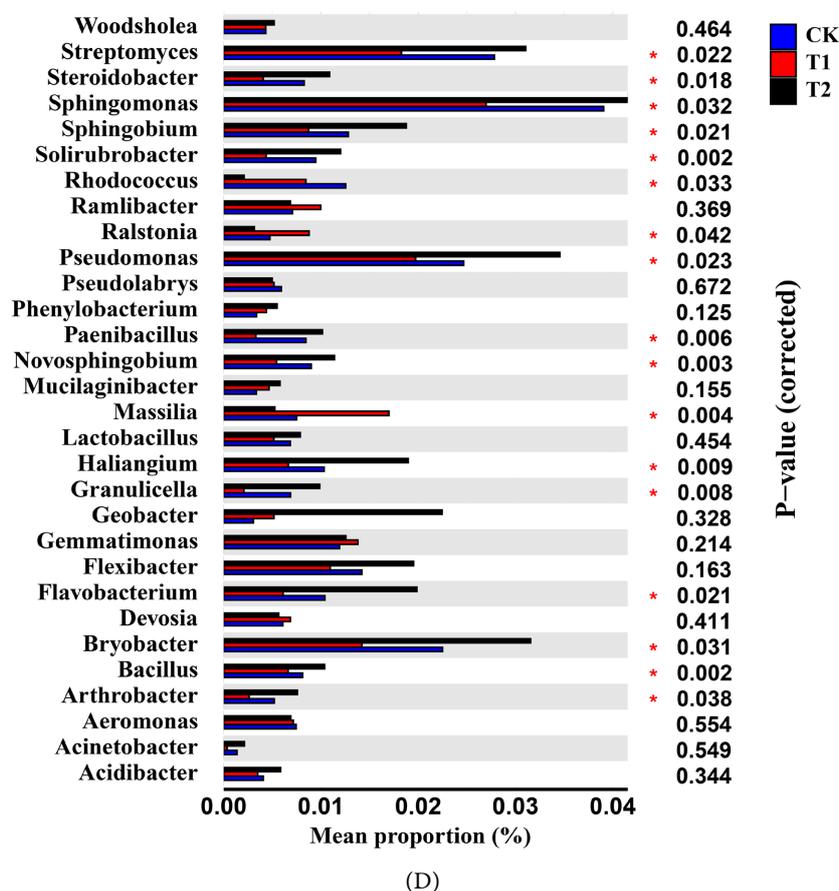


Figure 3. Soil bacterial communities of the three treatments. (A) The Principal coordinate analysis (PCoA) of soil bacterial community; (B) The relative abundance of bacterial phyla in soil samples; (C) Hierarchical cluster analysis of predominant bacterial genera. (CK: ddH₂O treatment; T1: *R. solanacearum* treatment; T2: *B. amyloliquefaciens* ZM9 treatment); (D) The relative abundances of the top 30 classified bacterial genera among different treatments. The abundances of different genera were analyzed by one-way ANOVA. * $p < 0.05$, ** $p < 0.01$.

Table 1. Alpha diversity index of bacteria of three treatments at 45 d, 75d and 105d post-transplanted, respectively.

Samples	Times	OTUs	Shannon	Chao1
CK	45 d	3642.48 ± 240.25	9.19 ± 0.18	3668.33 ± 68.11
	75 d	3710.03 ± 253.33	9.81 ± 0.14	4104.01 ± 131.3
	105 d	3767.25 ± 96.51	9.42 ± 0.13	4093 ± 188.51
T1	45 d	3510.25 ± 82.89	8.89 ± 0.11	3402.01 ± 167.76
	75 d	3524.27 ± 123.43	9.25 ± 0.13	3526.33 ± 123.58
	105 d	3634.41 ± 106.30	8.93 ± 0.18	3262.33 ± 45.74
T2	45 d	3870.33 ± 48	9.37 ± 0.27	3962.02 ± 59.03
	75 d	3882 ± 95.25	9.31 ± 0.06	4239.35 ± 142.98
	105 d	3961 ± 160.22	10.19 ± 0.24	4267.91 ± 92.51

The observed OTUs, Shannon diversity and Chao1 richness of bacterial communities in the soil are expressed as mean ± SE. CK: ddH₂O treatment, T1: *R. solanacearum* treatment; T2: *B. amyloliquefaciens* ZM9 treatment.

T1 and T2 groups were clustered together, and the bacterial communities in different treatment groups were separated from each other (**Figure 3(B)**). There were differences in bacterial community structure among different treatments in heat map. The abundance of Acidibacter, Bryobacter, Geobacter, Arthrobacter, Flavobacterium, Ralstonia, Aeromonas and Flexibacter increased with the treatment of *R. solanacearum*; Devosia, Streptomyces, Acinetobacter, Sphingomonas, Bacillus, Pseudomonas, Sphingobium and Rhodococcus were added to the treatment of *B. amyloliquefaciens* ZM9. These results indicate that the application of different microorganisms has an effect on the structure of bacterial community (**Figure 3(C)**). The distribution of the first 30 abundance bacterial genera in the three treatments is shown in the figure. Among them, Sphingobium, Pseudomonas, Haliangium and Bryobacter were dominant in the treatment of *B. amyloliquefaciens* ZM9, while the proportion in T1 was lower (**Figure 3(D)**).

3.4. Fungal Diversity in Soil

In total 679,451 high-quality raw sequences with the average length of 252 bps for fungus were obtained from rhizospheric soil samples after quality filtering. The OTUs, Chao1 and Shannon indexes were used to evaluate and compare the richness and diversity of fungal communities under different treatments (**Table 2**). It can be seen from the data that the indexes of OTUs and Shannon in the rhizosphere soil treated by *R. solanacearum* were slightly lower than those of the CK and T2 groups. Compared with CK group, Shannon and Chao1 index slightly lower *B. amyloliquefaciens* ZM9 process. The results show that the addition of different microorganisms affects the richness and diversity of soil fungal communities to a certain extent.

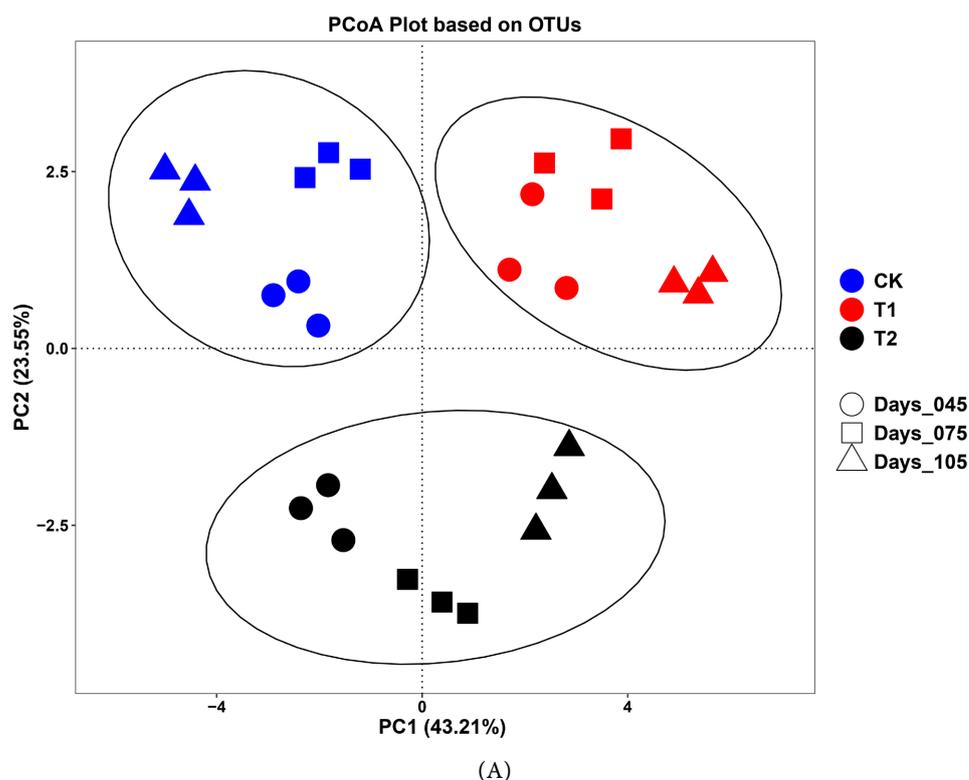
Table 2. Alpha diversity index of fungal of three treatments at 45 d, 75d and 105d post-transplanted, respectively.

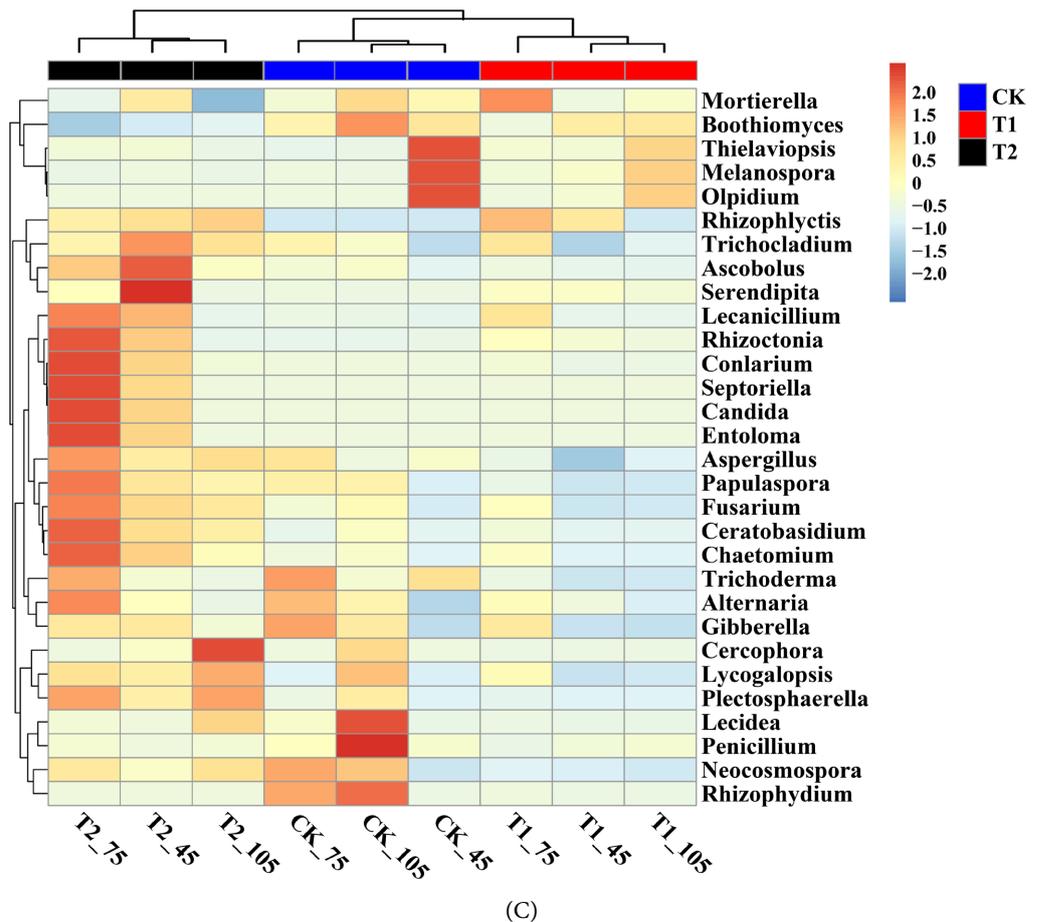
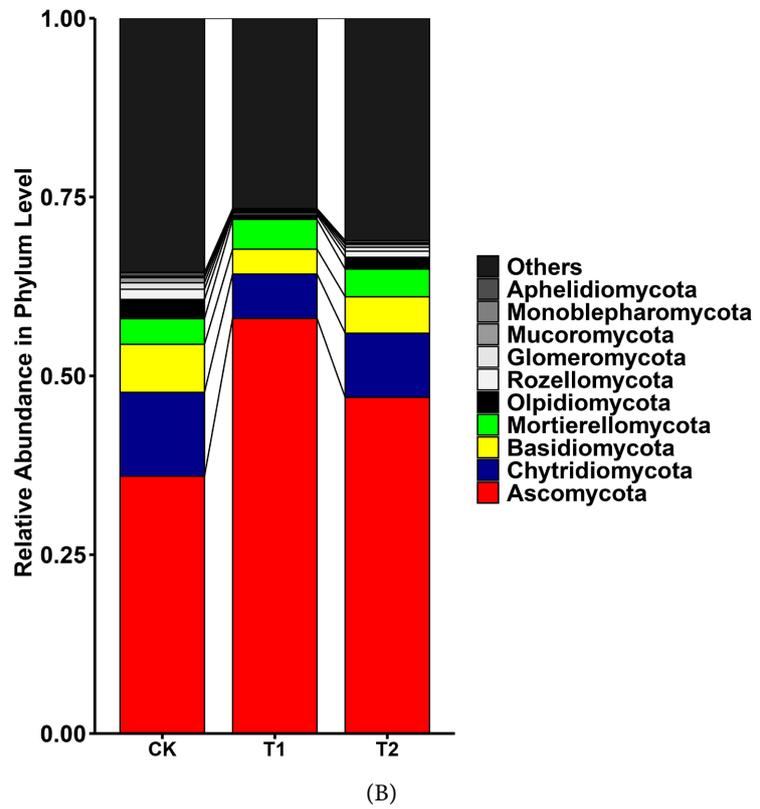
Samples	Times	OTUs	Shannon	Chao1
CK	45 d	551.67 ± 38.63	5.24 ± 0.38	536.52 ± 35.48
	75 d	625.33 ± 4.93	6.09 ± 0.13	631.31 ± 13.64
	105 d	665.33 ± 11.85	5.15 ± 0.16	740.29 ± 22.31
T1	45 d	471.67 ± 33.02	4.24 ± 0.32	550.88 ± 32.73
	75 d	523 ± 47.29	4.61 ± 0.26	456.81 ± 23.46
	105 d	460.33 ± 36.9	3.81 ± 0.17	480.17 ± 13.38
T2	45 d	548.67 ± 47.48	4.88 ± 0.28	457.71 ± 60.68
	75 d	559.67 ± 23.63	5.08 ± 0.54	518.96 ± 49.89
	105 d	552.67 ± 38.59	4.71 ± 0.76	624.2 ± 39.09

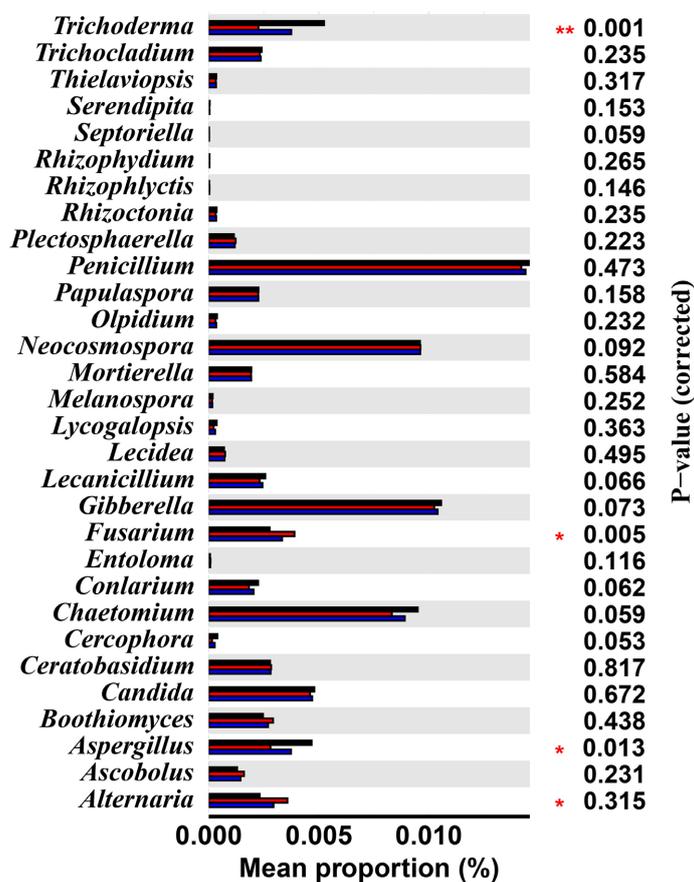
The observed OTUs, Shannon diversity and Chao1 richness of Fungal communities in the soil are expressed as mean ± SE. CK: ddH₂O treatment; T1: *R. solanacearum* treatment; T2: *B. amyloliquefaciens* ZM9 treatment.

3.5. Fungal Composition in Soils

In tobacco soil samples with different treatments, *Ascomycota* accounted for 35.12% - 62.8% of the top 10 fungi phyla. Followed by *Aphelidiomycota* (6.52% - 12.64%), *Basidiomycota* (4.34% - 8.9%), *Mortierelomycota* (2.12% - 3.61%), *Olpidiomycota* (1.02% - 1.23%), *Rozellomycata*, *Glomeromycota*, *Mucoromycota*, *Monoblepharomycota* and *Aphelidiomycota*. Among the 10 dominant bacteria, the relative abundance of *Ascomycota*, *Aphelidiomycota* and *Basidiomycota* exceeded 4% (Figure 4(A)). Using the weighted UniFrac distance for principal coordinate analysis (PCoA) in different treatments, PC1 and PC2 explained 66.76% of the total fungal community. The fungal communities of the CK group at different time periods gathered together, the corresponding fungal communities of the T1 and T2 groups at different time periods gathered together, and the fungal communities of different treatment groups were separated from each other (Figure 4(B)). There are differences in the fungal community structure between different treatments in the heat map. The treatment of *R. solanacearum* did not make much difference in the fungal community; while the treatment of *B. amyloliquifaciens* ZM9 increased the abundance of *Ascobolus*, *Serendipita*, *Rhizoctonia*, *Conlarium*, *Papulasporiella*, *Candida*, *Entoloma*, *Entoloma*, *Ceratobasidium* and *Chaetomium*. The two treatments are very different from the CK group. These results indicate that different microorganisms have a greater impact on the structure of the fungal community (Figure 4(C)). Further analysis was carried out at the genus level, and the different distributions of the first 30 abundance fungal genera in the three treatments are shown in the figure. There







(D)

Figure 4. Soil fungal communities of the three treatments. (A) Principal coordinate analysis of soil fungal communities (PCoA); (B) Relative abundance of fungi in soil samples; (C) Hierarchical cluster analysis of main fungal genera (CK: ddH₂O treatment; T1: *R. solanacearum* treatment); T2: *B. amyloliquefaciens* ZM9 treatment); (D) The relative abundance of the first 30 fungal genera among different treatments. The abundance of different genera was analyzed by one-way analysis of variance. * $p < 0.05$, ** $p < 0.01$.

were significant differences in 4 fungal genera among the 3 treatments, namely *Trichoderma*, *Fusarium*, *Aspergillus* and *Alternaria* (Figure 4(D)).

3.6. The Relationship between Root Exudates and Microbial

Redundant analysis (RDA) was used to analyze the relationship between root exudates and microbial community structure under different treatments. The results showed that the community variation of bacteria and fungi was 39.85% and 43.96%, respectively (Figure 5). Benzoic acid, propionic acid, sebacic acid, caprylic acid, palmitic acid, oxalic acid, myristic acid, and 3-hydroxybutyric acid are positively correlated with the bacteria *Ralstonia*, and negatively correlated with the bacteria *Sphingobium*, *Sphingomonas*, *Haliangium*, *Flavobacterium*, *Paenibacillus*, and *Bacillus* (Figure 5(A)). Sphingosine, oxalic acid, benzoic acid, sebacic acid, propionic acid, myristic acid, 3-hydroxybutyric acid, palmitic acid are positively correlated with the fungi *Fusarium* and *Alternaria*, and negatively

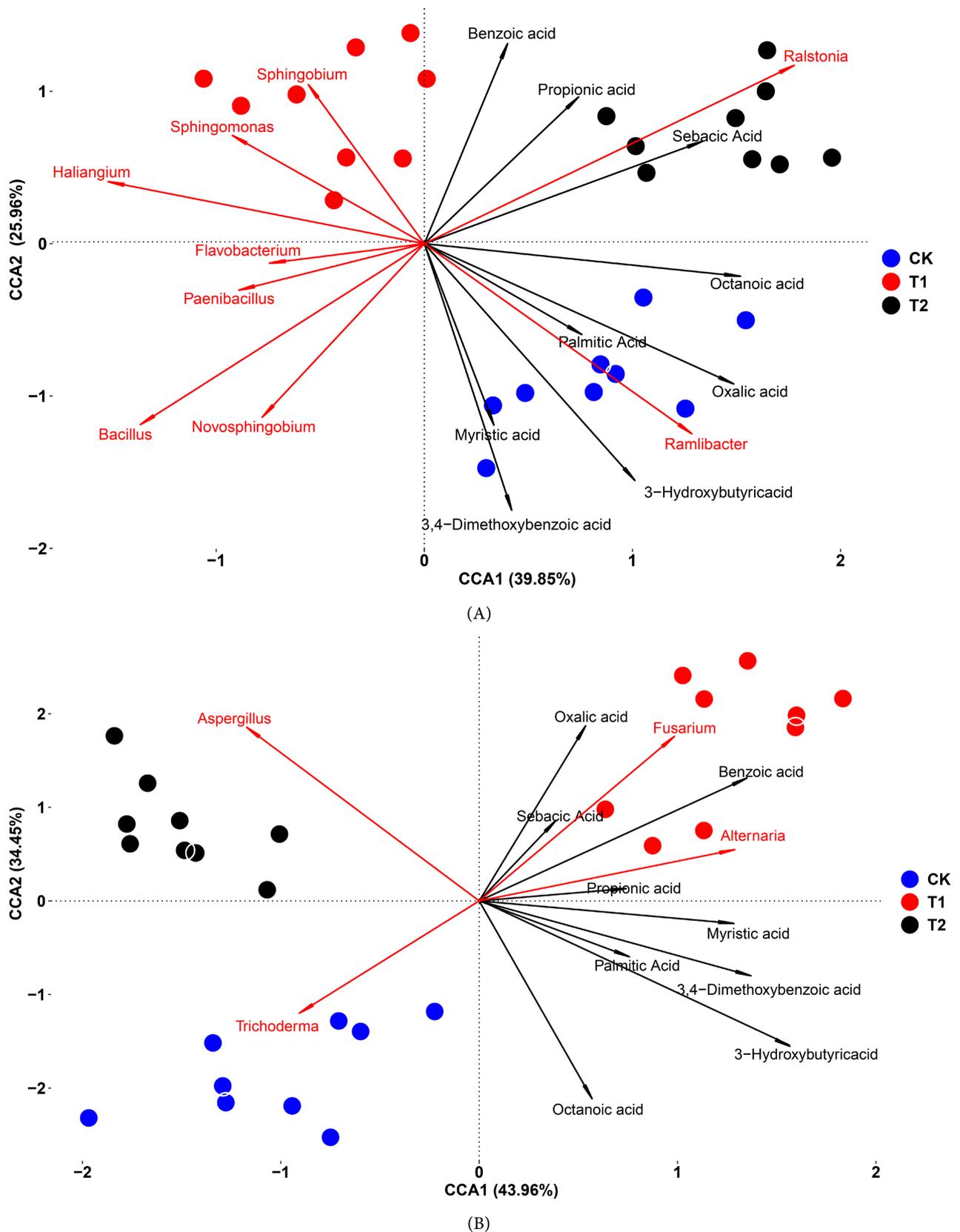


Figure 5. Redundancy analysis of root exudates and rhizosphere microbial communities under different treatments ((A) bacterial community; (B) fungal community).

correlated with the fungi *Aspergillus* and *Trichoderma* (Figure 5(B)). Redundancy analysis shows that the microbial community has a greater impact on soil root exudates.

4. Discussion

Plant-root exudates-rhizosphere microbes form a cyclic interaction relationship [23]. Rhizosphere microorganisms transform the organic nutrients of root exudates into inorganic nutrients to promote plant absorption and utilization, thereby affecting the comprehensive indicators of plants [24] [25] [26] [27]. By analyzing the changes in tobacco root exudates under the action of different microorganisms, the study found that when the pathogenic microorganisms in tobacco rhizosphere microorganisms increase, tobacco root exudates increase significantly. When the abundance of bacterial wilt in the rhizosphere increases, organic acids and amines increase greatly. When adding *B. amyloliquefaciens* ZM9, the composition of root exudates changed significantly, adding 24 kinds of ingredients, but thiodiglycol, 1-butanol, sebacic acid, benzimidate, hydrochloride, valtrate, dicarboxylic acid and cyclopropane disappeared completely. Although the mechanism of action of propionic acid, myristic acid, ethyl benzoate and sebacic acid is not clear, studies have confirmed that terephthalic acid, isophthalic acid and benzoic acid have allelopathic and autotoxic effects on many crops. The significant increase in pathogen abundance is an important factor leading to continuous cropping obstacles [28] [29] [30] [31] [32]. It is speculated that the possible mechanism is the increase in pathogen abundance [33], which induces crops to produce allelopathic autotoxins, thereby providing harmful bacteria, forming a vicious circle, and ultimately leading to continuous cropping obstacles. Although this conjecture needs to be further confirmed, this study provides new clues for exploring tobacco continuous cropping obstacles and soil-borne diseases.

5. Conclusion

Comprehensive treatment results of *R. solanacearum* and *B. amyloliquefaciens* ZM9 found that the change of root exudates has little to do with the time of tobacco transplantation, and is mainly affected by microorganisms. Comprehensive analysis found that stearic acid, propionic acid, oxalic acid, caprylic acid, myristic acid, itaconic acid, isophthalic acid, dodecanoic acid, benzoic acid, 3-hydroxybutyric acid and 3,4-dimethoxy Benzoic acid is greatly affected by different microorganisms. Redundant analysis (RDA) analysis results show that benzoic acid, propionic acid, sebacic acid, caprylic acid, palmitic acid, oxalic acid, myristic acid and 3-hydroxybutyric acid are positively correlated with the pathogens of *R. solanacearum*. GC-MS analysis results also show that bacterial wilt pathogens can significantly increase the content of acids and amines, while *B. amyloliquefaciens* ZM9 can eliminate some acids and amines. The results of the bacterial alpha diversity index show that the treatment of *R. solanacearum*

affects the richness and diversity of the soil bacterial community to a certain extent, and the treatment of its probiotic *B. amyloliquefaciens* ZM9 can increase the richness and diversity of the soil bacterial community. The research results show that the root exudates caused by the pathogen of *R. solanacearum* contain a variety of allelopathic substances, and *B. amyloliquefaciens* ZM9 can eliminate some allelopathic substances and enrich the soil microbial community.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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