ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Biotechnology

ISSN 1682-296X
DOI: 10.3923/biotech.2019.32.41

Research Article
Effects of Mycorrhiza and Drought Stress on the Diversity of Fungal Community in Soils and Roots of Trifoliate Orange

Jia-Dong He, Qiang-Sheng Wu and Ying-Ning Zou

College of Horticulture and Gardening, Yangtze University, Jingzhou, Hubei, 434025, China

Abstract

Background and Objective: Citrus is highly dependent on soil arbuscular mycorrhizal fungi (AMF) for vigor growth. Either drought stress (DS) or AMF modifies soil fungal diversity, a bio-indicator towards the maintenance of soil ecosystem and soil fertility. The present work was to explore the effects of both AMF and drought stress on fungal communities in rhizosphere soils and roots of host plants. **Materials and Methods:** In this study, trifoliate orange seedlings in pots were inoculated with *Funneliformis mosseae* and subjected to well-watered and DS for 8 weeks. The internal transcribed spacer fragment sequencing was used to analyze fungal diversity in soils and roots. **Results:** The results showed that a significantly higher operational taxonomic unit (OTU) number was found in roots than in soils. The AMF colonization increased root OTU number under both well-watered and DS and soil OTU number under DS. The AMF inoculation promoted the increase of *Sordariomycetes* in roots and the relative abundance of *Chytridiomycota* in rhizosphere soils and *Ascomycota* in roots. The stability of the fungal community in rhizosphere soils was better than in roots. The AMF and DS had a much less impact on the abundance and diversity of the fungal community in soils than in roots. **Conclusion:** It concluded that AMF modulated superior fungal diversity in roots, but not in soils, to enhance plant tolerance to drought.

Key words: Arbuscular mycorrhizal fungi, citrus, drought stress, fungal diversity, OTU

Citation: Jia-Dong He, Qiang-Sheng Wu and Ying-Ning Zou, 2019. Effects of mycorrhiza and drought stress on the diversity of fungal community in soils and roots of trifoliate orange. Biotechnology, 18: 32-41.

Corresponding Author: Ying-Ning Zou, College of Horticulture and Gardening, Yangtze University, Jingzhou, Hubei, 434025, China

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Soil drought stress (DS), one of the atrocious natural phenomena, is defined as a prolonged deficiency of rainfall usually for a season or more[1]. It leads to water scarcity in soils which is detrimental to agricultural production, including citrus, one of the leading fruit trees grown in more than 140 countries, with China as the biggest citrus producing country[2]. Earlier studies indicated that DS affected not only the physical and chemical parameters of soil fertility, but also the microbiological parameters to degenerate soil ecosystem and structure in the long term[3]. Recently, several studies have been carried on the impact of DS on the abundance, composition and metabolized production of microorganisms[4-6]. It seemed that the intensification of DS weakened the microbial activity in the soil. The effect of DS on plants has been studied in depth, while the information is relatively scarce in the influences of DS on soil microbial ecosystems. This is primarily due to the inability to cultivate soil microbes in lab conditions. Due to recent advances in sequencing technology, it has now become feasible to mass sequence and analyzes vast amounts of genetic data from environmental samples. This has delivered the tools to analyze soil for taxonomic and phylogenetic perspectives of microbial ecology, providing a means to compare soil ecology between sites[7].

Trifoliate orange (*Poncirus trifoliata* (L.) Raf.) is a kind of citrus rootstocks widely used in southeast Asia. The plant is highly sensitive to DS and also is strongly dependent on a kind of beneficial soil microorganisms, arbuscular mycorrhizal fungi (AMF), which are able to establish arbuscular mycorrhizas (AMs) with roots[8]. There are abundant AMF communicates in citrus rhizosphere as many 45 species[9]. The AMs played an important role in soil ecosystems[10]. Also, AMs can improve plant health through a more specific increase in water and mineral absorption[10]. Earlier studies had shown the positive effect of AMF on drought tolerance of trifoliate orange[8,11], whereas the information regarding the diversity changes of fungal community in rhizosphere caused by mycorrhization are poorly known under DS. The objective of the present work was to clarify the effect of AMF and DS on the diversity of fungal community in rhizosphere of trifoliate orang seedlings.

MATERIALS AND METHODS

Plant set-up and experimental design: The three-leaf-old trifoliate orange seedlings grown in autoclaved sands were planted in 3.6 L pots that were supplied with autoclaved (0.11 MPa, 121°C, 2 h) growth substrate with soils and sands (5 : 2, v/v). When the seedlings were transplanted, 1200 spores of an arbuscular mycorrhizal fungus *Funneliformis mosseae* (Nicol. and Gerd.) It was inoculated in the pot as the mycorrhizal treatment. The non-mycorrhizal group also received the same amounts of sterilized inoculums. Subsequently, these AMF and non-AMF seedlings were maintained in 75% of maximum water holding capacity in the substrate (WW) for 12 weeks. Half of seedlings were modified to 55% of maximum water holding capacity in the substrate for 8 weeks as DS treatment, whilst the other was still kept on soil WW status for 8 weeks. A total of 20 weeks was arranged. The experiment contained four treatments in a completely randomized arrangement with the inoculation with or without *F. mosseae* (+Fm; -Fm) and water treatments with WW and DS: WW+Fm, DS+Fm, WW-Fm and DS-Fm. Each treatment replicates 4 times with 3 seedlings per pot.

After 8 weeks of DS treatment, the experiment was ended and the AMF and non-AMF seedlings were harvested. Roots and rhizosphere soils were collected and quickly stored in -80°C for the analysis of fungal diversity.

DNA extraction and PCR amplification: Total genome DNA was extracted from the plant roots and soils using the DNA purification ELISA kits (ZYMO Research Company, Irvine, USA) and then checked by the 0.8% agarose gel for the quality level. The ITS genes were amplified using the specific primer with 12 nt unique barcode: ITS3_KYO2 (5'-GATGAAGAACGYAGYR AA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3')[12]. The PCR mixture (25 μL) contained 1×PCR buffer, 1.5 mM $MgCl_2$, 0.4 μM deoxynucleoside triphosphate, each primer at 1.0 μM, 0.5 U of KOD-Plus-Neo (TOYOBO) and 10 ng template DNA. The PCR amplification program consisted of initial denaturation at 94°C for 1 min followed by 30 cycles (denaturation at 94°C for 20 sec, annealing at 50°C for 30 sec and elongation at 72°C for 30 sec) and a final extension at 72°C for 5 min and the whole PCR was implemented in Applied Biosystems® Gene Amp® PCR System 9700. Three replicates of PCR reactions for each sample were combined together and then, PCR products that were mixed with 1/6 volume of 6X loading buffer were loaded on 2% agarose gel for detection. Samples with bright main strip between 200-400 bp were chosen for further experiments. Subsequently, the electrophoresis band was purified using OMEGA Gel Extraction Kit (Omega Bio-Tek, USA). The PCR products from different samples were pooled with equal molar amount.

Miseq library preparation and sequencing: Sequencing libraries were generated using TruSeq DNA PCR-Free Sample Prep Kit following manufacturer's recommendations and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. At last, the library was applied to paired-end sequencing (2×250 bp) with the Illumina Miseq apparatus Kit v2 at Rhonin Biosciences Co., Ltd.

Sequencing data analysis: Paired-end reads from the original DNA fragments theoretically were merged using FLASH[13], which is a very fast and accurate analysis tool. Paired-end reads was assigned to each sample according to the unique barcode. The sequences with high quality (length >200 bp, without ambiguous base 'N' and average base quality score >30) were screened for chimeras checking using Uchime algorithm[14]. Sequences were clustered into operational taxonomic units (OTUs) at 97% identity threshold using UPARSE-OTUref algorithms[15]. Representative sequences were picked for each OTU. Representative sequences were picked for each OTU. Taxonomy were assigned using the UNITE database (https://unite.ut.ee/) and uclust method. In case of the influences of sequencing depth on community diversity, the OTU table was rarified to make all samples holding the same sequence number. All data analyses were performed using Python (https://www.python.org/). Phylogenetic diversity (Faith's PD18) was calculated using Picante[16]. Weighted and Unweighted Unifrac distances were calculated in GUniFrac. Other alpha and beta-diversity metrics were calculated in Vegan. Rarefaction curves were generated based on these three metrics. Principal component analysis was applied to reduce the dimensions of original community data. Principal coordinate analysis and non-metric multi-dimensional scaling were performed using package Ape22 and Vegan, respectively. Hierarchical cluster analysis was done using R function hclust. To identify if there were significant differences among different groups, Permutational multivariate analysis of variance was performed based on the Bray-Curtis dissimilarity matrix.

RESULTS

Fungal diversity: In this study, 318, 339, 335 and 306 OTUs were observed in the SSU rRNA clone library of rhizosphere soils under WW+Fm, DS+Fm, WW-Fm and DS-Fm conditions, respectively (Fig. 1). It showed that DS

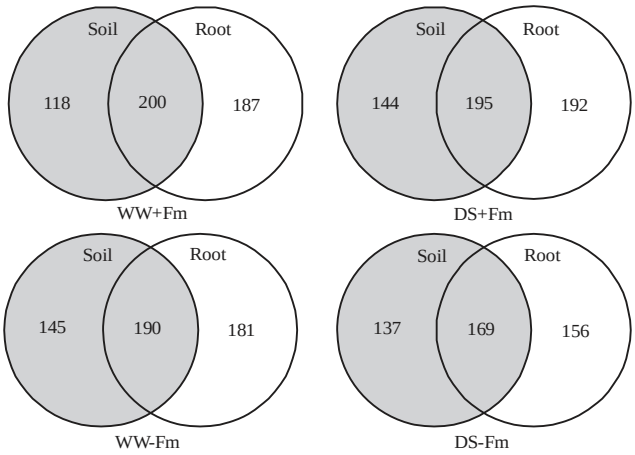

Fig. 1: Effect of *Funneliformis mosseae* on quantity of operational taxonomic units (OTUs) in roots and rhizosphere soils of trifoliate orange seedlings under well-watered and drought stress

WW: Well-watered, DS: Drought stress, +Fm: Inoculation with *Funneliformis mosseae*, -Fm: Inoculation without *Funneliformis mosseae*

reduced soil OTU number of non-AMF seedlings but increased in AMF-seedlings. In addition, AMF inoculation notably increased soil OTU number under DS but reduced it under WW. And 387, 387, 371 and 325 OTUs were observed in the SSU rRNA clone library of roots under WW+Fm, DS+Fm, WW-Fm and DS-Fm conditions, respectively (Fig. 1). The changes in OTUs of roots caused by mycorrhization and DS were similar with soil OTUs. Hereinto, 200, 195, 190 and 169 OTUs were overlapped in both soils and roots under WW+Fm, DS+Fm, WW-Fm and DS-Fm conditions, respectively. On the other hand, a significantly higher OTU number was found in roots than in soils in all of those experimental conditions.

The observed number of species (Observed), Chao1 and Shannon index was used to make dilution curves. The rarefaction curves showed that the curves became flatter when the number of the clones rose to 260-280 and 260-340 OTUs in rhizosphere soils and roots, respectively (Fig. 2). Meanwhile, for both Chao 1 and Shannon value, a relatively higher alpha diversity in fungal species was shown in roots than in rhizosphere soils as observed at 97% similarity level. As showed in Fig. 1-3, in AMF-inoculated seedlings, higher OTUs and greater fungal diversity were found under DS conditions. On the contrary, OTUs and fungal diversity in WW conditions were higher than those in DS in non-AMF-inoculated seedlings. Similarly, in rhizosphere soils, the results were consistent with those in root systems.

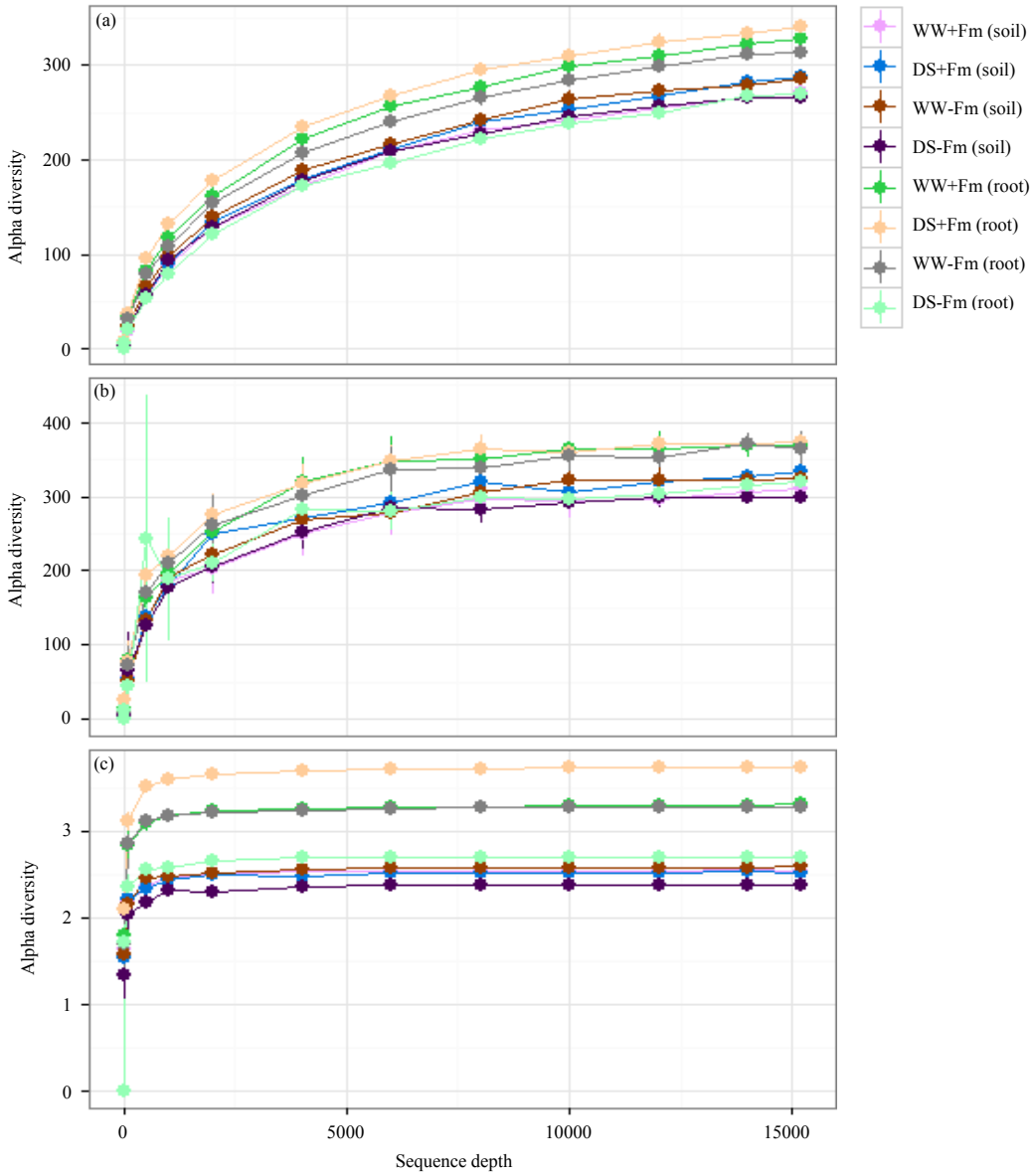

Fig. 2(a-c): Rarefaction curve of operational taxonomic units (OTUs) based on alpha diversity of sequence depth in roots and rhizosphere soils of *Funneliformis mosseae*-colonized trifoliate orange seedlings under well-watered and drought stress, (a) Observed, (b) Chao1 and (c) Shannon

WW: Well-watered, DS: Drought stress, +Fm: Inoculation with *Funneliformis mosseae*, -Fm: Inoculation without *Funneliformis mosseae*

Fungus community at phylum, class and genus levels: The AMF community at the phylum level was shown in Fig. 4. *Ascomycota* was the dominant population in both rhizosphere soils and roots. Overall, DS decreased the relative abundance of *Ascomycota* to different degrees and it was most significant in roots of non-AMF-inoculated seedlings under DS. Under both WW and DS conditions, the AMF inoculation increased the relative abundance of *Chytridiomycota* in rhizosphere soils and *Ascomycota* in roots. However, it decreased *Ascomycota* in rhizosphere soils and *Basidiomycota* in roots.

Figure 5 showed the fungus community at the class level. Hereinto, *Sordariomycetes* and *Dothideomycetes* counted for the most in rhizosphere soils and roots, respectively. In rhizosphere soils, DS increased the relative abundance of the relative abundance of *Sordariomycetes* and *Chytridiomycetes* but resulted in the reduction of the relative abundance of *Eurotiomycetes*, *Leotiomycetes* and *Pezizomycetes* in both

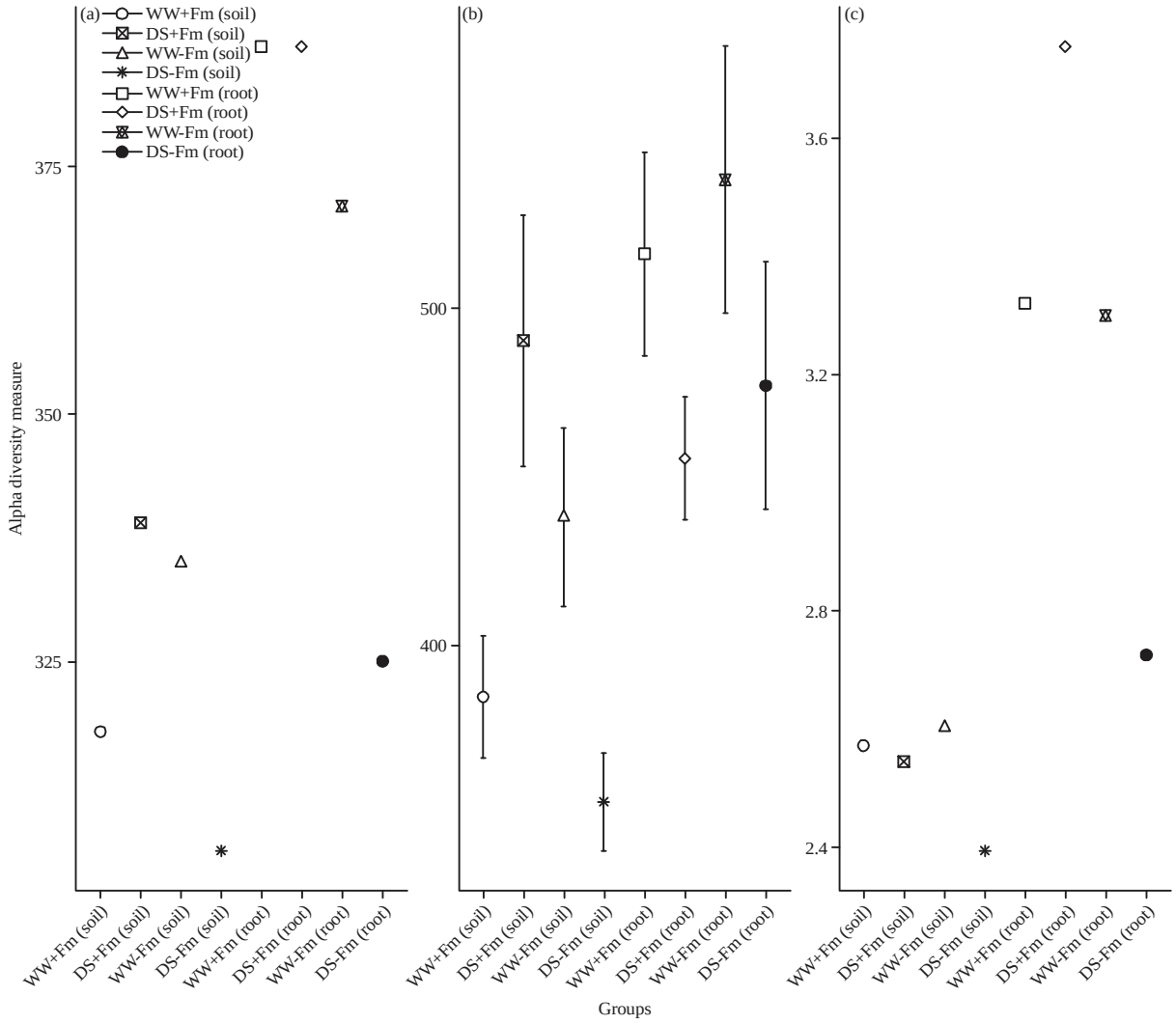

Fig. 3(a-c): Alpha diversity index in roots and rhizosphere soils of *Funneliformis mosseae*-colonized trifoliate orange seedlings under well-watered and drought stress, (a) Observed, (b) Chao1 and (c) Shannon

WW: Well-watered, DS: Drought stress, +Fm: Inoculation with *Funneliformis mosseae*, -Fm: Inoculation without *Funneliformis mosseae*

AMF-inoculated and non-AMF-inoculated seedlings. Under WW condition, inoculation of AMF increased the relative abundance of *Sordariomycetes*, *Leotiomycetes*, *Chytridiomycetes* and *Pezizomycetes*, while reducing the *Eurotiomycetes*. Under DS condition, the relative abundance of *Leotiomycetes* and *Chytridiomycetes* increased in AMF-inoculated seedlings, while *Sordariomycetes*, *Agaricomycetes* and *Eurotiomycetes* decreased.

In the roots of AMF-inoculated seedlings, DS reduced the relative abundance of *Dothideomycetes*, *Agaricomycetes* and *Pezizomycetes*, but increased the relative abundance of *Sordariomycetes* and *Eurotiomycetes*. In the roots of non-AMF-inoculated seedlings, DS reduced the relative abundance of *Sordariomycetes* and *Dothideomycetes*, while *Agaricomycetes* and *Wallemiomycetes* increased. Under WW condition, the relative abundance of *Dothideomycetes* and *Pezizomycetes* were increased, while *Sordariomycetes* and *Eurtiomycetes* reduced. Under DS condition, inoculation of AMF increased the relative abundance of *Sordariomycetes* and *Dothideomycetes* but reduced the relative abundance of *Agaricomycetes* and *Wallemiomycetes* (Fig. 5). The top 50 highest read abundance of the fungal community at the genus level was shown in Fig. 6. In rhizosphere soils, the top three fungus clones were *Ascomycota Ascobolus*, *Ascomycota Geomyces* and *Chytridiomycota Spizellomyces* in WW+Fm treatment, *Chytridiomycota Spizellomyces*,

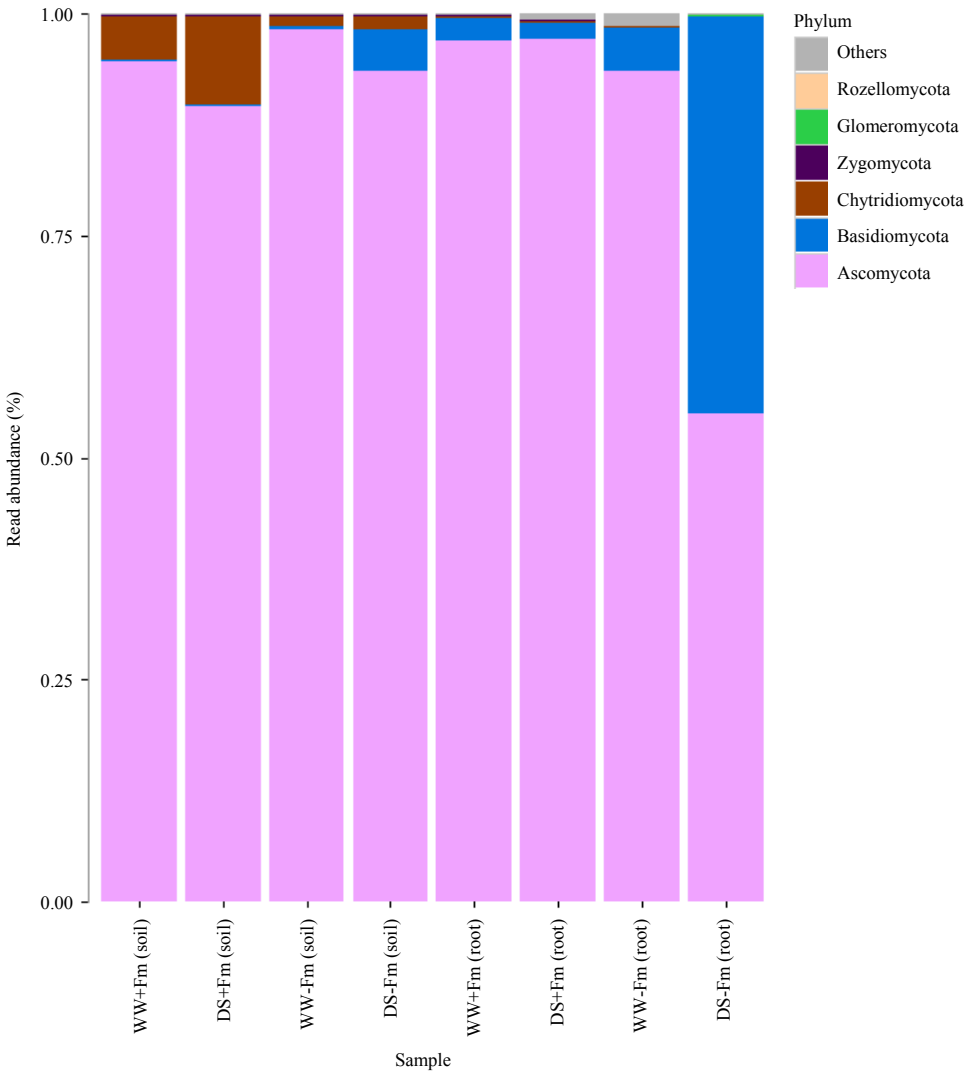

Fig. 4: Relative abundance of fungal community in the phylum level in roots and rhizosphere soils of *Funneliformis mosseae*-colonized trifoliate orange seedlings under well-watered and drought stress
WW: Well-watered, DS: Drought stress, +Fm: Inoculation with *Funneliformis mosseae*, -Fm: Inoculation without *Funneliformis mosseae*

Ascomycota Geomyces and *Ascomycota Ascobolus* in DS+Fm treatment, *Ascomycota Aspergillus*, *Ascomycota Geomyces* and *Ascomycota Spizellomyces* in WW-Fm treatment and *Basidiomycota Lycoperdon*, *Chytridiomycota Spizellomyces* and *Ascomycota Aspergillus* in DS-Fm treatment (Fig. 6). In roots, the top three fungus clones were *Ascomycota Toxicocladosporium*, *Ascomycota Alternaria* and *Ascomycota Ascobolus* in WW+Fm, *Ascomycota Toxicocladosporium*, *Ascomycota Alternaria* and *Ascomycota Aspergillus* in DS+Fm, *Ascomycota Toxicocladosporium*, *Ascomycota Aspergillus* and *Ascomycota Arthrobotrys* in WW-Fm and *Ascomycota Alternaria*, *Ascomycota Cercospora* and *Ascomycota Aspergillus* in DS-Fm (Fig. 6).

Clustering analysis: Based on the calculation results of the distance between samples, the samples were clustered to determine the degree of similarity of each sample. Cluster analysis of all samples showed that the closer the sample was and the shorter the branch length was indicating that the species composition of the two samples was more similar (Fig. 7).

The clustering analysis also showed that the four rhizosphere soil samples were divided into two taxonomic similarities (+Fm and -Fm) with the database sequences. In four root samples, the two treatments of WW-Fm and DS+Fm were listed in the taxonomic similarity with the database sequences and then, the outward branches were WW+AMF and DS-AMF, respectively.

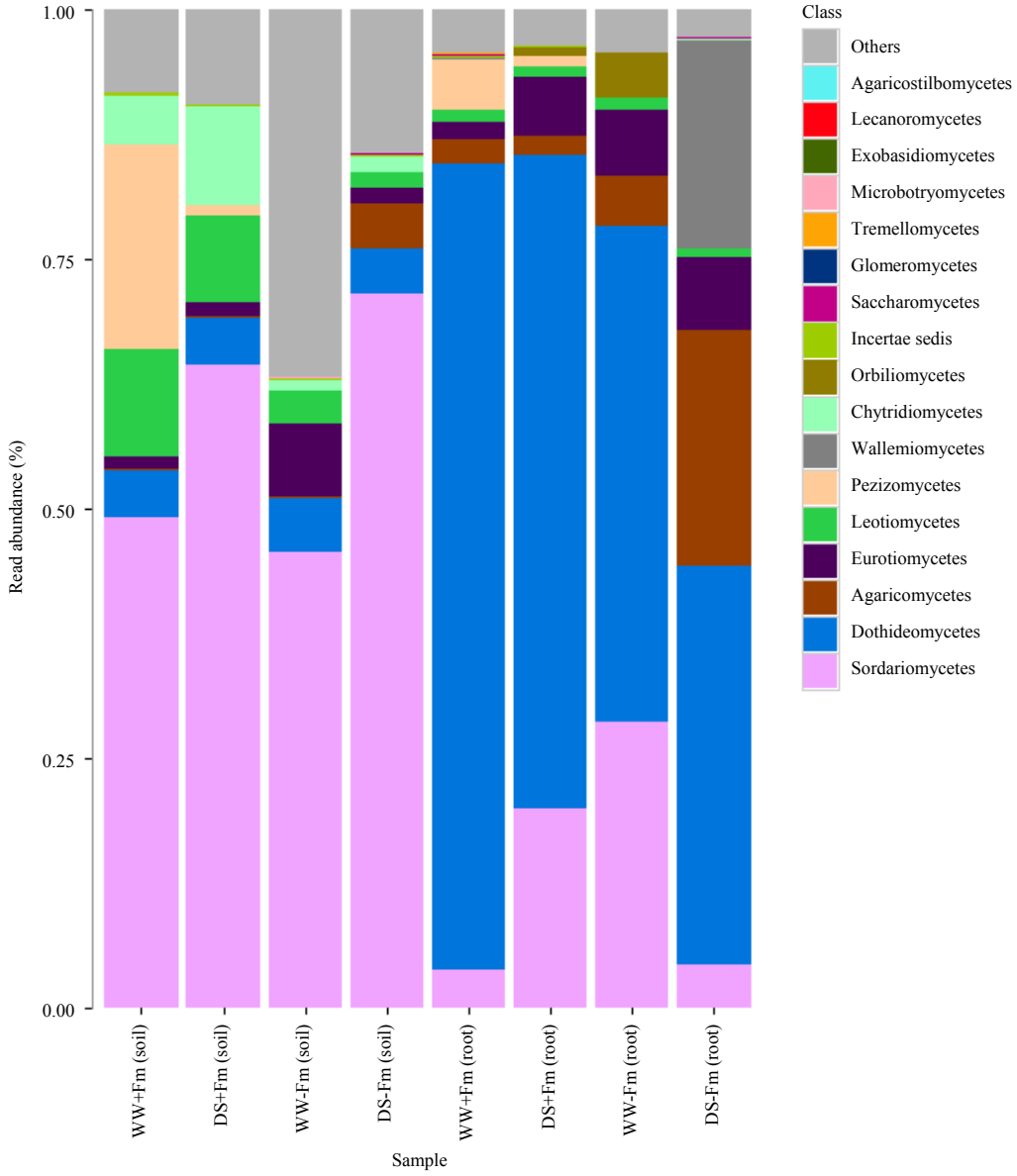

Fig. 5: Relative abundance of fungal community in the class level in roots and rhizosphere soils of *Funneliformis mosseae*-colonized trifoliate orange seedlings under well-watered and drought stress

WW: Well-watered, DS: Drought stress, +Fm: Inoculation with *Funneliformis mosseae*, -Fm: Inoculation without *Funneliformis mosseae*

DISCUSSION

OTUs, based on the rRNA gene, are clustered to evaluate fungal and bacterial species[17,18]. Each OTU is corresponding to a species of fungi and bacterium. In this study, DS heavily reduced soil OTU numbers and fungal diversity of non-AMF seedlings but increased in AMF-seedlings. Meanwhile, AMF inoculation notably increased soil OTU number under DS but reduced it under WW. It might be due to the fact that moderate DS promoted metabolism process in the root system, activated its defense mechanisms and made the root system having more OTUs and more fungal diversity, thus resisting the DS from the outside.

In the Venn diagram, OTUs of rhizosphere soils and roots overlapped in each treatment suggesting the high similarly of fungal diversity between soils and roots in trifoliate orange seedlings. In addition, the significant higher OTUs in roots than in soils implied that roots might contain more fungal diversity than soils. It is in agreement with the findings of Wu *et al.*[17] and Sun *et al.*[19] in citrus plants. It also showed that

Fig. 6: Read abundance of the top 50 fungal community in the genus level in roots and rhizosphere soils of *Funneliformis mosseae*-colonized trifoliate orange seedlings under well-watered and drought stress

WW: Well-watered, DS: Drought stress, +Fm: Inoculation with *Funneliformis mosseae*, -Fm: Inoculation without *Funneliformis mosseae*

fungal diversity in roots varied more widely than that in rhizosphere soils. This indicated that the effect of DS and AMF on fungal diversity of trifoliate orange seedlings was greater in roots than in rhizospheric soils. Possibly, indigenous fungi in the soil had a more stable system and were less susceptible to external factors than the root system.

In this study, AMF and water stress had different effects on rhizosphere fungi at phylum, class and genus levels. On the whole, it could be seen that there were more species of fungi in roots than in rhizosphere soils, which was consistent with the results of Venn diagram, dilution curve and alpha diversity index. Interestingly, at phylum level and class level, a large number of fungi was found belonging to *Ascomycota* and *Sordariomycetes*, respectively. It is well documented that *Ascomycota* is a very common fungal phylum, previously reported to dominate fungal groups in plant tissues and different soil types and fertilizers[20,21] and members of *Sordariomycetes* can grow in soil, dung, leaf litter and decaying wood as decomposers as well as being fungal parasites and insect, human and plant pathogens[22,23]. The present study showed that the inoculation of *F. mosseae* strongly promoted the increase of *Sordariomycetes*, which is beneficial to the resistance of roots to external diseases, as well as to the improvement of soil health.

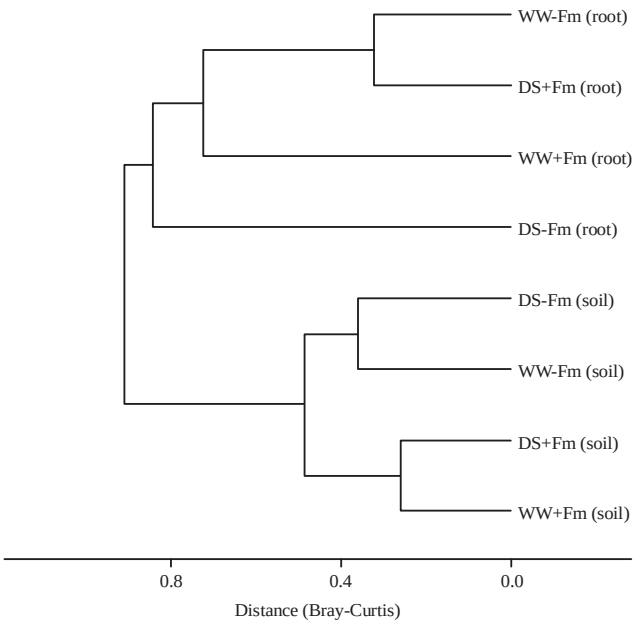

Fig. 7: Neighbor-joining tree in terms of small subunit rRNA (SSU rRNA) sequences in roots and rhizosphere soils of *Funneliformis mosseae*-colonized trifoliate orange seedlings under well-watered and drought stress
WW: Well-watered, DS: Drought stress, +Fm: Inoculation with *Funneliformis mosseae*, -Fm: Inoculation without *Funneliformis mosseae*

The clustering method used in this study was UPGMA (Unweighted Pair Group Method with Arithmetic Mean)[24]. The present work indicated that the rhizosphere soil was divided into two branches by whether or not inoculated with AMF, indicating that the inoculation of AMF was the main factor for changing the fungal community of rhizosphere soils. In the clustering analysis of fungal communities in root samples, a similar conclusion was gotten. Root fungal communities were more sensitive to external soil water than rhizosphere soils.

CONCLUSION

The inoculation with *F. mosseae* and moisture stress collectively affected the abundance and diversity of fungal communities in rhizosphere soils and roots of trifoliate orange seedlings to varying degrees. At the same time, the stability of the fungal community in rhizosphere soils was better than in roots. External environmental factors, such as AMF and DS, had a much less impact on the abundance and diversity of the fungal community in rhizosphere soils than in roots. As a result, in arid region, more attention should be paid for fungal community and diversity in roots than in soils in citriculture.

SIGNIFICANCE STATEMENT

Drought stress can reduce the abundance and diversity of fungi in rhizosphere soils and roots to some extent. This study indicated that the inoculation of an arbuscular mycorrhizal fungus *Funneliformis mosseae* could increase the abundance and diversity of fungi in rhizosphere soils and roots, especially under drought stress. Such result can provide a basis to mitigate the damage of soil water deficit in plants. It was also of great significance for the field fertility management and the improvement of soil structure and nutrition.

ACKNOWLEDGMENTS

This study was supported by the Key Project of the Science and Technology Research, Hubei Provincial Department of Education (D20171304), the Plan in Scientific and Technological Innovation Team of Outstanding Young Scientists, Hubei Provincial Department of Education (T201604) and the Outstanding Master Dissertation Cultivation Program of Yangtze University in 2018.

REFERENCES

1. Trenberth, K.E., A. Dai, G. van der Schrier, P.D. Jones, J. Barichivich, K.R. Briff and J. Sheffield, 2014. Global warming and changes in drought. Nature Climate Change, 4: 17-22.
2. Srivastava, A.K. and S. Singh, 2008. Citrus nutrition research in India: Problems and prospects. Indian J. Agric. Sci., 78: 3-16.
3. Rousk, J., A.R. Smith and D.L. Jones, 2013. Investigating the long-term legacy of drought and warming on the soil microbial community across five European shrubland ecosystems. Global Change Biol., 19: 3872-3884.
4. Manzoni, S., J.P. Schimel and A. Porporato, 2012. Responses of soil microbial communities to water stress: Results from a meta-analysis. Ecology, 93: 930-938.
5. Davidson, E. A., S. Samanta, S.S. Caramori and K. Savage, 2012. The dual Arrhenius and Michaelis-menten kinetics model for decomposition of soil organic matter at hourly to seasonal time scales. Global Change Biol., 18: 371-384.
6. Moyano, F.E., S. Manzoni and C. Chenu, 2013. Responses of soil heterotrophic respiration to moisture availability: An exploration of processes and models. Soil Biol. Biochem., 59: 72-85.
7. Sharma, S.B. and T.A. Gobi, 2016. Impact of Drought on Soil and Microbial Diversity in Different Agro Ecosystems of the Semi Arid Zones. In: Plant, Soil and Microbes: Implications in Crop Science, Hakeem, K.R., M.S. Akhtar and S.N.A. Abdullah (Eds.)., Springer, Cham, USA., pp: 149-162.

8. He, J.D., T. Dong, H.H. Wu, Y.N. Zou and Q.S. Wu, 2019. Mycorrhizas induce diverse responses of root *TIP*aquaporin gene expression to drought stress in trifoliate orange. Sci. Hortic., 243: 64-69.
9. Wu, Q.S., A.K. Srivastava, Y.N. Zou and S.K. Malhotra, 2017. Mycorrhizas in citrus: Beyond soil fertility and plant nutrition. Indian J. Agric. Sci., 87: 427-443.
10. Borde, M., M. Dudhane and J.P. Kaur, 2010. Diversity of AM fungi in some tree species from dry land area of central Maharashtra (India). Arch. Phytopathol. Plant Prot., 43: 1796-1808.
11. Wu, Q.S., A.K. Srivastava and Y.N. Zou, 2013. AMF-induced tolerance to drought stress in citrus: A review. Scient. Hortic., 164: 77-87.
12. Toju, H., A.S. Tanabe, S. Yamamoto and H. Sato, 2012. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. PloS One, Vol. 7.
13. Magoc, T. and S.L. Salzberg, 2011. FLASH: Fast length adjustment of short reads to improve genome assemblies. Bioinformatics, 27: 2957-2963.
14. Edgar, R.C., B.J. Haas, J.C. Clemente, C. Quince and R. Knight, 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics, 27: 2194-2200.
15. Edgar, R.C., 2013. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. Nature Meth., 10: 996-998.
16. Kembel, S.W., P.D. Cowan, M.R. Helmus, W.K. Cornwell and H. Morlon *et al*., 2010. Picante: R tools for integrating phylogenies and ecology. Bioinformatics, 26: 1463-1464.
17. Wu, Q.S., P. Sun. and A.K. Srivastava, 2017. AMF diversity in citrus rhizosphere. Ind. J. Agric. Sci., 87: 653-656.
18. Koeppel, A.F. and M. Wu, 2013. Surprisingly extensive mixed phylogenetic and ecological signals among bacterial operational taxonomic units. Nucleic Acids Res., 41: 5175-5188.
19. Sun, P., Y.C. Zhang, Y.L. Shen, C.Q. Feng and Q.S. Wu, 2017. Identification of fungal community in citrus rhizosphere by ITS gene sequencing. Biotechnology, 16: 85-91.
20. Al-Bulushi, I.M., M.S. Bani-Uraba, N.S. Guizani, M.K. Al-Khusaibi and A.M. Al-Sadi, 2017. Illumina MiSeq sequencing analysis of fungal diversity in stored dates. BMC Microbiol., Vol. 17. 10.1186/s12866-017-0985-7.
21. Kazeeroni, E.A. and A.M. Al-Sadi, 2016. 454-pyrosequencing reveals variable fungal diversity across farming systems. Front. Plant Sci., Vol. 7. 10.1016/j.apsoil.2012.02.012.
22. Berbee, M.L. and J.W. Taylor, 1992. Two ascomycete classes based on fruiting-body characters and ribosomal DNA sequence. Mol. Biol. Evol., 9: 278-284.
23. Celio, G.J., M. Padamsee, B.T.M. Dentinger, R. Bauer and D.J. McLaughlin, 2006. Assembling the fungal tree of life: Constructing the structural and biochemical database. Mycologia, 98: 850-859.
24. Sokal, R.R. and C.D. Michener, 1958. A statistical method for evaluating systematic relationships. Univ. Kansas Sci. Bull., 38: 1409-1438.