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Original article

Resilience of root and soil bacteria to drought stress depends on host plant's colonization affinity towards arbuscular mycorrhiza fungi

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ABSTRACT

Water deficit is one of the most important climate events that has strong effect on agricultural ecosystem functionality comprising soil microbial communities and their functions. Arbuscular mycorrhizal fungi (AMF) are widely known for their roles in combating drought, including facilitation of drought-tolerant bacteria. However, differences in cultivar/variety affinity for mycorrhization have never been considered as influencing factors. In the present study, we evaluated the influence of mycorrhizal affinity of two durum wheat (T. turgidum subsp. Durum (Desf.)) varieties, Iride and Ramirez (high and low, respectively) on root and soil bacteriomes under well-watered and drought conditions. We used the 16S metagenomics approach (amplicon sequencing) to assess the bacterial communities of root and soil samples. The suppression effect of drought was evident across a wide range of bacterial taxa, including drought-tolerant taxa, especially in the non-inoculated plants. Nevertheless, the protective effect of AMF was also shown, especially in the Iride variety (high AMF colonization affinity) in both compartments (root and soil), as the relative abundance of drought-depleted taxa, such as Planctomycetes, Bacteroidetes and Verrucomicrobia, was either similar under well-watered and water deficit conditions or increased under water deficit conditions. Moreover, drought reduced the network complexity of root and soil bacteria, especially in Ramirez variety which has a lower AMF colonization affinity. Together, our results suggest that not only AMF colonization, but also host plant colonization affinity is one of the regulating factors in alleviating drought-induced changes in wheat plants by altering plant-fungal-bacterial interactions.

1. Introduction

Climate change is continuously increasing the intensity and frequency of extreme climate events [1], which have a strong impact on global ecosystem function. Understanding ecosystem responses to these extreme events is essential for societal adaptation and mitigation [2]. Agroecosystem is being one of the most affected sector by climate extreme events [3]. Crop health fundamentally depends on the plant-soil-microbiome relationships through the formation of a defined biosphere termed the rhizosphere, in which all the activities of interest to the plant are performed [4,5]. Plant vitality is thereby derived from nutrient supply and abiotic stress resistance enhanced by their microbiome (root endosphere and rhizosphere) [6,7]. Hence, interaction between above- and belowground biota have potential to modify ecosystem response to climate change.

Over the past few years, drought has been one of the most frequent and adverse climate events in relation to agricultural productivity, and is expected to intensify in the future [8,9]. Drought considerably decreases plant growth and production by negatively affecting the physiological, biochemical, and molecular traits of plants [10]. On top of that, drought substantially modifies the biomass, diversity and structure of the endosphere and rhizosphere microbial communities, often leading to changes or disturbances in ecosystem processes and plant community dynamics [2,7,11]. Drought further reduces the co-occurrence of microbial network by destabilizing their network properties such as centrality (an estimation on how important a node or edge is for the connectivity or the information flow of the network), modularity (is a measure of the structure of networks which measures the strength of division of a network into modules) etc. [12].

Plant growth-promoting microbes, especially root endophytes, play a

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prominent role in alleviating drought-induced changes through the activation of various defence mechanisms, which could further help in post-drought recovery [7,11,13]. Among these beneficial endophytes, arbuscular mycorrhizal fungi (AMF), which form symbioses with most terrestrial plants, have gained considerable attention for alleviating drought-induced damage. This is due to their ability to ameliorate plant water and nutrient use efficiency, antioxidant enzyme activity, stomatal conductance and reducing the risk of soil compaction by improving root and hyphal growth [14–18]. Despite the fact that AMF also substantively alters the diversity and structure of root and soil microbial communities [19–22], relatively little attention has been given to understanding the plant-AMF-bacterial interactions under drought conditions. A recent finding by Hestrin et al. [23] showed that AMF presence increases bacterial resilience to water deficit conditions and may promote post-drought recovery. Moreover, the authors reported suppressive effect of AMF on bacterial growth potential under well-watered conditions. This indicates that fungal-bacterial interactions are not always mutualistic but context-dependent.

Ganugi et al. [24] has screened genetic diversity of 127 accessions belonging to different T. turgidum subspecies in relation to their level of AMF colonization (defined as AMF colonization affinity) and reported the significant differences in AMF colonization affinity of these varieties. In our latest study, we showed that differences in AMF colonization affinity of wheat varieties (high and low) play a focal role in determining AMF responsiveness to water deficit [25]. Furthermore, soil bacterial networks are less stable under drought conditions than fungal networks, and changes in bacterial communities are more strongly linked to soil functioning during recovery than changes in fungal communities [12]. Following to these, we experimentally investigated the response of root and rhizosphere bacteriomes in two durum wheat (T. turgidum subsp. Durum (Desf.)) varieties, Iride and Ramirez, under both well-watered and water-deficit conditions. Varieties Iride and Ramirez have high and low mycorrhizal affinities, respectively. We hypothesized that (i) host plant mycorrhizal colonization affinity (high vs low) is a key factor in mitigating drought-induced changes; (ii) higher bacterial resilience could be seen in roots and soil of Iride compared to Ramirez variety due to high AMF affinity of Iride variety.

2. Material and methods

2.1. Experimental design and sample collection

The experiment was conducted in a growth chamber at the Department of Agriculture (DAGRI), University of Florence, Italy. We used two varieties of Triticum turgidum ssp. Durum, "Iride" and "Ramirez", which we previously determined to differ in the extent of AMF colonization affinity [24]. In particular, Iride shows high mycorrhizal affinity, whereas Ramirez shows low mycorrhizal affinity [24]. Both selected varieties were modern accessions and genetically uniform (all seeds within the variety belonged to the same genotypes). Silty-clay agricultural soil (Table 1) was collected from a farm located in Grosseto, Italy (42°53′04″N11° 16′17″ N). Soil was sampled at a depth between 0 and 10 cm at 3 different points, 20 m away from each other, along the diagonal of a 500 m^2 plot, to obtain 3 independent replicates. Subsequently, it was transferred to the laboratory to be air drying, crushing, sieving (pore size: 2 mm), and then mixed with silica sand (0.2–1 mm size) and peat (Terriccio Universal, Tuttofiori, TerComposti, Brescia, Italy) (3.5:3:3.5, w/w) to improve aeration and drainage. The resulting soil mixture (Table 1) was homogenized, and 1 kg of soil was placed in square pots (22 cm × 10.5 cm).

Table 1
Principal characteristics of the collected soil (Flo_01) and the resulting soil mixture (Flo_02).

Soil	Sand %	Silt %	Clay %	pH H2O
Flo_01	29.72	39.43	30.85	7.92
Flo_02	51.7	14.7	7	7.88

Subsequently, all pots were transferred to a climatic chamber with a cycle of 16 h light and 8 h dark at a temperature of 15 °C during the light cycle and 25 °C during the night cycle. The experiment was conducted on two wheat varieties (Iride and Ramirez) with two AMF treatments (AMF and non-AMF for AMF-inoculated and non-inoculated plants, respectively) and two water regimes (W and D for well-watered and drought stress, respectively). The experiments were arranged in a full factorial design, with four seeds per pot and nine replicates per condition (D_AMF [drought stress plus AMF inoculated], D_nonAMF [drought stress plus nonAMF inoculated], W_AMF [well-water plus AMF inoculated], and W_nonAMF [well-water plus non-AMF inoculated]), amounting to a total of 72 pots (36 for Iride and 36 for Ramirez). The plant seeds were subjected to mycorrhizal treatments before sowing. Seeds were inoculated with the commercial product, MICOSAT F® SEMI wp (CCS AOSTA Srl. Italy) with 2 a.m. fungal species (Rhizoglomus irregulare and Funneliformis mosseae) at a concentration of 460 sp/g (230 sp/g of each). Furthermore, the commercial product also contained beneficial rhizosphere bacteria (1 × 107 CFU/g) and saprophytic fungi (Trichoderma koningii; 3 × 108 sp/g). One gram of the product (for each cultivar) was dissolved in 250 mL of sterilized water according to the manufacturer's instructions; 144 seeds of each variety were tanned, and four seeds were sown into each pot. After 3 months of growth and regular watering (60% of field water-holding capacity), watering was stopped in pots allocated to drought treatments (D_AMF and D_nonAMF). Sampling was performed to detect the first mild phenotypic symptoms of drought stress in plants, which started to appear on the 15th day of irrigation interruption.

All root and soil samples from each pot were immediately frozen in liquid nitrogen and stored at −80 °C for downstream analysis. Hereafter, the comparison between the root and soil samples is termed the compartment niche. Root samples from four plants grown in each pot were pooled into one sample. All root samples were subjected to check for mycorrhiza colonization [25]. The samples did not show mycorrhizal colonization under the AMF treatment, and samples showing mycorrhizal colonization under the non-AMF treatment were discarded before further analysis. Thus, three out of the nine individual replicates (48 samples in total) were selected for further downstream analysis.

The percentages of AMF colonization were estimated using the gridline intersect method with a dissecting microscope (Wild, Leica, Milano, Italy) at 25x and 40× magnification, after clearing with 10% KOH and staining with 0.05% trypan blue in lactic acid [26]. Further, roots were washed only with sterilized distilled water and used for root microbial community analyses. In this study, we therefore define the "root bacteriome" as the combined bacterial communities of the root endosphere and root surface since the sample collection method did not discriminate between these two compartments. Moreover, as the root system of the four plants grown in each pot completely occupied the available space, we defined the soil as ectorhizospheric. All soil microbial analyses were performed in DAGRI, University of Florence, Italy while soil chemical analyses were performed by Demetra snc laboratory (Pescia, Italy).

2.2. DNA extraction and amplicon sequencing

Total genomic DNA was extracted from 0.5 g of rhizosphere soil or root powder that was obtained by grinding with liquid nitrogen using the Fast DNA™SPIN Kit for soil (MP Biomedicals, Santa Ana, California, USA) according to Ref. [27]. Briefly, DNA were extracted using mechanical - chemical cell lysing using sodium phosphate buffer and FastPrep instrument. Extracted DNA were purified by washing two time with guanidine (5.5 M) and SEWS-M, respectively. Purified DNAs were eluted with 100 μl of DES. Quality control and DNA yield were checked by 1.5% agarose gel electrophoresis and spectrophotometer (Picodrop limited, Hinxton, UK).

The V3–V4 region of 16 S rRNA gene was amplified using the Illumina barcoded primer pair S-D-BACT-0341F/S-D-BACT-0785 (Klindworth et al., 2013) by using a TProfessional thermal cycler (Biometra, biomedizinische Analytik GmbH). The PCR reaction mix (50 μl) contained: 40 ng of template DNA, 1X (plus MgCl2 20 mM) Dream Taq reaction buffer (Thermo Scientific, Carlsbad, CA, USA), 0.04 μg μl−1 of BSA, 0.05 Units μl−1 of Taq DNA Polymerase (ThermoFisher Scientific), 0.4 μM of each primer, 0.4 mM of dNTPs. PCR running conditions were: 3 min denaturation at 95 °C, followed by 30 sequential cycles each consisting of 30 s at 95 °C, 30 s at 55 °C, 30 s at 72 °C, followed by a final extension step at 72 °C for 7 min. PCR products (amplicon size ~550 bp) were purified using a NucleoSpin® Gel and PCR Clean-up Kit (MACHEREY-NAGEL, Germany) and then quantified by an Invitrogen™ Qubit™ 2.0 Fluorometer (ThermoFisher Scientific). Purified amplicons were used for library preparation and sequencing, according to the Illumina 16 S Metagenomic Sequencing Library Preparation guide (downloaded from https://support.illumina.com/content/dam/illuminasupport/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf). Paired-end sequencing (2 × 300 bp) was conducted by using a MiSeq System (Illumina, California, USA). Sequencing was conducted at IGA Technology Services (Udine, Italy).

2.3. Sequencing data processing and data analysis

Raw demultiplexed sequences (R1 and R2) were downloaded from Illumina BaseSpace website and were analyzed using QIIME 2 (Quantitative Insight Into Microbial Ecology) version 2021.2 (Bolyen et al., 2019). Demultiplexed sequences were trimmed, joined and denoised for quality control using DADA2 plugin (Callahan et al., 2016), available in QIIME 2, to generate a feature table of obtained ASVs (Amplicon Sequence Variant). Then, sequences were aligned with MAFFT plugin, available in QIIME 2 to generate phylogeny. Taxonomic composition of ASVs from kingdom to species level was determined using a pre-trained naïve-Bayesian classifier on the SILVA database version 138. After quality filtering a total of 2,366,848 - 16 S rRNA sequences and 13,454 ASVs were used for further analysis. Rarefaction and alpha diversity of ASVs were performed on resampled datasets with the same number of sequences randomly selected from all samples (33,000 sequences). Illumina datasets were submitted to the European Nucleotide Archive (ENA) under the study accession number PRJEB65004.

ASVs with low number of sequences (≤5 of total count) were eliminated from ASVs feature table for further analysis. All data were normalized by dividing the number of sequences belonging to each phylogenetic group by the total number of sequences in the given sample and transformed into the relative abundance so that the sum of ASVs in each sample is one. PAST 4.06 (Hammer et al., 2001) and R Statistical Environment (R development Core Team 2008) were used for all statistical analysis. Alpha diversity indices were analyzed by three-way ANOVA (AMF treatment [AMF vs nonAMF], water regime [well-watered vs drought stress] and compartment niche [root vs soil]) to check any significant effect of AMF treatment, drought stress and compartment niche and their interaction on variability of data for each plant varieties. Further, multiple pairwise comparisons of means were done by Tukey's honestly significant difference (HSD) test at $p < 0.05$ level of significance to analyze the individual effects of each factor. Principal coordinate analysis (PCoA) and PERMANOVA test (using the 'adonis' function of the VEGAN package in R (Oksanen et al., 2007)) were conducted based on Bray-Curtis's similarity distance to determine the distribution of diversity and statistical significance of beta-diversity, respectively.

2.4. Network analysis

Co-occurrence of the 500 most abundant ASVs of each treatment (Mycorrhiza treatment, drought stress and compartment niche with 12 samples for each network) for both plant varieties were analyzed by calculating Spearman's rank coefficients (P) using the R package "Hmisc" (Harrell 2008). Subsequently, those significant (FDR adjusted P value < 0.01) and robust ($P \geq 0.6$) correlations between ASVs were exported as a GML format network file using R package igraph (Csardi and Nepusz 2006). Network visualization was conducted using the Fruchterman-Reingold layout of the interactive platform Gephi version 0.9.2. The network complexity was defined according to previous studies (Pimm 1984, Wagg et al., 2019, Xiong et al., 2021). Nodes with higher Betweenness and closeness centrality values were identified as hub nodes in co-occurrence networks.

2.4.1. Chemical analysis

Soil pH, organic substances, Total nitrogen, micronutrients and C/N ration of soil samples were determined by official methods of chemical analysis of soil [28]. Soil pH were determined in water extracts (sample: deionized water ratio of 1:1, w/w) using a pH meter. Total nitrogen was determined by titrimetric method. Mineral-fraction content (Ca, Cu, Fe, K, Mg, Mn, and Zn) was detected using an inductively coupled plasma optical emission spectrometer method. To determine Potassium, Magnesium and Calcium exchangeable, the soil was extracted with $BaCl_2$ and trietanolammine solution while to determine Fe, Mn, Cu and Zn, the soil was extracted with Na ditionite citrate (Fe) and Aqua regia (Mn, Cu and Zn).

To determine Fe, Mn, Cu and Zn, the soil was extracted with Na ditionite citrate (Fe) and Aqua regia (Mn, Cu and Zn). The cations were determined on extracted solution using spectrophotometer atomic absorption.

3. Results

3.1. Evaluation of AMF affinity

The level of AMF affinity assessed by dissecting microscopy confirmed the presence of root symbiosis in the mycorrhized samples and the absence of fungi in non-mycorrhized samples, for both varieties and under both water regime conditions (Supplementary Fig. 1) [25]. The two varieties showed significantly different affinities for mycorrhization, with Iride and Ramirez roots presenting a high (mean = ~65%) and a low (~17%) percentage of AMF colonization, respectively. Furthermore, drought increased AMF colonization affinity in both varieties especially in Iride variety (6.52%).

3.2. Soil chemical properties

The effects of water regime and AMF treatments on different soil chemical properties are shown in Table 2. AMF addition did not have any significant effect on soil pH, whereas drought significantly reduced soil pH in Iride variety. Neither factor had a significant effect on organic substances, Total nitrogen, and C/N. Furthermore, there no significant differences were observed in micronutrients except for assimilable Fe (iron) and Cu (copper). Drought significantly reduced the amount of assimilable Fe and Cu in both wheat varieties.

3.3. Bacteria alpha diversity

The rarefaction curve reached saturation for all samples, indicating that the sequencing depth was sufficient to cover detectable species in all samples (Supplementary Fig. 1). Alpha diversity (within-sample species richness and evenness) was measured by calculating the Faith's phylogenetic diversity (Faith PD), evenness, and Shannon index (Tables 3 and 4). All three factors AMF treatments, water regimes and compartment niche, and their interactions have different effects on alpha diversity in both varieties (Table 3). Alpha diversity was significantly lower in the root compartment than in the soil (Table 4). Faith PD significantly decreased in the root compartment whereas increased in

Table 2
Changes in chemical properties (mean ± SD, n = 3) in rhizosphere soils of both wheat cultivars under different treatments (W = well-watered, D = drought, AMF = AMF addition, AMF = no AMF addition). Different letters indicate significant differences within each cultivar (Tukey's HSD at P < 0.05).

	Iride				Ramirez			
	W_AMF	W_nonAMF	D_AMF	D_nonAMF	W_AMF	W_nonAMF	D_AMF	D_nonAMF
pH(H_2O)	8.13 ± 0.03^{a}	8.13 ± 0.03^{a}	7.93 ± 0.03^{b}	7.93 ± 0.03^{b}	8.03 ± 0.03	8.07 ± 0.03	7.97 ± 0.03	7.93 ± 0.03
Organic substances (%)	7.47 ± 0.46	8.27 ± 1.07	6.12 ± 0.38	7.5 ± 0.44	6.52 ± 0.2	7.86 ± 0.6	7.07 ± 0.24	7.31 ± 0.09
Total Nitrogen (g/kg)	3.1 ± 0.06	3.22 ± 0.12	3.06 ± 0.23	2.99 ± 0.01	3.02 ± 0.09	3.38 ± 0.12	2.99 ± 0.33	2.71 ± 0.32
C/N	14 ± 0.92	14.83 ± 1.39	11.63 ± 0.13	14.53 ± 0.85	12.57 ± 0.49	13.5 ± 0.81	14.13 ± 1.91	16.1 ± 1.91
Exchangeable K (mg/kg)	1463.33 ± 25.83	1329 ± 182.87	1445.33 ± 190.79	1346.33 ± 31.8	1416.67 ± 98.4	1574 ± 134.76	1291.67 ± 116.83	1302.67 ± 117.7
Exchangeable Ca (mg/kg)	1765 ± 86.23	1903.33 ± 31.48	1809 ± 105.95	1821 ± 44.24	1887.67 ± 20.09	1990.67 ± 98.15	1763.33 ± 149.42	1885 ± 173.49
Exchangeable Mg (mg/kg)	357.33 ± 27.24	396.33 ± 6.96	348.67 ± 27.17	360.67 ± 9.35	342.33 ± 13.48	389.67 ± 30.12	320 ± 35.53	352.67 ± 40.92
Assimilable Fe (mg/kg)	95.47 ± 10.76^{ab}	127.73 ± 28.33^{a}	46.67 ± 2.24^{b}	44.53 ± 0.58^{b}	113.33 ± 29.99^{a}	95.47 ± 24.95^{a}	38.27 ± 4.81^{b}	36.93 ± 2.89^{b}
Assimilable Mn (mg/kg)	43.6 ± 6.96	81.47 ± 23.76	28.93 ± 1.76	25.87 ± 0.48	67.2 ± 26.58	54 ± 17.26	21.33 ± 3.58	21.2 ± 1.74
Assimilable Cu (mg/kg)	3.84 ± 0.26^{ab}	4.57 ± 0.5^{a}	3.09 ± 0.5^{ab}	2.63 ± 0.04^{b}	4.53 ± 0.73^{a}	4.01 ± 0.51^{ab}	2.29 ± 0.21^{b}	2.32 ± 0.19^{b}
Assimilable Zn (mg/kg)	12.8 ± 0.46	12.47 ± 1.22	15 ± 1.03	13.73 ± 0.24	12 ± 0.35	13.6 ± 0.53	12.13 ± 1	13 ± 1.3

Table 3
Main effects due to AMF colonization (AMF), Drought stress (D) and Compartment niche (CN) and their interaction on the variability of selected alpha diversities. Values are *F*-values (*F*-stat) from three-dimensional ANOVA (treatment x time x plant presence) with the corresponding *P* level and statistical significance. § Significance: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns: not significant.

Factor	df[#]	Iride			Ramirez		
		Faith PD	**Evenness**	**Shannon**	**Faith PD**	**Evenness**	**Shannon**
AMF colonization	1	0.29^{ns}	61.63***	2.52^{ns}	0.22^{ns}	1.63^{ns}	0.01^{ns}
Drought Stress	1	0.01^{ns}	254.24***	25.99***	0.10^{ns}	1285.01***	43.85***
Compartment niche	1	141.00***	2444.13***	327.20***	22.70***	6690.44***	275.11***
AMF colonization x Drought	1	0.63^{ns}	7.63*	0.002^{ns}	0.00^{ns}	919.67***	23.23***
AMF colonization x Compartment niche	1	0.27^{ns}	19.12***	0.75^{ns}	4.55*	19.05***	0.79^{ns}
Drought x Compartment niche	1	11.40**	76.65***	0.02^{ns}	0.97^{ns}	1029.24***	59.43***
AMF colonization x Drought x Compartment niche	1	0.21^{ns}	0.00^{ns}	1.36^{ns}	2.66^{ns}	1436.01***	80.25***

Table 4
Changes in alpha diversity indices of bacterial community (mean ± SD, n = 3) in roots and rhizosphere soils of both wheat cultivars under different treatments (W = well-watered, D = drought, AMF = AMF addition, AMF = no AMF addition). Different letters indicate significant differences within each cultivar (Tukey's HSD at P < 0.05).

Plant	Index	Root				Rhizosphere			
		W_AMF	W_nonAMF	D_AMF	D_nonAMF	W_AMF	W_nonAMF	D_AMF	D_nonAMF
Iride	Faith PD	86.05 ± 1.65	88.97 ± 3.15	80.89 ± 5.0	78.04 ± 5.69	105.64 ± 1.58^{b}	108.86 ± 1.12^{ab}	113.97 ± 0.48^{ab}	115.66 ± 3.32^{a}
	Evenness	0.85 ± 0.00^{c}	0.87 ± 0.00^{b}	0.88 ± 0.00^{b}	0.9 ± 0.00^{a}	0.93 ± 0.00^{b}	0.92 ± 0.00^{c}	0.93 ± 0.00^{b}	0.94 ± 0.00^{a}
	Shannon	8.76 ± 0.04	8.94 ± 0.06	9.09 ± 0.13	9.15 ± 0.1	9.79 ± 0.03^{bc}	9.77 ± 0.04^{c}	9.99 ± 0.03^{ab}	10.08 ± 0.07^{a}
Ramirez	Faith PD	83.4 ± 7.02	87.04 ± 1.37	79.93 ± 1.98	95.47 ± 11.51	106.42 ± 3.9	106.37 ± 0.8	107.73 ± 0.64	95.54 ± 3.65
	Evenness	0.82 ± 0.00^{b}	0.73 ± 0.00^{c}	0.82 ± 0.00^{b}	0.9 ± 0.00^{a}	0.91 ± 0.00^{c}	0.93 ± 0.00^{a}	0.93 ± 0.00^{a}	0.92 ± 0.00^{b}
	Shannon	8.42 ± 0.16^{b}	7.47 ± 0.03^{c}	8.46 ± 0.04^{b}	9.53 ± 0.23^{a}	9.61 ± 0.04^{b}	9.84 ± 0.02^{a}	9.83 ± 0.01^{a}	9.45 ± 0.06^{b}

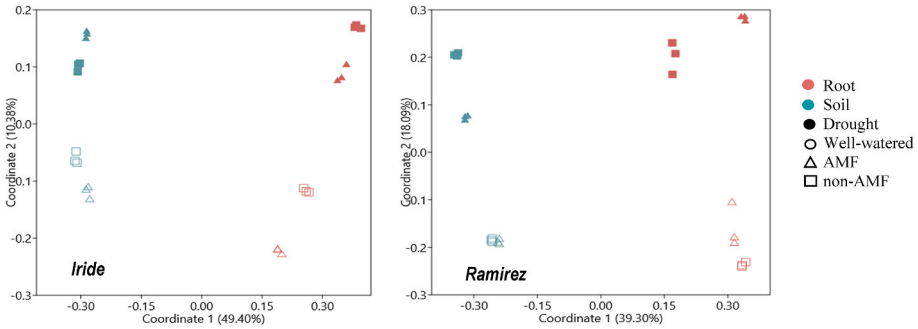
the soil under drought stress condition in the Iride variety (Table 4), while the opposite trend was observed in the Ramirez variety. Evenness and Shannon indices significantly increased under drought stress conditions in both compartments of the Iride variety (Table 4). A similar trend was observed in Ramirez only in the root compartment whereas evenness and Shannon index significantly decreased in the soil compartment (Table 4). Moreover, AMF addition did not have a significant effect on alpha diversity in the Ramirez variety (Table 4).

3.4. Bacterial beta diversity

PCoA of Bray-Curtis's distance was used to analyze the variation in the bacterial community as affected by mycorrhiza colonization affinity, drought stress, and compartment niche (soil vs. root) (Fig. 1). The first two principal coordinators explain a high percentage of variance (~60% for both plant varieties) with distinction in community structure associated with all three factors. Plots revealed that the community clustered differently under all three factors, especially under the compartment niche (soil vs. root compartment). PERMANOVA results confirmed a significant effect of compartment niche (F = 37.112 and 35.332 for Iride and Ramirez respectively, p = 0.0001), water regimes (F = 7.926 and 16.202 for Iride and Ramirez respectively, p = 0.001), and AMF treatments (F = 2.891 and 4.636, p = 0.04 and p = 0.004 for Iride and Ramirez, respectively). The strongest influence was shown by the compartment niche, followed by drought stress and AMF addition. Furthermore, the interaction of all three factors also significantly affected the bacterial community structure (Fig. 1).

3.5. Taxonomic composition of bacterial community

To analyze the effect of all factors on bacterial composition, we assessed relative abundance of bacteria at the phylum and order levels (Fig. 2 and Supplementary Fig. 2). More than 35 phyla were identified in the present study; however, we only showed those present at > 1%



Factor	df[#]	Iride	Ramirez
Stress	1	7.926***	16.202***
Mycorrhiza	1	2.891*	4.636**
Compartment Type	1	37.112***	35.332***
Stress: Mycorrhiza	1	3.054*	4.342**
Stress: Compartment Type	1	4.547**	7.451**
Mycorrhiza: Compartment Type	1	3.204*	4.035**
Stress: Mycorrhiza: Compartment Type	1	2.492[ns.]	4.385**

Fig. 1. Principal Coordinate Analysis (PCoA) based on Bray–Curtis similarity distance of ASVs of roots and rhizosphere soils bacterial community of two Wheat cultivars under different treatments. Significant differences detected by permutational MANOVA (PERMANOVA).

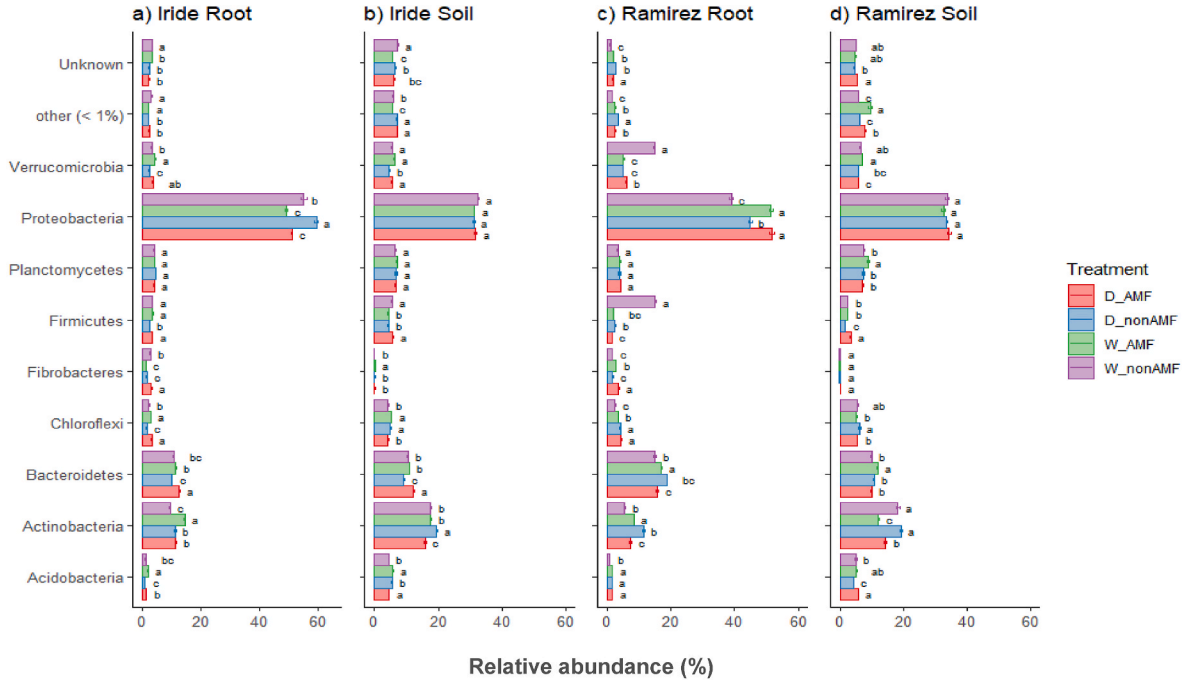


Fig. 2. The variation in bacterial community composition (mean ± SD, n = 3) in roots and rhizosphere soils bacterial community at phylum level soils bacterial community of two Wheat cultivars under different treatments. (a) Iride root, (b) Iride rhizosphere soil, (c) Ramirez root and (d) Ramirez rhizosphere soil. Different letters indicate significant differences among mycorrhiza and drought stress treatments (Tukey's HSD at $P < 0.05$). (W = well-watered, D = drought, AMF = AMF addition, AMF = no AMF addition).

(Fig. 2). Overall, the phylum Proteobacteria (~50%), followed by Actinobacteria, Bacteroidetes, Chloroflexi, and Firmicutes were the most abundant phyla in all samples. Up to 55% of the root bacteriome was composed of Proteobacteria in both varieties. The abundance of Bacteroidetes and Fibrobacteres was higher in the root compartment than in the soil compartment. At the same time, the relative abundance of Actinobacteria, Acidobacteria, and Chloroflexi increased in the soil compartment. AMF addition and drought stress had stronger effects on root bacterial community than on the soil bacterial community (Fig. 2). Drought stress increased the relative abundance of Fibrobacteres in the roots of both varieties. Drought also significantly increased the abundance of Bacteroidetes in the root and rhizosphere soil of the Iride variety. Relative abundance of Actinobacteria significantly decreased in the rhizosphere soil of both cultivars under interaction of drought stress and AMF addition. Furthermore, the effect was more profound in the Ramirez variety, which had a lower AMF colonization affinity than the Iride variety (higher AMF colonization affinity) (Fig. 2). Relative abundance of Firmicutes and Verrucomicrobia significantly reduced in Ramirez root under drought stress and AMF addition.

3.6. Network analysis of bacterial community and identification of keystone bacterial hubs

Six networks per each wheat variety were generated to evaluate the effects of AMF treatments, water regimes and compartment niche (Figs. 3 and 4). All three factors had different effects on bacterial community co-occurrence patterns. Network topological properties, such as betweenness centrality, modularity, average path distance, clustering coefficient, and positive/negative interactions, markedly differ under all factors (Table 5). Comparing the compartment niche, network complexity was higher in roots (with an average betweenness centrality of 63.14 in Iride and 63.10 in Ramirez) compared to rhizosphere soil (2.23 in Iride and 42.07 in Ramirez). The number of "hub nodes" (nodes with high values of betweenness centrality (>60) and closeness centrality (>0.3) in the network) decreased from root to rhizosphere soil in the Iride variety, while the opposite trend was observed in Ramirez. AMF addition markedly increased the network complexity in both varieties, especially in Iride as the betweenness centrality, average path distance, and number of hub nodes were higher the in Iride variety under AMF addition than in Ramirez. Conversely, drought stress decreased the network complexity in both varieties, as betweenness centrality, modularity, and average path distance were markedly lower under drought stress conditions compared to well-watered conditions. The number of hub nodes was higher under well-watered condition compared to drought condition in Iride variety, whereas the opposite trend was observed in the Ramirez variety.

Proteobacteria followed by Actinobacteria, Bacteroidetes were the most abundant bacterial taxa in all treatments (Figs. 3 and 4). There was not much difference in the taxonomic composition of networks among the different treatments, especially at the phylum level. However, substantial differences were observed in hub nodes/network hubs (keystone bacterial species) under the different treatments. Phenotypes belonging to the phylum Bacteroidetes family Cytophagaceae (ASV 116 and 278), and Class Alphaproteobacteria (ASV 254) were the three main network hubs in the Iride roots (Table S1). While phenotypes belonging to Phyla Bacteroidetes Family Cytophagaceae (ASV 21), Actinobacteria (ASV 46), and Class Alpha-proteobacteria (ASV 57) were keystone species in Ramirez roots (Table S2). No hub nodes identified in the rhizosphere of the Iride variety whereas phenotypes belonging to the Phyla Actinobacteria Family Streptosporangiaceae (ASV 8 and 22) and ASV 57 (unknown bacterium) were the main network hubs in the Ramirez rhizosphere (Table S3).

Remarkable differences were observed in hub nodes under non-AMF and AMF treatments in both varieties (Tables S4–7). Taxa belonging to Class Alphaproteobacteria (ASV 114) and Phyla Actinobacteria (ASV 76 – Streptomyces and ASV 34) were three main keystone species in Iride variety under AMF colonization (Table S4) while ASV 206 (Family Chitinophagaceae – Phyla Bacteroidetes), ASV 81 (Genus Stenotrophomonas belongs to Proteobacteria) and ASV 10 belonging to Phyla Actinobacteria were main hub nodes under non-AMF treatment (Table S6). In Ramirez cultivar, first three hub nodes belonging to Gammaproteobacteria (ASV 147), Deltaproteobacteria (ASV 37 belongs to Class Myxococcales) and Acidobacteria (ASV 163 – Chloracidobacterium) under AMF addition (Table S5). In the non-AMF treatment, main hubs belonged to Euryarchaeota (ASV 147 – Genus Methanosarcina), Actinobacteria (ASV 48) and Deltaproteobacteria (ASV 156 belonging to Class Myxococcales) (Table S7).

Drought stress also caused differences in hub nodes in both varieties compared to the well-watered condition (Tables S8–10). Taxa belonging to Class Alphaproteobacteria Family Hyphomicrobiaceae (ASV 6), Gammaproteobacteria (ASV 25) and Phyla Bacteroidetes Family Rhodothermaceae (ASV 19) were three main keystone species in Iride variety under drought stress (Table S8) while ASV 181 (Family Comamonadaceae – Class Betaproteobacteria), ASV 159 (Deltaproteobacteria) and ASV 34 belongs to Phyla Actinobacteria were main hub nodes under well-watered condition (Table S10). In Ramirez variety, first three hub nodes belonging to Gammaproteobacteria (ASV 39 belongs to Family Sinobacteraceae), Alphaproteobacteria (ASV 28 belonging to Family Hyphomicrobiaceae) and Acidobacteria (ASV 10) under drought stress (Table S9). No hub nodes were identified under well-watered condition in Ramirez variety. Remarkably, there were no common or shared hub nodes among all treatments, indicating specific selection of keystone taxa by specific treatment.

4. Discussion

All three factors, compartment niche, AMF addition and water limitation had distinctive effects on the bacterial community in terms of structure and diversity. Strongest effect was manifested by the compartment niche (root *vs.* rhizosphere) followed by drought stress and AMF addition. This indicates that microbiome assembly (here bacterial) at the crop level is primarily determined by the compartment niche

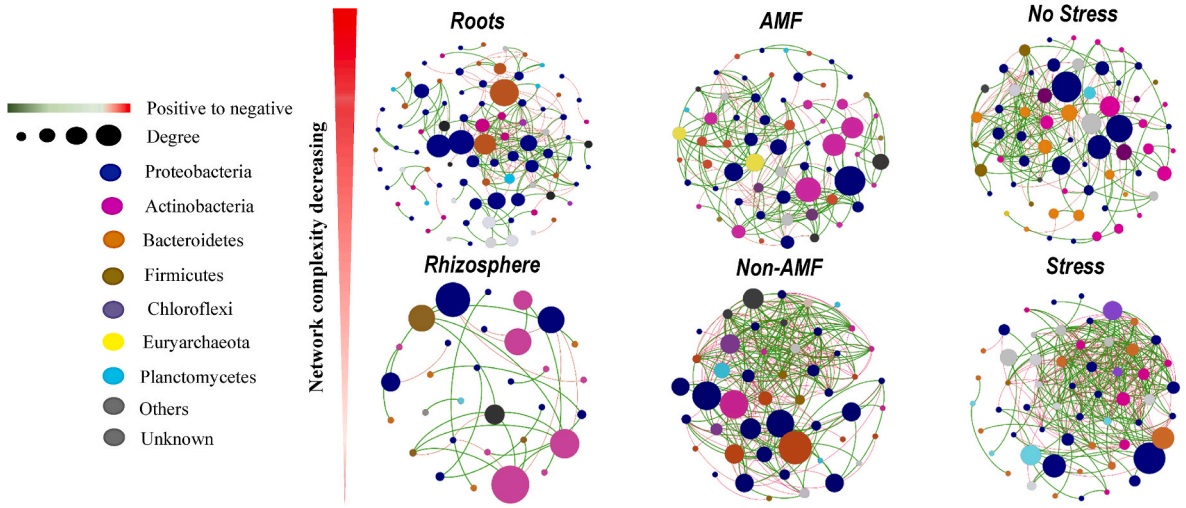


Fig. 3. Network of co-occurring ASVs (500 most abundant) of Iride cultivar in both compartment niche (root and rhizosphere), mycorrhiza colonization (AMF and non-AMF) and drought stress (Stress and control) based on correlation analysis. Each edge stands for a strong (Spearman's $\rho > 0.6$) and significant ($p < 0.01$) correlation. The size of each node is proportional to the number of connections (i.e., degree). The nodes are also coloured by taxon classification at phylum level. The thickness of the edges is proportional to the robustness of a given Spearman'sρ, ranging from 0.6 to 0.9. Green and red colour of edges shows positive and negative correlations, respectively.

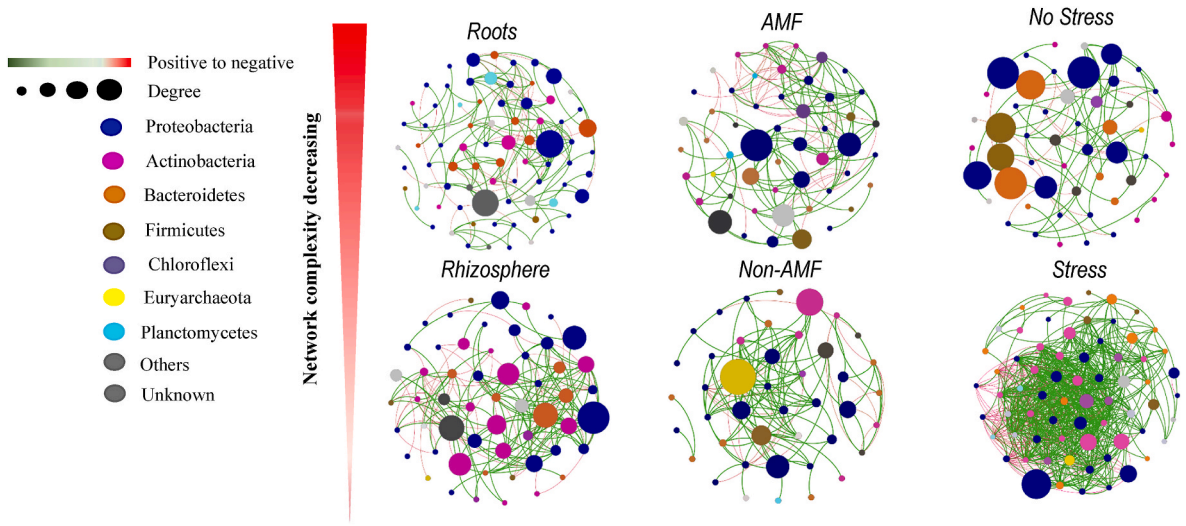


Fig. 4. Network of co-occurring ASVs (500 most abundant) of Ramirez cultivar under cultivar under compartment niche (root and rhizosphere), mycorrhiza colonization (AMF and non-AMF) and drought stress (Stress and control)), based on correlation analysis. Each edge stands for a strong (Spearman's $\rho > 0.6$) and significant ($p < 0.01$) correlation. The size of each node is proportional to the number of connections (i.e., degree). The nodes are also coloured by taxon classification at phylum level. The thickness of the edges is proportional to the robustness of a given Spearman'sρ, ranging from 0.6 to 0.9. Green and red colour of edges shows positive and negative correlations, respectively.

Table 5
Bacterial co-occurrence network characteristic of both wheat cultivars. In both compartment niche (root and rhizosphere), mycorrhiza colonization (AMF and non-AMF) and drought stress (Stress and control).

Niche	Node	Positive edge	Negative edge	Betweenness centrality	Modularity[a]	Average clustering coefficient[b]	Average path distance[c]	Hub node[d]
Iride								
Root	91	143	111	63.14	0.52	0.526	3.637	22
Rhizosphere	31	21	9	2.39	0.662	0.308	1.974	0
AMF	63	145	61	55.71	0.527	0.504	3.272	20
non-AMF	49	164	159	21.67	0.323	0.692	2.071	4
Stress	56	174	162	35.73	0.216	0.616	2.449	10
No Stress	65	117	83	65.52	0.422	0.445	3.128	29
Ramirez								
Root	77	121	47	60.03	0.69	0.57	4.35	8
Rhizosphere	55	133	64	42.07	0.43	0.58	2.81	15
AMF	51	112	44	43.43	0.60	0.65	3.41	11
non-AMF	43	93	27	32.40	0.53	0.70	3.20	8
Stress	68	584	142	32.68	0.19	0.71	2.07	12
No Stress	52	53	20	94.17	0.70	0.51	6.15	0

[a] Degree of nodes tending to differentiate into different network modules.
[b] Degree of nodes tending to cluster together.
[c] Network path distance is the length of the shortest path between two nodes within the network.
[d] Hub node is defined as a node with high values of Betweenness centrality (>60) and closeness centrality (>0.3) in the network.

rather than by other environmental factors [29].

Drought stress and AMF addition had marginal and inconsistent effects on alpha diversity. These inconsistencies may be dependent on drought context and intensity, variability in AMF affinity or lineage, as well as the experimental method employed [22,23,30,31]. After accounting for compartment niche changes, drought was found to have a particularly large influence on beta diversity, followed by AMF addition. This corresponds to previous findings, suggesting that abiotic factors have a stronger influence on the bacterial community structure compared to biotic interactions [32,33]. Furthermore, alteration in the bacteriome structure under AMF addition was more evident under drought stress conditions especially in the rhizosphere compartment. Stress-dependent alteration under AMF addition could be due to the focal role of AMF mitigating water stress by up and down regulation of various metabolomic pathways [25,34], which could further alter the root and soil microbiome due to variations in root exudation patterns. When comparing the community structure between both wheat varieties, the effect intensity of drought stress followed by AMF addition and their interaction have stronger influence (almost two folds, Fig. 1) on the Ramirez variety, which has a lower affinity for AMF colonization. Results showed that the mycorrhizal protective effect could be strongly linked to plant colonization affinity. Higher colonization affinity of plant/variety may increase mycorrhizal-bacterial synergisms, which may further restrain the negative influence of drought stress or any other environmental stress [23,35].

Similarly, to community structure, taxonomic composition was initially formed by compartment niche rather than environmental factors (Fig. 2). Root bacteriome was mainly formed by Proteobacteria (~60%) whereas the rhizosphere bacteriome was more diverse and dispersed with various bacterial taxa. These results are in line with the pervious findings of [36], who showed that the root endophytic bacterial community is typically dominated by Proteobacteria, which further

confirmed that roots are effective habitat filters and have restrictive taxa selection compared to soil compartment [37].

The effect of drought repression was evident across a wide range of bacterial taxa, including the drought-tolerant taxa. The most commonly perceived phenomenon under drought conditions is an increase in the ratio of monoderm (gram-positive i.e. Phyla Firmicutes and Actinobacteria) to diderm (gram-negative i.e. Phyla Proteobacteria, Verrucomicrobia and Bacteroidetes) [7,38–41]. The oligotrophic vs. copiotrophic lifestyle (substrate preference and metabolic capacities) of monoderm and diderm bacteria can explain their discrete drought responsiveness [7]. We only found this phenomenon in Ramirez roots, where the relative abundance of Proteobacteria and Verrucomicrobia was decreased under water-limited condition. Furthermore, we found a depletion in the relative abundance of drought-tolerant taxa such as Actinobacteria, which is in line with previous studies that found similar trends [38,42]. Hence, changes in bacterial taxonomy are to an extent- and context-dependent [7] and it is worth noting that the phenomenon of monoderm/diderm is not universal.

Protective effect of AMF affinity under drought condition was also observed, especially in the Iride variety (high AMF affinity variety) as the relative abundance of drought-depleted taxa, such as Planctomycetes, Bacteroidetes, and Verrucomicrobia [38,42] was either similar under well-watered and drought conditions or even increased under drought conditions. Our results are in line with the recent findings of Hestrin et al. [23] who also showed that ASVs belonging to these drought-depleted phyla have similar growth potentials in AMF-inoculated soils, irrespective of moisture treatments. This indicates that presence of AMF could alter soil edaphic properties which further leads to bacterial resilience to water limitations [23]. AMF produce glomalin, a hydrophobic and thermo-tolerant protein that stabilizes soil aggregates and confers resistance of soil aggregates under water-limited conditions [20,43,44]. Furthermore, the AMF mycelia network persistently renews itself and dead mycelia contribute to the stocks of organic matter and physical binders involved in soil aggregation [45,46]. These mechanisms show that AMF could reduce the risk of soil compaction under water-limited conditions and continue to facilitate microbial transport, thus reducing microbial dormancy and death under low water availability [23]. In contrast, AMF reduces the abundance of taxa such as Proteobacteria and Firmicutes (Copiotrophics) in control (well-watered) conditions, especially in the roots of Iride, due to competition for macronutrients [21,47], which could decrease the growth of other microbial decomposers [48,49].

We observed that bacterial network complexity was reduced from the root to the soil compartment, especially for the Iride variety, which suggests that despite the host plant controlling the selection of its own microbiota, there are other biotic and abiotic factors that might influence microbial selection and their interactions [37,50]. AMF presence and colonization affinity could have direct or indirect effects by changing root exudate patterns as well as water stress, which could increase or decrease bacterial network complexity. However, our results are contradicting recent findings of Xiong et al. [29] who found that network complexity gradually reduced from soil to root to leaf compartments. This inconsistency could be due to differences in host genetics [29] and other linked abiotic and biotic factors. Overall positive effects of AMF were observed in both varieties as the network complexity of the bacterial community were enlarged under AMF colonization, which indicates a stimulatory interaction between soil bacteria and AMF fungi. While comparing varieties, Iride with high AMF affinity had higher bacterial network complexity than Ramirez, which has a lower AMF affinity. Higher AMF affinity of the Iride variety could enlarge the hyphal network in roots which could probably lead to higher availability of C compounds and root exudates [51,52]. These additional food resources further stimulate the growth of bacterial communities and increase their network complexity. In contrast, drought negatively influences bacterial networks. Drought promotes the destabilization of properties in bacterial networks thus reducing network complexity (by means of reduced centrality, modularity, and having lower negative: positive cohesion) [2,12,35]. When comparing both varieties, drought had a stronger impact on the bacterial networks of the Ramirez variety. The lower AMF colonization affinity of Ramirez could reduce the protective effect of AMF towards the associated microbial community, which may lead to higher destabilization of community networks. This clearly indicates that not only the AMF colonization, but host's colonization affinity also plays vital role in alleviating drought-induced stress.

Appealingly, we have also observed similar trends in the root metabolome (our recently published data) [25] and bacteriome of root and rhizosphere soil (current results) in countering drought stress, especially under AMF colonized plants. This indicates that there is an interlinked correlation between the two omics profiles. For example, plants generally accumulate more proline than other amino acids under drought conditions. Nonetheless, we observed downregulation of proline biosynthesis in plants inoculated with AMF under stress conditions, which indicates that AMF-inoculated plants experienced less stress under drought conditions [25]. Moreover, the degree of protective effect of AMF addition was also different in both wheat varieties, especially under drought conditions. For example, the concentration and accumulation of phenylpropanoids and terpenoids (specialized metabolites in mitigating plant water scarcity) was significantly higher in Iride (with high AMF affinity) than in Ramirez with low AMF affinity [25]. A similar protective effect of AMF addition and host colonization affinity was observed on the root and rhizosphere bacteriome, as stated above, which clearly indicates that not only AMF inoculation but plant colonization affinity also play an important role in mitigating drought-induced stress by altering plant-fungal-bacterial interaction. However, combined studies of plant metabolomics and soil microbial communities require the integration of advanced wet lab techniques with statistical and modelling techniques to understand the complex mechanism of plant-soil-microbe interactions.

5. Conclusion

Our study not only addressed the importance of AMF presence, but also showed that plant AMF colonization affinity plays a focal role in alleviating drought-induced changes. The severity of the drought-induced effect on diversity, composition and network complexity of the bacterial community was more pronounced in Ramirez variety (low AMF affinity) than in the Iride cultivar, which has higher AMF colonization affinity. This suggests that mycorrhiza secureness is directly linked to its host plant colonization affinity and can be a limiting factor in mitigating drought-induced effects. Wheat cultivars with high AMF colonization affinity have shown higher resilience responses to drought-induced changes in terms of altering the root and soil bacteriome (current study) as well as root metabolites [25]. Therefore, designing agriculture systems/rotations including cultivars with high AMF colonization affinity, could be a one of the developing strategies to combat drought events in this high climate change scenario.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejsobi.2023.103540.

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