

# Effect of different continuous cropping years on the rhizosphere soil microbial community structure of *Tussilago farfara* L.

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## Research Article

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# Abstract

The rhizosphere soils from 1, 2, and 3 years of *Tussilago farfara* L. continuous cropping were used as the research objects. The region of bacterial 16S rRNA and fungal ITS was analyzed by Illumina MiSeq high-throughput sequencing technology. The effect of continuous cropping years on the microbial community structure and diversity in *Tussilago farfara* L. soil was investigated. The result shows that rhizosphere soil fast-acting nutrient content, alkaline phosphatase, and sucrase activity gradually decreased, urease activity increased first and then decreased, and catalase activity did not change significantly with the increase of cropping years. The number and diversity of rhizosphere soil bacteria increased first and then decreased, while the fungal species increased by 22.5% after continuous cropping for two years compared with rotational cropping. Among the bacteria, the relative abundance of Proteobacteria and Acidobacteria decreased significantly after continuous cropping, and the relative abundance of Actinobacteria and Chloroflexi increased; among the fungi, the relative abundance of Ascomycota and Basidiomycota decreased significantly after continuous cropping, while the relative abundance of Mortierellomycota increased. The population type gradually changed from bacterial to fungal, and the relative abundance of beneficial genera such as *Ralstonia*, *Nitrospira*, and *Trichoderma* gradually decreased, while harmful genera such as *Mortierella*, *Fusarium*, and *Tricharina* accumulated in large quantities. This study reflects the dynamic balance of rhizosphere soil bacterial-fungal communities of *Tussilago farfara* L. in different continuous cropping years, and the changes of nutrient content, enzyme activity, and genera reflect the possible genera associated with the occurrence of cropping obstacles of *Tussilago farfara* L., as well as the relevant groups of bacteria to maintain the stability of microbial communities, which provides a scientific reference basis for further investigation of cropping obstacles and screening of beneficial genera.

## 1 Introduction

*Tussilago farfara* L. belongs to Asteraceae perennial herb, whose dry flower buds can be used as medicine <sup>[1–2]</sup>. It mainly contains sesquiterpenes, flavonoids, sterols, and other components <sup>[3]</sup>, Pharmacological research shows that Farfarae flos has the function of suppressing cough, lining the lungs, resolving phlegm and anti-inflammatory <sup>[4]</sup>, which is an important raw material for a variety of proprietary Chinese medicines, and is known as “the most ancient and modern prescriptions for treating cough with warm lung” <sup>[5]</sup>. Because of its important medicinal value, it is cultivated in Gansu, Sichuan, Shanxi, Hubei, and other places in China <sup>[6–8]</sup>, and is one of the genuine medicinal materials in Gansu, with a total planting area of about 1 600 hm<sup>2</sup> in 2020 and annual market demand of 1 550 t, the supply of Farfarae flos occupies more than 80% of the total national demand, with large market demand and good economic benefits <sup>[9]</sup>.

Due to the over-harvesting and depletion of wild resources in recent years, most of them are now sporadically distributed. Artificial planting is also not optimistic, because the production process requires a lot of manual labor digging, sorting, picking buds, drying, etc., high production costs, time-consuming

and labor-intensive, the area of artificial cultivation has decreased year by year, according to the report, the planting area of *Tussilago farfara* L. in Gansu Province in 2020 is about 30% less than in 2019 <sup>[10]</sup>. At the same time, it is difficult to eradicate the rhizomes after harvesting, farmers often choose to bury them underground after harvesting and harvest them again the following year. In the long run, the same plot is planted repeatedly in the same year, and the continuous cropping obstacle of *Tussilago farfara* L. has become increasingly prominent, seriously affecting its yield and quality, and becoming a major constraint and urgent problem in production. At present, the research on *Tussilago farfara* L. mainly focuses on morphological observation, pharmacological efficacy and cultivation improvement of cultivation measures, the causes and internal mechanisms of cropping obstacles have not been reported, there are numerous studies have shown that rhizosphere soil microbial community structure is one of the main causal factors for the development of cropping obstacles <sup>[11–12]</sup>. In this study, 16S rDNA and ITS sequencing were performed on rhizosphere soil using a high-throughput sequencing platform to reveal the changes in rhizosphere soil microbial community in different continuous cropping years, which is of great significance for the investigation of the causes and internal mechanisms of continuous cropping obstacles.

## 2 Material and methods

### 2.1 Overview of the test area

The experimental site is located in elevated Kan Village, Majiaji Town, Weiyuan County (E 103°6'47", N 35°49'41"), with a high cold and shady climate, a n altitude of 2 352 m, an average annual precipitation of 600–700 mm, a frost-free period of 130 d, and a soil texture of chestnut-calcium soil.

### 2.2 Test materials

The planting material is a high-quality rhizome of *Tussilago farfara* L. with three nodes, white color, and tender without diseases and pests, which purchased from the medicinal seed seedling market in Huichuan Town, Dingxi City and identified as *Tussilago farfara* L. in the family *Asteraceae* by Jin Xiaojun, a researcher from the College of Agronomy, Gansu Agricultural University.

### 2.3 Experimental design

In this experiment, three plots that had not been planted *Tussilago farfara* L., planted *Tussilago farfara* L. for one year, and planted *Tussilago farfara* L. for two years were selected as the sample plots for planting *Tussilago farfara* L., forming three treatments of rotational cropping (T1), continuous cropping for one year (T2), and continuous cropping for two years (T3), with adjacent plots and 15° slope, and the three treatments were divided into three plots of equal size (15 m × 10 m), totaling nine plots. Before planting, the conventional fertilization amount (N:288 kg·hm<sup>-2</sup>, P:211.5 kg·hm<sup>-2</sup>, K:210 kg·hm<sup>-2</sup>) was applied as the base fertilizer, and the direct planting technique with the rows were spaced 25 cm apart, the plants were spaced 15 cm apart, the holes were 10 cm deep was followed in mid-to-late March 2021 <sup>[13]</sup>, the soil

was mulched and harrowed immediately after planting with 1 m protection rows around. Other field managements were the same.

## 2.4 Collection of rhizosphere soil samples

After harvesting (November 26, 2021), the rhizosphere soil was collected from the rotational cropping, continuous cropping for one year, and continuous cropping for two years using "S" type five-point sampling, mixed thoroughly in quadratic method, and divided into two parts by removing stones and dead leaves in sterile bags: one part was stored in the refrigerator at -80°C for subsequent microbiological determination; the other part was placed at room temperature and dried naturally for the determination of soil nutrients and enzyme activity.

## 2.5 Soil nutrient and enzyme activity determination

Soil nutrients: pH by pH meter potentiometric method (water-soil ratio 5:1); organic matter by potassium dichromate volumetric external heating method; alkali-hydrolyzed nitrogen by alkaline diffusion method; available phosphorus by molybdenum antimony anti-colorimetric method; available potassium by ammonium acetate leaching - flame photometric method.

Soil enzyme activity: soil catalase activity by potassium permanganate titration method; soil urease activity by sodium phenol-sodium hypochlorite colorimetry; soil sucrase activity by 3,5-dinitro salicylic acid colorimetry; soil alkaline phosphatase activity by sodium benzyl phosphate colorimetry.

## 2.6 Sample DNA extraction and high-throughput sequencing

The composition and diversity of rhizosphere soil bacterial and fungal communities were determined by 16S rDNA and ITS amplicon sequencing techniques<sup>[14–16]</sup>. The following methods are as follows:

Total genome DNA from rhizosphere soil samples was extracted using CTAB method. DNA concentration and purity was monitored on 1% agarose gels. According to the concentration, DNA was diluted to 1 ng/μL using sterile water. The specific primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3') were selected for the V3 + V4 variable region of the bacterial 16S rRNA gene, the ITS1-1F-F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS1-1F-R (5'-GCTGCGTTCTTCATCGATGC-3') specific primers targeting the internal transcribed spacer (ITS) I region of the fungal ribosomal coding gene PCR amplification was performed (Bio-rad T100, Burroughs, USA). Amplification procedure: 1 min pre-denaturation at 98°C, 30 cycles (10 s denaturation at 98°C, 30 s annealing at 50°C, 30 s extension at 72°C), 5 min single extension at 72°C, termination and storage at 4°C. PCR reaction systems were 15 μL Phusion Master Mix (2 × ) buffer, 10 μL gDNA (1 ng/μL), 1 μL Forward Primer (0.2 μM/μL), 1 μL Reverse Primer (0.2 μM/μL) and ddH<sub>2</sub>O to 30 μL. PCR reactions were performed in triplicate, and PCR products were purified and identified using 2% agarose gel electrophoresis (1 × TAE) (The product purification kit was Qiagen Gum Recovery Kit (Qiagen, USA)). The PE libraries (Illumina's TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA)) were constructed using the standard protocol of Agilent 2100 Technologies Co., Ltd. (USA), and the libraries were

sequenced after being quantified and tested by Qubit. The DNA extraction and PCR amplification were performed at Shenzhen Weikemen Technology Group Co. (Shenzhen, China) using the Illumina Novaseq platform for sequencing analysis.

## 2.7 Data analysis

After sequencing, the raw data were denoised, spliced and de-chimerized by QIIME2 system using qiime tools import program(Callahan et al. 2016), and subjected to OTU clustering analysis, Alpha diversity analysis, species composition analysis, Beta diversity analysis, and species difference analysis(Bokulich et al. 2018). Among them, sequence splicing was performed using FLASH, sequence filtering using QIME, and clustering using UPARSE pipeline software. IBM SPSS Statistics 26.0 software was used for multiple comparisons and significance analysis, and Excel 2019 was used for the statistical organization of data and graphical analysis, and data in tables and graphs are expressed as mean  $\pm$  standard error (Mean  $\pm$  SE), and different lowercase letters are used to indicate significant differences between treatments in the graphs ( $P < 0.05$ ).

## 3 Results and analysis

### 3.1 Analysis of rhizosphere soil nutrient content of *Tussilago farfara* L. in different continuous cropping years

As shown in Table 1, the differences in the content of each nutrient in rhizosphere soil under continuous cropping conditions were statistically significant ( $P < 0.05$ ). The rhizosphere soil pH, organic matter, and available phosphorus contents decreased year by year with the increase of continuous cropping years, among which T3 decreased by 3.18%, 27.52%, and 34.85%, respectively, compared with T1; the contents of available potassium and alkali-hydrolyzed nitrogen showed an overall decrease with the increase of continuous cropping years, T2 decreased by 52.42% and 43.69%, respectively, compared with T1, and T3 was slightly higher than T2, but did not reach a significant level ( $P > 0.05$ ).

Table 1  
Effects of different continuous cropping years on the nutrient content in rhizosphere soil of *Tussilago farfara* L.

| Treatment | pH           | Organic matter (g·kg <sup>-1</sup> ) | Available Phosphorus (mg·kg <sup>-1</sup> ) | Available Potassium (mg·kg <sup>-1</sup> ) | Alkali-hydrolyzed nitrogen (mg·kg <sup>-1</sup> ) |
|-----------|--------------|--------------------------------------|---------------------------------------------|--------------------------------------------|---------------------------------------------------|
| T1        | 8.39 ± 0.01a | 23.38 ± 1.28a                        | 20.86 ± 2.44a                               | 110.00 ± 2.31a                             | 144.20 ± 4.04a                                    |
| T2        | 8.33 ± 0.04a | 22.05 ± 0.44b                        | 17.34 ± 0.82b                               | 52.33 ± 4.81b                              | 81.20 ± 4.04b                                     |
| T3        | 8.12 ± 0.17b | 16.95 ± 0.95c                        | 13.59 ± 1.03c                               | 52.67 ± 3.38b                              | 83.53 ± 2.33b                                     |

### 3.2 Analysis of rhizosphere soil enzyme activity of *Tussilago farfara* L. in different continuous cropping years

As shown in Table 2, the soil sucrase and alkaline phosphatase activities decreased year by year with the increase of continuous cropping years, and the average annual decline rate reached 54.41% and 8.00%, respectively; catalase activity did not show significant changes and remained between 1.89 ~ 1.99 mL (g·h)<sup>-1</sup>; urease activity increased first and then decreased, T2 increased by 16.79% compared with T1, and T3 decreased by 58.75% compared with T2. Among them, both catalase and urease activities increased in the first year of continuous cropping and decreased significantly in the second year of continuous cropping; sucrase and alkaline phosphatase activities were the lowest in the plots in the second year of continuous cropping.

Table 2  
Effects of different continuous cropping years on the enzyme activities in rhizosphere soil of *Tussilago farfara* L.

| Treatment | Sucrase activity (mg·g <sup>-1</sup> ) | Catalase activity [mL·(g·h) <sup>-1</sup> ] | Urease activity [mg·(g·d) <sup>-1</sup> ] | Alkaline phosphatase activity (mg·g <sup>-1</sup> ) |
|-----------|----------------------------------------|---------------------------------------------|-------------------------------------------|-----------------------------------------------------|
| T1        | 1.36 ± 0.11a                           | 1.97 ± 0.02ab                               | 90.95 ± 2.73b                             | 2.62 ± 0.03a                                        |
| T2        | 0.71 ± 0.07b                           | 1.99 ± 0.01a                                | 106.22 ± 0.51a                            | 2.33 ± 0.06b                                        |
| T3        | 0.60 ± 0.21b                           | 1.89 ± 0.03b                                | 43.82 ± 2.97c                             | 2.21 ± 0.24c                                        |

3.3 Changes of rhizosphere soil bacterial community structure of *Tussilago farfara* L. in different continuous cropping years

#### 3.3.1 Quality analysis of rhizosphere soil sequencing results in different continuous cropping years

As shown in Table 3, 680 542 effective sequences of bacteria were measured in the nine soil samples, of which 425 074 (62.46%) were high-quality sequences with an average length of 430 bp, which were consistent with the bacterial 16S rDNA-V3 + V4 sequences length. The fungal effective sequences were 767 453, of which 582 343 (76.12%) were high-quality sequences with an average length of 261 bp, matching the length of fungal ITS sequences. The dilution curves were able to illustrate the depth of sequencing. Using the observation feature method to plot the dilution curves after categorizing rhizosphere soil bacteria and fungi OTUs with 97% similarity (Fig. 1). The results showed that both bacteria and fungi leveled off with increasing sequencing depth, indicating that the sequencing data were close to saturation and the amount of data was reasonable, which could truly reflect the diversity contained in the soil samples.

| Table 3<br>High-quality sequence statistics of rhizosphere soil in different continuous cropping years |                |                       |              |                |                       |              |
|--------------------------------------------------------------------------------------------------------|----------------|-----------------------|--------------|----------------|-----------------------|--------------|
| Treatment                                                                                              | Bacteria       |                       |              | Fungi          |                       |              |
|                                                                                                        | Valid sequence | High-quality sequence | Proportion/% | Valid sequence | High-quality sequence | Proportion/% |
| T1                                                                                                     | 72 289         | 44 243                | 61.27        | 86 236         | 65 999                | 76.44        |
| T2                                                                                                     | 80 784         | 51 961                | 63.81        | 83 916         | 65 380                | 78.06        |
| T3                                                                                                     | 73 774         | 45 487                | 60.97        | 85 665         | 62 736                | 73.88        |
| Total                                                                                                  | 680 542        | 425 074               | 62.46        | 767 453        | 582 343               | 76.12        |

### 3.3.2 Bacterial OUT distribution of rhizosphere soil in different continuous cropping years

The OTU clustering analysis of the sequencing results of the rhizosphere soil bacterial community of *Tussilago farfara* L. (Fig. 2-A) showed that the three treatment soil samples had a total of 866 OTUs, accounting for 10.18%; T1, T2, and T3 had 2 202, 2 298, and 1 883 specific OTUs, respectively; T1 and T2 had 659 OTUs (accounting for 7.75%), T1 and T3 had 255 OTUs (accounting for 3.00%), and T2 and T3 had 341 OTUs (accounting for 4.01%). The OTU clustering analysis of the fungal community sequencing results showed (Fig. 2-B) that the three treatment soil samples had a total of 192 OUT, accounting for 16.64%; T1, T2, and T3 had specific OTU numbers of 225 253, and 318, respectively; T1 and T2 had a total of 40 OTUs (accounting for 3.47%), T1 and T3 had a total of 52 OTUs (accounting for 4.50%), and 74 OTUs were shared by T2 and T3 (6.41%).

#### 3.3.3 Alpha diversity analysis of rhizosphere soil bacteria and fungi of *Tussilago farfara* L. in different continuous cropping years

The Alpha diversity analysis of rhizosphere soil bacterial and fungal communities of *Tussilago farfara* L. in continuous cropping was conducted by using the Goods\_coverage index, Ace index, Chao index,

Shannon index, and Simpson index (Table 4), the coverage of bacterial flora detected in all samples reached more than 99%, Chao index and Ace index increased first and then decreased with the increased of continuous cropping years. The Shannon and Simpson indices showed  $T2 > T1 > T3$ , but the differences were not significant ( $P > 0.05$ ). This indicates that the bacterial diversity and abundance of each soil sample increased first and then decreased with the increase of continuous cropping years, and  $T3$  was significantly lower than  $T1$  ( $P < 0.05$ ). On the contrary, the fungal flora coverage detected in all samples reached more than 100% (Tables 4), and the community abundance Chao and Ace indices both showed  $T3 > T2 > T1$ , which increased with the increase of continuous cropping years, and  $T3$  was significantly different from  $T1$  and  $T2$  ( $P < 0.05$ ); As with the abundance index, both the Shannon and Simpson indices increased with the continuous cropping years, but the changes between  $T2$  and  $T3$  were not significant ( $P > 0.05$ ). It showed that the fungal diversity and abundance of each soil sample increased with the increase of cropping year, and  $T3$  was significantly higher than  $T1$  ( $P < 0.05$ ).

Table 4  
Alpha diversity of bacteria and fungi in rhizosphere soil of *Tussilago farfara* L. with different continuous cropping years

| Treatment      | Bacteria  |           |           | Fungi   |         |         |
|----------------|-----------|-----------|-----------|---------|---------|---------|
|                | T1        | T2        | T3        | T1      | T2      | T3      |
| Goods_coverage | 0.99a     | 0.99a     | 0.99a     | 1.00a   | 1.00a   | 1.00a   |
| Chao           | 1 820.06b | 1 966.82a | 1 628.20c | 248.67b | 255.67b | 296.00a |
| Ace            | 1 821.88b | 1 970.68a | 1 632.24c | 272.00b | 280.00b | 322.00a |
| Shannon        | 9.24ab    | 9.88a     | 8.92c     | 4.41b   | 5.24a   | 5.69a   |
| Simpson        | 0.98a     | 0.99a     | 0.98a     | 0.78b   | 0.93a   | 0.95a   |

### 3.3.4 Changes in rhizosphere soil bacterial community structure of *Tussilago farfara* L. in different continuous cropping years

As shown in Fig. 3-left, the rhizosphere soil bacterial community of *Tussilago farfara* L. at the phylum level mainly consisted of *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Nitrospirae*, *Chloroflexi*, WS3, *Bacteroidetes*, and *Firmicutes*. The relative abundance of *Proteobacteria* ranked first, decreasing first and then increasing with the increase of continuous cropping years, which was 45.39%, 28.93%, and 31.99%, respectively. The relative abundance of *Acidobacteria*, *Nitrospirae*, and WS3 showed an increase first and then a decrease, but the changes between  $T1$  and  $T2$  were not significant, and  $T3$  decreased by 75.95%, 78.53%, and 74.41%, respectively, compared with  $T1$ , reaching significant differences ( $P < 0.05$ ). The relative abundance of *Gemmatimonadetes* was 7.52%, 6.46%, and 1.95%, respectively, decreasing year by year. In contrast to *Gemmatimonadetes*, *Actinobacteria*, *Chloroflexi*, *Bacteroidetes*, and *Firmicutes* showed a yearly increase with the increase of cropping years, and the relative abundance of each phylum in  $T3$  increased by 1.94, 4.22, 0.34, and 4.47 times compared with  $T1$ , respectively.

Further analysis of the rhizosphere soil bacterial community at the genus level of *Tussilago farfara* L. (Fig. 3-right) showed that the top 10 genera mainly included *Ralstonia*, *Neisseria*, *Veillonella*, *Arthrobacter*, *Sphingomonas*, *Nitrospira*, *Kaistobacter*, *Skermanella*, *Haemophilus*, and *Methylibium*. With the increase of continuous cropping years, the relative abundance of *Ralstonia* and *Nitrospira* decreased among which *Ralstonia* changed most significantly, T3 decreased by 76.86 times compared with T1. The relative abundance of *Arthrobacter*, *Sphingomonas*, and *Skermanella* increased with the increase of cropping years, and T3 increased by 3.69, 7.74, and 7.08 times, respectively, compared with T1. The relative abundance of *Haemophilus*, *Neisseria*, and *Veillonella* in T1 was less than 0.1%, increased with the increase of cropping years, and T3 increased 167.25 and 68.75 times, respectively, compared with T1. The relative abundance of *Methylibium* increased first and then decreased with the increase of continuous cropping years, with the highest in T2.

### 3.3.5 Changes in rhizosphere soil fungal community structure of *Tussilago farfara* L. in different continuous cropping years

As shown in Fig. 4-left, the rhizosphere soil fungal community at the phylum level mainly consisted of *Ascomycota*, *Mortierellomycota*, *Basidiomycota*, *Fungi\_unclassified*, *Chytridiomycota*, *Zoopagomycota*, *Glomeromycota*, and *Olpidiomycota*. The relative abundance of *Zygomycota* was ranked first and decreased year by year, which was 65.96%, 54.10%, and 46.80%, respectively. The relative abundance of *Chytridiomycota* and *Zoopagomycota* was 0.58%, 0.42%, 0.49%, and 0.08%, 0.06%, and 0.21%, respectively, which also showed a decrease first and then increase with the increase of cropping years, and the relative abundance of *Chytridiomycota* was not significant in T1, T2, and T3, while *Zoopagomycota* increased 162.27% in T3 compared with T1. The relative abundance of *Mortierellomycota*, *Fungi\_unclassified*, and *Olpidiomycota* increased gradually with the increase of cropping years, reaching 24.29%, 1.61%, and 0.20% in T3, respectively, and the relative abundance of *Olpidiomycota* changed the most, increasing 5.67 times in T3 compared with T1. The relative abundance of *Basidiomycota* and *Glomeromycota* was the opposite of *Ascomycota*, showing an increase first and then a decrease with the increase of cropping years, with the relative abundance of 12.41%, 30.19%, 18.89%, and 0.04%, 0.28%, 0.14%, respectively, all reaching the highest in T2.

As shown in Fig. 4-right, the top 20 genera of rhizosphere soil fungal communities of *Tussilago farfara* L. include *Mortierella*, *Trichoderma*, *Solicoccozyma*, etc. Among them, the relative abundance of *Trichoderma* and *Didymella* changed the most with the increase of cropping years, and the relative abundance of *Trichoderma* decreased from 25.24% in T1 to 0.61% in T3, which decreased by 40.38 times; the relative abundance of *Didymella* increased from 0.07% in T1 to 3.80% in T3, which increased by 53.29 times, indicating that *Trichoderma* and *Didymella* have a higher sensitivity to continuous cropping. The relative abundance of *Mortierella*, *Unspecified\_Nectriaceae*, *Mrakiella*, *Fusarium*, *Unspecified\_Didymellaceae*, *Titaea*, *Unspecified\_Fungi*, and *Paraphoma* increased with the increase of cropping years, with T3 increased by 21.30%, 92.20%, 157.39%, 168.75%, 916.67%, 124.14%, 75.58%, 196.97%, respectively, compared with T1, and all differed significantly from T1 ( $P < 0.05$ ), with the relative abundance of *Unspecified\_Didymellaceae* changing nearly 10 times. In contrast to the *Mortierella*, the

relative abundance of the *Trichocladium* and *Pseudombrophila* in T1 were significantly higher than in T2 and T3, but the variation between T2 and T3 did not reach significance. The relative abundance of *Solicoccozyma*, *Tausonia*, *Thelebolus*, Unspecified\_Sebacinales, and *Tricharina* all showed increased first and then decreased with the increase of continuous cropping years, but each genus was still significantly higher than T1 after the decrease.

### 3.3.6 Beta diversity analysis of rhizosphere soil bacteria and fungi in different continuous cropping years

PCA analysis of rhizosphere soil bacteria and fungi sequencing results showed (Fig. 5-a,b) that the structure of rhizosphere soil bacterial and fungal communities in different continuous cropping years of *Tussilago farfara* L. was zoned without overlapping parts, and the heat map of cluster analysis based on  $\beta$ -diversity-Weighted Unifrac distance showed (Fig. 5-c,d) that the bacterial and fungal communities in T1 and T2 were more similar and clustered into one group, while T3 clustered into one group alone, indicating that the microbial community structure among rhizosphere soil in different continuous cropping years differed significantly, and the differences between T1 and T2 were small and became larger with the increase of continuous cropping years.

## 4 Discussion

### 4.1 Increasing continuous cropping years affect rhizosphere soil bacterial diversity and community structure of *Tussilago farfara* L.

Soil microorganism is an important part of maintaining soil structure and guaranteeing the stability of micro-ecosystems. They function through different microflora to maintain ecosystem stability, which is one of the factors affecting normal plant growth and development <sup>[17–18]</sup>, and there are significant differences in microbial community structure between different soils or different plots of the same soil type <sup>[19]</sup>. The diversity of the rhizosphere soil bacterial community has been used as an important indicator to assess soil quality and fertility <sup>[20–21]</sup>, and plays an important role in regulating soil fertility, nutrient distribution, and disease suppression. Gao <sup>[22]</sup> studied the soil microbial community of tobacco under continuous cropping found that the bacterial  $\alpha$ -diversity index decreased with the increase of continuous cropping years. Na <sup>[23]</sup> found that the  $\alpha$ -diversity of rhizosphere soil bacterial community in continuous cropping of *Lycium chinense* was lower than that in the control. Li <sup>[24]</sup> found that soil respiration in long-term continuous cropping was limited by soil organic carbon and total nitrogen, which reduced the number and community diversity of soil bacteria of *Chrysanthemum morifolium*.

In this study, the analyzed the rhizosphere soil samples from different continuous cropping years of *Tussilago farfara* L. revealed that the  $\alpha$ -diversity of the bacterial community increased first and then decreased with the increase of continuous cropping years, and the bacterial abundance and diversity index in soil samples from continuous cropping for two years were the smallest and significantly lower

than the rotational cropping, which was consistent with the results of Gao and Na <sup>[22–24]</sup>. Comparison of bacterial composition and abundance in rhizosphere soil of different continuous cropping years showed that the main dominant bacterial phyla in the samples were all *Proteobacteria*, *Actinobacteria*, and *Acidobacteria*, but the relative abundance of each phylum varied greatly among treatments. *Proteobacteria* and *Actinobacteria* are beneficial in soil, which can help plant growth and participate in the carbon and nitrogen cycle, and also can effectively degrade pesticide residues in soil and restore the soil environment. In this study, the relative abundance of *Proteobacteria* was significantly lower than the rotational cropping, which was consistent with the changes in *Chrysanthemum morifolium* <sup>[24]</sup> and selenium sand melon <sup>[25]</sup> under continuous cropping, indicating that the *Proteobacteria* bacteria are highly adaptable to the changes in soil environment under continuous cropping conditions. Unlike *Proteobacteria*, the relative abundance of *Actinobacteria* increased with the increase of continuous cropping years, presumably because the soil environment was still suitable for their growth and reproduction during the short continuous cropping years, and might decrease with the extension of cropping years. It has been shown that *Acidobacteria* can provide nutrients to the soil by degrading complex lignin <sup>[26]</sup> and cellulose <sup>[27]</sup>. In this study, the relative abundance of *Acidobacteria* decreased gradually with the increase of cropping years, indicating that the change of soil environment under continuous cropping would inhibit the growth and reproduction of *Acidobacteria*, and the energy provided by soil microorganisms would decrease. Among the top 10 genera, the relative abundance of *Ralstonia* and *Nitrospira* decreased significantly with the increase of continuous cropping years. As environment-friendly microorganisms for the degradation of benzene substitute pollutants and PHB synthesis, *Ralstonia* has great potential and advantages in environmental pollution management <sup>[28]</sup>. *Nitrospira* is often found in neutral or weakly alkaline environments as nitrifying bacteria in soil, which can oxidize nitrite to nitrate in soil and promote nitrogen cycle <sup>[29]</sup>; *Neisseria* bacteria can ferment a variety of sugars and produce acid but not gas; *Veillonella* bacteria can participate in fermentation metabolism, fermenting pyruvic acid, oxalic acid, malic acid, and producing acetic acid, propionic acid, CO<sub>2</sub>, and H<sub>2</sub>. Therefore, the relative abundance of *Nitrospira* decrease with the increase of continuous cropping years may be related to the decrease of soil pH due to acid production by bacteria of the genera *Neisseria* and *Veillonella*. Nitrifying bacteria can directly oxidize and utilize ammonia, which will be converted to ammonium under acidic conditions, thus making it impossible for nitrifying bacteria to survive. *Sphingomonas* can degrade a variety of macromolecular organic compounds <sup>[30]</sup> and is resistant to a variety of pesticides and herbicides <sup>[31]</sup>. The relative abundance of *Sphingomonas* in rhizosphere soil significantly increases in continuous cropping for two years may be related to the use of the resistance of pesticides in the previous planting process that has made them resistant.

The PCA analysis showed that the soil bacterial communities in different quadrants and far apart in different continuous cropping years, indicating that there were significant differences in bacterial community structure with increasing cropping years. Meanwhile, the number of rhizosphere soil bacteria OUT decreased significantly by 16.00% compared with the rotational cropping after continuous cropping for two years, indicating that the number of bacteria decreased significantly after continuous cropping for

two years, which was consistent with the clustering analysis in which the samples were grouped into one category, This indicates that the rhizosphere soil bacterial community did not change significantly in the early stage, but the number decreased and the structure of the bacterial community changed with the extension of the continuous cropping years.

#### 4.2 Increasing continuous cropping years affect the rhizosphere soil fungi diversity and community structure of *Tussilago farfara* L.

Soil microbial balance is a sign of soil health, and soil fungi are among the main microorganisms that play an important role in the whole microorganism, accounting for a relatively large number and species, maintaining the balance and stability of the whole ecosystem together with other microorganisms, and participating in the material cycle and energy flow<sup>[32]</sup>. In this study, it was found that continuous cropping of *Tussilago farfara* L. disrupted the soil microecological balance and changed the fungal diversity, and the number of fungal species in rhizosphere soil increased with the increase of continuous cropping years, especially the number of fungal species reached 180 in two years of continuous cropping, which was 22.45% higher than the rotational cropping. The  $\alpha$ -diversity analysis of rhizosphere soil fungal communities in different cropping years showed that both abundance and diversity indices increased with the increase of continuous cropping years, which was consistent with the studies of Mu<sup>[33]</sup>, in which the overall rhizosphere soil fungal diversity increased with the increase of continuous cropping years in Taibai Bayberry. Xu<sup>[34]</sup> found that the rhizosphere soil fungal diversity of roasted tobacco was higher than that at three years of continuous cropping, and Gao<sup>[22]</sup> found that the rhizosphere soil fungal diversity of roasted tobacco increased to some extent after continuous cropping. In the study of Gao<sup>[22]</sup>, the diversity of soil fungi in rhizosphere soil was increased to a certain extent after continuous cropping.

The differences in cropping growth and cropping years will lead to changes in the soil microcosm environment, fungal community structure, composition, and abundance<sup>[35]</sup>. In this study, we found that the main dominant fungal phyla in the rhizosphere soil of *Tussilago farfara* L. were the same and remained stable, with the highest relative abundance of *Ascomycota* (46.80%-65.69%), followed by *Mortierellomycota* (20.00%-24.29%) and *Basidiomycota* (12.41%-18.89%). Most of the *Ascomycota* are nutrient-dependent fungi, while most of the *Basidiomycota* are nutrient-poor fungi that grow and reproduce more slowly, both associated with organic matter degradation<sup>[36]</sup>. When the soil nutrients and enzyme activity is gradually decreased after continuous cropping, the relative abundance of *Ascomycota* are decreased, which is sensitive to the soil environment. Because of the slow growth and reproduction of *Basidiomycota*, soil nutrients are still sufficient after one year of continuous cropping, in the same time the cropping growth process will secrete a lot of metabolic substances to provide nutrients for *Basidiomycota*, but with the increase of continuous cropping years, the supply of soil nutrients is insufficient and the organic matter content decreases, therefore, the relative abundance of *Basidiomycota* showed an increase and then decrease after continuous cropping. Among the top 10 fungal genera, the relative abundance of *Mortierella* and *Fusarium* increased with the increase of continuous cropping years. It has been shown that *Mortierella* is endophytic fungi of many plant roots<sup>[37]</sup>, and most of them

are harmful that can cause plant and animal diseases and cause some losses in production <sup>[38]</sup>. *Fusarium* is relatively large among fungi, and can cause plant wilt, root rot through infestation, evolving into wilt and root rot, which seriously affects production and profitability <sup>[39]</sup>. Among them, *Fusarium acuminatum*, which is a fungus belonging to the genus *Fusarium*, is a major disease in the production of *Tussilago farfara* L., causing the plant to wilt and die <sup>[40]</sup>. The relative abundance of *Fusarium* in the rhizosphere soil of continuous cropping for two years was 1.69 times higher than the rotational cropping. Zhang <sup>[41–42]</sup> showed that the imbalance of microbial community structure due to the massive accumulation of pathogenic bacteria is the core mechanism causing continuous cropping obstacles. *Trichoderma* can produce bioactive substances that are antagonistic to a variety of plant pathogens, regulating plant growth, development, and metabolism through nutritional competition and microparasitism <sup>[43–45]</sup>, which plays an important role in ensuring the structural balance of soil microbial communities. In this study, the relative abundance of *Trichoderma* decreased significantly with the increase of continuous cropping years, and the relative abundance of this genus in the rhizosphere soil continuous cropping for two years decreased by 40.38 times compared with the rotational cropping, indicating that the structure of the *Tussilago farfara* L. fungal community has been imbalanced under continuous cropping conditions, and *Trichoderma* could not play a corresponding role.

## 5 Conclusion

The normal growth and development of the rhizosphere soil microbial community are closely related to the continuous cropping, which will cause the rhizosphere soil pH value to develop in the acidic direction, nutrient imbalance, enzyme activity changes, and soil environment from "bacterial" to "fungal" change, beneficial bacteria genus decline and harmful bacteria accumulation.

## Declarations

### Author contributions

WX and JXJ conceived and designed the research. WX performed the experiments, analyzed the results, interpreted the data and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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### Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Figures

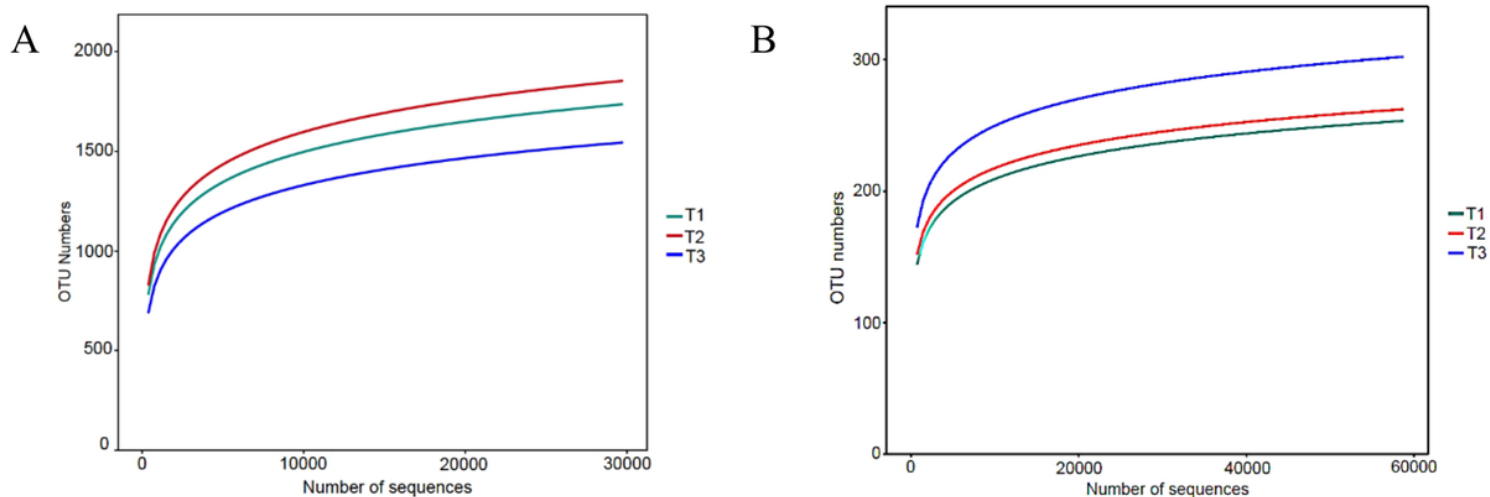


**Figure 1**

OTU dilution curve of bacteria and fungi in rhizosphere soil of *Tussilago farfara* L. with different continuous cropping years (A-Bacteria, B-Fungi)

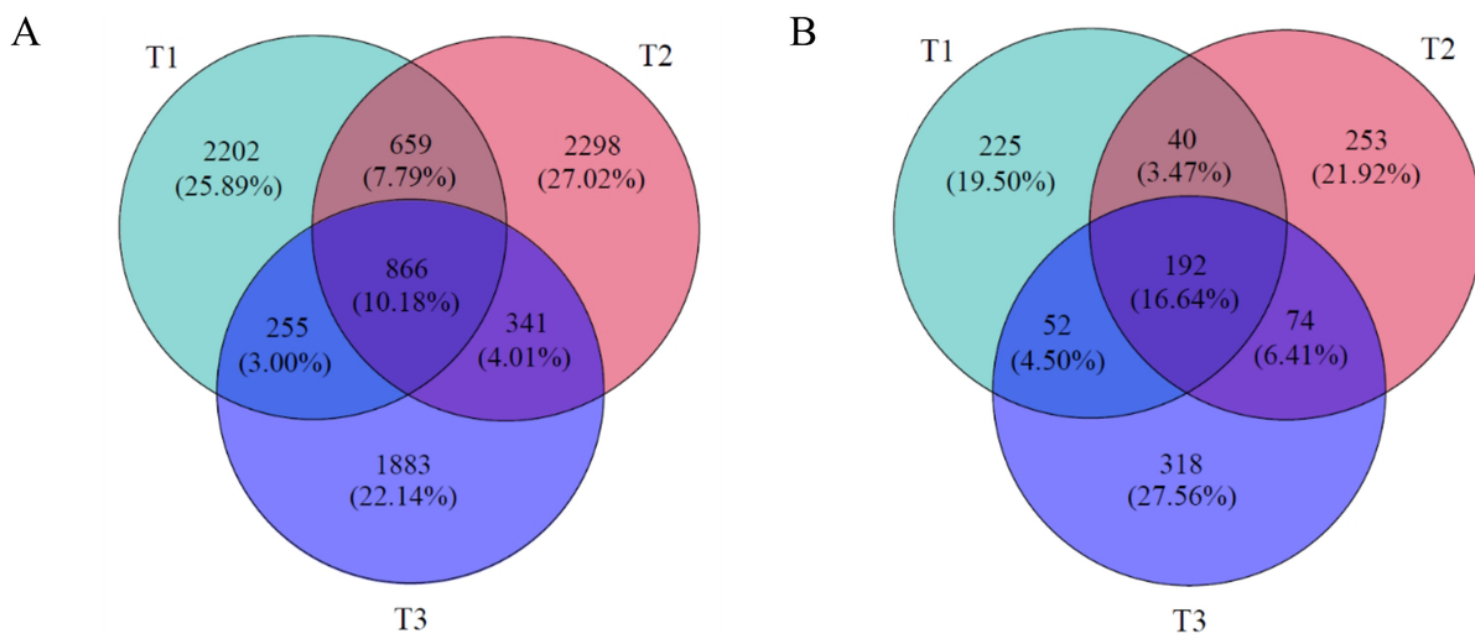


**Figure 2**

Venn diagram of bacterial and fungi OTU distribution in rhizosphere soil of *Tussilago farfara* L. with different continuous cropping years (A-Bacteria, B-Fungi)

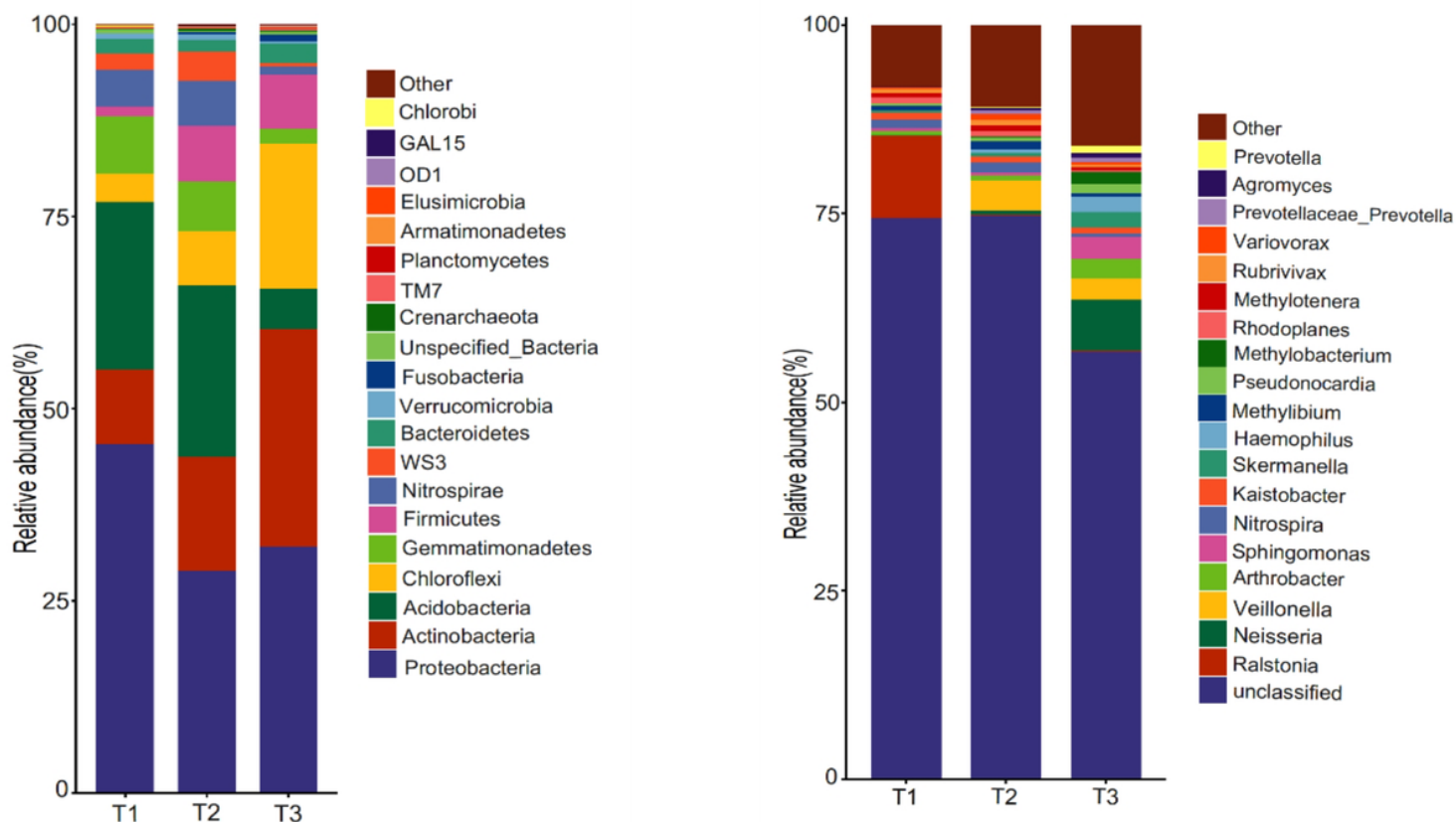


**Figure 3**

Relative abundance of bacterial species at phylum level and genus level in rhizosphere soil of *Tussilago farfara* L. with different continuous cropping years(left: phylum level; right: genus level)

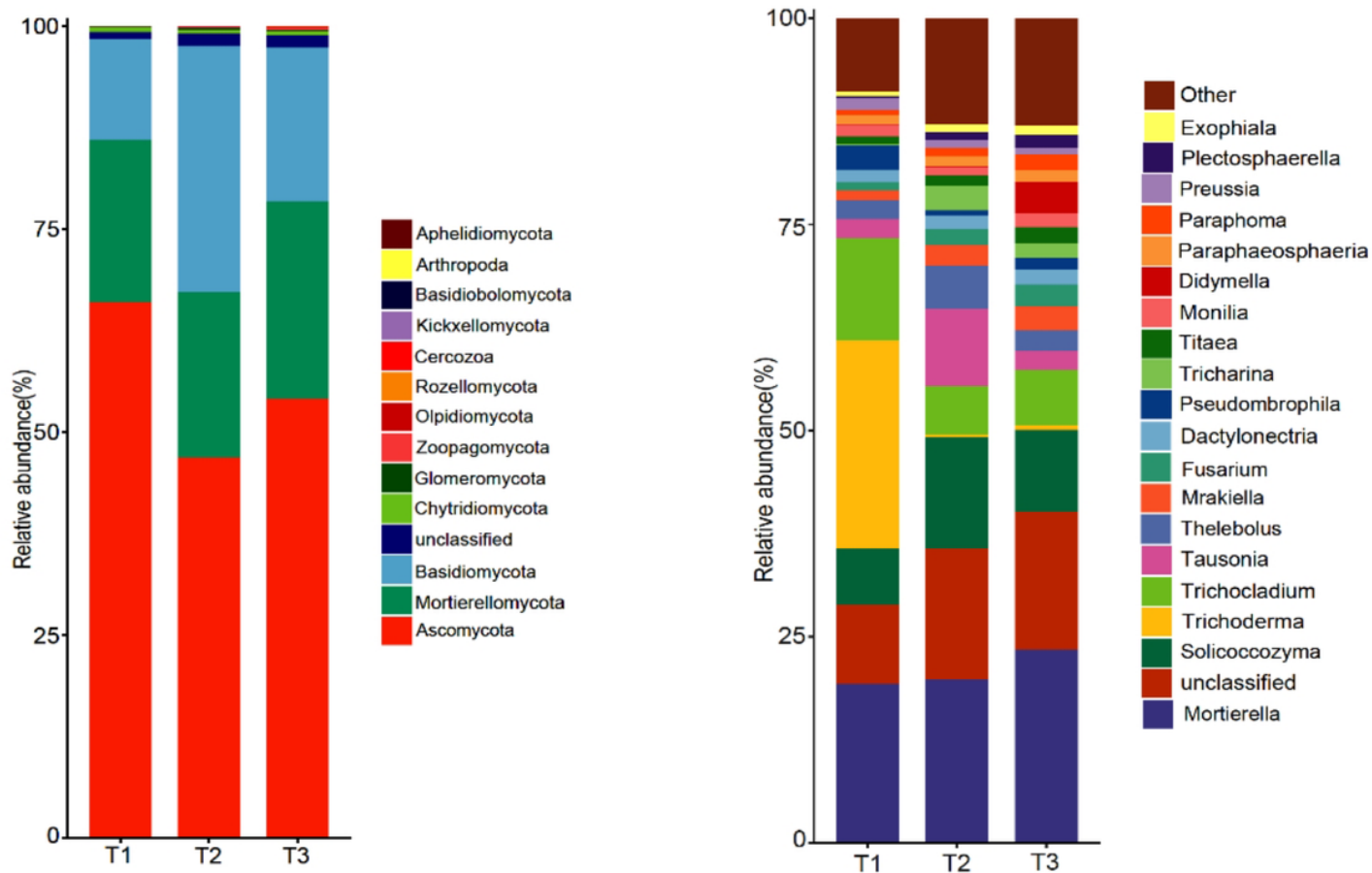


**Figure 4**

Relative abundance of fungi species at phylum level and genus level in rhizosphere soil of *Tussilago farfara* L. in different continuous cropping years (left: phylum level; right: genus level)

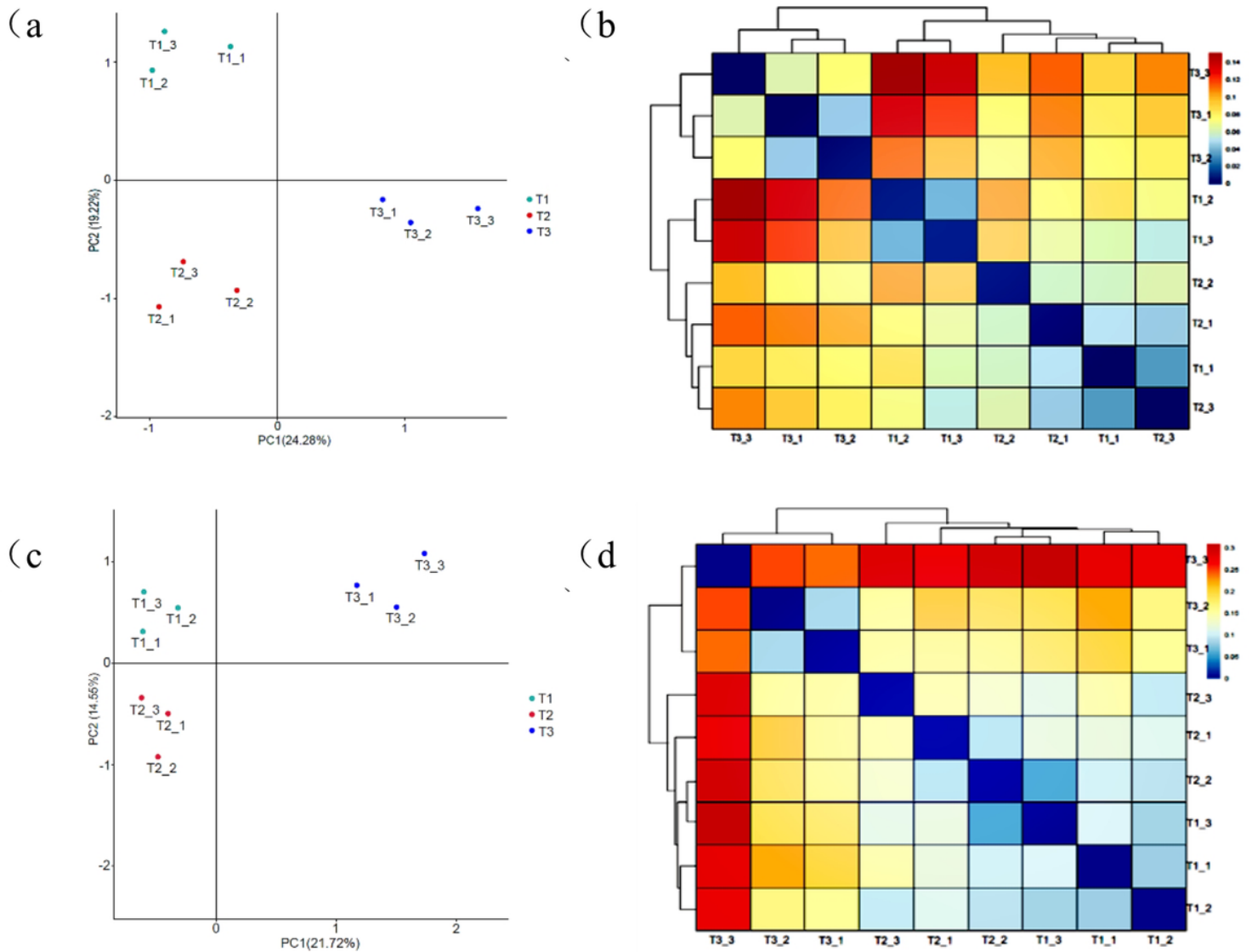


**Figure 5**

$\beta$ -diversity-PCA analysis and Weighted Unifrac heat map of bacterial and fungal community in rhizosphere soil of *Tussilago farfara* L. with different continuous cropping years (a and b are bacterial, c and d are fungi)

## Supplementary Files

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- [Rawdatad1.zip](#)