

Effects of different years of natural recovery of *Gastrodia elata* on the community structure of bacteria and fungi in rhizosphere soil

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Research Article

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Abstract

Background: There are serious obstacles to continuous cropping of *Gastrodia elata*. After continuous cropping of *Gastrodia elata*, the yield will be greatly reduced, or even no harvest. Soil nutrients and microorganisms play an important role in it. There are few related studies on the effects of natural restoration of the rhizosphere soil of *Gastrodia elata* on the structure of soil bacteria and fungi for different years.

Results: In this study, a combination of Illumina Miseq library preparation and high-throughput sequencing technology was used. The soil that had not been planted with *Gastrodia elata* was used as a control to study the characteristics of soil microbial communities in the rhizosphere of *Gastrodia elata* from 0 to 5 years of natural restoration, and Correlation between soil characteristics and fungal and bacterial communities. The results showed that (1) The dominant bacteria in the rhizosphere soil of *Gastrodia elata* are roteobacteria, *Actinobacteria* and *Acidobacteria*, and the most important bacterial genera are *norank_f_Xanthobacteraceae* and *Bradyrhizobium*. The dominant fungal phyla are *Basidiomycota* and *Ascomycota*, and the main fungal genera are *Mortierella* and *Trichoderma*. The bacterial and fungal community composition in the rhizosphere soil of *Gastrodia elata* and the soil without *Gastrodia elata* planted are not much different, but the community structure and relative abundance are quite different. (2) The Chao index, Shannon index and Invsimpson index of *Gastrodia elata* rhizosphere soil show an increasing trend with the increase of the natural restoration period, indicating that natural restoration for a certain period of time will increase the diversity and abundance of bacteria and fungi in the rhizosphere soil of *Gastrodia elata* Increase. (3) With the increase of the natural restoration period, the total nitrogen and pH content of the rhizosphere soil of *Gastrodia elata* showed an increasing trend. total nitrogen has the greatest influence on the bacterial community structure, and the influence of pH on the fungal community structure ranks No. Second, in the top three phyla with relative content, total nitrogen is positively correlated with *Actinobacteria*, and pH is positively correlated with *Mortierellomycota*.

Conclusion: After 3 years of natural restoration, the soil environment gradually returns to normal, and the microbial community tends to balance; in addition, the Spearman rank correlation coefficient analysis shows that total nitrogen has the greatest impact on the bacterial community structure in the rhizosphere soil of *Gastrodia elata*, and total phosphorus has the greatest impact on the fungal community structure. The results provide a theoretical basis for the development of new type microbial fertilizers and soil amendments for *Gastrodia elata* to alleviate continuous cropping obstacles.

Keywords: Soil physical and chemical properties; bacterial community; fungal community; rhizosphere soil; *Gastrodia elata*

Background

Gastrodia elata belongs to the *Orchidaceae* family. It is a traditional and precious Chinese medicinal material in my country. Its dried underground tubers are used as medicine [1]. It is a commonly used and more expensive Chinese medicine. Mainly used for polio, epilepsy convulsions, tetanus, headache, dizziness, hand and foot failure, numbness of the limbs, rheumatic arthralgia, etc.[2,3]. *Gastrodia elata* is known as one of the "Three Treasures of Guizhou". "Guizhou *Gastrodia elata*" has entered the National Health and Family Planning Commission's new food raw material review process. If it is listed as a new food raw material, it is expected to form a *Gastrodia elata* industry worth tens of billions [4]. In recent years, *Gastrodia elata* has

moved from a medicinal plant to the ranks of health care products with the same medicine and food. The market demand is large [5], and wild resources have been in short supply. *Gastrodia elata* is a heterotrophic higher plant, which has strict requirements on the climate environment and terrain and altitude. It is often planted in hillside forests, shrubs or grasses. The growth and development of *Gastrodia elata* must rely on honey fungus infecting wood to provide nutrition for it. Therefore, the artificial cultivation of *Gastrodia elata* is inseparable from forest resources [6].

With the gradual expansion of the artificially planted *Gastrodia elata*, the forest resources suitable for the cultivation of *Gastrodia elata* in several major producing areas in Guizhou are gradually decreasing. *Gastrodia elata* is a serious non-continuous cropping crop. The old ground for continuous cultivation of *Gastrodia elata* will cause serious fungal infections. The fungus is parasitized by Trichoderma. The production of *Gastrodia elata* has been cut by half for two years of continuous cropping, and almost no harvest for three years of continuous cropping. In addition, the serious occurrence of diseases and insect pests in the old land has caused the quality of *Gastrodia elata* to decrease [7]. A large number of studies have confirmed that the rhizosphere soil micro-ecosystem is unbalanced after continuous cropping, and with the increase of continuous cropping years, the types and numbers of bacteria decrease, and pathogenic microorganisms are abundantly enriched [8,9]. Soil microorganisms play an important role in promoting soil nutrient conversion, organic matter decomposition, soil structure improvement, fertility evolution, degradation of harmful substances in soil and soil purification [10]. The rhizosphere is the soil area near the roots of plants, where soil microorganisms and plant root systems are very strong [11,12]. Rhizosphere soil microorganisms are closely related to the absorption and transformation of soil nutrients. Therefore, their community structure is the main factor affecting plant growth, development and health [13-16]. Many reports indicate that continuous cropping has changed the structure of the rhizosphere. Soil microorganisms [17-20]. These changes further lead to serious continuous cropping obstacles [21]. Therefore, the relationship between continuous cropping and soil microorganisms has become a research hotspot [22].

At present, there are few reports on the rotation of other crops in *Gastrodia elata*. Xu Jiao et al. [23] studied the influence of *Gastrodia elata* and winter fungus rotation pattern on soil microbial community structure and concluded that the influence of winter fungus on soil microbial community structure is shown by inhibiting the growth of armillaria, thereby making the soil microbial community structure restored, but it is unclear whether new secretions will be produced and the growth of Armillaria will be inhibited by planting *Gastrodia elata* after winter sun rotation. There are no reports on the microbial changes of *Gastrodia elata* soil after a certain period of natural restoration, and the changes in the physical and chemical properties and microbes of *Gastrodia elata* rhizosphere soil for different periods of natural restoration have not yet been clarified. Therefore, this study uses amplicon sequencing (16s and ITS) technology to sequence specific genetic material, prokaryotic and eukaryotic microorganisms in the microbial cells in the soil without *Gastrodia elata* and natural recovery from 0 to 5 years in the rhizosphere soil of *Gastrodia elata*, So as to fully and fully reflect the microbial diversity of bacteria and fungi in the soil, the types and the law of growth and decline of dominant populations with different recovery years, and the relative abundance between them. The results of this study provide a theoretical basis for clarifying the soil physical and chemical properties and the changes of microbial communities in the natural recovery of *Gastrodia elata* in Dejiang from 0 to 5 years.

Results

***Gastrodia elata* rhizosphere soil physical and chemical properties**

Table 1 shows the determination results of pH, organic matter (abbreviated as OM, the same

below), total nitrogen (abbreviated as TN, the same below), TP (abbreviated as TP, the same below), and total potassium (abbreviated as TK, the same below) of *Gastrodia elata* rhizosphere soil. With the increase of the natural recovery period, pH, TP and TK show a trend of first decline and then rise; TN and OM show an overall upward trend. Compared with CK, the pH value of *Gastrodia elata* rhizosphere soil showed an upward trend. The soil pH in R0-R5 years was 1.20, 1.34, 1.19, 1.13, 1.24 and 1.28 times that of CK, and they were all significantly higher than CK. The content of OM in the rhizosphere soil of *Gastrodia elata* in R0-R5 years was higher than that in CK soil, which were 2.14, 2.33, 2.33, 3.12, 2.90, and 4.16 times that of CK, except that the difference in R0, R1 and R2 was not significant, and the differences among the other samples were significant. The change trend of TN content is: Except for treatments R1 and R2, the TN content of other treatments is significantly higher than that of CK. Among them, R5 has the highest TN content, which is 1.81 times that of CK. And the difference between R0 and R3 is not significant. The change trend of TK content is: in addition to R3, compared with CK, the soil TK content of R0, R1, R2, R4, R5 increased by 12.40%, 11.0%, 13.10%, 10.40%, 11.7%, and R3 soil TK content is lower than that of CK, but the difference is not significant. The change trend of TP content is: Except for R0, the TP content of the other treated soils is lower than or equal to CK soil. Among them, the TP content of R0 soil was significantly higher than that of the soil naturally restored for 1-5 years, and the difference in other treatments was not significant.

Tab. 1 The physicochemical properties of different soil samples

样品	pH	OM/(g/kg)	TN/(g/kg)	TP/(g/kg)	TK/(g/kg)
R0	5.87±0.07bc	17.89±1.77c	0.19±0.00c	0.65±0.05a	12.99±0.17b
R1	6.53±0.03a	19.45±0.67c	0.14±0.00e	0.36±0.06b	11.49±0.19d
R2	5.80±0.01c	19.95±1.71c	0.08±0.00f	0.33±0.00b	13.67±0.10a
R3	5.50±0.11d	26.04±0.24ab	0.19±0.00c	0.31±0.00b	9.99±0.86f
R4	6.03±0.03bc	24.19±0.73ab	0.22±0.00b	0.38±0.00b	10.86±0.20e
R5	6.23±0.03b	34.67±0.94a	0.29±0.00a	0.37±0.01b	12.23±0.09c
CK	4.87±0.07e	8.34±0.40d	0.16±0.00d	0.38±0.00b	10.45±0.08ef

Values are mean±standard error of triplicate determinations. Different letters in the same column indicate the significant differences in natural restoration of the rhizosphere soil of *Gastrodia elata* in different years, using Duncan's multiple range test, $P < 0.05$; OM:organic matter, TN:total nitrogen; TP :total phosphorus ;TK :total potassium. R0 represents the rhizosphere soil that has been naturally restored for 0 years after the *gastrodia* stubble; R1 represents the natural recovery for 1 year; R2 represents the natural recovery for 2 years; R3 represents the natural recovery for 3 years; R4 represents the natural recovery for 4 years ; R5 means natural recovery for 5 years. The same as following.

Analysis of Alpha diversity index of *Gastrodia elata* Rhizosphere soil bacteria and fungi

The sparse curves of all samples are nearly flat (Figure 1), indicating that the sequencing depth of this study is good. The taxonomic unit (OTU) of the sample is 3864-4232 for bacteria and 971-1866 for fungi. In order to better obtain the diversity information of the species in the samples,

all sequences were divided into OTUs at a similarity level of 97%. The bacteria in the soil samples of CK, R0, R2, R3, R4, and R5 were obtained by 3911, 4071, 4232, 4199, 3984, 3864, 4134 OTUs, fungi got 971, 1790, 1332, 1841, 1866, 1781, 1262 OTUs, respectively. With the increase of the natural recovery period, the number of bacterial OTUs increased first and then decreased. Among them, the number of bacterial OTUs of R2 was the largest, and the number of bacterial OTUs of R5 was the smallest. The number of fungal OTUs showed an overall upward trend. Among them, R4 had the largest number of fungal OTUs, and R0 had the smallest number.

Alpha diversity refers to the diversity within a specific area or ecosystem, which is often measured by species richness, including Chao value, Invsimpson value, and Shannon index. Chao, Shannon and Invsimpson indexes are all calculated based on bacterial OTU. It can be seen from Table 2 that the Shannon index and Invsimpson index of R2 soil bacteria are significantly higher than CK, R0, R1, R3-R5, and the Chao index of R3 is significantly higher than CK, R1, R2, R4, R5. The Chao index, Shannon index and Invsimpson index of R0 soil fungi were significantly lower than CK, R1-R5, and the Chao index, Shannon index and Invsimpson index of R3 were the largest. This result shows that with the increase of the natural recovery period, the abundance and diversity of soil bacterial and fungal communities show an overall increasing trend.

Table 2 Comparison of the estimated OTU richness and diversity indices of the 16S rRNA and its gene libraries for clustering at 97% identity, as obtained from the pyrosequencing analysis

Type	Sample	0.97			
		OTUs	Shannon	Invsimpson	Chao
Bacteria	R0	3911	6.46±0.02bc	150.20±0.97b	3688.59±1.05d
	R1	4071	6.36±0.00d	90.34±0.48d	3763.21±4.20cd
	R2	4232	6.69±0.04a	191.20±4.71a	3862.12±30.02b
	R3	4199	6.54±0.02b	131.55±4.77c	3981.95±2.38a
	R4	3984	6.58±0.00b	150.00±4.80b	3714.32±2.87d
	R5	3864	6.55±0.01b	141.65±10.11bc	3828.30±2.93bc
	CK	4134	6.58±0.01b	142.18±2.53bc	3838.75±21.21bc
Fungi	R0	971	2.77±0.17c	3.11±0.39e	753.08±9.86e
	R1	1790	4.85±0.03a	48.17±0.44a	1169.85±9.52b
	R2	1332	3.59±0.00b	11.54±0.07d	1021.16±45.89c
	R3	1841	4.99±0.03a	48.33±1.39a	1329.18±27.18a
	R4	1866	4.74±0.06a	32.54±1.16c	1408.38±18.60a
	R5	1781	4.91±0.03a	42.05±0.86b	1319.92±20.92a
	CK	1262	3.66±0.02b	8.94±0.79d	901.66±34.20d

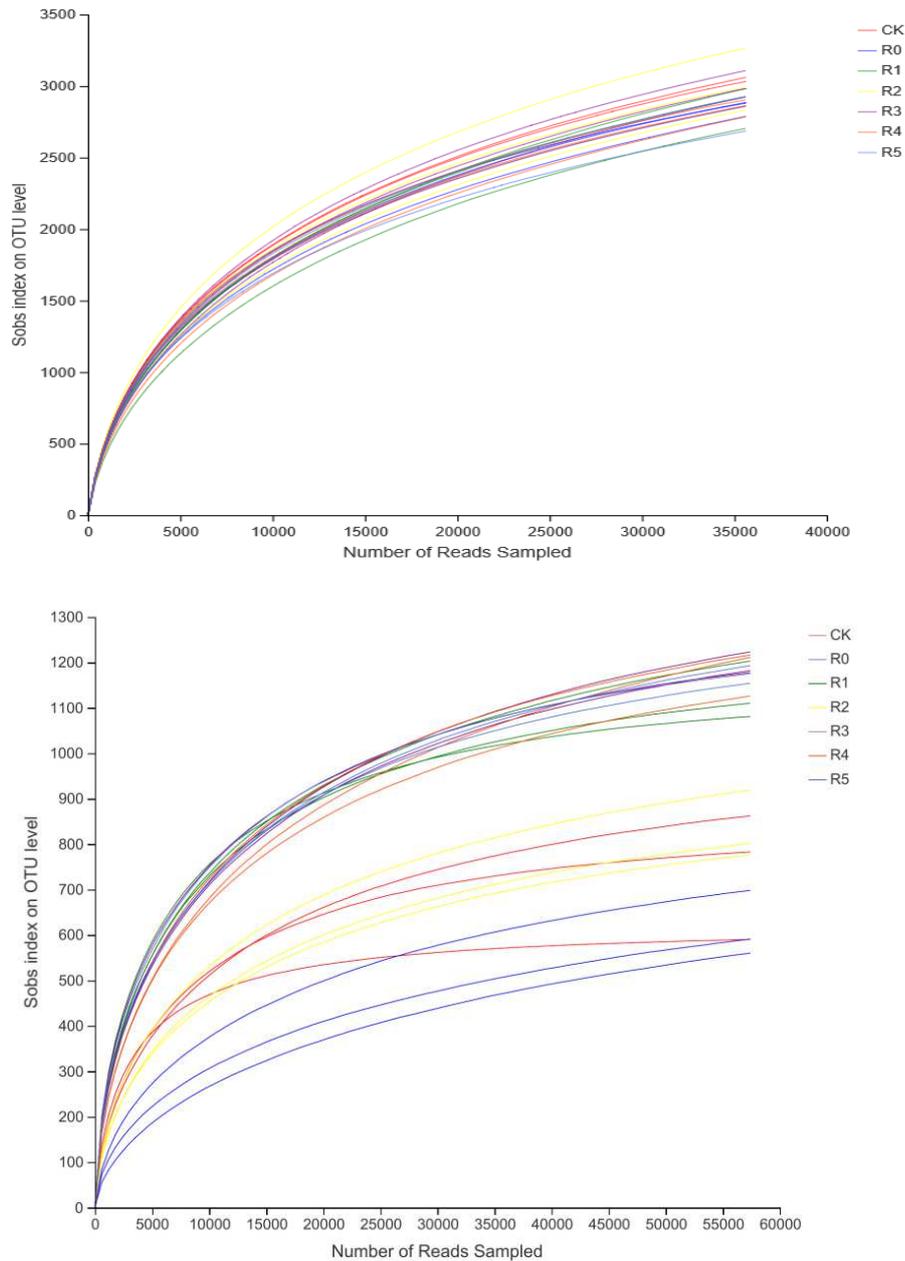


Fig. 1 Rarefaction curves of all samples. CK represents the soil that has not been planted with *Gastrodia elata* ; The picture above shows bacteria; the picture below shows fungi, all charts below are the same.

Analysis of bacterial and fungal community composition in the rhizosphere soil of *Gastrodia elata*

The level of bacteria and fungi (Fig.2), the bacterial dominance of all samples is *Proteobacteria* (31.77%~39.07%), *Actinobacteria* (12.53~21.22%), *Acidobacteria* (12.18%~20.38%), *Chloroflexi* (5.01%~13.74) %, *Myxococcota* (2.70%~4.21%), *Bacteroidetes* (2.02%~5.34%), which together accounted for 85.75% of the total bacterial sequence. In addition, a small amount of *Verrucomicrobia*, *Methylomirabilota*, *Gemmatimonadato*, *Planctomycetota*, *Firmicut-es*, *Desulfobacterota*, *Nitrospirota* accounted for 10.57% of the total bacterial sequence. Among them, *Proteobacteria* has the largest number, followed by *Actinobacteria* and *Acidobacteria*. The soil bacteria phylum *Proteobacteria* accounted for the largest proportion of each sample, being the

first dominant bacterial group, and *Actinobacteria* was the second dominant bacterial group in each sample. There is no big difference between R0-R5 and CK soil in the composition of bacterial community, but there is a big difference in community structure and relative abundance. *Proteobacteria* has the highest abundance of bacteria in the rhizosphere soil of *Gastrodia elata* after natural recovery for 4 years, and the lowest abundance of bacteria in CK soil. Compared with CK, the number of *Proteobacteria* treated with R0-R5 increased by 10.18%, 16.44%, 16.22%, 8.81%, 18.68% and 12.19%.

Four fungal phyla were detected from seven *Gastrodia elata* rhizosphere soil samples (Fig. 2), including *Basidiomycota* (45.64%), *Ascomycota* (37.56%), *Mortierellomycota* (7.43%), *Rozellomycota* (3.41%), accounting for all soil fungi 94.04% of the total number of sequencing. Among them, *Basidiomycota* and *Ascomycota* accounted for the largest proportion, occupying a dominant position in the soil fungal community structure. Among the dominant fungi, *Basidiomycota* accounted for 26.92%~68.85% in each sample, and *Ascomycota* accounted for 23.03%~49.40% in each sample. *Ascomycota* is a large group of fungi, with a wide variety of species and various metabolic forms. It plays an important role in maintaining the function of the soil ecosystem. In this study, *Ascomycota* has an absolute advantage in all samples. *Ascomycota* has the highest relative abundance in R3 soil, and the lowest proportion of CK. As the natural recovery period increases, the proportion of *Ascomycota* in each sample gradually increases.

It can be seen from Figure 3 that the fungal genera in the soil mainly include *norank_f_Xanthobacteraceae* (6.19%~11.30%), *Bradyrhizobium* (3.48%~5.97%), *norank_f_norank_o_Vicinamibact* (1.93%~4.15%). Compared with R0, *norank_f_Xanthobacteraceae* in R1~R5 and CK soil increased by 45.22%, 14.86%, 30.61%, 23.38% and 18.23%. Compared with CK, *Bradyrhizobium* in R1, R2, R4, R5, R6 soil increased by 14.06%, 58.36%, 22.81%, 28.65%, 23.08%, and R3 decreased by 8.33%. Compared with CK, *norank_f_norank_o_Vicinamibacterales* in R0, R1, R2, and R4 soils decreased by 172.09%, 30.36%, 32.95%, and 33.71%, while R3 and R5 increased by 32.90% and 24.79%.

It can be seen from Figure 3 that at the genus level, the most important fungi in the soil samples are *Mortierella* (1.63%~11.63%), *Trichoderma* (2.24%~14.54%), *Saitozyma* (1.53%~10.35%). Among them, *Mortierella* is a beneficial microorganism to the soil. This fungus can repair soil fungal communities and can effectively prevent crop root rot. The *Mortierella* of R0 soil is the smallest, and *Mortierella* of R1~R5 and CK soil is 3.82 of R0, 7.13, 5.01, 3.99, 5.47, 4.39 times. In this study, *Phallus* was only detected in the treatment of CK, R5 and R6, which were 7.29%, 6.16% and 8.38%. In addition, only a small amount of *Phallus* was detected in R2. *Psathyrella*, this genus of fungi often grows in the litter layer with high humus content in summer and autumn. In swamps, farmland, grassland, and even underwater, you can see *Psathyrella* fungi. Growth, which was only detected in R0 in this study, was 15.32%.

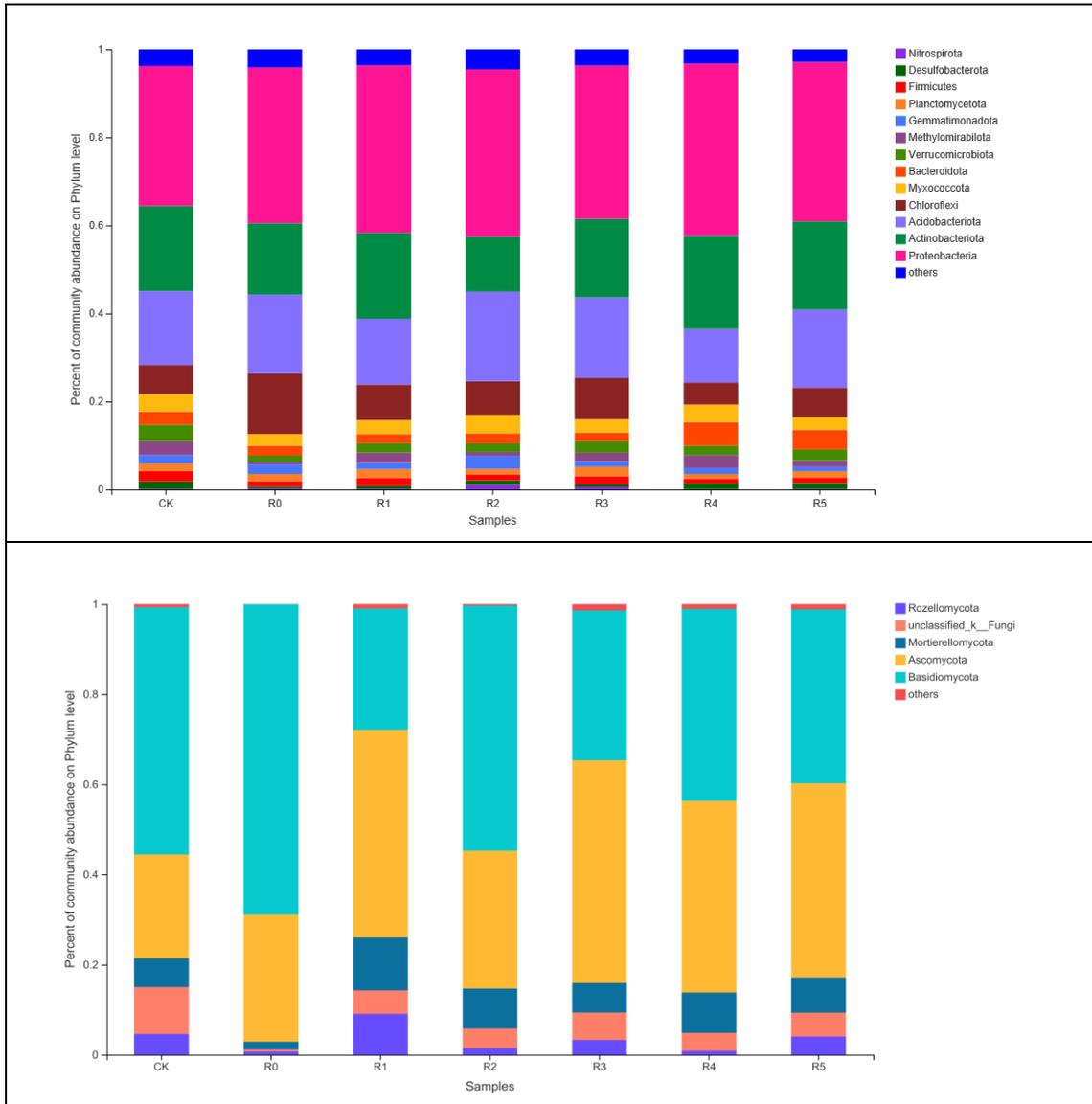


Fig. 2 The relative abundance of bacteria and fungi in rhizosphere soil.



Fig. 3 The relative abundance of bacteria and fungi in rhizosphere soil

OUT Venn analysis of bacteria and fungi in the rhizosphere soil of *Gastrodia elata*

The Venn diagram reveals the common and unique OTUs of all samples (Fig. 4). Under the 97% sequence similarity level, the OTUs of the soil bacteria in each treatment were summarized and divided, and the number of unique and shared OTUs of each treatment was displayed. As shown in Figure 2, the unique OTUs of R0, R1, R2, R3, R4, R5 and CK for species classification are 269, 105, 156, 143, 102, 113, and 163, respectively, and the total number of OTUs is 1,607. It shows that the bacterial community in the rhizosphere soil of *Gastrodia elata* occurred in 1 and 5 years of natural recovery. Compared with CK, the number of unique OTUs in soils R1~R5 decreased by 35.58%, 4.29%, 12.27%, 37.42%, and 30.67%.

Based on the 97% sequence similarity level, the OTUs of each soil fungus were summarized and divided, and the number of unique and shared OTUs for each treatment was displayed (Fig. 4). It can be seen from the figure that the number of unique OTUs for R0~R5 and CK are 141, 407, 200, 374, 434, 444, and 279, of which there are 169 OTUs intotal. Obviously, with the increase of

the natural recovery period, the unique OUT number of *Gastrodia elata* rhizosphere soil fungi showed an increasing trend. Three years after the natural recovery of *Gastrodia elata* soil, the soil fungal community was larger than CK, indicating that after planting *Gastrodia elata* and after a certain period of natural recovery, the soil fungal community became rich.

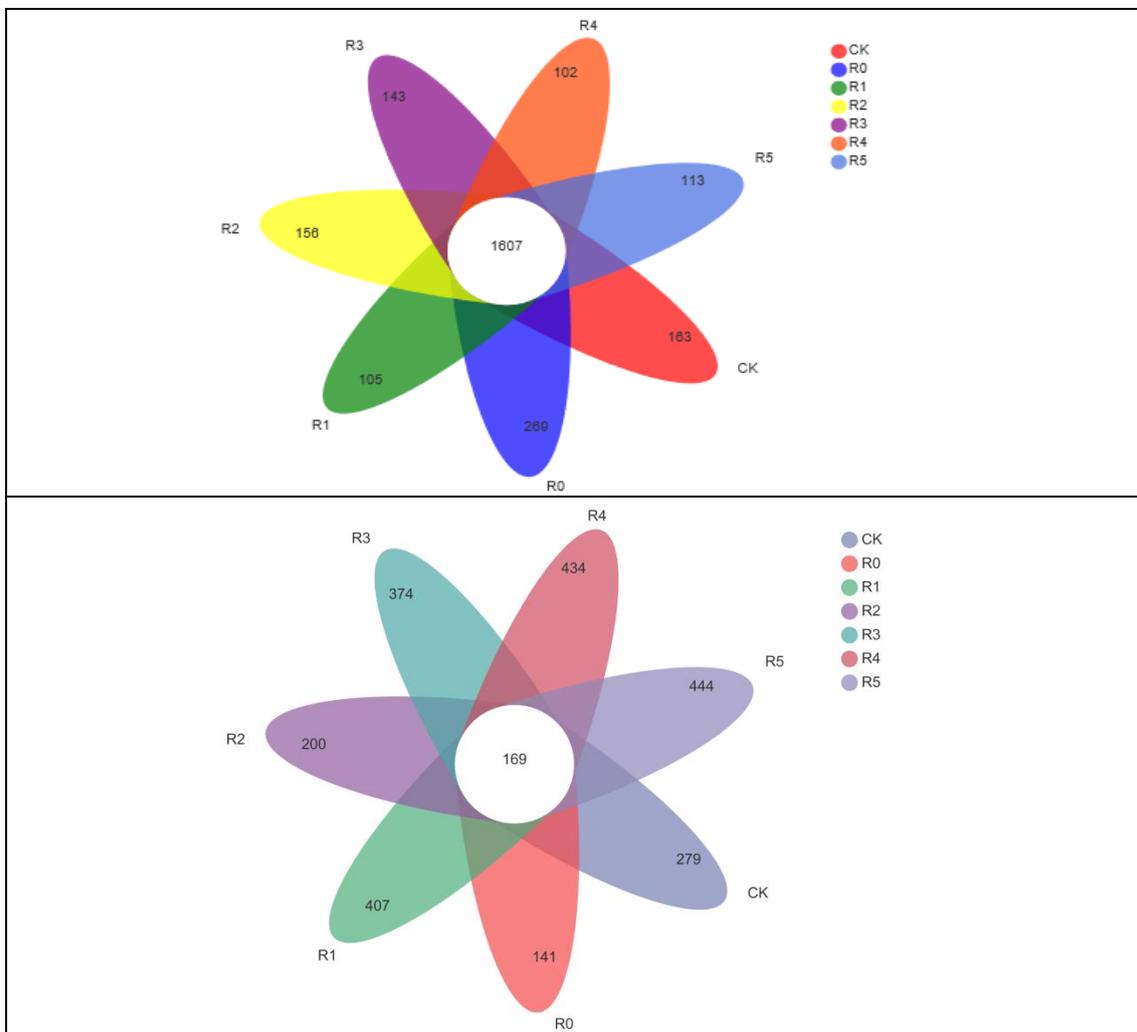


Fig.4 Number of common and unique OTUs based on Wenn analysis

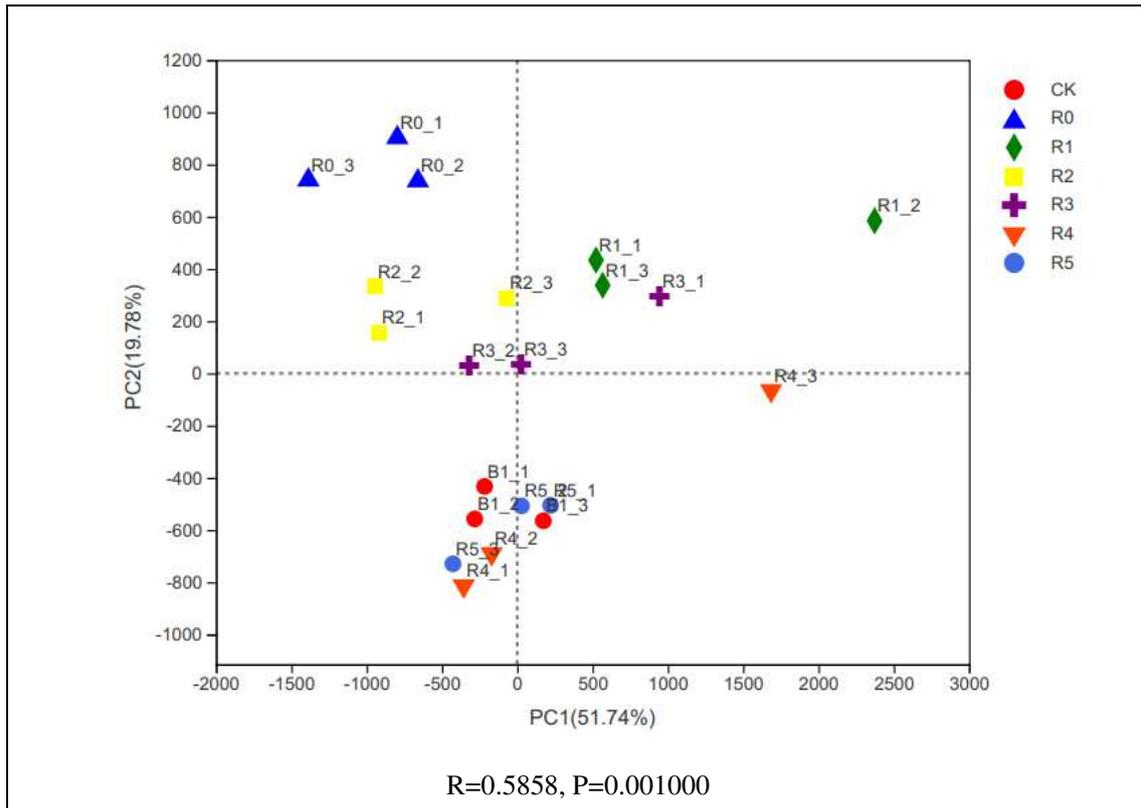
Principal component analysis of bacteria and fungi in the rhizosphere soil of *Gastrodia elata*

PCA was performed on the OTU of R0-R5 and CK. The two principal components extracted explained 71.52% of the total variation (Fig.5). Further analysis shows that along the PC2 axis, the distance between R0 and R4 is the farthest, indicating that the bacterial community composition of the two is obviously different. Compared with other groups, R3,R4, R5 and CK are closer, indicating that their bacterial community composition is more similar. R0, R1, R2 and CK are far apart, indicating that there are greater differences between their bacterial communities.

It can be seen from Fig.5 that the PCA analysis found that the coefficients of variation of the first principal component (PCA1) and the second principal component (PCA2) were 22.91% and 43.21%, and the total variation was 66.12%, indicating that they can represent the composition of the soil fungal community Characteristics. Further analysis showed that along the PC1 axis, CK and R0 are the farthest apart, indicating that their fungal community composition is quite different,

R3, R4, R5 and the other groups are far apart, indicating that the fungal community composition is quite different from other groups. Along the PC2 axis, R3, R4, and R5 co-located on the negative semi-axis, and the distances are close, indicating that there is a greater similarity in the composition of the fungal community.

In general, the results show that with the increase of the natural recovery period, the soil bacterial and fungal community structure in the rhizosphere of *Gastrodia elata* changes; secondly, when the natural recovery period is greater than 3 years, the soil bacterial and fungal community structure tends to be stable.



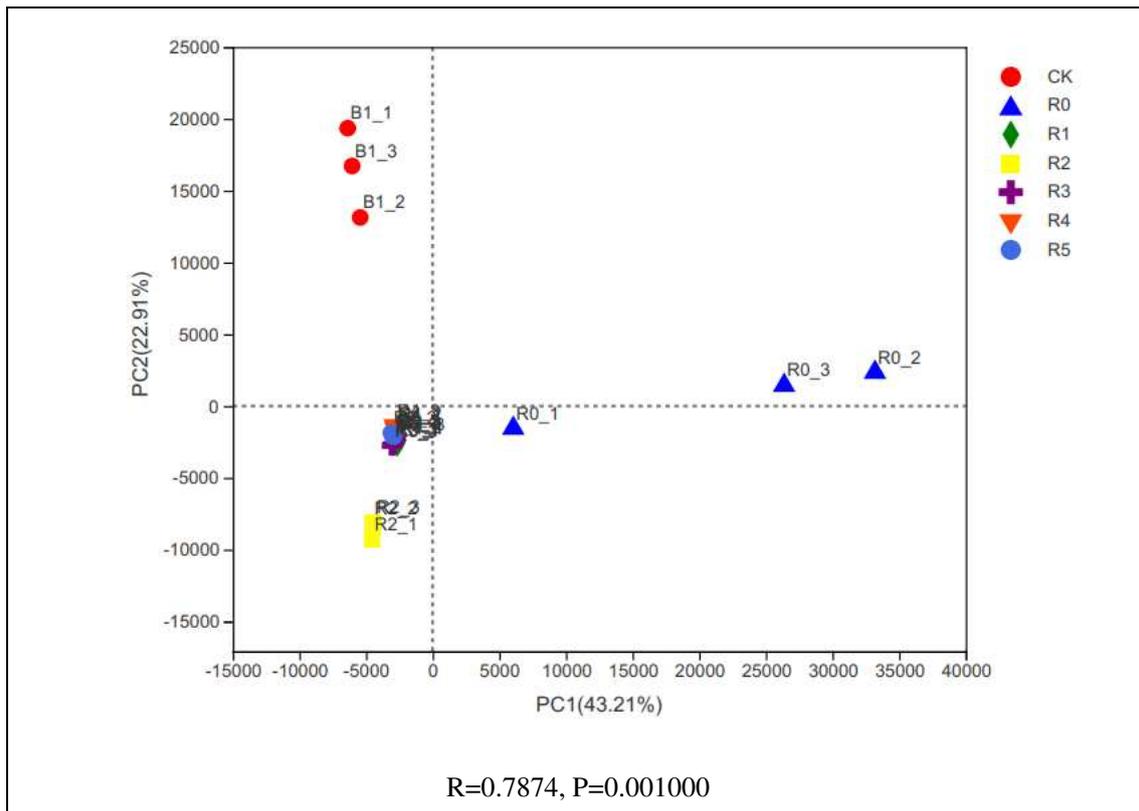


Fig. 5 Principal component analysis (PCA) of OTUs

Relationship between *Gastrodia elata* rhizosphere soil bacteria and fungi and physical and chemical properties

The results of the correlation analysis (RDA) between the top ten bacterial phyla and environmental factors in the rhizosphere soil of *Gastrodia elata* after natural restoration of different years are shown in Figure 6. RDA1 and RDA2 explained 7.78% and 19.47% of the total variation, respectively. The degree of influence of soil properties on bacterial community structure is in the order of TN>TK>TP>OM>pH. The results showed that TK, TN, and OM were significantly related to the bacterial community, and TN was a strong predictor of natural restoration of the soil bacterial community composition of the rhizosphere soil of *Gastrodia elata* over different years. In addition, the results of Spearman's correlation coefficient analysis are as follows (Fig.7). TN was positively correlated with *Actinobacteria* and *Bacteroidetes*, but *Gemmatimonadato* was significantly negatively correlated; TK was positively correlated with *Gemmatimonadato*, and was negatively correlated with *Methylomirabilota* and *Actinobacteria*; OM was significantly negatively correlated with *Gemmatimonadato*.

The correlation analysis (RDA) of the top ten fungal phyla and environmental factors in the rhizosphere soil of *Gastrodia elata* with different years of natural restoration, the results are shown in Figure 6. RDA1 and RDA2 explained 5.13% and 69.85% of the total variation, respectively. The degree of influence of soil properties on bacterial community structure is in the order of TP>PH>TK>OM>TN. The results showed that TK and OM were significantly related to the bacterial community, and TP was a strong predictor of the natural restoration of the soil bacterial community composition in the rhizosphere of *Gastrodia* over different years. In addition, the results of Spearman's correlation coefficient analysis are as follows (Figure 7). TK was positively correlated with *Basidiomycota*, but was significantly negatively correlated with

Chytridiomycota and *Glomer-omycota*; OM was positively correlated with *Ascomycota* and *Kickxellomycota*; PH was positively correlated with *Mortierellomycota*; TP was positively correlated with *Basidiomycota* and negatively correlated with *Kickxellomycota*. At the same time, it can be seen from Figure 7 that the physical and chemical properties of soil are divided into two groups, TP and TK are integrated into one group, and the rest are gathered into another group, indicating that TP and TK have similar effects on bacteria, but are different from other physical and chemical properties.

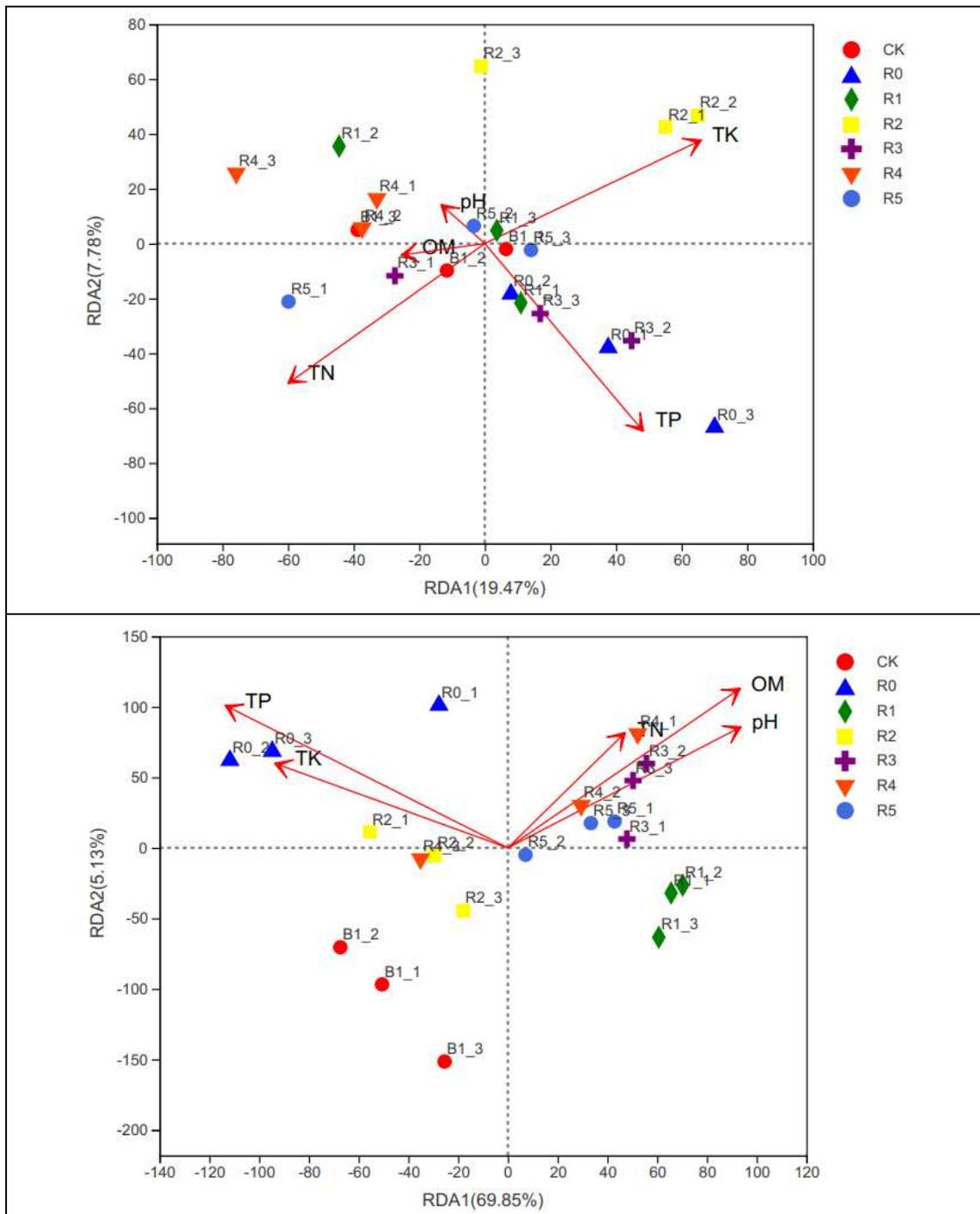


Fig. 6 Redundancy analysis of the 10 dominant bacterial phyla and soil physicochemical properties.

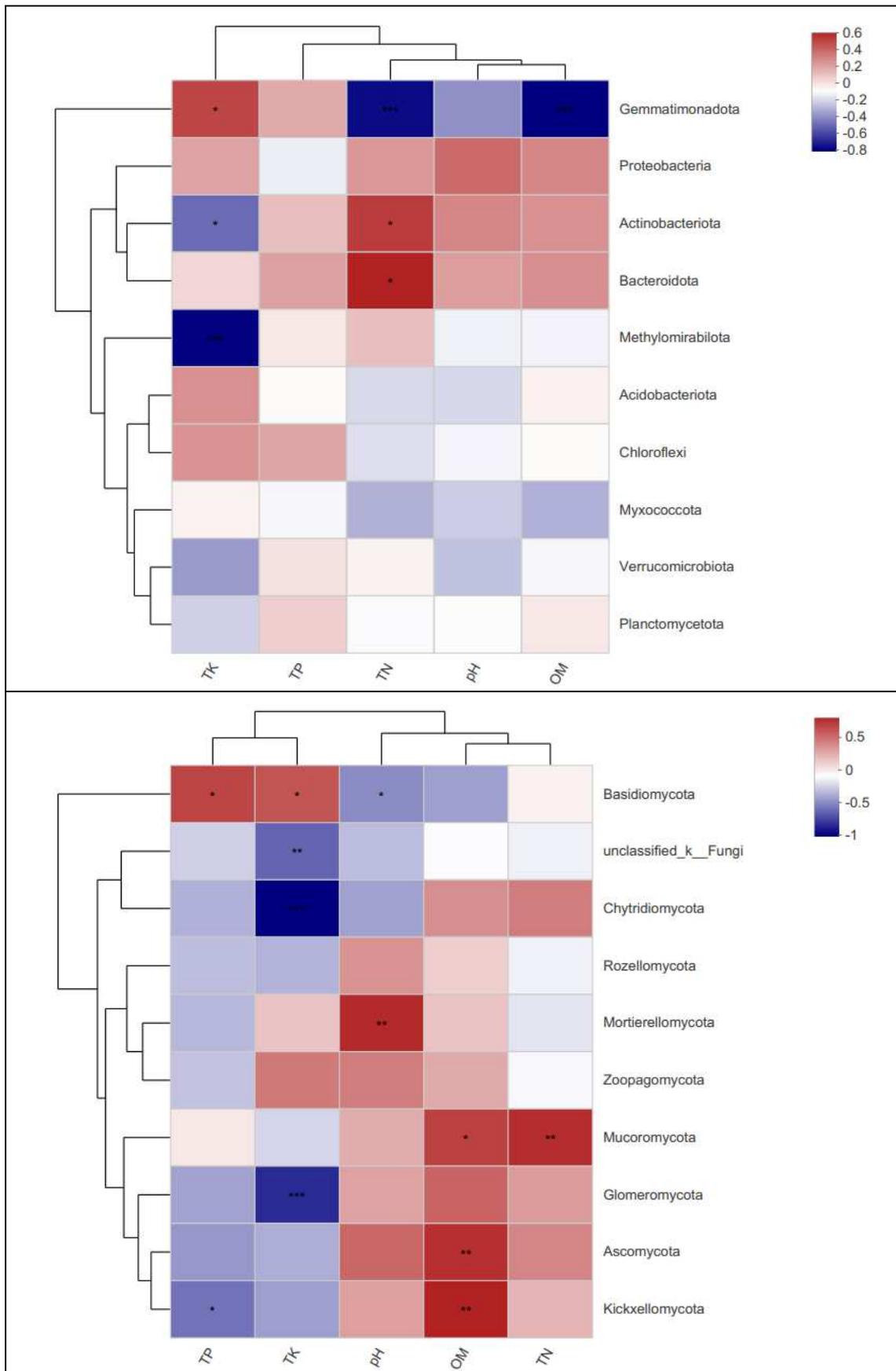


Fig.7 Correlations between the 10 dominant bacterial phyla and soil properties. *, $P < 0.05$.**, $P < 0.01$.***, $P < 0.001$

Discussion

Studies have shown that both *Gastrodia elata* and *Armillaria* will produce continuous cropping and allelopathic effects during the cultivation process [23], resulting in serious reductions in *Gastrodia elata* production. Therefore, land-exchange cultivation methods are often used in production to solve the problem, resulting in the use of wood, land and other resources. The rate is not high, the ecological imbalance of soil microorganisms and other issues [24]. Continuous cropping obstacles refer to the phenomenon of planting the same crops in the same plot for many years, which leads to deterioration of soil physical and chemical properties, nutrient imbalance, crop yield and quality reduction, and aggravation of pests and diseases [25,26,27]. In order to have a more comprehensive understanding of the bacterial community structure in the rhizosphere soil of *Gastrodia elata* from 0 to 5 years of natural restoration, this study used the Illumina MiSeq method [28,29]. The V4-V5 hypervariable region was chosen as the sequencing region because previous studies have shown that this region is the best sequenced region among the 9 hypervariable regions [30].

Through this study, it was found that the Chao index, Shannon index and Invsimpson index showed an increasing trend with the increase of the natural recovery period, indicating that natural recovery for a certain period of time would increase the bacterial diversity and abundance in the rhizosphere soil of *Gastrodia elata*. However, the Shannon index and Invsimpson index of R1 are lower than R0, which may be caused by differences in soil environmental conditions. With the increase of the natural recovery period, the unique OUT number of *Gastrodia elata* rhizosphere soil fungi showed an increasing trend. Three years after the natural recovery of *Gastrodia elata* soil, the soil fungal community was larger than CK, indicating that after planting *Gastrodia elata* and after a certain period of natural recovery, the soil fungal community became rich.

Natural recovery the most important bacterial phyla in the rhizosphere soil of *Gastrodia elata* from 0 to 5 years are *Proteobacteria* and *Actinomycetes*, which are consistent with the results of [31-34]. The main function of *Proteobacteria* is to decompose organic matter and promote plant growth [35,36]. With the increase of the natural recovery period, the relative abundance of the *Proteobacteria* phylum increased first and then decreased; the *Acidobacteria* phylum gradually decreased and then slightly increased; these differences may be due to differences in soil environmental conditions, leading to soil microbial growth Different changes [37-39]. As we all know, *Actinomycetes* can produce antibiotics, hydrolytic enzymes and other active substances. It is the bacteria that mainly participate in the transformation of substances and produce antibiotics [40]. With the increase of natural recovery years, the relative abundance of *Actinomycetes* decreases first and then increases. On the contrary, *Gemmatimonadetes* is a harmful bacteria that can cause N loss and reduce crop growth [41]. With the increase of the natural recovery period, the relative abundance of *Gemmatimonadetes* in the rhizosphere soil of *Gastrodia elata* gradually decreases, indicating that the natural recovery of *Gastrodia elata* soil for a certain number of years is harmful The bacterial phylum has a certain mitigation effect. Analyze at the subordinate level. With the increase of the natural recovery period, the main genus *norank_f_Xanthobacteraceae* in the rhizosphere soil of *Gastrodia elata* showed a gradually increasing trend, while the *Bradyrhizobium* had a decreasing trend. Among them, the *Bradyrhizobium* was a symbiotic nitrogen-fixing bacteria [42], if the continuous cropping of *Gastrodia elata* is continued after the

harvest of *Gastrodia elata*, the increase of the flora may lead to an increase in soil nitrogen content and accelerate soil compaction.

The most important fungal phyla in the rhizosphere soil of *Gastrodia elata* from 0 to 5 years of natural recovery is *Basidiomycota* and *Ascomycota*, which account for the largest proportion, dominate the soil fungal community structure, and are the most important fungal group. With the increase of the natural recovery period, the proportion of *Ascomycota* fungi showed an upward trend. *Ascomycota* is a large group of fungi, with a wide variety of species and various metabolic forms. It plays an important role in maintaining the function of the soil ecosystem. In this study, *Ascomycota* has an absolute advantage in all samples. At the level of genus analysis, some beneficial fungi, such as *Mortierella* and *Trichoderma*, appear in the rhizosphere soil of *Gastrodia elata*. With the increase of the natural recovery period, the proportion of *Mortierella* fungi is gradually increasing. *Mortierella* is a beneficial microorganism to the soil. This fungus can repair soil fungal communities and effectively prevent crop root rot. It shows that the natural restoration of the rhizosphere soil of *Gastrodia elata* for a certain period of time can improve the beneficial fungi to a certain extent.

With the increase of the natural recovery period, the physical and chemical properties of the rhizosphere soil of *Gastrodia elata* also changed. In this study, TN was found to have the greatest impact on the structure of bacterial community, and pH's impact on the structure of fungal community ranked second. Soil pH will affect the physiological metabolism of soil microorganisms, change the competitive relationship within the microbial community, and inhibit the growth of non-adapted microorganisms [43], all of which will affect the structure of the microbial community. Among the top three bacterial phyla with relative content, TN is positively correlated with *Actinobacteria*. In the top three fungal phyla with relative content, pH was positively correlated with *Mortierellomycota*.

In general, compared with CK, the relative content of harmful bacteria in the soil after planting *Gastrodia elata* increased, and the structure of the soil microbial community in the rhizosphere soil of *Gastrodia elata* changed, which may be an important aspect of continuous cropping obstacles. Therefore, maintaining the balance of the rhizosphere soil micro-ecosystem is essential to alleviate continuous cropping obstacles. In addition, continuous cropping obstacles are related to many factors. It is necessary to further study the mechanism of continuous cropping obstacles of *Gastrodia elata*.

Conclusion

There are various reasons for the continuous cropping obstacles of *Gastrodia elata*. It is the result of the combined effects of soil physical and chemical properties and microorganisms. *Gastrodia elata* planting obviously affects the bacterial community structure and physical and chemical properties of rhizosphere soil, increasing the diversity of bacteria and fungi. Comprehensive analysis shows that the community structure of the rhizosphere soil of *Gastrodia elata* after natural recovery more than 3 years tends to be stable. In this study, the dominant soil bacterial populations, *Proteobacteria* and *Chloroflexi*, were higher than those before planting *Gastrodia elata*. *Ascomycota* and *Mortierellomycota* belonged to the soil dominant fungi phylum, compared to planting *Gastrodia elata*. Compared with the previous soil, the proportion of these two fungi in the soil was increased after planting *Gastrodia elata*. With the increase of the natural recovery period, the species of these two fungi gradually increased, and the biggest change was at 3 years after the natural recovery. Naturally recovering to a certain number of years, the structure

and composition of the soil microbial community will tend to balance. This study shows that the soil environment can indeed be improved after 3 years of natural restoration. Further research is needed for the rotation of other crops in *Gastrodia elata* to alleviate the obstacles to continuous cropping of *Gastrodia elata*.

Method

General situation of test site and test soil

The test soil was taken from the *Gastrodia elata* planting base of Lvtong, Dejiang County, Tongren City, Guizhou Province (108°8'33.07"E, 28°7'31.4"N, 1049m above sea level). It is a barren sloping land and no crops have been planted. The experimental treatments included natural restoration of the soil in the hemp hole after the harvest of *Gastrodia elata* elata, 0 years (R0), 1 year (R1), 2 years (R2), 3 years (R3), 4 years (R4), 5 years (R5) and *Gastrodia elata* planting. For the former soil (CK), the soil around the fungus materialis taken from 0-20cm per treatment, and the soil is yellow loam.

Experimental design and soil sample collection

Set up 7 treatments, namely: (1) soil that has not been planted with *Gastrodia elata* in the same sampling site (CK); (2) soil that has been restored naturally after *Gastrodia elata* stubble for 0 years (R0); (3) soil that has been restored naturally after *Gastrodia elata* stubble for 1 year (R1); (4) Naturally restored soil for 2 years after *Gastrodia elata* stubble (R2); (5) Naturally restored soil for 3 years after *Gastrodia elata* stubble (R3); (6) Naturally restored soil for 4 years after *Gastrodia elata* stubble (R4); (7) Naturally restored soil for 5 years after *Gastrodia elata* stubble (R5); 3 replicates for each treatment, a total of 21 samples. Choose the same points of natural restoration and different years of numbing points, and use the five-point sampling method. First remove the 0~5cm topsoil, collect soil samples from the periphery of the fungus sticks, collect 7 soil samples, mix the soil samples, remove the visible impurities, remove the excess soil with the quarter method, take 1kg into a ziplock bag, and label it. Take it back to the laboratory for natural drying and sieving for analysis of physical and chemical properties. Take another 10g into a sterile EP tube, label it, store it in an ice bag, and bring it back to the laboratory for storage at -80°C for use in nucleic acid extraction.

Analysis of soil physical and chemical properties

Refer to the Baoshidan method [44], the organic matter is measured by the potassium dichromate volumetric method and the external heating method; the soil water extract is measured by the pH acid meter; the TN is measured by the nitrogen analyzer; the TP is measured by the molybdenum antimony colorimetric method; The TK is determined by flame photometry.

DNA extraction, amplification and sequencing

Entrusted Shanghai Meiji Biomedical Technology Co., Ltd. to complete DNA extraction, Illumina library preparation and high-throughput sequencing according to the MP-soil soil DNA extraction kit instructions. Primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') were used to amplify the bacterial 16S V3+V4 variable region; primers ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS2R (GCTGCGTTCTTCATCGATGC) were used to amplify the fungal ITS1 region. Amplification program: 95°C pre-denaturation 3min, 27 cycles (95°C denaturation 30s, 55°C annealing 30s, 72°C extension 30s), then stably extended at 72°C for 10 minutes, and finally stored at 10°C (PCR instrument: ABI GeneAmp® Type 9700). The PCR reaction system is: 5×TransStart FastPfu buffer 4μL, 2.5mM dNTPs 2μL, upstream primer (5uM) 0.8μL, downstream primer (5uM) 0.8μL,

TransStart FastPfuDNA polymerase 0.4μL, template DNA 10ng, make up to 20μL. Each treatment was repeated 3 times, and high-throughput sequencing was performed on the Illumina Miseq PE300 platform [45].

Data processing and statistical analysis

The Chao, Shannon, and Simpson indices use Mothur (1.30.2 https://www.mothur.org/wiki/Download_mothur) to calculate alpha diversity. OTU uses Uparse (7.0.1090 <http://www.drive5.com/uparse/>) to cluster with a similarity cutoff of 97%, and the chimeric sequences are identified and removed using UCHIME. Use Qiime (1.9.1 "<http://qiime.org/install/index.html>) to generate a water abundance table for each taxonomy. The RDP 11.5 classifier is used to analyze the sequence of each 16S rRNA gene. SILVA 138 analyzes the phylogeny of the database The affiliation relationship, the confidence threshold is 70%. SPSS11.0 software is used to perform variance analysis and LSD multiple comparison.

Abbreviation

CK: The soil that has not been planted with Gastrodia; R0~R5: The rhizosphere soil of Gastrodia naturally restored for 0~5 years after stubble; OTU: Operational Taxonomy Unit; PCA: Principal Component Analysis; RDA: Redundancy Analysis; Analysis of Variance: One-way Analysis of Variance

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Author's contribution

LFL and LJ designed this experiment. HJL and WYF conducted sample collection. HJL, WYF and LLP performed experiments and analyzed the data. LLP completed the manuscript. The final manuscript read and approved by all authors.

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Availability of data and materials

Ethical approval and consent to participate

not applicable.

Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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