NOTE

Contrasting Responses of Arbuscular Mycorrhizal Fungal Families to Simulated Climate Warming and Drying in a Semiarid Shrubland

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Abstract

We carried out a 4-year manipulative field experiment in a semiarid shrubland in southeastern Spain to assess the impacts of experimental warming (W), rainfall reduction (RR), and their combination (W + RR) on the composition and diversity of arbuscular mycorrhizal fungal (AMF) communities in rhizosphere soil of H. syriacum and G. struthium shrubs using single-molecule real-time (SMRT) DNA sequencing. Across climate treatments, we encountered 109 AMF operational taxonomic units (OTUs) that were assigned to four families: Glomeraceae (93.94%), Gigasporaceae (2.19%), Claroideoglomeraceae (1.95%), and Diversisporaceae (1.92%). AMF community composition and diversity at OTU level were unaffected by the climate manipulation treatments, except for a significant decrease in AMF OTU richness in the W treatment relative to the control. However, we found a significant decrease of AMF family richness in all climate manipulation treatments relative to the control treatment. Members of the Gigasporaceae and Diversisporaceae families appeared to be highly vulnerable to intensification of heat and drought stress, as their abundances decreased by 67% and 77%, respectively, in the W + RR treatment relative to current ambient conditions. In contrast, the relative abundance and dominance of the Glomeraceae family within the AMF community increased significantly under the W + RR treatment, with Glomeraceae being the indicator family for the W + RR treatment. The interaction between warming and rainfall reduction had a significant effect on AMF community structure at family level. These findings provide new insights to help in the conservation of the soil biodiversity facing climate change in dryland ecosystems.

Keywords Arbuscular mycorrhizal fungi · Diversity · SMRT sequencing · Drought stress · Warming · Drylands

Arbuscular mycorrhizal fungi (AMF) are important symbiotic partners of the majority of terrestrial plant species and depend on the photosynthetic carbon provided by the host plant for their growth and function [1]. In return, AMF can enhance host plant nutrient and water uptake and resistance to environmental stresses such as drought. It has been shown that coexisting AMF show host plant preferences [2], and are functionally distinct [3] and that AMF community diversity can affect plant community diversity and productivity and, therefore, ecosystem stability and sustainability [4].

It is accepted that AMF associations are differently influenced by environmental variation across ecosystem types, showing no general patterns in the majority of environmental manipulation experiments conducted until now in natural conditions [5]. The semiarid Mediterranean ecosystems in southeastern Spain are frequently exposed to severe drought episodes and heatwaves, erosive processes during heavy rainstorms, and vegetation cover reductions. These processes
lead to degraded habitats which are among the most fragile and endangered by environmental changes such as global warming [6]. The key role of AMF in improving the physiological functioning and performance of dryland plant communities and soil quality is well established [7–10]. However, the extent to which ongoing climatic changes could affect the composition and structure of AMF communities in dryland ecosystems is still unknown, since most research has focused on plant community responses to climate change drivers [11, 12]. Investigating the response of AMF communities to simulated climate warming and drying and identifying the major factors driving AMF community assembly in soils are necessary to help the management and conservation of the biodiversity and functions of semiarid ecosystems.

We performed a 4-year manipulative field experiment in a semiarid shrubland in southeastern Spain (Sorbas, Almería). We simulated future warmer and drier climate conditions, to investigate the effect of increased temperature (W, warming), rainfall reduction (RR), and their combination (W + RR) on shrubland vegetation and AMF community composition and species richness. Details on the experimental design and treatment impacts on climatic conditions within the experimental plots are provided in the supplementary information section (S1) [12–14]. Warming and rainfall reduction strongly reduced plant nutrient contents, photosynthesis, and water use efficiency, aboveground biomass growth and drought survival in this semiarid shrubland community by >20–40%, especially in the W + RR treatment [12].

We collected 45 rhizosphere soil samples under H. syriacum and G. struthium shrubs across climate treatments, extracted genomic DNA from 300 mg of each individual rhizosphere soil sample employing the FastDNA spin kit for soil (MP Biomedicals), and quantified the AMF communities by single-molecule real-time (SMRT) DNA sequencing. We followed the molecular and bioinformatic protocols described in Schlaeppi et al. [15]. We sequenced 3 SMRT®Cells at the Functional Genomic Centre Zurich (Zurich, Switzerland; http://www.fgcz.ch). Operational taxonomic units (OTUs) were clustered at a level of 97% sequence similarity and classified by AMF family using specific AMF taxonomy [16].

To ascertain whether the AMF community structure was affected by the climate manipulation treatments, we conducted a permutational multivariate analysis of variance (perMANOVA) using the adonis function (vegan R package, [17]) based on Bray–Curtis as measure of dissimilarity and 999 permutations. A non-metric multidimensional scaling (NMDS) ordination using Bray–Curtis dissimilarities was performed to visualize differences in AMF community structure among treatments (metaMDS function, vegan package in R). Alpha diversity indices (richness, S; Shannon–Weaver, H’) were calculated for the AMF community at both OTU and family levels (specnumber and diversity functions, vegan R package). The influence of the climate manipulation treatments on H’ and S was determined by a two-way ANOVA analysis and a post hoc Tukey’s HSD test. An indicator species analysis was conducted using the family abundance data (indicspecies R package, [18]). All statistical analyses were performed in R (v4.0.4; [19]).

A total of 67,222 reads were obtained from 45 samples which were assigned to 278 OTUs. We normalized the data by rarefying the samples to the sequence number of lowest sample. After removal of singletons and non-glomeromycota sequences, 109 AMF OTUs (26,768 sequences) were assigned to four families: Glomeraceae (93.94%), Gigasporaceae (2.19%), Claroideoglomeraceae (1.95%), and Diversisporaceae (1.92%).

The climate manipulation treatments had no significant effects on AMF community structure or diversity indices at OTU level (every term in perMANOVA: pseudo$F_{1,44} < 1.2, p > 0.1, R^2 ≤ 0.02$) (Table S1, Fig. S1), except for a significant decrease in AMF richness in the W treatment relative to the control. However, AMF family richness decreased significantly and consistently in all the climate manipulation treatments relative to the control treatment (W, RR, and W + RR; $F_{3,41} = 3.42, p < 0.026$) (Table 1). The relative abundance and dominance of the Glomeraceae family within the AMF community increased significantly under the W + RR treatment (Fig. 1), with Glomeraceae being the indicator family for the W + RR treatment ($IndVal = 0.61; p < 0.05$). Moreover, the interaction between warming and rainfall reduction had a significant effect on AMF community structure at family level (perMANOVA: pseudo$F_{1,44} = 4.7, p = 0.005, R^2 = 0.09$) (Table S1). The Shannon diversity index did not show significant differences between treatments for OTUs or families.

| AMF OTUs | Richness | Shannon–Wiener |
|----------|----------|----------------|
|          | Mean     | SD             | Mean     | SD       |
| Control  | 28.19b   | 10.56          | 2.26a    | 0.63     |
| RR       | 23.75ab  | 15.40          | 1.94a    | 0.84     |
| W        | 16.10a   | 10.90          | 1.78a    | 0.73     |
| W + RR   | 26.18ab  | 12.97          | 2.18a    | 0.51     |
| AMF families |      |                |          |          |
| Control  | 3.25b    | 0.77           | 0.31a    | 0.30     |
| RR       | 2.37a    | 1.19           | 0.22a    | 0.15     |
| W        | 2.30a    | 0.67           | 0.33a    | 0.33     |
| W + RR   | 2.45a    | 0.93           | 0.16a    | 0.18     |
Decreased AMF family richness under simulated climate change could be consequence of shifts in belowground carbon flow pathways, as climatically stressed host plants with greatly reduced photosynthesis could favor their more drought-adapted or beneficial AMF partners through preferential carbon allocation, at the expense of AMF that become less beneficial under warmer and drier conditions [20]. Alternatively, decreased AMF family richness under a warmer and drier climate could be the result of increased competition among AMF taxa for a dwindling carbon supply by host roots, with some high carbon demanding AMF being outcompeted by less carbon demanding AMF or more aggressive AMF competitors for root space [21] and/or carbon supply [22]. Among the four AMF families detected in our study, Gigasporaceae and Diversisporaceae were the most sensitive to the intensification of heat and drought stress, as their abundances in the W + RR treatment decreased by 67% and 77%, respectively, relative to current ambient conditions (Fig. 2). By contrast, Glomeraceae showed highest abundance under the most stressful climate conditions (W + RR) with an average 20% increase over the control, and was also the unique indicator family for the W + RR treatment. These patterns may reflect functional differences among AMF taxa that have been proposed to rely on different life history strategies [21]. AMF taxa within the Glomeraceae and Claroideoglomeraceae families tend to allocate more biomass to root colonization and intraradical structures than to external hyphae, whereas those in the Gigaporaceae and Diversisporaceae display the opposite tendency [3]. Treseder et al. [23] suggested that when carbon allocation to AMF decreases as consequence of global change stressors, members of the Gigaporaceae tend to show decreasing abundance linked to their higher carbon demand to support the growth and maintenance of their profuse external mycelial network [3]. In contrast, the Glomeraceae strategy is based on extensive root colonization and randomly connected hyphae with limited production of extraradical mycelium, which has been interpreted as
an adaptive strategy to avoid and resist drought conditions in soil [3, 24]. Previous surveys of AMF community composition along aridity gradients found that the presence of Gigasporaceae greatly decreased under dry conditions, and that the most arid sites showed a predominance of Glomeraceae species [25].

In conclusion, this study improves our understanding of AM fungal community responses to climate warming and drying, and is thus relevant to help the conservation of soil biodiversity under the climate change scenarios predicted for Mediterranean drylands during the second half of the twenty-first century.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00248-021-01886-6.

Author Contribution JIQ designed the study; JIQ and MMA collected the samples; MMA, KS, MVH, and ALG analyzed and interpreted data; MMA and JIQ wrote the manuscript, with input from all co-authors.

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Data Availability The raw sequencing data is available from the European Nucleotide Archive (http://eutils.ncbi.nlm.nih.gov/entrez/query.fcgi?db=nuccore) with the sample ID SAMEA4711908 under the study accession PRJEB21595.

Declarations

Ethics Approval Not applicable.

Conflict of Interest The authors declare no competing interests.

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