

Organic Mulching Can Suppress Litchi Downy Blight Through Modification of Soil Microbial Community Structure and Functional Potentials

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Abstract

Organic mulching is an important management practice in agricultural production to improve soil quality, control crop pests and diseases and increase the biodiversity of soil microecosystem. However, the information about soil microbial diversity and composition in litchi plantation response to organic mulching and its attribution to litchi downy blight severity was limited. This study aimed to investigate the effect of organic mulching on litchi downy blight, and evaluate the biodiversity and antimicrobial potential of soil microbial community of litchi plantation soils under organic mulching. Our results showed that organic mulching could decrease the disease incidence in the litchi plantation. As a result of high-throughput 16S rRNA and ITS rDNA gene illumine sequencing, higher bacterial and fungal community diversity indexes were found in organic mulching soils, the relative abundance of norank f norank o Vicinamibacterales, norank f Vicinamibacteraceae, norank f Xanthobacteraceae, Unclassified c sordariomycetes, *Aspergillus* and *Thermomyces* were significant more than that in control soils. Isolation and analysis of antagonistic microorganism showed that 29 antagonistic bacteria strains and 37 antagonistic fungi strains were unique for mulching soils. Thus, we believe that organic mulching has a positive regulatory effect on the litchi downy blight and the soil microbial communities, and so, is more suitable for litchi plantation.

Introduction

As one important management practice in agricultural production, organic mulching is mainly used for soil improvement and environmental protection. The application of mulching derived from plant residues could not only increase water infiltration [1], prevent soil nutrient loss [2] and suppress weed germination [3], but also control crop pests and diseases [4, 5] and increase the biodiversity of soil microecosystem [6].

Soil microbial communities play a critical role in nutrient transformation and cycling, and soil aggregation. The diversity and structure of soil microorganism was associated with soils of varying texture, N content, P content, carbon availability and soil PH, and changes in microbial communities and structure response to agricultural practices can be used as early indicator of soil “health” and “quality” [7, 8]. Moreover, soil microorganism was sensitive and easily affected by the changes of soil physicochemical properties created by mulching process, and close ties between the composition of soil microbial communities and mulching pattern was found [9]. Previous studies reported strong changes in richness and diversity of soil microbial community regulated by organic mulching in vegetable and tea plantation [10, 11].

While soil microorganism consisted of large community, such as endophytes, symbionts, pathogens, and plant growth promoting rhizobacteria. Along with improving soil health and increasing plant’s responses to abiotic stress by altering defense and metabolic pathways, the soil microbiota also provides an important role in suppressing plant disease [12, 13]. The ability of a soil to suppress disease is of key core in measuring soil productivity. There are many studies focused on the disease suppression linked to

the soil microbial diversity. For example, the decrease of soil microbial diversity was responsible for the development of soil-borne bacterial wilt diseases of tomato [14], otherwise, the high functional redundancy in soil microbial diversity enables wilt resistance in tomato [15].

Litchi (*Litchi chinensis* Sonn.), a tropical and subtropical fruit species that widely cultivated in Southeast Asia. Litchi crops are subject to downy blight during blooming and fruiting stages, which leads to great economic losses [16, 17]. Organic mulching has been reported to involved with improvement of soil physicochemical properties in litchi orchard [18]. However, to our knowledge, the information about soil microbial diversity and composition in litchi plantation with organic mulching and its effect on litchi downy blight was limited.

In the present study, one field trail was performed to investigate the litchi downy blight under different management methods. Additionally, based on 454-pyrosequencing of the fungal internal transcribed spacer (ITS) region and the bacterial 16S rRNA gene, a comparative microbiome analysis of soils was investigated in the same litchi plantation treated with/without organic mulching. Moreover, the relationship between soil bacterial, fungal community structure and disease-suppression were discussed. This study not only explore the efficacy of organic mulching on the control of litchi downy blight, but also provided theoretical support for the application of organic mulching in litchi plantation.

Materials And Methods

Field experiments

The field experiments were conducted from 2018 to 2020 at Xili Orchard in Shenzhen of China. The cultivated litchi is 'Nuomici'. The field was divided into two blocks and the treatments were: (1) the control group (CK): bare soil in conventional tillage methods, (2) the mulching group (T): soil covered with litchi shredded branches and in 8–10 cm depth. To discover disease incidence and shifts in the soil microbiome, same conventional cultivation was carried out in the control group and mulching group.

Disease incidence discovery

In 2018 and 2020, disease incidence was investigated by calculating the disease incidence of dropped fruits and the litchi fruit on trees. During April and May of 2018, dropped fruits in the control field and mulching field were collected and put into plastic boxes to keep humidity. After 2–3 d, fruitlets covered with white mold were counted and the disease incidences were examined. Each treatment was conducted by collecting fruits from 5 different trees for one repeat, and at least 200 fruits for one repeat was calculated. In June 2020, 15 trees in the control group and mulching group were randomly selected and each repeat contained 5 trees. Disease incidence of the trees were determined by calculating 30 fruits in each of the four directions (east, south, west and north) of each tree, and diseased fruit with downy white sporangiophores were counted.

Soil sampling collection

For each treatment, 4 different soil samples from different trees were collected. The soils from 15 cm depth were sterile plastic bags and transported to the laboratory in an icebox immediately. The samples were stored at -80 °C until high-throughput sequencing and analysis.

DNA extraction

Aliquots (0.25 g) of the soil samples were processed using a MOBIO PowerSoil® kit. The extracted DNA samples were analyzed using a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The DNA quality was confirmed by 1% agarose gel electrophoresis. The extracted DNA samples were selected and used to conduct microbial community analysis by PCR using primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWT CTAAT-3') for 16S rDNA in bacteria, and primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') for ITS in fungi. The PCR reactions were performed in triplicate, using 20 µL mixtures containing 4 µL 5× FastPfu buffer, 2 µL 2.5 mM dNTPs, 1 µL primer mix (5 µL), 0.4 µL FastPfu polymerase, and 5 ng extracted DNA as the template. The PCR products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). The products were quantified using QuantiFluor-ST (Promega, Madison, USA). Purified amplicons were then pooled in equimolar concentrations and paired-end sequenced (2 × 300) using the Illumina MiSeq platform (Illumina, San Diego, CA, USA) according to the standard protocols of Shanghai Majorbio Bio-pharm Technology Co., Ltd. Raw sequences were filtered using FASTX Toolkit 0.0.12 software to remove low quality reads with Q value < 20 and less than 35 bp.

Illumina sequencing and processing of sequencing data

The purified amplicons were pooled on the Illumina MiSeq platform (Illumina, San Diego, USA) of equal molecular weight and paired-end sequencing (2 × 300) according to the standard protocol of MajorbioBio-Pharm Technology Co. Ltd. (Shanghai, China). The original sequencing sequence was controlled using Trimmomatic software and merged by FLASH software. The specific criteria are consistent with previous study [19]. UPARSE (version 7.1; <http://drive5.com/uparse/>) was used to cut the similarity of the operational classification units (OTUs) to 97%. <http://drive5.com/uparse/> uses a novel “greedy” algorithm that could perform chimera filtering and OTU clustering at the same time. Using the confidence threshold of 70%, the classification of each 16S rRNA gene sequence was analyzed against the Silva database (Release132; <http://www.arb-silva.de>) through the RDP classifier algorithm (<http://rdp.cme.msu.edu/>). Using the confidence threshold of 70%, the classification of each ITS sequence was analyzed against the Unite database (version 7.2; <http://unite.ut.ee/index.php>) through the RDP classifier algorithm.

Selection and identification of biocontrol bacteria and fungi

Soils from litchi plantation treated with organic mulching for 2 years or without organic mulching were collected and used for bacteria and fungi isolation. Serial dilution method was used to isolate bacteria by dissolving soil samples (25 g) with sterile water (100 mL) in a 250 mL sterilized conical flask and shaking for 30 min using a rotary shaker at 150 rpm, then the resulting solutions were serially diluted up to 10^{-3} ,

spread on LB plates and incubated at 30 °C for 24 h for bacteria inoculation, and incubated on PDA plates for 3 d at 25 °C for fungi inoculation [20]. Singal colonies were transferred to new LB plates (for bacteria) or PDA plates (for fungi) and used for antagonistic microorganism selection.

The antagonistic abilities of tested bacteria isolates were determined by primary and second screening as shown in Fig. 6A, and the potential antagonistic activity of fungi isolates were measured as shown in Fig. 7A. After incubating on PDA plates for 6 d, the diameters of the pathogen zone of mycelium growth inhibition around bacteria were measured and recorded.

Then, the bacteria or fungi with markedly inhibitory activity were selected and identified by amplifying 16S rRNA gene sequence of tested bacteria using universal primers 27F and 1492R, and amplifying internal transcribed spacer (ITS) of tested fungi using universal primers ITS1 and ITS4 [21, 22]. The obtained PCR products were sequenced and the sequences were subjected to Basic Local Alignment Search Tool (BLAST) searches using the National Center for Biotechnology Information (NCBI) database (<http://www.ncbi.nlm.nih.gov>) to compare with other sequences deposited in GenBank.

Results

Organic mulching could suppress litchi downy blight

To explore the effect of organic mulching on the inhibition of litchi downy blight, disease incidence of litchi planation under organic mulching or conventional tillage methods were investigated. The results showed a significant decrease in the disease incidence of litchi downy blight after the application of organic mulching (Fig. 1). In the investigation on April and May of 2018, the disease incidence of dropped fruits was significantly lower in mulching group than that of in control group (CK), which presented the reducing value of 7.19 % and 8.70 %, respectively. For the disease investigation on the fruit on the tree, the disease incidence in the control field (3.61 %) was significantly higher than that in the organic mulching field (1.51 %). Our results inferred that organic mulching could delayed the development of litchi downy blight, which maybe attributed the modification of soil microbial community.

Changes in the diversity of soil bacterial and fungal community

In order to determine the response of soil microorganism to organic mulching, the bacterial and fungal diversity of soil samples treated with/without organic mulching were assessed using phylotype taxonomy. A total of 627,647 high-quality reads of bacteria and 740,047 high-quality reads of fungi were remained in the dataset with the average length of 417 bp and 244 bp, respectively. Through clustering operations, the optimized sequences were classified into operational taxonomic units (OTUs) according to their similarity. With a 3% dissimilarity threshold, the sequences were classified into 8632 and 2175 OTUs in bacterial and fungal communities using the Ribosomal Database Project (RDP) classifier. The Venn diagrams showed that mulching soils under different period (T1, T2 and T3) exhibited a greater

number of OTUs than control soils (CK) (Fig. 2). In bacterial communities, the numbers of OTUs in T1, T2 and T3 were more than that in CK, and that in T2 was the highest (Fig. 2A). In fungal communities, the numbers of OTUs in three mulching treatments (T1, T2 and T3) were significantly greater than that in CK, and the highest number of fungal OTUs was 1308 which detected in T3 (Fig. 2B).

To quantify the diversity and richness of microbial community of soils among different treatments, the microbial community α -diversity were evaluated by the Ace, Chao1, Shannon and Simpson within a single microbial ecosystem, is shown in Table 1. The coverage indexes from 24 soil samples were greater than 0.97, showed that the sequencing capacity were acceptable. The richness (Ace and Chao1 indices) and diversity (Shannon and Simpson) of bacteria and fungi calculated based on the rarefied sequences showed that mulching treatment and different mulching period affected the bacterial and fungal communities. In bacterial communities, the richness (Ace and Chao1 indices) and Shannon diversity of T3 was higher than that in CK, T1 and T2 soils; while in fungal communities, the richness (Ace and Chao1 indices) of CK was higher that in mulching soils (T1, T2 and T3). However, the Simpson diversity of CK in bacterial community was slight higher than that in mulching soils (T1, T2 and T3), while in fungal community, the Simpson diversity of CK was significantly lower than that in mulching soils (T1, T2 and T3).

Table 1
The diversity and richness indices of soil bacterial and fungal communities

	CK	T1 (1 year)	T2 (1.5 years)	T3 (2 years)
Bacteria				
Ace	3965.47 ± 60.20	4920.88 ± 66.58	5004.96 ± 61.42	5388.96 ± 56.98
Chao1	3889.78 ± 37.22	4863.81 ± 50.06	5025.71 ± 11.38	5086.18 ± 44.44
Shannon	6.67 ± 0.05	6.98 ± 0.03	6.95 ± 0.01	7.05 ± 0.06
Simpson (%)	0.33 ± 0.01	0.22 ± 0.01	0.25 ± 0.01	0.22 ± 0.01
Coverage (%)	97.65 ± 0.14	97.12 ± 0.11	97.16 ± 0.45	97.07 ± 0.09
Fungi				
Ace	983.32 ± 19.59	764.41 ± 33.17	732.07 ± 35.70	888.89 ± 9.81
Chao1	989.24 ± 17.79	778.13 ± 51.10	748.22 ± 38.41	886.31 ± 10.85
Shannon	4.63 ± 0.16	3.25 ± 0.39	4.11 ± 0.20	3.97 ± 0.24
Simpson (%)	2.42 ± 0.51	16.49 ± 2.09	4.67 ± 0.76	5.47 ± 0.30
Coverage (%)	99.81 ± 0.03	99.69 ± 0.02	99.81 ± 0.06	99.77 ± 0.04

To get a better insight into the differences of the soil microbial communities, the principal coordinates analysis (PCoA) based on the Bray-Curtis distance was applied to evaluate the microbial community β -

diversity. As shown in Fig. 3A, samples of CK and T3 were distributed separately at 52.63% and 15.57% on the pCoA vector x and y axes for the bacterial community, while T2 and T3 were contiguous but distinct from CK and T3. Likewise, the pCoA variation (39.61% for PC1 and 19.44% for PC2) accounted for the fungal community across all samples (Fig. 3B). All soil samples distinct from the others except for T1 and T2, demonstrated that large microbial community differences affected by organic mulching and different mulching period.

Bacterial communities in the Soil

Obvious differences in the composition and diversity of the bacterial communities were found across the soil. As shown in Fig. 4A, Proteobacteria, Actinobacteria, Acidobacteria and Chloroflexi, in rank order, were the abundant phyla in both treatments, and Proteobacteria, Acidobacteria and Bacteroidota in mulching soils (T1, T2 and T3) were significantly larger than in control soil (CK). At the genus level, relative abundance of top 10 bacterial communities was identified, and top 3 microbial communities (norank_f_norank_o_Vicinamibacterales, Norank_f__Vicinamibacterace -ae, Norank_f__Xanthobacteraceae) significantly increased in response to organic mulching (Fig. 4B).

Fungal communities in the Soil

To dissect the taxonomic composition of fungal communities in the soil, relative abundance of the dominant fungal communities at phylum and genus levels were aligned. At the phylum level, Ascomycota was significantly dominated across both treatments, and its relative abundance in T2 and T3 treatment significantly higher compared to control soil (CK) (Fig. 5A). Notably, organic mulching markedly increased the relative abundance of *Thermomyces*, *Aspergillus* and *Acremonium* in the soil, whereas, the relative abundance of *Neocosmospora* in fungal community were significantly decreased with the increasing period of mulching treatment (Fig. 5B).

Identification of antagonistic bacteria contributing to antimicrobial activity

The richness and species of antagonistic bacteria were positively associated with the survival of pathogen. From the primary and second screening, a total of 50 antagonistic bacteria strains were obtained, with 18 strains were common in both treatments, and 29 and 3 strains were unique for mulching soils and control soils (CK), respectively (Fig. 6B). Based on the sequence similarities to the 16S rRNA gene sequences, 8 different species of antagonistic bacteria with excellent antimicrobial efficacy were identified. These were *Burkholderia gladioli*, *Leuconostoc mesenteroides*, *Paenibacillus polymyxa*, *Bacillus subtilis*, *B. altitudinis*, *B. velezensis*, *B. amyloliquefaciens*, and *B. vallismortis* (Fig. 6C).

Identification of antagonistic fungi contributing to antimicrobial activity

To evaluate the antimicrobial activity of enrichment fungal cultures against *P. litchii*, primary and second screening test were conducted. In total, 52 antagonistic fungal strains were obtained, with 13 strains were common in both treatments, and 37 and 2 strains were unique for mulching soils and control soils (CK), respectively (Fig. 7B). As a result of ITS sequences alignment, eight major species of antagonistic fungi with excellent antimicrobial efficacy were identified from organic mulching soils, which belonging to *Trichoderma* sp. and *Penicillium* sp. (Fig. 7C).

Discussion

Organic mulching was widely adopted in agricultural cultivation for a long time. The general effect of mulching on soils has also been fully recognized [23]. However, in terms of the influence of organic mulching on the control of disease and the composition of microorganisms in soils, the depth of understanding is far from enough [6].

The results of the present study indicated that organic mulching application led to a significant decline in the disease incidence of litchi downy blight, along with an increase in the abundance of soil bacterial and fungal community. The findings were in line with numerous studies on other crops that practices of bio-organic fertilizer application raised soil microbial diversity and enhance plant disease suppression [24, 25]. Therefore, the modification of soil microbial community composition and functional potentials might contribute to the increase of disease suppression on litchi downy blight under organic mulching application.

The structure and functions of soil microbial community are closely related to soil quality and ecosystem stability and sustainability, which are crucial for plant health and productivity [26]. Various studies demonstrated the alteration of soil microbial community was closely associated with soil suppressiveness to soilborne pathogenic fungi [27, 28]. Wu et al. highlighted the close association between replant disease and the variations in structure and potential functions of rhizosphere bacterial community [29]. Yang et al. indicated that soil microbial diversity had a strong effect on tobacco wilt disease level [30]. In addition, any modifications in soil microbial community assemblages will have a cascade of effects on soil structure and nutrient cycling, including soil aggregate stability and decomposition processes [31, 32].

Soil microbiota is considered to be a critical factor to regulate soil quality and sustainability [33], its community and diversity are significant involved with soil-plant health via triggering different functional roles, which including decomposing organic matter, ecosystem regulators, and biological antagonism [34, 35]. In this study, we found that organic mulching application could increase the soil bacterial community diversity and enhance the populations of beneficial microorganisms. Similarly, the phyla Proteobacteria, Actinobacteria, Acidobacteria and Chloroflexi, which are consistently predominant bacteria in tillage soil [36], are dominant bacteria in our study. Proteobacteria plays an important role in straw decomposition and soil nutrient uptake [37], and Acidobacteria is considered to have extensive metabolic and genetic functions [38]. Therefore, the relative abundance of phyla Proteobacteria and Acidobacteria increased

after organic mulching application might be attribute to organic matter incorporation. The fungal taxonomic composition and richness of functional fungi in the soils under organic mulching methods also significantly higher than that in bare soils with conventional tillage methods. The dominant *Aspergillus* and *Thermomyces* genera were observed under organic mulching application. Certain affiliated genera including *Thermomyces* spp. are the key contributors to the hemicellulose hydrolysis during root-surrounding decomposition, which supply nutrients to microbial system in the soils [39].

Litchi downy blight caused by *P. litchii* is one of the most destructive diseases in litchi planation. As the important origin of primary infection, residual pathogens on dropped-blossoms and fruits play crucial role in epidemic of litchi downy blight [40]. Our findings indicated that organic mulching application reduced disease incidence and manipulated the soil microbial community structure, especially the increasing bacterial families such as *Burkholderia*, *Bacillus*, and *Paenibacillus*, which have been proved to possess antagonistic activities and contribute to disease suppressiveness [41–43]. Moreover, the frequent occurrence of specific beneficial fungal species, such as *Trichoderma* sp. and *Penicillium* sp., reflect the survival of *P. litchii* in planting system. Several studies revealed the excellent beneficial effects of *Trichoderma*, *Aspergillus* and *Penicillium* for pathogen control [44–47]. Their fungal abundance may be also related to the suppression of litchi downy blight through mycoparasitism fabrication of lytic enzymes and antimicrobial chemicals and competing for space and food, which was consistent with mentioned results of the reduction of litchi downy blight in organic mulching soils. On the other hand, organic mulching application led to a significant decrease in the relative abundance of the genus *Fusarium*, which is the major crop pathogens in the world and attribute to many soilborne disease through colonizing root surfaces, producing some plant irritant and some secondary metabolite during growth and metabolism [48], exhibited lower abundance in organic mulching soils in comparison with control soils. Therefore, organic mulching might have exerted positive feedback effects on litchi planation and resistance through inhibiting potential soil borne pathogens and fostering plant-beneficial microbes which targeting the causal agent *P. litchii*.

In conclusion, the findings in this study revealed that the litchi downy blight was closely associated with dysbiosis of soil microbiota. The organic mulching application could delay this disease mainly depend on reshaping the soil microbial community and modifying the potential functions microbes that harbor antagonistic activities against *P. litchii* and contribute to soil suppressiveness. Our results reinforce the influence of organic mulching on disease control and soil microbial diversity in litchi plantation from the aspects of microbial structure and ecological function. Further work is needed to investigate the survival of causal agent in the presence of beneficial microorganisms and to link the potential functions to organic mulching application.

Conclusions

Organic mulching is capable of modifying soil microbial community by contributing to higher bacterial and fungal community diversity, and lower the abundances of plant-harmful microbes which potential as plant pathogens, thus improved soil-plant growth productivity. Moreover, litchi downy blight was highly

delayed after the application of organic mulching, which attributed to the improvement of abundances of plant-beneficial microbes that significantly contributed to antagonizing *Peronophythora litchii*. Our work further demonstrated the structural alteration of soil bacterial and fungal communities and thus explained the positive correlation with suppression of litchi downy blight in response to organic mulching, providing a suitable method for litchi plantation.

Declarations

Declarations

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Figures

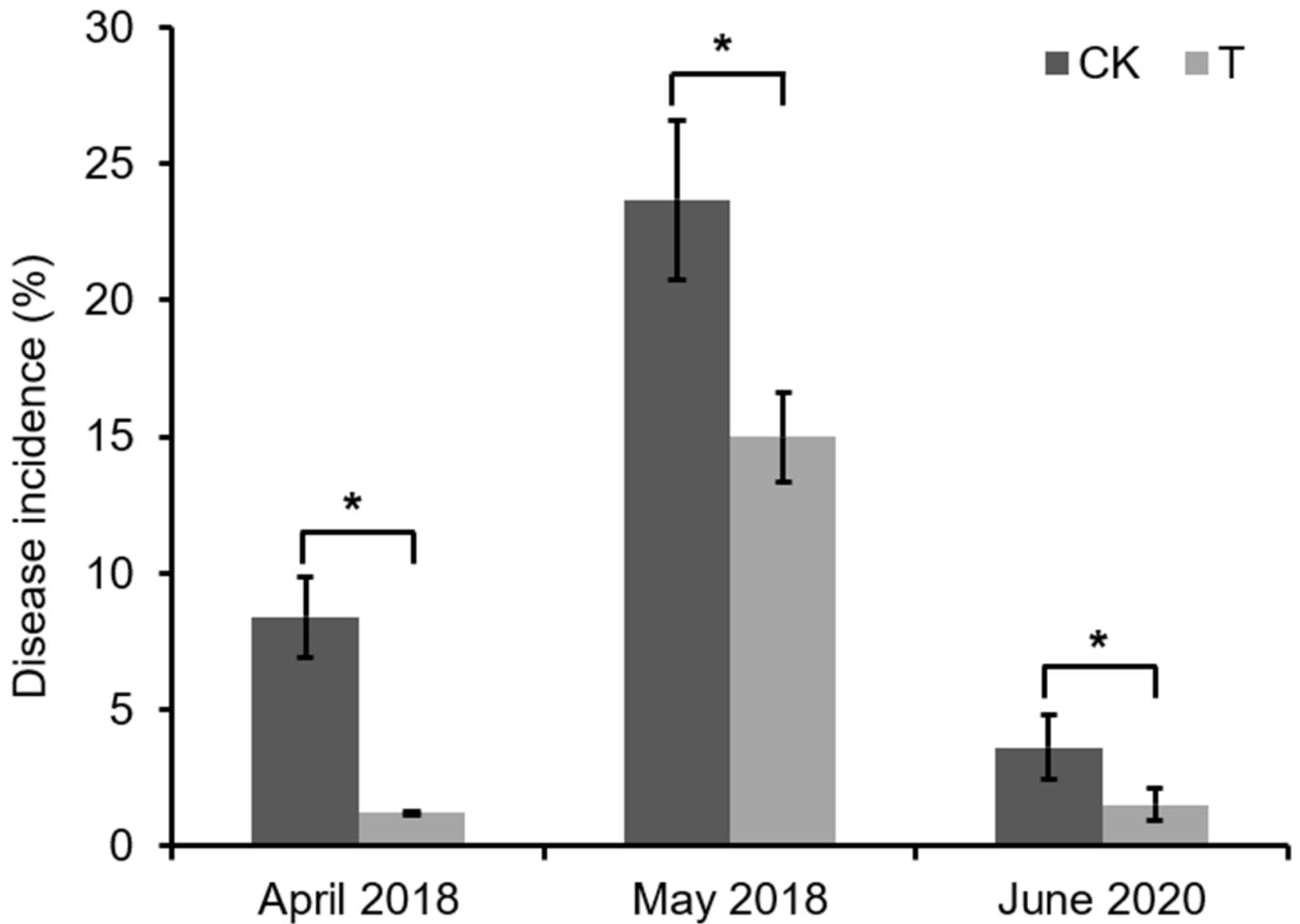


Figure 1

Disease incidence of litchi downy blight from control group (CK) and mulching group (T) in 2018 and 2020. For 2018, disease incidence of dropped fruit was investigated on April and May. For 2020, disease incidence of fruit on the tree in different group was conducted on June. Results are represented as the mean of all replicates \pm standard error (SE). The asterisks (*) indicate significant difference between different treatments.

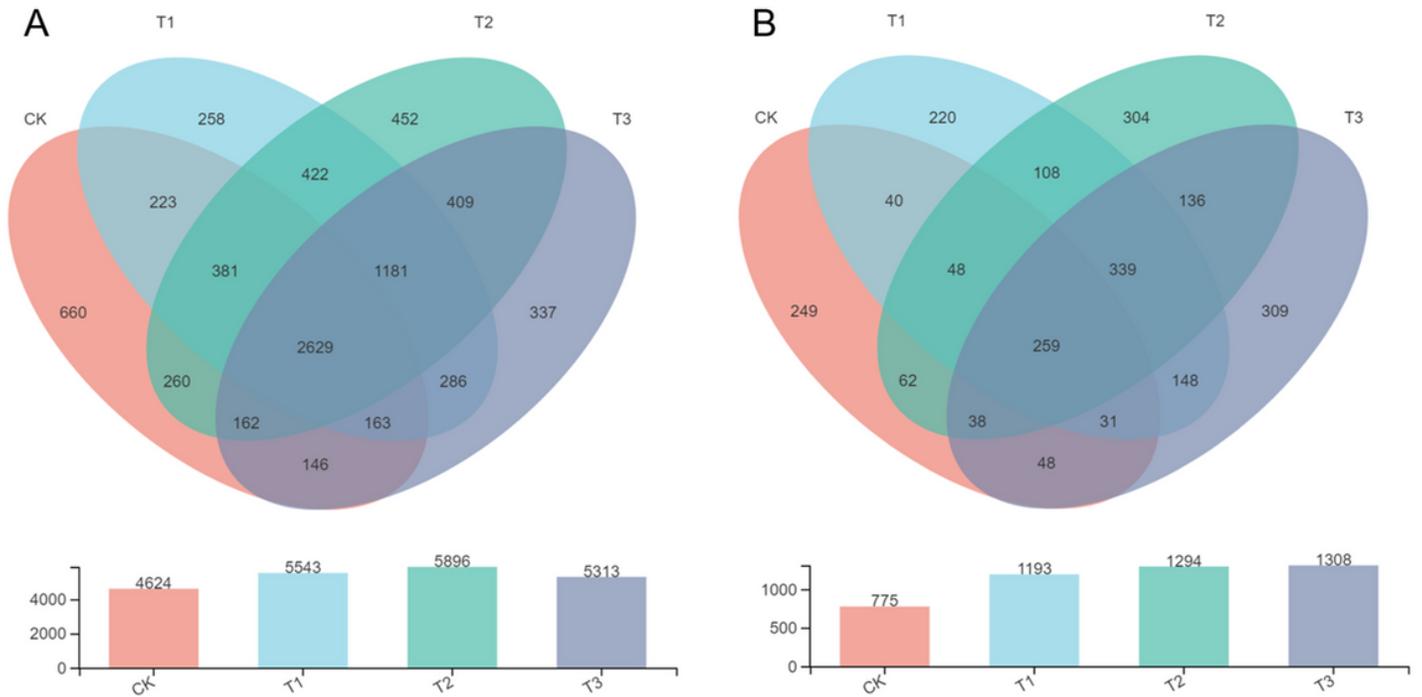


Figure 2

The Venn diagram of microbial communities in soils under different treatments. A, the number of bacterial OTUs in soils under different treatments; B, the number of fungal OTUs in soils under different treatments. CK, bare soil in conventional tillage methods; T1, treated with mulching for 1 year; T2, treated with mulching for 1.5 years; T3, treated with mulching for 2 years.

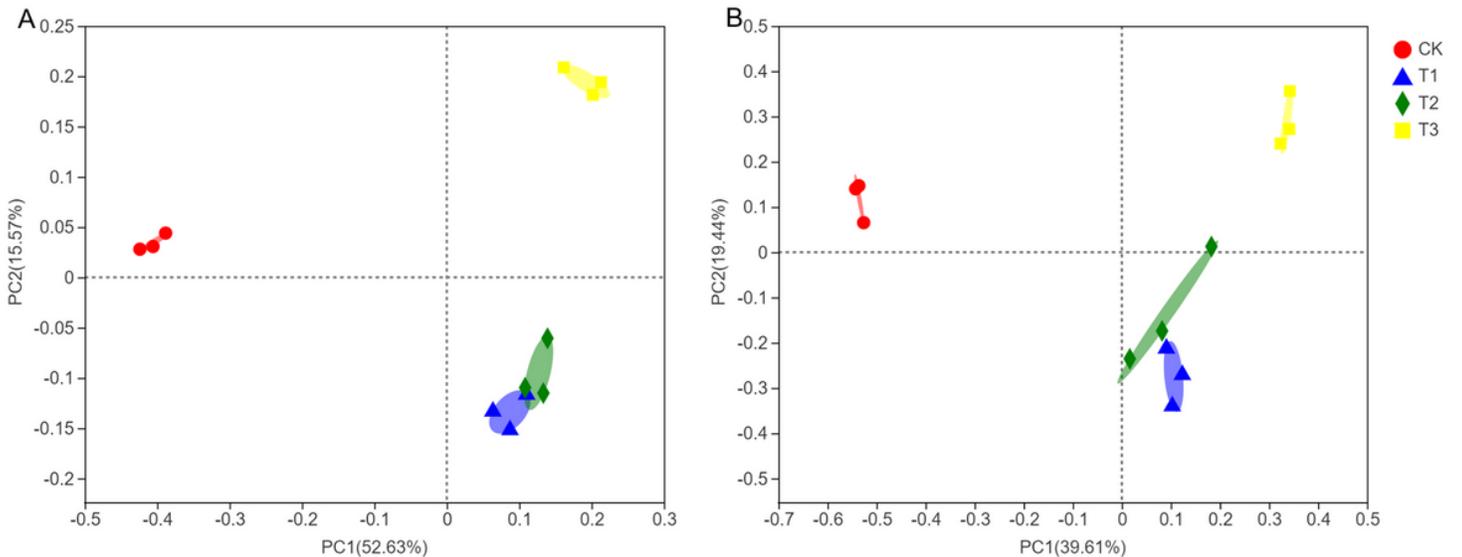


Figure 3

The principal co-ordinates analysis (pCoA) based on the Bray-Curtis distance in microbial communities in soils under different treatments. A, the distribution of bacterial communities in soils under different treatments; B, the distribution of fungal communities in soils under different treatments. CK, bare soil in

conventional tillage methods; T1, treated with mulching for 1 year; T2, treated with mulching for 1.5 years; T3, treated with mulching for 2 years.

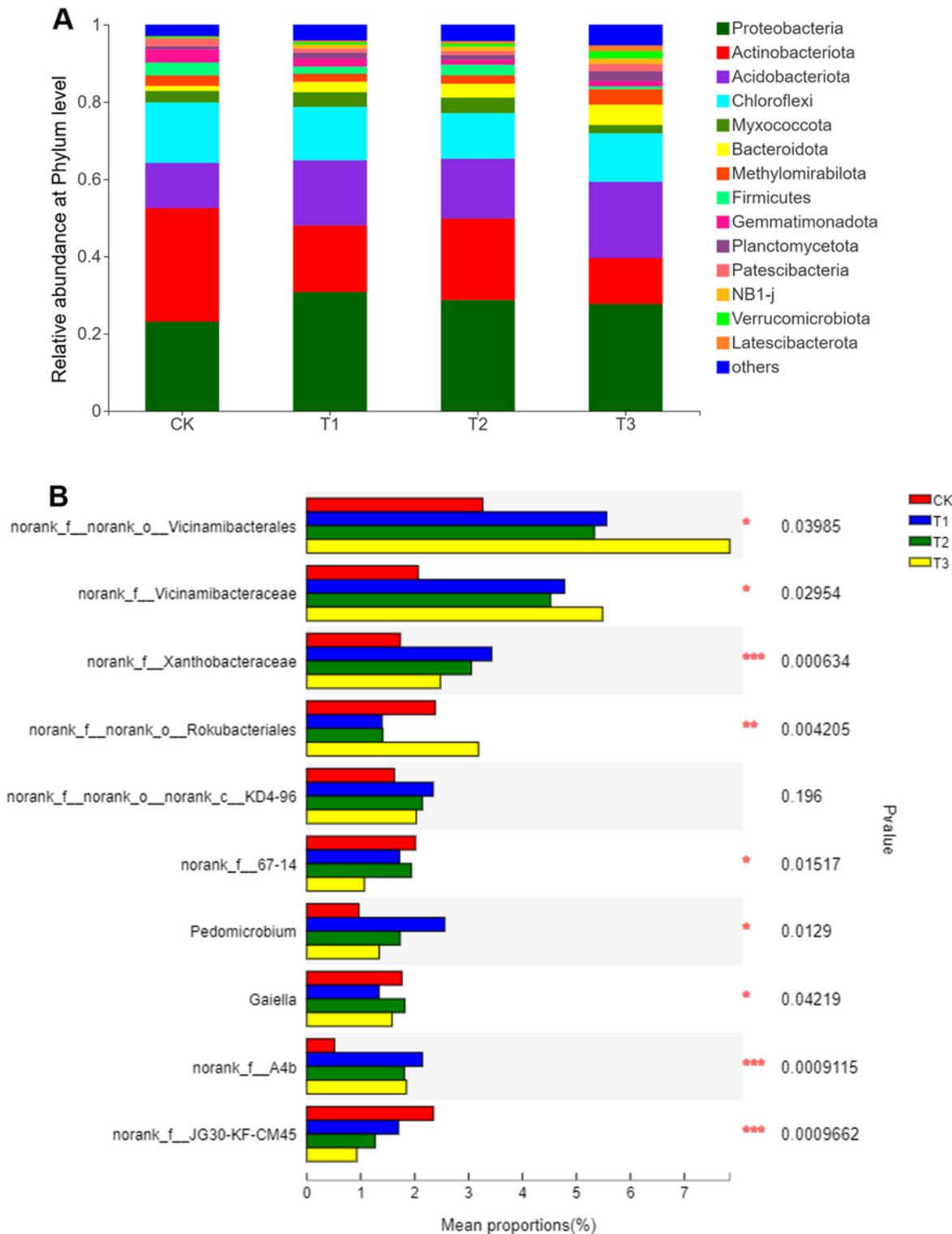


Figure 4

The relative abundance of main bacterial communities in soil under different treatments. A, relative abundance of the dominant bacterial community at phylum level; B, relative abundance of top 10 bacterial community at genus level. The “others” comprise the unclassified and low-abundance phyla. CK,

bare soil in conventional tillage methods; T1, treated with mulching for 1 year; T2, treated with mulching for 1.5 years; T3, treated with mulching for 2 years. The Scheffé's value cutoff was 0.95, $***p \leq 0.001$, $**0.001 < p \leq 0.01$, and $*0.01 < p \leq 0.05$.

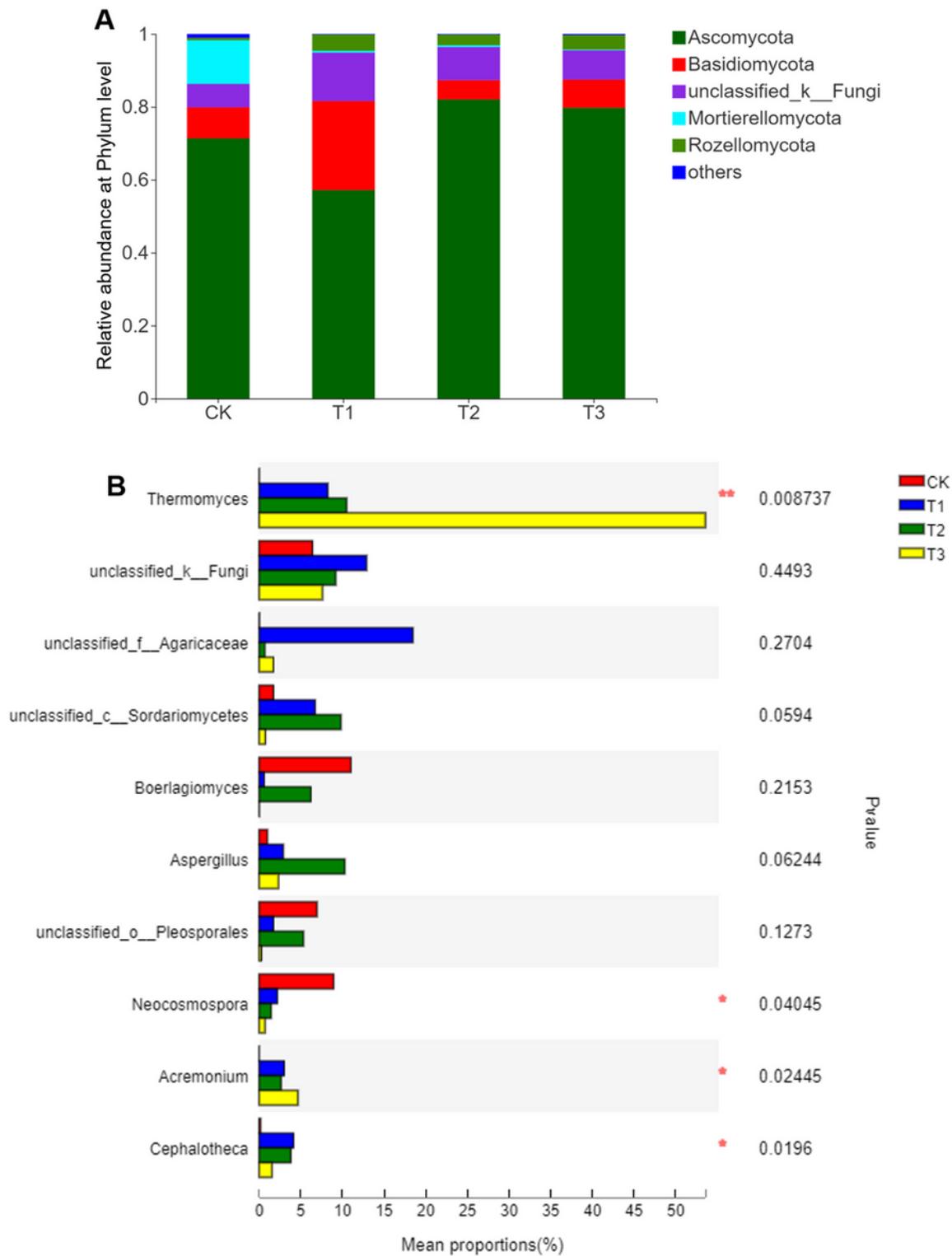


Figure 5

The relative abundance of main fungal communities in soil under different treatments. A, relative abundance of the dominant fungal community at phylum level; B, relative abundance of top 10 fungal

community at genus level. The “others” comprise the unclassified and low-abundance phyla. CK, bare soil in conventional tillage methods; T1, treated with mulching for 1 year; T2, treated with mulching for 1.5 years; T3, treated with mulching for 2 years. The Scheffe’s value cutoff was 0.95, *** $p \leq 0.001$, ** $0.001 < p \leq 0.01$, and * $0.01 < p \leq 0.05$.

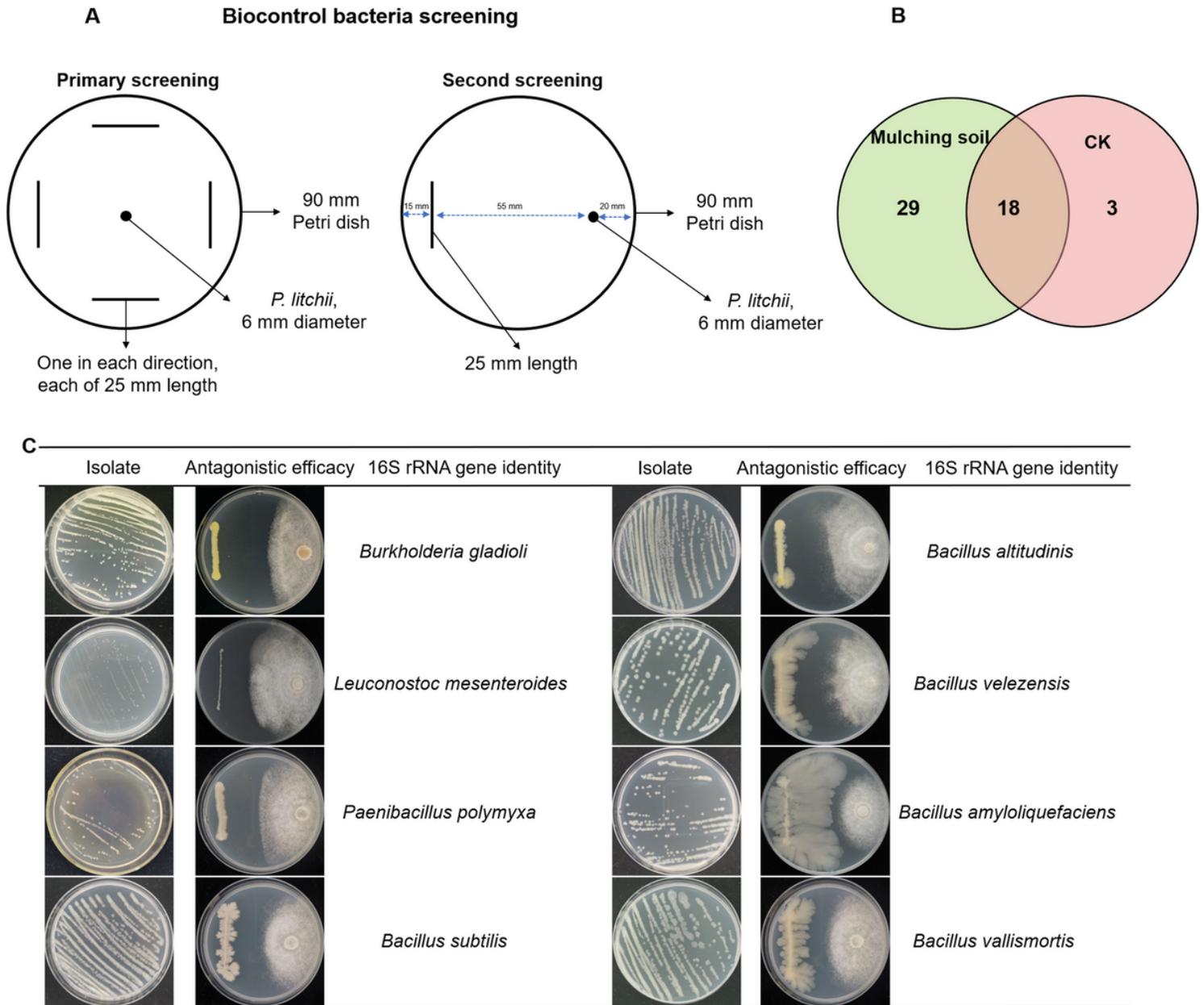


Figure 6

Screening and characterization of antagonistic bacteria isolates detected in this study. A, the method of biocontrol bacteria screening; B, Venn diagram for common and unique strains detected from mulching soils (mulching for 2 years) and control soils (CK, bare soil in conventional tillage methods); C, Characterization of antagonistic bacteria isolates detected in this study.

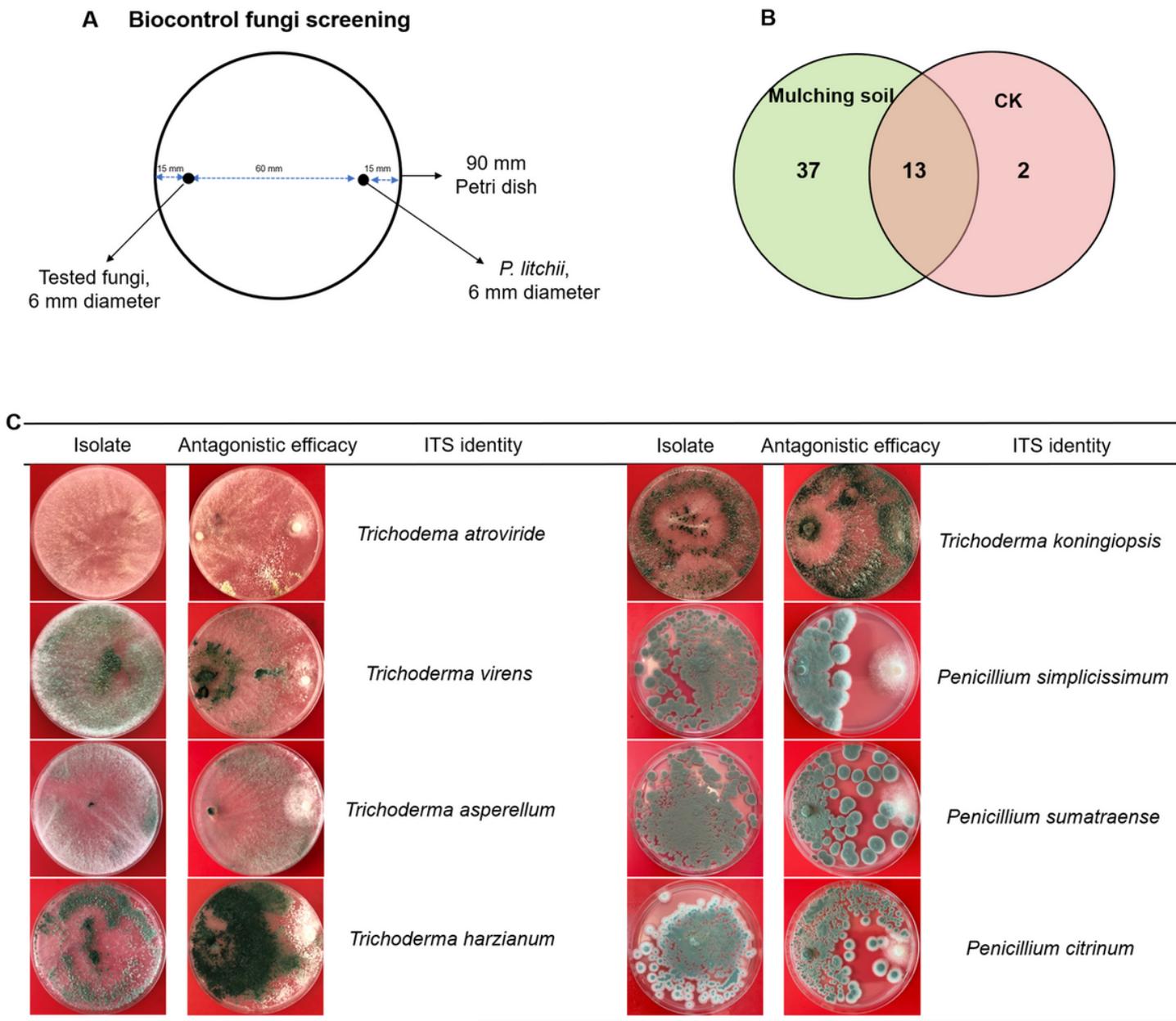


Figure 7

Screening and characterization of antagonistic fungi isolates detected in this study. A, the method of biocontrol fungi screening; B, Venn diagram for common and unique strains detected from mulching soils (mulching for 2 years) and control soils (CK, bare soil in conventional tillage methods); C, Characterization of antagonistic fungi isolates detected in this study.