Comparative study of the rhizospheric arbuscular mycorrhizal fungi from three medicinal licorice plants from Xinjiang

Tiantian Gao
Shihezi University
Zhongke Wang
Shihezi University
Xinhua Lv
Shihezi University
Guifang Li
Shihezi University
Yaling He
Shihezi University
Li Zhuang (519206056@qq.com)

Research article

Keywords: Licorice; Arbuscular mycorrhizal fungi; High throughput sequencing 41

Posted Date: May 7th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-23918/v1

Comparative study of the rhizospheric arbuscular mycorrhizal fungi from three medicinal licorice plants from Xinjiang

Tiantian Gao[1], Yaling He[1], Guifang Li[1], Xinhua Lv[1], Zhongke Wang[1*], Li Zhuang[1*]

* Correspondence: zhuangliii@163.com

[1] College of life science, shihezi university, north fourth road, shihezi city, xinjiang weiwu autonomous region 832003, China

Abstract

Background: Glycyrrhiza is mainly used as medicine by roots and rhizome, However, the underground biomass of the active components of cultivated Glycyrrhiza is lower than that of wild. Arbuscular mycorrhizal fungi(AMF) can increase the content of flavonoids and other effective components in licorice; increase the underground biomass of licorice root. Therefore, We collected the rhizosphere from these licorice plants, from the soil layer with a depth of 0-20 cm, 20-40 cm, and 40-60 cm in the Xinjiang region, and employed the Illumina Miseq high-throughput sequencing platform to investigate the structure and diversity of these AM fungal communities.

Results: In this study, we isolated a total of 34 AM fungi that encompassed a phylum, a class, five (orders, families, and genus), and the Glomus and Paraglomus emerged as the dominant genus. We observed the highest diversity in the AM fungi in Glycyrrhiza uralensis Fisch. The Glycyrrhiza inflata showed the highest richness, whereas the Glycyrrhiza glabra showed the lowest richness and diversity in each soil

layer. The plant species influenced the AM fungi more than the soil depth. The total phosphorus, available phosphorus, and organic matter in the soil prominently impacted the distribution of soil AM fungi. In contrast, the soil ammonium and nitrogen content had the lowest-impact on the AM fungi distribution. The genus Glomus was was found to be positively correlated with total phosphorus ($P<0.001$), and negatively correlated with total potassium ($P<0.001$), total salt ($P<0.01$), soil water content, and organic matter ($P<0.05$). The genus Paraglomus was found to be negatively correlated with the total phosphorus ($P<0.001$), whereas positively correlated with total potassium ($P<0.001$), total salt ($P<0.001$), organic matter ($P<0.05$), and fast-acting potassium ($P<0.05$). Besides, we found a positive correlation between the genus Diversispora and ammonium nitrogen ($P<0.05$), whereas the available phosphorus ($P<0.05$) and total salt ($P<0.05$) were negatively correlated with the genus Diversispora.

Conclusions: In this study, The genus Glomus emerged as the dominant genus, The distribution of the rhizospheric AM fungal communities was significantly affected by the host plant species, but it was least affected by the soil depth. The total phosphorus, available phosphorus, total salt, and organic matter affected the rhizospheric AM fungi to a certain extent. The correlations between different AM fungi and the soil's physical and chemical properties were found to be distinct.

Keywords: Licorice; Arbuscular mycorrhizal fungi; High throughput sequencing

Background: Glycyrrhiza belongs to the family Leguminosae. It has a characteristic

feature of drought, salt, and alkali resistance. Besides, it shows the presence of an evolved root system that can withstand strong wind currents, conserve soil and water, improve saline as well as alkaline land, and helps in sand fixation [1]. These plants are best suited for the desserts. Glycyrrhiza is globally distributed, and in China, it is majorly distributed in temperate desert and grassland areas of the northwest arid regions. Due to the extended climatic zone, it shows its presence in a long-to-west long and narrow north-south zone. Currently, there are around 29 species and six variants of the Glycyrrhiza genus across the globe, out of which 18 species and three variants were reported to be present in China. However, only Glycyrrhiza uralensis Fisch., Glycyrrhiza inflata Bat, and Glycyrrhiza glabra L. are included in the Pharmacopoeia of The People's Republic of China （Ch.P.）. Among them, the Glycyrrhiza uralensis Fisch. is widely distributed across the northwest, northeast, and north region of China. The Glycyrrhiza inflata Bat and the Glycyrrhiza glabra L. are primarily found in the Xinjiang and Gansu regions, respectively [2] [3]. The licorice plant parts, i.e., roots and rhizomes, are widely used. It is a commonly used herbal medicine in China. Alternatively, it is also is referred to as “guolao” and has anti-inflammatory [4], anti-oxidant [5], antiviral [6], antitumor [7], and many other medicinal properties. Apart from medicine, it has broader applications in other industries as well, for instance, animal husbandry, chemical industry, and so on. At present, the main problems of cultivated licorice are that the active components and underground biomass of the cultivated licorice are not as good as the wild licorice [2], which creates a steep disequilibrium between the supply and demand of licorice.

Therefore, improvement of the cultivated licorice biomass, as well as the quality of its effective component and meeting its demand in the international market, are the crucial issues that need to be addressed by the research studies dealing with the licorice cultivation. The AM fungi are the primary component of the microbial community in the rhizosphere of natural ecosystems, and it has received widespread attention for its ecological characteristics. Previous studies have found that AM fungi have a symbiotic relationship with the majority of the higher plants as it promotes the absorption of nutrients such as phosphorus [8] [9] and nitrogen in the host plant. Besides, it also enhances the sustainability of the host plant in challenging environmental conditions such as the presence of drought [10] [11], high temperature [12], saline-alkali conditions [13], and heavy metal content [14]. It changes the root system structure by increasing the number of adventitious roots, lateral roots, lower plant part biomass [14], and active ingredient content such as glycyrrhizic acid [15].

Rhizospheric AM fungi have a complex interaction with soil and host plants. Along with the rhizosphere, an in-depth strategic investigation of the correlation between AM fungi, soil, and host plants might improve the yield and quality of licorice. The research studies on the wild medicinal licorice rhizosphere AM fungi are insufficient. In the current study, we have examined the Glycyrrhiza uralensis, Glycyrrhiza inflata, and Glycyrrhiza glabra from the Xinjiang region. The genomic DNA from the soil samples was used as the template for PCR amplification and high-throughput sequencing. In this paper, we have also discussed three types of licorice, their rhizospheric AM fungi community structure, and diversity, as well as their

relationship with the host plant, soil depth, physical and chemical properties. A study on the role of AM fungi in licorice roots from the Xinjiang's unique ecological environment will have a theoretical and practical impact on the improvement of the licorice cultivation and ecological protection.

Results

1、Physical and chemical properties of licorice rhizosphere soil

Table 1.

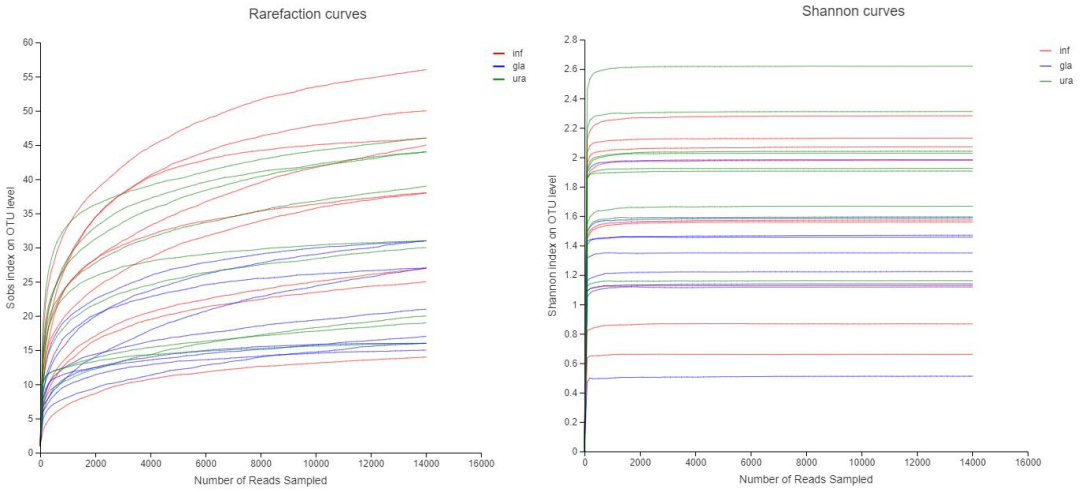
species	depth	OSC（g/kg）	TN（g/kg）	TP（g/kg）	TK（g/kg）	N3（mg/kg）	N4（mg/kg）	AP（mg/kg）	AK（mg/kg）	TS（g/kg）	PH	SWC（%）
inf	0-20	27.85±5.91ac	0.94±0.65a	0.56±0.17a	21.55±0.75a	20.56±3.70ac	5.31±0.75ac	11.56±5.57a	207.02±29.47a	8.49±4.60a	8.48±0.18a	4.48±0.98
	20-40	28.38±8.82a	0.75±0.06a	0.55±0.10a	21.30±2.08a	14.06±8.29a	5.30±1.47a	10.73±10.45a	161.30±81.31a	6.12±7.42acd	8.34±0.12a	4.94±1
	40-60	27.74±5.31ah	0.60±0.11a	0.50±0.11ah	22.73±0.60ah	7.98±3.73a	3.99±0.89ah	6.74±4.70a	171.78±91.96a	2.48±1.51ah	8.53±0.31a	5.35±0.79
	mean value	27.99A	0.76	0.54A	21.86A	14.2A	4.87A	9.68	180.03A	5.7AB	8.45	4.92AB
gla	0-20	11.71±2.18ad	0.88±0.21a	0.68±0.01a	20.88±0.58a	4.67±2.54ad	3.26±0.38ad	7.62±2.68a	276.74±13.72a	4.01±2.84a	8.81±0.58a	6.95±1.42
	20-40	11.88±2.03a	0.69±0.06ab	0.68±0.04a	20.65±0.71a	3.22±1.56a	3.15±0.37a	5.38±1.00a	256.86±2.94af	4.27±0.64ac	8.89±0.87a	7.65±1.61
	40-60	7.90±0.11ai	0.50±0.03b	0.64±0.04ahi	20.79±0.43ai	2.77±1.35a	3.56±0.83ah	2.87±0.55a	285.68±116.18	6.41±1.91ai	8.79±0.91a	9.35±1.46
	mean value	10.5B	0.69	0.67B	20.77AB	3.55B	3.33B	5.29	273.09B	4.89A	8.83	7.98A
ura	0-20	15.89±3.52ad	0.71±0.23a	0.72±0.06a	19.69±1a	7.9±9.15acd	6.17±0.74ad	3.74±2.57a	88.78±31.69ae	1.13±0.18a	8.48±0.3a	2.91±0.19
	20-40	14±3.96a	0.91±0.18a	0.71±0.06a	19.54±1.03a	8.03±8.84a	5.63±1.28a	4.14±4.37a	79.06±35.8ag	0.98±0.07ad	8.57±0.04a	3.54±0.29a
	40-60	14.35±7.31ai	0.88±0.37a	0.71±0.07ai	20±0.75ai	6.85±6.48a	6.26±0.68ai	3.22±2.64a	75.78±29.21a	1±0.4ah	8.55±0.26a	4.31±0.51ab
	mean value	14.74B	0.83	0.71B	19.74B	7.59AB	6.02C	3.7	81.21C	1.03B	8.53	3.58B

As depicted in Table 1, the physical and chemical properties of the soil vary according to the varieties? and soil layers. The rhizospheric soil pH value of the three licorice varieties was above 8.3 (8.31-8.84), which indicates the presence of alkaline soil. The total nitrogen content of the Glycyrrhiza inflata and the Glycyrrhiza glabra decreased with the soil depth. The total nitrogen content of the Ural licorice was highest in the 20-40 cm soil layer. The water content increased with soil depth, and the water content of Glycyrrhiza glabra L. was the highest. We did not observe a significant difference between the pH, total nitrogen, and available phosphorus content among different Glycyrrhiza plants at different soil layers. The total salt content was between

1.0-2.4 g/kg, and the mean value of the total salt content of the Glycyrrhiza glabra was significantly higher than the other licorice varieties ($P<0.05$). The mean values of soil organic carbon, total phosphorus, potassium, nitrate, and nitrogen of the Glycyrrhiza inflata Bat, were significantly higher than the Glycyrrhiza uralensis and Glycyrrhiza glabra L. ($P<0.05$). There was no significant difference in potassium availability between different soil layers of the same glycyrrhiza , but there was significant difference between the same soil layers of different glycyrrhiza ($P<0.05$) . Moreover, we observed a significant difference between the available potassium and ammonium nitrogen content among the three licorice plants ($P<0.05$). Apart from the significant difference in the total nitrogen content in the soil layers of the Glycyrrhiza inflata, the differences of other indicators were not significant, which indicates that the physical and chemical properties of soil were influenced more by the plant species than by the soil layer.

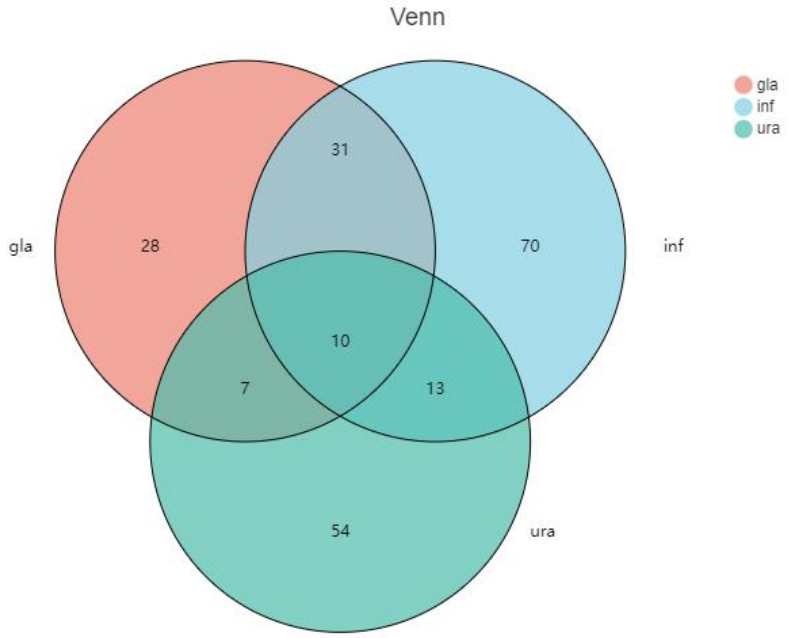
1. Arbuscular mycorrhizal fungal community structure

Figure 1.



(a) (b)

Figure 2.



We obtained a total of 1,258,294 original sequences from the rhizospheric soil of three licorice species. After screening, a total of 629,147 valid sequences were obtained Later, we performed the OTU cluster analysis on the obtained sequences with the criteria of 97% similarity. As depicted in Figure 1., the dilution curve of the three licorice rhizosphere soil samples was flat, which indicates a reasonable amount of sequencing data of the AM fungi in each soil group. The Venn (Figure 2) diagram of the number of AM fungi OTUs from the three different licorice roots rhizospheric soil showed that a total of 215 OTUs were obtained for the three types of licorice roots. The number of unique OTUs in Glycyrrhiza inflata, Glycyrrhiza uralensis, and Glycyrrhiza glabra decreased to 71, 55, and 26, respectively. These three licorice varieties had a total of 13 common OTUs. Furthermore, the Glycyrrhiza inflata and

the Glycyrrhiza glabra showed 31 common OTUS. The Glycyrrhiza inflata and Glycyrrhiza uralensis had 12 common OTUs. The Glycyrrhiza glabra and Glycyrrhiza uralensis had seven common OTUs.

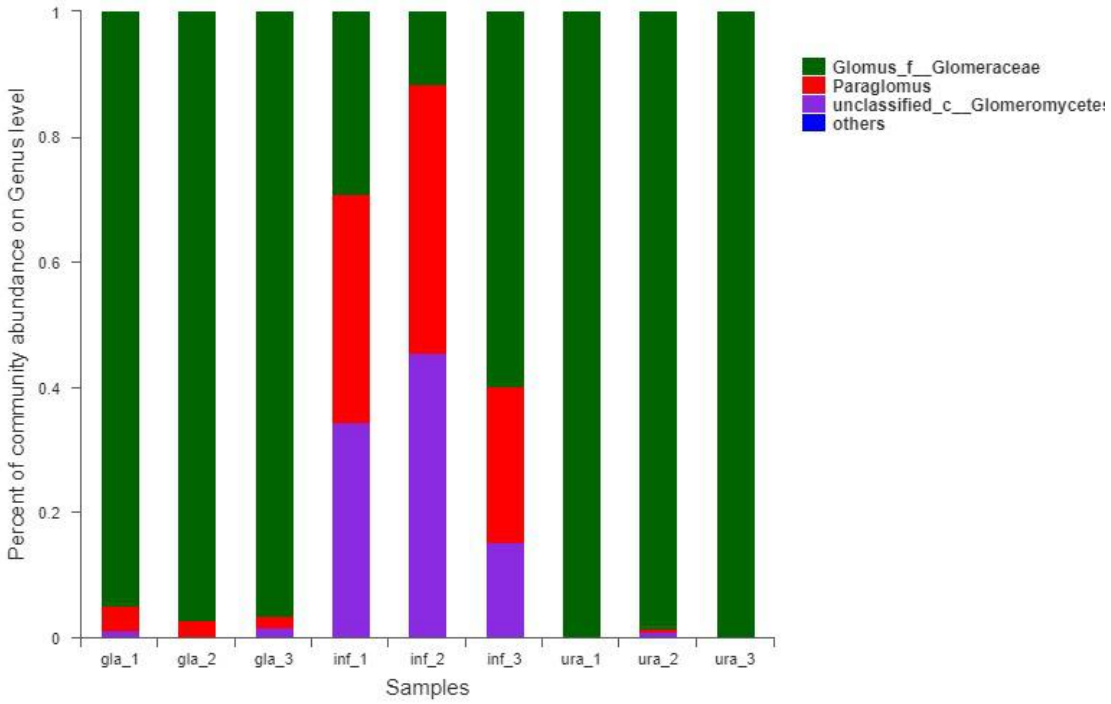
A higher number of common OTUs between the two licorice species indicate a higher similarity in their fungal community composition. Thus, the Venn diagram analysis indicates that the Glycyrrhiza glabra -Glycyrrhiza inflata had a higher similarity in the rhizospheric AM fungi composition as compared to the Glycyrrhiza inflata - Glycyrrhiza uralensis, and Glycyrrhiza uralensis -Glycyrrhiza glabra

We isolated a total of 34 AM fungi belonging to a phylum, a class, and 5 (orders, families, and genera) in this experiment. The rhizospheric AM fungi community composition of different varieties of licorice was different, but they were relatively consistent as far as the soil depths are concerned (Figure 3). The three types of rhizospheric AM fungi from the licorice root were the same at the genus level, but their proportion was different. The genus Glomus was found to be the dominant genus in the AM fungi, which accounted for 77.59% of the total OTUs, whereas 11.53% of the total OTUs belonged to genus Paraglomus.

The relative abundance of the genus Glomus in the three-layer of Ural licorice was above 98%, and it was more than 94% in the Glycyrrhiza glabra. The relative abundance of the Glycyrrhiza inflata was 11.6% in the 20-40 cm layer., which was reported to be the lowest. Moreover, the genus Paraglomus showed a maximum relative abundance of 42.9% in Glycyrrhiza biloba at 20-40 cm. However, this genus

was not found in the Glycyrrhiza urals at 0-20 cm and 40-60 cm.

Figure 3.



Besides, we isolated a total of 14 AM fungi from the rhizosphere of the Glycyrrhiza inflata, 13 AM fungi from the Glycyrrhiza glabra, and 31 AM fungi from the Glycyrrhiza uralensis. In contrast to the other two Glycyrrhiza species, Glycyrrhiza uralensis showed the presence of 18 AM fungal species, all of which belonged to genus Glomus. Among them, s__Diversispora-spurca-VTX00263 was found at the molecular level classification in the 40-60 cm soil layer from Glycyrrhiza glabra and Glycyrrhiza uralensis.

2. Diversity of arbuscular mycorrhizal fungi

Table 2.

species	depth	sobs	shannon	simpson	ace	chao	coverage %
inf	0-20	36.33±18.50	1.43±0.71	0.39±0.19	40.23±14.41	37.79±18.01	99.97
	20-40	39.67±13.32	1.58±0.70	0.36±0.22	48.29±17.63	48.06±19.50	99.96
	40-60	35.33±8.62	1.75±0.54	0.26±0.14	48.44±15.85	43.31±6.3585	99.95
	meam value	37.67±13.44A	1.58±0.59AB	0.34±0.17AB	43.67±15.99A	41.45±13.41A	99.960
ura	0-20	31.77±12.58	1.79±0.75	0.29±0.16	38.88±11.12	35.25±12.20	99.96
	20-40	30.33±13.50	2.0816±0.19	0.18±0.02	34.33±11.62	33.17±13.48	99.97
	40-60	33.00±13.08	1.88±0.19	0.26±0.11	36.49±14.63	36.61±15.688	99.96
	meam value	32.11±11.76A	1.92±0.42A	0.24±0.11A	39.09±12.14A	36.47±13.32AB	99.97
gla	0-20	23.33±6.66	1.27±0.19	0.41±0.12	28.59±2.28	24.90±6.84	99.97
	20-40	23.33±7.51	1.53±0.43	0.30±0.13	30.72±10.99	27±10.44	99.96
	40-60	22±8.66	1.14±0.56	0.48±0.28	26.75±9.39	25.72±9.06	99.97
	meam value	22.33±6.69B	1.32±0.40B	0.39±0.18B	28.06±9.35B	26.81±11.54B	99.98

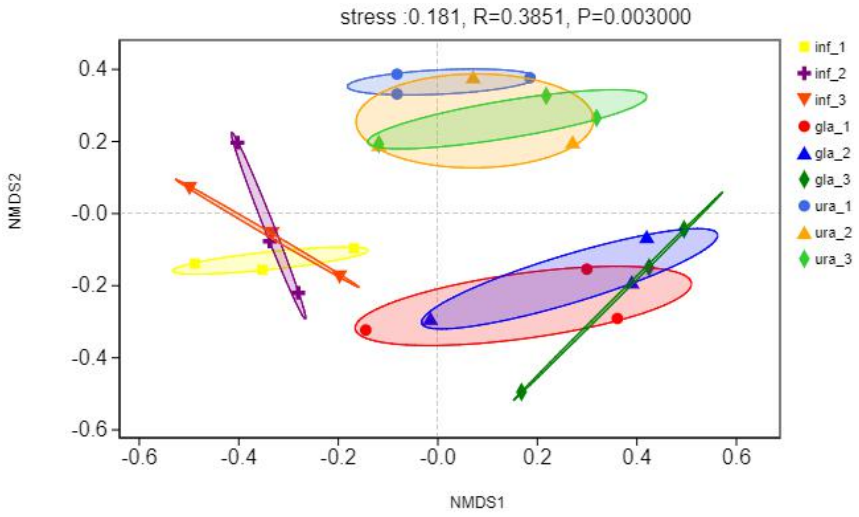
Table 2 demonstrates the diversity index of the three licorice rhizospheric AM fungi, with the coverage index of above 99%, which demonstrates a high probability of gene sequence detection in the soil samples. Also, it reflects the three licorice rhizospheric AM fungal community species and structure.

We comprehensively analyzed the Chao1 and ACE richness index as well as the Shannon and Simpson diversity index. The outcome of this analysis demonstrated that the AM fungal community diversity in the three licorice rhizosphere soils could be ranked from high to low as Glycyrrhiza uralensis > Glycyrrhiza inflata Bat > Glycyrrhiza glabra; the rhizosphere AM fungal of Glycyrrhiza uralensis was significantly higher than the Glycyrrhiza glabra ($P<0.05$). The rhizosphere AM fungal richness index could be ranked from high to low as Glycyrrhiza inflata> Glycyrrhiza uralensis > Glycyrrhiza glabra. The Chao1 and ACE richness index was significantly higher for the Glycyrrhiza inflata AM fungi as compared to that of Glycyrrhiza glabra AM fungi ($P<0.05$), which indicates that the diversity of the rhizospheric AM fungal community was significantly different in different Glycyrrhiza species ($P<0.05$). The

rhizosphere AM fungal diversity index and richness index of Glycyrrhiza inflata increased with the depth of the soil layer. The Glycyrrhiza glabra showed the highest diversity index and the richness index scores at the 20-40 cm soil layer, whereas Glycyrrhiza uralensis showed the highest value of diversity index at the soil layer of 20-40 cm. The soil layer of 0-20 cm showed the maximum richness index value. We did not observe any significant difference between different soil layers of the same licorice variety.

The diversity of Ural licorice in different soil layers was higher as compared to Glycyrrhiza inflata, and Glycyrrhiza glabra. The richness of Ural licorice at 0-20 cm was higher than that of Glycyrrhiza inflata, and Glycyrrhiza glabra; however, the richness of Glycyrrhiza inflata in different soil layers was higher than the Ural licorice. Glycyrrhiza glabra showed the lowest richness in all the soil layers. The richness did not vary significantly between the different licorice variety with the same soil layer.

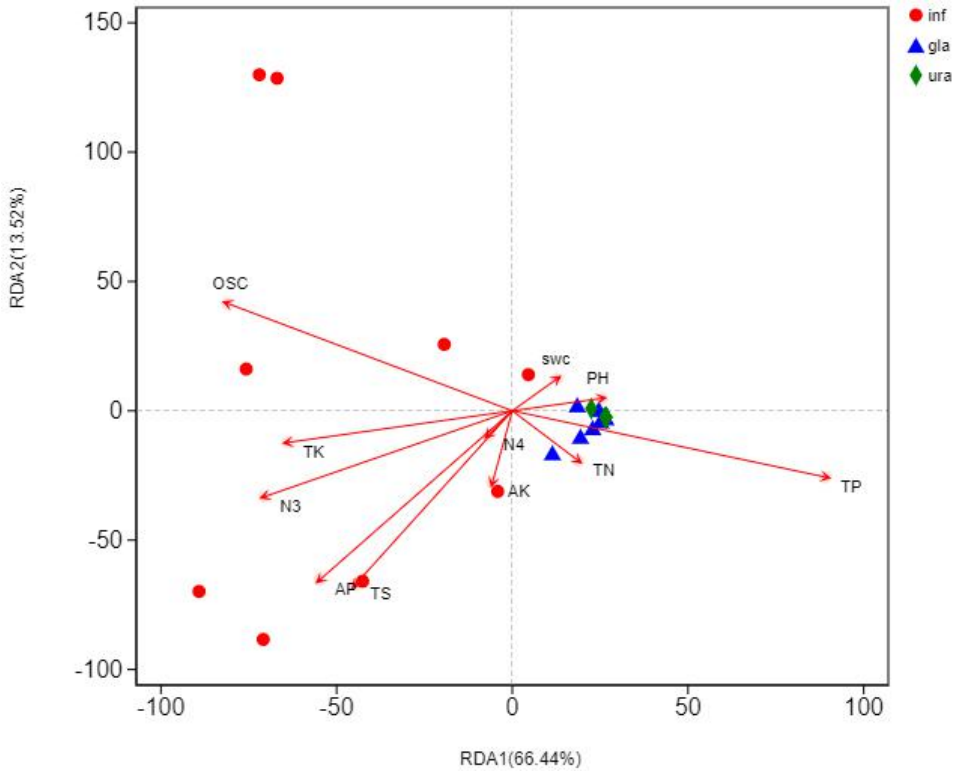
Figure 4.



The PerMANOVA analysis demonstrated that the AM fungal communities were significantly affected by plant species (R2=0.60027, P=0.001) as compared to the soil depth. Non-metric multi-dimensional analysis (NMDS) (Figure 4) of the AM fungi showed that the rhizospheric AM fungal communities of these three licorice roots were utterly separated, but there was some overlap between different soil depths of the same licorice. Interestingly we observed that the difference between the three licorice variety was higher than the difference within the group (R-value of ANOSIM=0.181, P<0.01), which indicates that the effect of plant species on the composition of rhizospheric AM fungal community was higher than the soil depth.

3. Correlation between the soil's physical and chemical properties and arbuscular mycorrhizal fungi

Figure 5.



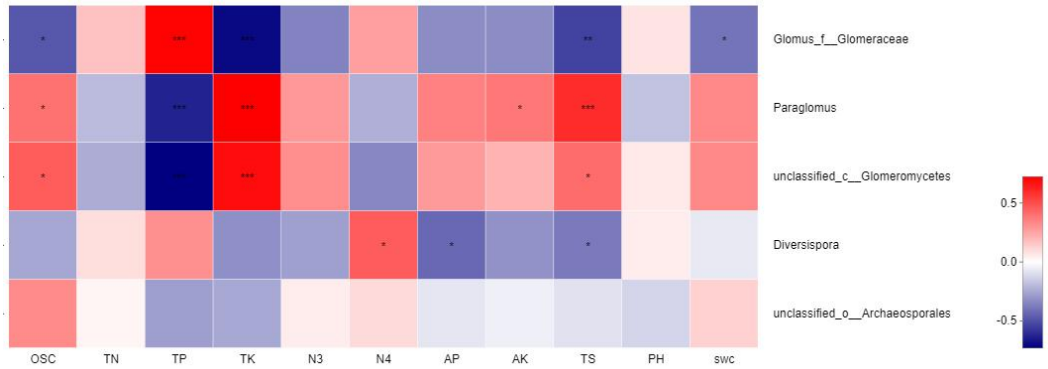
We conducted the redundancy analysis (RDA, Figure 5) of the physicochemical properties of soil and AM fungal communities to examine if the AM fungal communities are affected by the physical and chemical properties of soil. The first and second axes of the RDA analysis accounted for a total of 80.09% of the community change, where RDA1 and RDA2 contributed to the 6.55% and 13.54% of the community change, respectively.

The soil physicochemical factors that majorly influenced the distribution of the horizontal community of AM fungi in the licorice soil were total soil phosphorus ($r=0.5366$), organic matter ($r=0.4833$), and available phosphorus ($r=0.4725$), while ammonium nitrogen ($r=0.4725$) had the least influence on the distribution of AM fungi. Besides, the soil's physical and chemical properties are also correlated. The soil pH value is negatively correlated to the soil's physical and chemical properties. This is consistent with previous findings that an increase in soil pH limits the nutrient's availability [16].

We analyzed the correlation heat map and found a significant correlation between the fungal genus and various. The correlation heat map showed that the genus Glomus was positively correlated with TP ($P<0.001$), and negatively correlated with TK ($P<0.001$), TS ($P<0.01$), soil moisture ($P<0.05$), and OSC ($P<0.05$). The genus Paraglomus was negatively correlated with TP ($P<0.001$) and positively correlated with TK ($P<0.001$), TS ($P<0.001$), OSC ($P<0.05$), and AK ($P<0.05$). The genus Diversispora was positively correlated with ammonium nitrogen ($P<0.05$) and

negatively correlated with AP (P<0.05) and TS (P<0.05). Moreover, we found a negative correlation between the g__unclassified_c__Glomeromycetes and TP (P<0.001), and positive correlation with TK (P<0.001), OSC (P<0.05), and TS (P<0.05).

Figure 6.



Discussion

Effects of plant species on the AM fungi

The structure of the AM fungi, which are obligate symbiotic fungi, is greatly influenced by the host plant, and there is specific selectivity between the plants and fungi [17] [18]. Therefore, the AM fungal communities are different in different host plants [19]; He Xueli [20] et al. reported that the spatial distribution of AMF is closely related to plant species. In this experiment, a total of 215 OTUs were obtained, and the most significant number of unique OTUs were from the Glycyrrhiza inflata. The least number of unique OTUs were from the Glycyrrhiza glabra. There were 13 common OTUs between the three species, the most significant number of common

OTUs were in between the Glycyrrhiza glabra and Glycyrrhiza inflata. In contrast, the least number of common OTUs were in between the Glycyrrhiza inflata and Glycyrrhiza uralensis. Besides, the similarity between the rhizospheric AM fungal community in the Glycyrrhiza inflata and the Glycyrrhiza glabra was higher than the Glycyrrhiza glabra and the Glycyrrhiza uralensis. The different varieties of licorice show the presence of different AM fungi community structures. This finding was in line with the research results of farmland, grassland, and other soil ecosystems [21] [22] [23] [24]. This may be due to the presence of a large number of AM fungi species in these genera, and the strong diffusion ability and more extensive adaptability [23] [25]. The genus-level classification of AM fungi showed that the Glomus and Paraglomus are the dominant genus of AM fungi. The genus Glomus accounted for 99.56% in Glycyrrhiza uralensis, 96.19% in Glycyrrhiza glabra, and only 33.42% in Glycyrrhiza inflata. The relative abundance of the genus Paraglomus in Glycyrrhiza inflata was 34.85%, and it was relatively less in Glycyrrhiza uralensis and Glycyrrhiza glabra. There was a significant difference in the diversity and richness of AM fungi among the three licorice species. The richness and diversity of the Glycyrrhiza inflata were the lowest.

The NMDS and PerMANOVA analysis showed that the host plants have a significant influence on the rhizospheric AM fungi community structure due to selectivity in the symbiotic relationship between the host plants and fungi. Therefore, the composition and richness of the AM fungi in the root perimeters of different host plants were different. The host plants might influence the AM fungi structure through a variety of

potential factors, for instance, host species variety, root characteristics, mycorrhizal dependence, and change in the rhizosphere soil environment of host plants.

Effects of soil depth on AM fungi

In the current study, we found that the horizontal community composition of AM fungi did not change with the variation in soil depths, but its relative abundance changed. Some species were found only deep in the soil, such as s__Diversispora-spurca-VTX00263, s__unclassified_g__Diversispora were found at 40-60 cm of Glycyrrhiza uralensis and Glycyrrhiza glabra at the molecular classification level. The diversity and richness of AM fungi varied with the soil depths. The Glycyrrhiza uralensis and Glycyrrhiza glabra showed the highest AM at 20-40 cm soil layer, and the diversity index of Glycyrrhiza inflata increased with soil depth. The Glycyrrhiza inflata and Glycyrrhiza glabra richness index were higher at 20-40 cm, and the Glycyrrhiza uralensis richness index was lowest at 20-40 cm. In general, the richness and diversity index at 20-40 cm was relatively higher, while it was relatively lower at 40-60 cm. The AM fungi are aerobic fungi, which require oxygen for their growth and development, and the oxygen availability in the deep soil is low due to the low content of organic matter and limited availability of phosphorus. The AM fungal community composition may also be affected by the plant root morphology. The licorice roots are below 1.5 m from the soil surface, and the 0-50 cm is more exposed to the environment above the ground. In the present study, there was no consistent trend among the three soil depths, which may have influenced the different licorice

species.

Effects of soil on AM fungi

In this study, the RDA analysis of the soil physical and chemical properties of three licorice species and the AM fungi classification showed that the AM fungi community was affected by soil's physical and chemical properties at different degrees. Also, soil factors such as total phosphorus, organic matter, and so on had a significant effect on the AM fungi. The outcome of a previous study demonstrates that the soil phosphorus content such as total phosphorus (r=0.5366) and available phosphorus (r=0.4725) majorly influence the AM fungi community distribution in the soil. The correlation heat map depicted a significant positive correlation between the TP and the genus Glomus ($P<0.001$) and a significant negative correlation between the genus Paraglomus and g__unclassified_c__Glomeromycetes ($P <0.001$), as well as between the AP and the genus Diversispora ($P <0.05$). These findings indicate that the genus Glomus is more tolerant, while other AM fungi are sensitive to the high phosphorus content of the soil [26]. Also, a higher phosphorus concentration of the soil may decrease the AM fungal species diversity [27]. Tawaraya [26] et al. reported that a high phosphorus concentration in the soil might induce a change in the root exudates of the host plant, which in turn might affect the associated AM fungi. Besides, the soil potassium content also affects the distribution of AM fungi to a certain extent. The correlation between the total potassium, total phosphorus, and the AM fungi genus was analyzed. We found that the TK was negatively correlated with the genus Glomus

311 (P<0.001) but positively correlated with the genus Paraglomus and g__unclassified_c
312 __Glomeromycetes (P<0.001) and AK was positively correlated with the genus
313 Paraglomus (P<0.05). Further investigations are required to assess the underlying
314 mechanism behind the impact of soil factors on the AM. OSC (r=0.4833) also had a
315 high impact on the distribution of the soil AM fungal communities. OSC was
316 negatively correlated with the genus Glomus (P<0.05) and positively correlated with
317 the genus Paraglomus and g__unclassified_c_Glomeromycetes (P<0.05), which
318 indicates that OSC had a significant positive effect on these species .

319 OSC was positively correlated with the AM bacteria; a plausible explanation for this
320 could be the role of OSC in the soil, which acts as a substrate for the preservation of
321 mycelium. The AM fungi are saprophytic by nature, and the organic matter-rich soil
322 promotes their growth [28]. Few other studies have highlighted the significance of OSC
323 content in the soil and its impact on the AM fungi. These studies reported that within
324 a threshold range, OSC promotes the AM fungi growth, but above this threshold, the
325 OSC is no longer conducive for the growth and development of the AM fungi [29].

326 The effect of total salt (r=0.4833) on the distribution of soil AM fungal communities
327 cannot be ignored. Previous studies have pointed out that AM fungal spore density in
328 the rhizosphere of plants is significantly negatively related to soil salinity [30].
329 However, according to other reports, the AM fungi spore density is not correlated to
330 the soil salinity [31]. It is assumed that the salinity indirectly affects the AM fungi; for
331 instance, the Na+ from the salt content affects the AP, which in turn reduces the AM

fungi spore density, or it may influence other soil factors, which in turn increase AM fungi spore density [32] [33]. In this study, we found a positive correlation between the total salt and the genus Paraglomus, which indicates that this genus has a higher salt tolerance. The genus Paraglomus mostly occurs in the Glycyrrhiza inflata, which may impart a high salt and alkali tolerance to the Glycyrrhiza inflata.

The AM fungi species have optimal pH ranges, but the genus Glomus shows a more comprehensive optimal pH range [34]. The rhizospheric soil pH values of the three licorice roots in this study were above 8. The Glomus genus was the most abundant, followed by Paraglomus, which indicates that the genus Glomus is more adaptable to alkaline environments. The correlation heat map showed a negative correlation between pH and AM fungi, which was not significant, probably because of a close relationship between the acidic or alkaline conditions and the nutrient availability of the soil [35]. A pH value between 6.0 and 7.5 is associated with the maximum value for soil nutrient availability. An increase in soil pH limits the availability of the nutrients , which indirectly reduces the AM fungal richness.

In the present study, the SWC showed a minor effect on AM fungi, and it was negatively correlated only with the genus Glomus ($P<0.05$). The SWC affects the growth and development of plant roots and AM fungi. Previous studies suggest that the AM fungi and SWC are negatively correlated since, with the decline in SWC, plants rely more on the AMF to enhance their adaptability to arid environments [36]. In an appropriate range, AM fungal diversity increases with increasing SWC [37]. The

AM fungi are aerobic, but a high SWC leads to reduced soil aeration, which in turn affects the AM fungi growth [38].

Conclusion

In the current study, a total of 34 AM fungi belonging to a phylum, a class, 5 (orders, families, and genera) were isolated. The genus Glomus emerged as the dominant genus, probably due to a large number of AM fungal species it contains, and also due to the high diffusivity and adaptability of some AM fungal species that belong to this genus . The distribution of the rhizospheric AM fungal communities was significantly affected by the host plant species, but it was least affected by the soil depth. The AM fungi communities in the three Glycyrrhiza variety did not change with the vertical soil depth and, the richness, as well as diversity of the Glycyrrhiza glabra, were the lowest in each layer. The total phosphorus, available phosphorus, total salt, and organic matter affected the rhizospheric AM fungi to a certain extent. The soil's physical and chemical properties varied with the Glycyrrhizae species but not with the soil depths, which might explain the influence of host plant species on the AM fungi. The correlations between different AM fungi and the soil's physical and chemical properties were found to be distinct.

This study should be seen in the light of limitations since it was majorly focused on the different varieties of Glyrrihiza within a particular area of Xinjiang, which instead has a vast area with many licorice species with wide distribution. Ideally, in further investigation, we could focus on examining the differences between the AM fungal

communities in the different regions.

methods

1. overview of the research area

The study area is located in Hami, Xinjiang Uygur Autonomous Region, located between 96°23 '00 "~ 91°06' 33" and north latitude 40°52 '47 "~ 45°05' 33", with an altitude of 53 ~ 4886 m.Hami is a typical temperate continental arid climate. It is dry and less rainy and has more sunny days. The annual average temperature is 9.8 degrees, the extreme maximum temperature is 43°C, the extreme minimum temperature is -32°C, the annual precipitation is 33.8 mm, and the annual evaporation is 3300 mm. 3358 hours, 182 days without frost.

2. research methods

2.1 Sample collection

We performed the field investigation in May 2019, and a sampling area with good licorice growth was selected for the experiments. Healthy licorice plants with consistent growth were selected for the excavation in August 2019, a total of 27 samples were collected. Our samples were collected from private farms with the permission of the landowners. The samples (Glycyrrhiza uralensis Fisch., Glycyrrhiza inflata Bat, and Glycyrrhiza glabra L.) were identified by professor lu jiahui from the school of life sciences, shihezi university, the plant samples are kept in our laboratory. The rhizosphere was collected from the soil layer with a depth of 0-20 cm, 20-40 cm,

40-60 cm, and within 0.5 cm vicinity of the main root of licorice. The samples were collected in a 5 ml centrifuge tube and stored in a liquid nitrogen tank, all of which were used to extraction rhizospheric AM fungi. Additionally, the soil within the vicinity of 5 cm around the root system was collected, and about 500 g was taken by the diagonal quartet method. Some of these samples are used for the determination of the physical and chemical properties of the soil, and the rest are kept in the laboratory

2.2 Determination of soil physical and chemical properties

Naturally-dried soil was taken and sieved through a 100-mesh sieve for the determination of soil physical and chemical properties. The determination method was referenced from "Agrochemical Analysis of Soil" edited by Bao Shidan. The soil water content was determined by natural air-drying method; the soil pH was measured by a PHS-25 pH meter with a soil-to-water ratio of 2.5: 1; the Organic of soil content was measured by potassium dichromate external heating method; Flowserve 1035 full-automatic nitrogen determination; total phosphorus was determined by acid-molybdenum antimony colorimetry, Agilent CARY60 ultraviolet spectrophotometry; total potassium was determined by acid-atom absorption method using Thermo Scientific S series atomic absorption spectrometer ; Nitrate nitrogen and ammonium nitrogen: 0.01M calcium chloride extraction, BRAN + LUEBBE AA3 flow analyzer; fast-acting phosphorus was measured using sodium bicarbonate extraction-molybdenum antimony colorimetry, Agilent CARY60 UV spectrophotometer Fast-acting potassium was determined by ammonium acetate

extraction-atomic absorption method and Thermo Scientific S series atomic absorption spectrometer; total salt was determined by dry residue method.

2.3 Soil DNA extraction and inspection

The extraction kit is an Omega soil microbial DNA extraction kit. The total DNA is extracted according to the kit instructions. After the genomic DNA extraction is completed, the extracted genomic DNA is detected by 1% 1% agarose gel electrophoresis. The DNA samples were stored in the refrigerator at −20 ℃ for future use.

2.4 PCR amplification

AMV4-5NF (5'-AAGCTCGTAGTTGAATTTCG-3') 和 AMDG R (5'-CCCAACTA TCCCTATTAATCAT-3') primers were used for PCR amplification of AM fungi. The steps of amplification were:pre-denaturation at 95 ° c for 3 min, denaturation at 95 ° c for 30 s, annealing at 55 ° c for 30 s, extension at 72 ° c for 45 s, 32 cycles, and extension at 72 ° c for 10 min.The amplification system was Transstart Fastpfu DNA Polymerase, 20-phase reaction system, 4-phase L 5* Fastpfu buffer, 2-phase L 2. 5 mmol /L dNTPs, 0. 8 dl Forward Primer(5 mol /L), 0. 8 L Reverse Primer(5 mol /L), 0. 4 L FastPfu Polymerase, 2 L BSA, 10ng DNA Template, PCR using ABI Geneamp9700.PCR products from the same sample were mixed and detected by 2% agarose gel electrophoresis. The gel was recovered using AxyPrepDNA gel recovery kit (AXYGEN), and Tris_HCl was eluted. The PCR products were purified and quantified using Promega's QuantiFluor ™ -ST blue fluorescence quantification

system, and then mixed according to the required amount of sequencing for each sample.

2.5 construction and sequencing of Miseq library

The PCR products were composed into a sequencing library, and the construction and sequencing of the Miseq library were completed by Shanghai Meiji bio-pharmaceutical technology co., LTD. The sequencing platform was Illumina MiSeqPE300 /PE250.

2.6 biological information analysis

The original data is screened to obtain the effective sequence of each sample, and the effective sequence is filtered to obtain the optimized sequence. OTU (Operational Taxonomic Units) is formed at the sequence similarity level of 97%. Based on OTU cluster analysis results, generate dilution curves to detect sequencing depth, and perform a variety of diversity index analysis, including Chao index, Simpson index, Shannon index, etc .; Based on taxonomic information, annotate OTU species, and sample species composition and relative Histograms and Venn diagrams of abundance statistics results, non-metric multidimensional scaling (NMDS), etc., Redundancy analysis (RDA) of community composition and soil physical and chemical properties of multiple samples, etc.

Abbreviations

AMF: Arbuscular mycorrhizal fungi; Ch.P.: Pharmacopoeia of The People's Republic

of China; DNA: Deoxyribonucleic acid; PCR: Polymerase Chain Reaction; Inf: Glycyrrhiza inflata Bat.; Gla: Glycyrrhiza glabra L.; Ura: Glycyrrhiza uralensis Fisch.; OSC：Organic of soil content; TN: total nitrogen; TP: total phosphorus; TK: total potassium; N3: Nitrate nitrogen; N4: ammonium nitrogen; AP: available phosphorus; AK: available potassium; TS: total salt ; PH: hydrogen ion concentration; SWC: soil water content; OTU: Operational Taxonomic Units; Nonmetric multidimensional scaling (NMDS) ; Redundancy analysis(RDA)

DECLARATIONS

Ethics approval and consent to participate

Not applicable.

Consent for Publication

Not applicable.

Availability of data and material

The datasets used and analyzed within the current study are available from the corresponding author on reasonable request.

Competing interests

All authors declare that they have no competing interests.

Funding

The work was funded by the national natural science foundation of China regional

project (project number: 41561010; 2016-2019 and 31560177; 2016-2019). The funding agency has no role in the design, data collection, analysis or interpretation of the research or in the writing of the manuscript.

Authors' contributions

TG coordinated the study, collected data, conducted data analysis, interpreted data, curated the metagenomic data, drafts the manuscript and writes the manuscripts. YLH, GFL, and XHL coordinated the study, conducted data analysis, interpreted data. LZ and ZKW allocate funds, collect data and jointly analyze data, curated the metagenomic data, and critically analyze manuscripts. XHL,GFL, allocate funds and co-analysis the data. All authors read and approved the final manuscript.

Acknowledgements

We would like to thank Shanghai Meiji biomedical technology co., ltd. for its assistance in the analysis of the Illumina genome

Acknowledgements

We would like to thank Shanghai Meiji biomedical technology co., ltd. for its assistance in the analysis of the Illumina genome

Funding

The work was funded by the national natural science foundation of China regional

494 project (project number: 41561010; 2016-2019 and 31560177; 2016-2019). The
495 funding agency has no role in the design, data collection, analysis or interpretation of
496 the research or in the writing of the manuscript.

497 **Availability of data and materials**

498 The datasets used and analyzed within the current study are available from the
499 corresponding author on reasonable request.

500 **Authors' contributions**

501 TG coordinated the study, collected data, conducted data analysis, interpreted data,
502 curated the metagenomic data, drafts the manuscript and writes the manuscripts. YLH,
503 GFL, and XHL coordinated the study, conducted data analysis, interpreted data. LZ
504 and ZKW allocate funds, collect data and jointly analyze data, curated the
505 metagenomic data, and critically analyze manuscripts. XHL,GFL, allocate funds and
506 co-analysis the data. All authors read and approved the final manuscript.

507 **Ethics approval and consent to participate**

508 Not applicable.

509 **Consent for publication**

510 Not applicable.

511 **Competing interests**

512 All authors declare that they have no competing interests.

References

1. Ang, L., et al., *Effects of Glycyrrhiza uralensis vegetation on the ground surface microenvironment and soil physical.* Journal of Arid Land Resources and Environment, 2014. **28**(10): p. 128-132.

2. Mingjin, H., W.-q. wang, and W. shengli, *Investigation on medicinal plant resources of Glycyrrhiza uralensis in China and chemical assessment of its underground part.* China Journal of Chinese Materia Medica, 2010. **35**(08): p. 947-952.

3. Qiong, W., L. Jie, and SIMAYI.Hesilaiti, *Utilization Status and Protection Measures for Licorice Resources in Xinjiang.* 2018(02): p. 52-56.

4. Wang, C.-Y., et al., *Glycyrrhizic Acid and 18β-Glycyrrhetinic Acid Modulate Lipopolysaccharide-Induced Inflammatory Response by Suppression of NF-κB through PI3K p110δ and p110γ Inhibitions.* Journal of Agricultural\s&\sfood Chemistry. **59**(14): p. 7726-7733.

5. Gaiping, Z., Z. Huaze, and Y. Jianxiong, *Antioxidant activity of licorice extracts in vitro.* Chinese Journal of Bioprocess Engineering, 2010. **8**(03): p. 53-57.

6. Yeh, C.F., et al., *Water extract of licorice had anti-viral activity against human respiratory syncytial virus in human respiratory tract cell lines.* Journal of Ethnopharmacology. **148**(2): p. 466-473.

7. Yu-ting, H., et al., *Research progress on Licorice flavonoids and their antitumor activities.* Chinese Journal of New Drugs, 2017(13): p. 64-69.

8. Smith, S.E. and F.A. Smith, *Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth.* Mycologia. **104**(1): p. 1-13.

9. Srivastava, A.K., S. Singh, and R.A. Marathe, *Organic Citrus: Soil Fertility and Plant Nutrition.* Journal of Sustainable Agriculture. **19**(3): p. 5-29.

10. Augé, R.M., H.D. Toler, and A.M. Saxton, *Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis.* Mycorrhiza, 2015. **25**(1).

11. Jing, K., et al., *Effects of arbuscular mycorrhizal fungi on the drought resistance of the mining area repair plant Sainfoin.* International Journal of Mining Science and Technology, 2014. **24**(04): p. 485-489.

12. Zhu, X.C., et al., *Effects of arbuscular mycorrhizal fungus on photosynthesis and water status of maize under high temperature stress.* Plant & Soil. **346**(1-2): p. 189-199.

13. Garg, N. and R. Pandey, *Effectiveness of native and exotic arbuscular mycorrhizal fungi on nutrient uptake and ion homeostasis in salt-stressedCajanus cajanL. (Millsp.) genotypes.* Mycorrhiza. **25**(3): p. 165-180.

14. Shi-ji, H., et al., *Effects of biogas slurry and AM fungi on growth and heavy metal accumulation of licorice plants.* Journal of Agro-Environment Science, 2016. **35**(08): p. 1465-1472.

15. Xie, W., et al., *Improved phosphorus nutrition by arbuscular mycorrhizal symbiosis as a key factor facilitating glycyrrhizin and liquiritin accumulation in Glycyrrhiza uralensis.* Plant and Soil, 2019. **439**(1-2).

16. Asghar, M.N., S. Khan, and S. Mushtaq, *Management of treated pulp and paper mill effluent to achieve zero discharge.* World Pulp & Paper, 2008. **88**(4): p. 1285-1299.

17. Xian-gui, L. and H. Wen-ying, *MYCCRRHIZAL DEPENDENCY OF VARIOUS KINDS OF PLANTS.* Journal of Integrative Plant Biology, 1989(9): p. 721-725.

18. Xue-li, H. and L. Bin, *Selection research of VA mycorrhizal fungus and plant.* Acta Botanica Boreali-Occidentalia Sinica, 1999(3).

19. Eom, A.H., D.C. Hartnett, and G.W.T. Wilson, *Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie.* Oecologia, 2000. **122**(3): p. 435-444.

20. Mouratov, S., Y. Steinberger, and X. He, *Spatial Distribution and Colonization of Arbuscular Mycorrhizal Fungi under the Canopies of Desert Halophytes.* Arid Soil Research & Rehabilitation, 2002. **16**(2): p. 149-160.

21. Qin, H., et al., *Long-term fertilizer application effects on the soil, root arbuscular mycorrhizal fungi and community composition in rotation agriculture.* Applied Soil Ecology, 2015. **89**.

22. Beenhouwer, M.D., et al., *Changing soil characteristics alter the arbuscular mycorrhizal fungi communities of Arabica coffee (Coffea arabica) in Ethiopia across a management intensity gradient.* Soil Biology and Biochemistry, 2015. **91**.

23. Chun-xue, Y., et al., *Diversity characteristics of arbuscular mycorrhizal fungi in the rhizosphere of twenty six species of plants in Songnen saline-alkaline grassland.* Pratacultural Science, 2015. **32**(12): p. 2008-2020.

24. Yin, L.F., et al., *Study on Species Diversity of AMF Community Colonizing Loquat Roots by Constructing Clone Library.* Southwest China Journal of Agricultural Sciences, 2017.

25. Menezes, K.M.S., et al., *Arbuscular mycorrhizal fungal communities in buffelgrass pasture under intercropping and shading systems in Brazilian semiarid conditions.* Agriculture, Ecosystems and Environment, 2016. **230**.

26. Tawaraya, K., et al., *Effect of phosphate application to arbuscular mycorrhizal onion on the development and succinate dehydrogenase activity of internal hyphae.* Soil Science & Plant Nutrition, 1994. **40**(4): p. 667-673.

27. Alguacil, M.D.M., et al., *Phosphorus fertilisation management modifies the biodiversity of AM fungi in a tropical savanna forage system.* Soil Biology & Biochemistry, 2010. **42**(7): p. 1114-1122.

28. Joner, E.J. and I. Jakobsen, *Growth and extracellular phosphatase activity of arbuscular mycorrhizal hyphae as influenced by soil organic matter.* Soil Biology & Biochemistry. **27**(9): p. 0-1159.

29. Runjin, L., et al., *Ecological distribution of arbuscular mycorrhizal fungi in saline-alkaline soils of China.* Chinese Journal of Applied Ecology, 1999. **10**(6): p. 721-724.

30. Xinping, L., et al., *Effects of soil rhizosphere microbial community and soil factors on arbuscular mycorrhizal fungi in different salinized soils.* 生态学报, 2012. **32**(13): p. 4071-4078.

31. Cooke, J.C., R.H. Butler, and G. Madole, *Some Observations on the Vertical Distribution of Vesicular Arbuscular Mycorrhizae in Roots of Salt Marsh Grasses Growing in Saturated Soils.* Mycologia. **85**(4): p. 547-550.

32. Aliasgharzadeh, N., et al., *Occurrence of arbuscular mycorrhizal fungi in saline soils of the Tabriz Plain of Iran in relation to some physical and chemical properties of soil.* 2001. **11**(3): p. 119-122.

33. Barin, M., et al., *Abundance of arbuscular mycorrhizal fungi in relation to soil salinity around Lake Urmia in northern Iran analyzed by use of lipid biomarkers and microscopy.* Pedobiologia - International Journal of Soil Biology, 2013. **56**(4-6).

34. Brownell, K.H. and R.W. Schneider, *Roles of matric and osmotic components of water potential and their interaction with temperature in the growth of Fusarium oxysporum in synthetic media and soil.* 1985. **75**(1): p. 53-57.

35. AN, G., et al., ***Community structure of arbuscular mycorrhizal fungi associated with pioneer grass species Miscanthus sinensis in acid sulfate soils: Habitat segregation along pH gradients***. Soil Science & Plant Nutrition, 2008. **54**(4): p. 517-528.

36. Min, L., et al., *Seasonal Dynamics of Arbuscular Mycorrhizal Fungal Community in the Rhizosphere of Clematis fruticosa in the Daqingshan Mountains, Inner Mongolia.* Acta Botanica Boreali-Occidentalia Sinica, 2016(9): p. 1899.

37. Fa-Yuan, W. and L. Run-Jin, *Effects of environmental factors on the diversity of arbuscular mycorrhizal fungi.* Chinese Biodiversity, 2001. **09**(3).

38. Wolfe, B.E., et al., *Small-scale spatial heterogeneity of arbuscular mycorrhizal fungal abundance and community composition in a wetland plant community.* Mycorrhiza, 2007. **17**(3).

Table 1. Mean values of soil parameters under three host plants in the different soil depth.

Lowercase letters are significantly difference among three soil depth,capital letters are significantly difference among three plant species($P < 0.05$). Depth is cm.

Table 2. Mean values of Diversity indices under three host plants in the different soil depth.

Different letters within a row indicate statistical significances among the different soil depth and different plant species. Significant values are denoted as: different small letters, $P < 0.05$; different capital letters, $P < 0.01$.

Figure 1.the dilution curve of the three licorice rhizosphere soil samples

Rarefaction curve (A), Shannon-Wiener curve (B). Ura, Glycyrrhiza uralensis Fisch.; inf, Glycyrrhiza inflata Bat.; gla, Glycyrrhiza glabra L..

Figure 2. Venn diagram

Venn diagram showing overlap in AMF OTUs among the different plant species. Ura, Glycyrrhiza uralensis Fisch.; inf, Glycyrrhiza inflata Bat.; gla, Glycyrrhiza glabra L..

Figure 3. Column chart of the proportion of AM fungal genera at three plants in each soil layer.

Ura, Glycyrrhiza uralensis Fisch.; inf, Glycyrrhiza inflata Bat.; gla, Glycyrrhiza glabra L.. _1, the soil layers of 0–20 cm; _2, the soil layers of 20–40 cm; _3, the soil layers of 40–60 cm.

Figure 4. Nonmetric multidimensional scaling (NMDS)

Nonmetric multidimensional scaling (NMDS) analysis under three host plants in the different soil depth. (stress =0.181). Ura, Glycyrrhiza uralensis Fisch.; inf, Glycyrrhiza inflata Bat.; gla, Glycyrrhiza glabra L..Different colored circles represent different soil depths， _1, the soil layers

of 0–20 cm; _2, the soil layers of 20–40 cm; _3, the soil layers of 40–60 cm.

Figure 5. Redundancy analysis(RDA)

Redundancy analysis(RDA) of AMF community based on OTU matrix from different species. The different color points represent different species.The eleven arrows represent environment factors including OSC,TK,AP,TS,AK,TN,TP,SWC,PH,NH4+, NO3−. Ura, Glycyrrhiza uralensis Fisch.; inf, Glycyrrhiza inflata Bat.; gla, Glycyrrhiza glabra L..

Figure 6. Correlation heat map

Correlation heat map of the top five genera and soil properties. X and Y axis are environmental factors and genera. R in different colors to show, the right side of the legend is the color range of different R values. $0.01 < P \leq 0.05$ is marked with "*", $0.001 < P \leq 0.01$ is marked with "**", $P \leq 0.001$ is marked with "***".

Figures

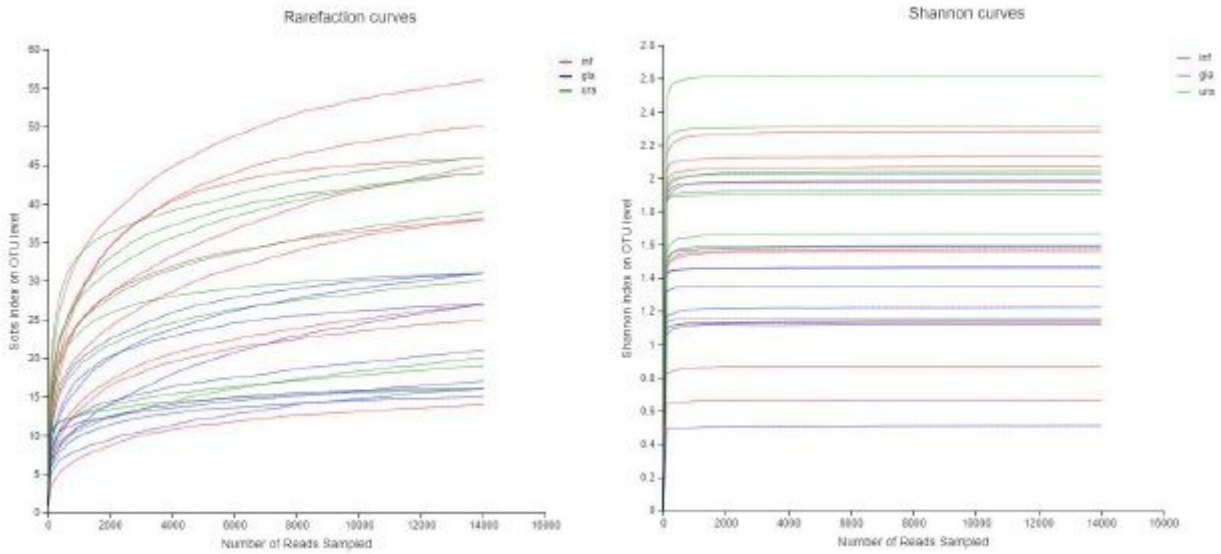


Figure 1

the dilution curve of the three licorice rhizosphere soil samples Rarefaction curve (A), Shannon-Wiener curve (B). Ura, Glycyrrhiza uralensis Fisch.; inf,Glycyrrhiza inflata Bat.; gla, Glycyrrhiza glabra L..

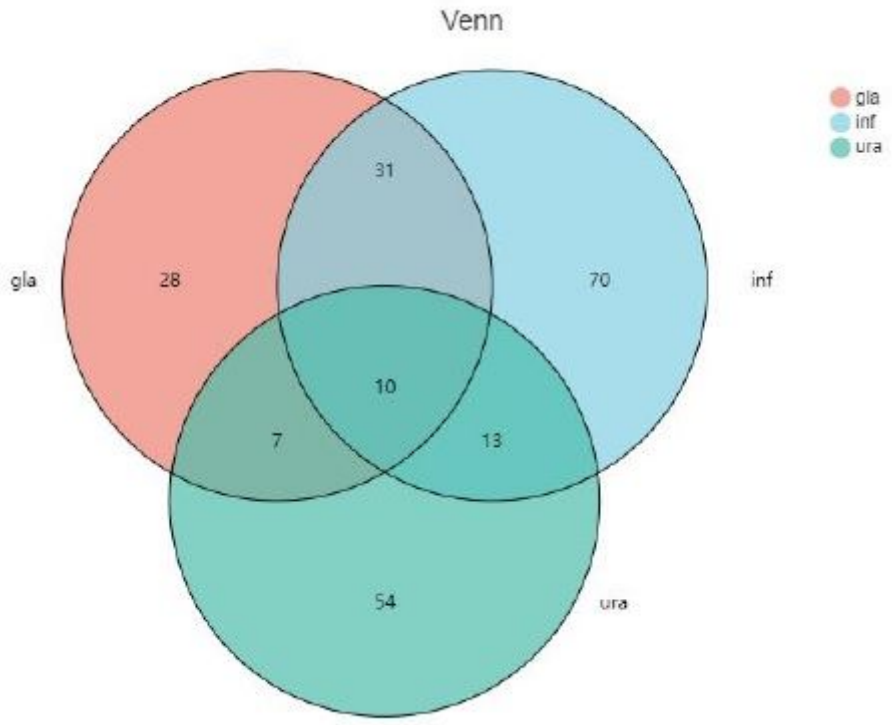


Figure 2

Venn diagram Venn diagram showing overlap in AMF OTUs among the different plant species. Ura, Glycyrrhiza uralensis Fisch.; inf, Glycyrrhiza inflata Bat.; gla, Glycyrrhiza glabra L..

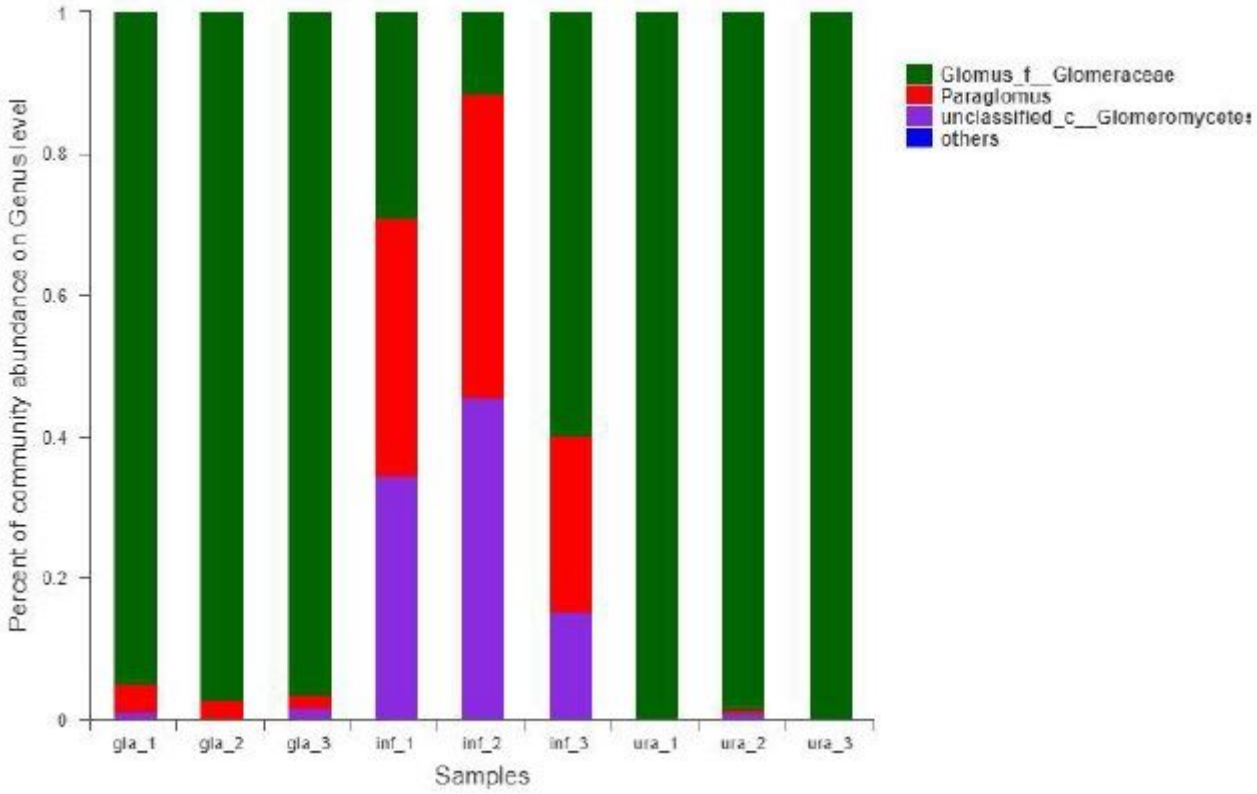


Figure 3

Column chart of the proportion of AM fungal genera at three plants in each soil layer.Ura, Glycyrrhiza uralensis Fisch.; inf, Glycyrrhiza inflata Bat.; gla, Glycyrrhiza glabra L.. _1, the soil layers of 0–20 cm; _2, the soil layers of 20–40 cm; _3, the soil layers of 40–60 cm.

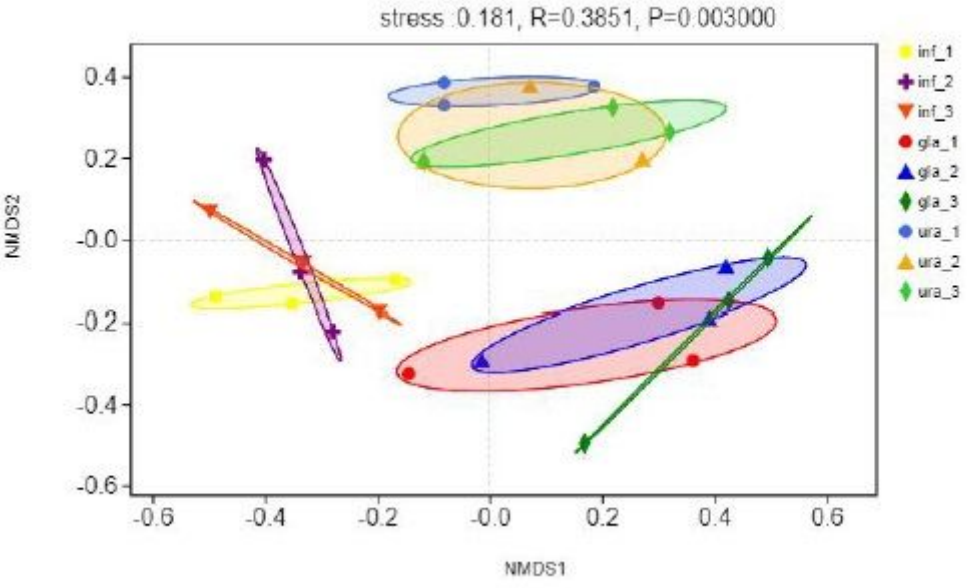
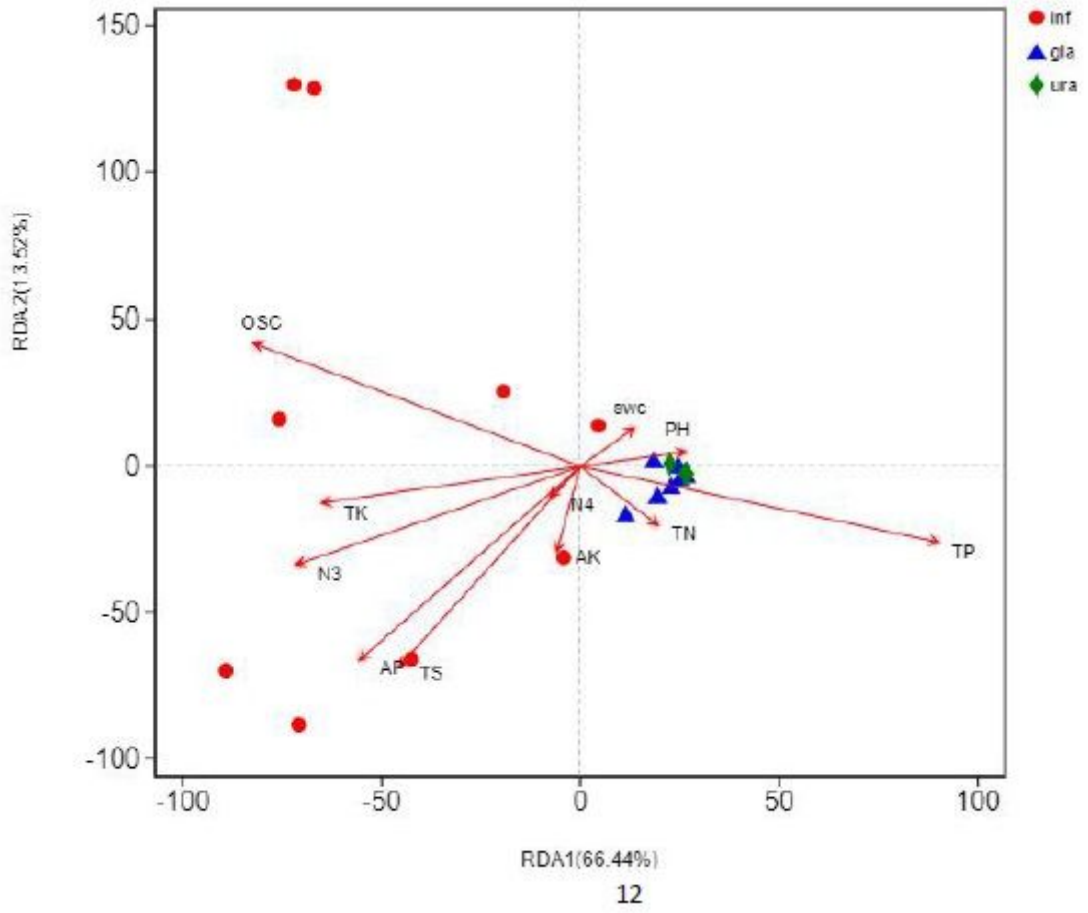


Figure 4

Nonmetric multidimensional scaling (NMDS)Nonmetric multidimensional scaling (NMDS) analysis under three host plants in the different soil depth. (stress =0.181). Ura, Glycyrrhiza uralensis Fisch.; inf, Glycyrrhiza inflata Bat.; gla, Glycyrrhiza glabra L..Different colored circles represent different soil depths _1, the soil layers 34 of 0–20 cm; _2, the soil layers of 20–40 cm; _3, the 638 soil layers of 40–60 cm.



12

Figure 5

Redundancy analysis(RDA)Redundancy analysis(RDA) of AMF community based on OTU matrix from different species. The different color points represent different species.The eleven arrows represent environment factors including OSC,TK,AP,TS,AK,TN,TP,SWC,PH,NH4+, NO3−. Ura, Glycyrrhiza uralensis Fisch.; inf, Glycyrrhiza inflata Bat.; gla, Glycyrrhiza glabra L..

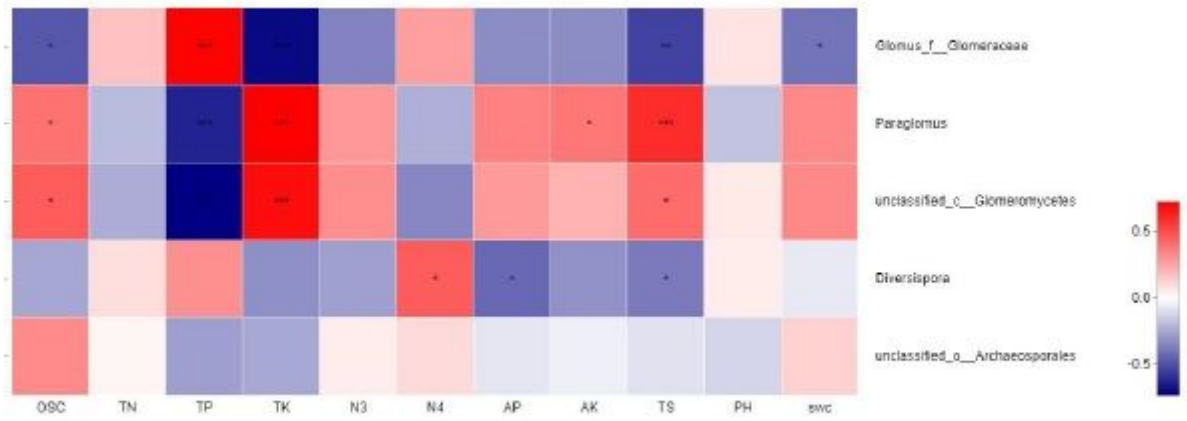


Figure 6

Correlation heat map Correlation heat map of the top five genera and soil properties. X and Y axis are environmental factors and genera. R in different colors to show, the right side of the legend is the color range of different R values. 0.01 < P ≤ 0.05 is marked with “*”, 0.001 < P ≤ 0.01 is marked with “**”, P ≤ 0.001 is marked with “***”.