# **Title:** Arbuscular mycorrhizal fungal community responses to drought and nitrogen fertilization in switchgrass stands

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## Abstract:

Anthropogenic global change is increasing the severity and frequency of abiotic stresses such as drought that are likely to affect soil communities. Arbuscular mycorrhizal fungi (AMF) play important roles in many soil processes, so it is important to understand how drought affects AMF biodiversity. This is especially relevant in agricultural systems where crops rely on AMF associations for water and nutrient uptake, and where management decisions such as crop selection and fertilizer application may influence how the AMF community responds to drought. In this study, we examined the effects of reduced precipitation and nitrogen fertilization on AMF richness, community composition, and root and soil colonization in monocultures of two cultivars of switchgrass (*Panicum virgatum*) grown for bioenergy feedstock. We conducted a two-year field experiment using rain-out shelters to manipulate precipitation in mature stands of switchgrass growing in a long-term nitrogen fertilization (0 or 56 kg N $ha^{-1}$) experiment at the W.K. Kellogg Biological Station Long-Term Ecological Research site in Michigan, USA. We expected that AMF richness and colonization would decrease due to drought, as predicted by the stress exclusion hypothesis. Contrary to our expectations, we found that drought stress increased AMF species richness in fertilized plots by 15%; there was no effect of drought on AMF richness in unfertilized plots. Drought also significantly altered AMF community composition, primarily due to increases in *Rhizophagus* taxa abundance*,* and reduced AMF root colonization in switchgrass by 6%. We also found variation in AMF richness and colonization across switchgrass cultivars as well as sampling dates. The changes in AMF richness and composition that we observed in this study may have implications for perennial bioenergy feedstock selection and management as changes in AMF communities may feedback to affect host plants.

**Key Words:** drought, nitrogen, arbuscular mycorrhizal fungi, biofuels, long-term ecological research, sequencing

**Abbreviations:** AMF, Arbuscular mycorrhizal fungi; CBDE, Cellulosic Biofuels Diversity Experiment; ERH, Extra-Radical Hyphae; KBS LTER, W.K. Kellogg Biological Station Long Term Ecological Research; N, Nitrogen; NMS, Non-metric Multidimensional Scaling; OTU, Operational Taxonomic Unit; PCR, polymerase chain reaction

## 1. Introduction

The severity and frequency of abiotic stresses on terrestrial systems from drought, heat, salinity, and flooding are expected to increase in the future due to anthropogenic global change (IPCC, 2014; Meehl and Tebaldi, 2004). Increased frequency and intensity of droughts is of particular concern for agricultural systems, as crop yields are often drastically reduced in response to drought (Wilhite, 2000). Many studies examining the effects of abiotic stress on biodiversity of animal and plant communities (e.g., Kottawa-Arachchi and Wijeratne, 2017; Vittoz et al., 2013) have not considered how belowground communities might respond to increases in abiotic stress. Soil communities play important roles in soil processes, especially in agricultural systems, because of their role in water purification, carbon sequestration, and nutrient cycling (Bardgett and van der Putten, 2014). Thus, it is important to understand how abiotic stresses associated with climate change, such as drought, might alter the composition and functioning of belowground communities in agricultural systems.

Arbuscular mycorrhizal fungi (AMF) are a major component of soil biodiversity and are known to form symbiotic associations with 80% of terrestrial plants (Smith and Read, 2008). AMF can benefit host plants directly through increased nutrient uptake and indirectly through increases in pathogen resistance and drought tolerance (Gosling et al., 2006; Harrier and Watson, 2004; Kivlin et al., 2013). AMF can also improve carbon storage and soil aggregation through the production of extraradical hyphal networks (Olsson and Johnson, 2005). Several studies have shown that drought and other abiotic stresses can alter the AMF-plant relationship (Kivlin et al., 2013; Latef et al., 2016), but very few studies have addressed how AMF communities themselves respond to abiotic stress. Soil microbial diversity often decreases in response to drought (Fahey et al., 2020) and the stress exclusion hypothesis (Millar and Bennett, 2016) predicts that AMF diversity should decrease due to abiotic stress filtering out less-tolerant AMF species. However, this prediction has not been widely tested. Additionally, the responses of AMF communities to stress are often closely linked to changes in the diversity of the host plant community (Cotton, 2018) making it difficult to determine how AMF communities themselves will directly respond to drought.

By studying the effects of drought on AMF communities in agricultural systems, it is possible to isolate AMF responses from plant community responses, as the host plant community composition is typically a monoculture, and particularly in perennial crops, is consistent over time (Brussaard et al., 1997; Verbruggen and Kiers, 2010). Grower decisions, such as crop selection and fertilizer application, can also affect AMF responses to drought. For example, in a previous study (Emery et al., 2017), nitrogen fertilization decreased AMF diversity and colonization associated with switchgrass, which may limit AMF community responses to drought due to species filtering (De Boeck et al., 2018). Perennial agricultural systems, such as bioenergy crops, may be particularly susceptible to drought because they are often planted on marginal lands with low fertility and can be more dependent on AMF than annual crops (Liang et al., 2012).

In this study, we investigated the effects of drought on AMF communities associated with two cultivars of switchgrass (*Panicum virgatum*) grown in monocultures as a bioenergy feedstock. Switchgrass, a native warm-season grass, is a leading lignocellulosic bioenergy crop in the USA due to its perenniality (10+ years of production) and efficient nutrient and water use (Parrish and Fike, 2005). Synthetic fertilizers, in particular nitrogen (N), are often applied to increase biomass production; however, fertilizer application comes with economic costs to growers (an estimated $37/ha; Hallam et al., 2001) and does not always deliver higher yields (e.g., Garten et al., 2011). Drought can cause up to a 35% increase in the cost of biofuel production from lignocellulosic bioenergy crops such as switchgrass due to reduced aboveground biomass production (Morrow et al., 2014), and so represents a real threat to the sustainability of these systems. Switchgrass also associates with AMF (Emery et al., 2018) and reductions in AMF diversity or colonization may affect switchgrass growth as well as ecosystem services associated with this perennial crop (Hoeksema et al., 2010). To better understand how drought and management may affect AMF communities, we established a two-year drought manipulation within a long-term experiment at W.K. Kellogg Biological Station Long Term Ecological Research (KBS LTER) site in Michigan, USA and asked: 1) Does drought reduce AMF richness, alter AMF community composition, or reduce AMF root and soil colonization in switchgrass monocultures? 2) Does synthetic N-fertilization alter AMF community responses to drought? And 3) Are these effects consistent in different cultivars of switchgrass? We expected that drought would reduce AMF richness and colonization associated with switchgrass, in support of the stress exclusion hypothesis (Millar and Bennett, 2016), while N-fertilization would reduce overall AMF richness but stabilize AMF communities to make them less responsive to drought stress (De Boeck et al., 2018). We did not expect AMF responses to vary between the two cultivars of switchgrass in this study, as our previous work found no differences in AMF communities associated with 12 different switchgrass cultivars (Emery et al., 2018).

## 2. Methods

### *2.1. LTER cellulosic biofuel diversity experiment design*

To characterize the effects of drought on AMF communities associated with switchgrass, we conducted a two-year study at the Cellulosic Biofuels Diversity Experiment (CBDE) at the

W.K. Kellogg Biological Station Long Term Ecological Research (KBS LTER) site in southwest Michigan USA (42°23’47” N, 85°22’26”W). This site averages 810 mm $yr^{-1}$ of precipitation and soils are Kalamazoo series fine loamy, mixed, mesic Typic Hapludalfs (Muñoz and Kravchenko, 2011). The CBDE was established as part of the KBS LTER in 2008 (7 years prior to our drought manipulation) to compare production of 12 different biofuel cropping systems varying in species composition and nitrogen input. For this study we focused on four of the 12 systems, which included four replicates each of two cultivars of switchgrass, (“Cave-in-Rock” and “Southlow”; each planted in 2008 at a rate of 3.9 kg seeds $ha^{-1}$) grown at two levels of fertilization (56 kg N $ha^{-1}$ $yr^{-1}$ and unfertilized).

Both Cave-in-Rock and Southlow are upland ecotypes of switchgrass, which are typically better adapted to drier conditions and cold temperatures compared to lowland ecotypes, and are recommended for planting in the upper Great Lakes region (Casler and Boe, 2003; Cassida et al., 2005). Cave-in-Rock was developed using seed collected from a single native remnant prairie in southern Illinois, while Southlow was developed from seeds collected from 11 native stands growing in southwest Michigan. There has been no intentional selection on either cultivar as a bioenergy feedstock, and both are commonly used for restoration of native grasslands as well as for bioenergy production (Stahlheber et al., 2020). In previous studies of these two cultivars, we found that Southlow can produce more root biomass than Cave-in-Rock and has a suite of physiological traits that may make it more drought tolerant than Cave-in-Rock (Emery et al., 2018; Stahlheber et al., 2020). The four switchgrass treatment combinations in this current experiment were planted in 9 m × 27 m plots, replicated four times in a randomized block design interspersed with the other eight treatments. Fertilizer (28% N; Urea + Ammonium nitrate) was applied to fertilizer treatment plots every year in the spring. Additional experiment details, including switchgrass biomass responses, can be found in Emery et al. (2020) and at: https://lter.kbs.msu.edu/datasets/109.

### *2.2. Precipitation manipulation*

In May 2015, we established a precipitation reduction ("drought") treatment in all of the switchgrass plots, by installing subplots containing 3 m × 2.5 m × 1.8 m rainout shelters placed in either the northwest or southwest corner of each plot (n = 16). The rainout shelters were based on the design of Yahdjian and Sala (2002) and removed 80% of ambient rainfall. Details of the design can be found in Emery et al. (2020). In the alternate west corner of each switchgrass plot, a 3 m × 2.5 m "ambient" subplot was also established. Shelters were installed May through October 2015 and 2016 and removed at the end of each growing season. Shelters reduced soil moisture by ~40% on average (soil water tension: -39.5 vs. -28.2 centibars; paired *t*-test $t_{1,23} = 11.68$, $p < 0.001$; Emery et al. 2020). For more details on measures of water stress under shelters see Emery et al. (2020).

### *2.3. AMF community composition and richness*

To measure changes in the AMF community, we collected soil cores (2 cm diameter x 15 cm deep; 10 per subplot) on three dates (July 2015, July and October 2016) from near the centers of each subplot. Cores from each subplot on each date were pooled, sieved through a 4mm sieve to remove rocks and large roots, and then stored at 4 °C until processed. We extracted DNA from 0.25 g of fresh soil subsampled from the pooled soil core samples and processed these for AMF community composition using methods for sequencing and bioinformatics detailed in Emery et al. (2017). Briefly, we used Powersoil DNA Extraction kits to isolate DNA (MOBIO

Laboratories, Carlsbad, CA, USA) and the 28S rRNA was targeted using AMF specific fusion primers [FLR3-FLR4, (Gollotte et al., 2004)]. PCR and MiSeq Illumina paired-end sequencing was conducted by the Research Technology Support Facility Genomics Core at Michigan State University, East Lansing, Michigan. Reads were assembled and quality filtered using USEARCH8 (http://drive5.com/usearch/). Sequences were dereplicated, clustered chimera checked, filtered, and clustered de novo into unique operational taxonomic units (OTUs, i.e., DNA sequences or amplicon types) based on 97% identity using the default settings in UPARSE implemented in UEARCH9 (Edgar, 2013, 2016). USEARCH quality filtering, chimera checking using UCHIME, and OTU clustering led to 554 OTUs and 8,517,983 reads. Representative sequences were then classified using the RDP naïve Bayesian classifier against the Fungal LSU training set 11 (Cole et al., 2014; Wang et al., 2007). Any sequences with bootstrap values below 60% match with Glomeromycota were removed from the dataset. Taxonomic filtering for AMF specific sequences resulted in 243 OTUs and 4,763,846 reads. We transformed OTU tables using variance stabilizing transformation (VST) in the DeSeq2 package (Love et al., 2014) in R (R Core Team, 2016) to control for biases in PCR amplification and to avoid biases due to rarefaction (McMurdie and Holmes, 2014). We used the vegan package in R (Oksanen et al., 2019) to calculate Chao1 richness based on OTUs in the untransformed community matrix.

### *2.4. AMF root and soil colonization*

To measure AMF colonization in plant roots, we extracted fine roots from 100 ml subsamples taken from the pooled soil cores described above from each subplot using a wet-sieve process [500 µm sieve (Milchunas, 2012)]. Roots were cleared with 10% KOH and stained using a 5% vinegar-ink solution using methods modified after Vierheilig et al. (1998). Visual estimation of percent root length colonization was made using 100 fields of view per sample under 200x magnification. To quantify extra-radical hyphae (ERH) growth, we extracted ERH from 20 ml subsamples taken from the pooled cores for each subplot. Soil subsamples were suspended in water, then stained and vacuum filtered through a 45 µm filter, following methods described in Staddon et al. (1999). AMF hyphae were distinguished from other soil fungi based on their generally aseptate hyphae with characteristic unilateral angular projections (Mosse, 1959). ERH length was estimated in 25 fields of view using the gridline intercept method at 100x magnification (Giovannetti and Mosse, 1980).

### *2.5 Data Analysis*

We evaluated the effects of drought, fertilization, and switchgrass cultivar on AMF richness (Chao1 index) and AMF root and soil colonization using three-factor general linear models with sample date and plot location as random block terms. These analyses were performed using Systat v. 12 (SYSTAT Software Inc, 2007). We used three-factor PERMANOVA (Anderson, 2001) with sample date and plot location as random block terms to examine overall differences in soil AMF community composition (based on OTU abundance) due to drought, fertilization, and switchgrass cultivar. To visualize differences in soil AMF community structure we performed non-metric multidimensional scaling (NMS) ordinations (McCune et al., 2002) with Bray-Curtis dissimilarity measures based on square-root transformed AMF OTU abundance data. OTU singletons were excluded from the PERMANOVA and NMS to improve resolution of analyses. PERMANOVA and NMS analyses were performed using Primer v. 6 (Anderson et al., 2008).

Finally, we conducted an indicator species analysis (Dufrene and Legendre, 1997) to identify any individual AMF OTUs that were uniquely associated with each significant treatment group identified in the PERMANOVA. Monte-Carlo randomizations (999 permutations) were used to test for indicator value significance. Indicator species analyses were conducted using the "indicspecies" package v. 1.7.9 and "multipatt" function (Cáceres and Legendre, 2009) in R.

## 3. Results

### *3.1. AMF Richness*

A total of 243 AMF taxa (OTUs) were identified from our soil samples. All of the OTUs were from five genera in two families in the order Glomerales. The genus with the most OTUs was *Rhizophagus* (102 OTUs), followed by *Septoglomus* (26 OTUs), *Funneliformis* (16 OTUs), *Glomus* (15 OTUs), and *Claroideoglomus* (13 OTUs). An additional 71 OTUs could only be identified to family (all Glomeraceae).

There were no main effects of fertilizer or cultivar on AMF OTU richness (Table 1). Instead, the effects of drought on AMF OTU richness (Chao1) depended on both fertilization treatment and switchgrass cultivar (Table 1). Drought did not have a significant ($p < 0.05$) effect on the number of AMF OTUs in the non-fertilized plots; however, in the fertilized plots AMF OTU richness increased by 15% in response to drought (Fig. 1a, Table S1). The effect of drought on AMF richness differed among the two switchgrass cultivars also: there was no significant effect of drought on AMF richness in Southlow, but a 14% increase in OTU richness in Cave-in-Rock (Fig 1b, Table S1). AMF richness also varied significantly across our three sampling dates (Table 1; Fig. S1), with richness 29-30% higher in July 2015 compared to the two later dates.

However, with only three sampling dates, we cannot evaluate causes of this observed variation in AMF communities over time.

### *3.2 AMF community composition*

AMF community composition differed significantly in response to drought, but these responses varied in the two cultivars (Fig. 2). The indicator species analysis showed that several *Rhizophagus* OTUs were significant indicators of the Cave-in-Rock drought treatment, and one *Septoglomus* OTU and an unknown Glomeraceae OTU were significant indicators of the Cave-in-Rock ambient precipitation treatment. There were no significant indicators of Southlow drought treatment, while four unknown Glomeraceae OTUs and one *Rhizophagus* OTU were significant indicators of Southlow under ambient conditions (Table 2).

The fertilizer treatment also altered AMF community composition, and this was independent of cultivar or drought treatments (PERMANOVA: fertilizer pseudo-F = 1.7, p = 0.04). AMF communities became more similar to one another with fertilization (Fig. 2). The indicator species analysis showed that 7 OTUs were significantly associated with fertilization (2 *Glomus*, 2 *Rhizophagus*, 1 *Septoglomus*, and 2 unknown Glomeraceae OTUs), while another 8 OTUs were significantly associated with the no fertilization treatment (1 *Septoglomus*, 1 *Rhizophagus*, 1 *Claroideoglomus*, and 5 unknown Glomeraceae OTUs) (Table 2).

### *3.3. AMF root and soil colonization*

Neither fertilizer nor cultivar treatments had significant effects on AMF root colonization (Table 1). The drought treatment had a significant direct effect on AMF root colonization in switchgrass (Table 1, Table S1), and across all treatments drought reduced root colonization by

6% (Fig. 1c). Neither fertilization nor switchgrass cultivar affected this response (Table 1, Table S1). There was no effect of any treatment on extra-radical hyphal length in soils associated with switchgrass (Table 1, mean ERH = 161.5 cm $g^{-1}$ soil across all treatments).

## 4. Discussion

In this study, we found that drought can alter AMF community richness, composition, and root colonization, though the magnitude and direction of these effects often depended on fertilization and host plant cultivar. Surprisingly, drought increased AMF richness in fertilized plots, especially for the Cave-in-Rock cultivar, which was counter to our predictions and conflicts with results from the few other studies that have examined effects of drought on AMF diversity (e.g., Deepika and Kothamasi, 2015; Wu et al., 2011). It is possible that AMF communities sampled in our study had adapted to the N-fertilization treatment; at the time we sampled these treatments had been in place for seven years. The long-term exposure to N-fertilization may have selected for AMF that were better able to positively respond to drought treatment as a secondary stress factor, as proposed by Millar and Bennett's (2016) stress adaptation hypothesis. It is also possible that fertilization selected for more ruderal species that are less competitive during times of increased environmental stress (Chagnon et al., 2013), allowing other AMF taxa to establish and coexist during short-term drought. Drought effects on AMF communities may have been stronger in Cave-in Rock compared to Southlow, as Southlow was developed from populations in southern Michigan, and so may be better adapted to local conditions than Cave-in-Rock (Durling et al., 2008). Notably, we detected significant changes in AMF communities after only two years of a drought manipulation, while other studies have suggested that it can take three or more years for AMF communities to respond to drought (Deveautour et al., 2020).

The shift in AMF community composition in response to drought was primarily due to increases in *Rhizophagus* taxa, especially associated with the Cave-in-Rock cultivar. Very little is known about the functions of specific genera of AMF, in part due to continuing revisions in the AMF phylogeny (Redecker et al., 2013). However, some research has demonstrated that AMF taxa vary in their functional roles, including their ability to provide pathogen protection, drought tolerance, or nutrient uptake, as well as life history traits, such as relative investment in intra- vs. extra-radical biomass (Chagnon et al., 2013; Klironomos et al., 2001; Powell et al., 2009). Taxa in Glomeraceae, including *Rhizophagus,* are common in agricultural systems (e.g., Borriello et al., 2012; Franke-Snyder et al., 2001) and are characterized as having a ruderal or stress-tolerator life history strategy (Chagnon et al., 2013). Research by Lenoir et al. (2016) showed that these taxa are tolerant to a wide range of abiotic stress, and a recent study in a vegetable cropping system found that *Rhizophagus spp.* increased in drought conditions compared to other taxa (Muller et al., 2019). However, further research into the stress tolerance of individual AMF species is warranted.

Our finding that drought decreased AMF colonization in switchgrass roots is consistent with findings from other studies, which have demonstrated that abiotic stresses often reduce root colonization, especially arbuscule and vesicle formation (Lenoir et al., 2016; but see Staddon et al., 2003). Drought also has been shown to reduce extraradical hyphae (ERH) elongation (Lenoir et al., 2016). While we did not observe any effect of drought on AMF ERH, this may be because species in Glomeraceae, which comprised the vast majority of taxa in our study, mostly produce intra-radical, not extra-radical, hyphae (Aldrich-Wolfe et al., 2020).

Nitrogen fertilization altered AMF community responses to drought and had direct effects on AMF community composition. While overall AMF OTU richness was not affected by long-term fertilizer application, AMF communities were more similar across cultivar and drought treatments in fertilized plots. Other studies have found that N-fertilization causes AMF community convergence in grassland systems due to loss of rare taxa and increases in ruderal taxa (Egerton-Warburton et al., 2007), and several AMF indicator taxa in our study are known to associate with N-enriched soils (Egerton-Warburton and Allen, 2000). It is surprising that we did not see a direct effect of N-fertilization on OTU richness itself. A number of other studies, including our own previous work (Emery et al., 2017), have shown that AMF abundance and diversity is reduced by nitrogen addition (Leff et al., 2015; Treseder, 2004; Zhang et al., 2018). The lack of effect of N-fertilization on AMF in this current study may be a consequence of the intensive agricultural land-use history of this site prior to the experiment (row-crop corn), which may have already excluded rare and disturbance-sensitive AMF taxa (Millar and Bennett, 2016).

Finally, while not the main focus of this study, we detected significant temporal variability in AMF communities across the three dates sampled in this experiment. Differences in AMF community composition between 2015 and 2016 were greater than differences due to the drought or fertilization treatments in each year, while we found no differences in AMF communities between the two samples taken in 2015. This suggests that interannual (between year) variation in climate could affect AMF communities more than intra-annual (seasonal) variation in weather or plant phenology. With only two years of sampling, we cannot attribute these differences to any specific climatic or environmental factor. However, the second year of the study (2016), was somewhat hotter and drier than the first (2015), with moderate drought conditions (D1) in July and August (National Integrated Drought Information System, 2008).

Switchgrass yield was also lower in 2016 than 2015 perhaps reflecting these conditions (Emery et al., 2020). On the other hand, we did not detect a significant decline in aboveground switchgrass biomass in response to our manipulation of drought in this experiment (Emery et al., 2020), and so it is not clear that changes in AMF communities are directly connected to changes in host biomass. Dirks and Jackson (2020) found that reduced switchgrass biomass was correlated with reduced AMF richness at the family level in a similar experiment in Wisconsin. This suggests that functional, rather than taxonomic, diversity of AMF is important for host growth. More long-term studies are needed to isolate causes and consequences of temporal variability in AMF communities.

The changes in AMF richness and composition that we observed in this study may have important implications for management and production of perennial bioenergy feedstock. Changes to AMF communities can feedback to affect host plant survival and growth (Deveautour et al., 2020) as AMF species differ in their abilities to aid in resource uptake (Verbruggen and Kiers, 2010), and AMF community composition can alter crop yield, disease and herbivore resistance, stress resistance, C storage, soil health, and other ecosystem functions (Cotton, 2018). While changes to AMF communities in our experiment do not seem to have direct effects on feedstock yield, changes may be associated with differences in feedstock quality via changes to cell wall composition (Emery et al. 2020). While there is still debate concerning the importance of AMF communities in agricultural production systems (Ryan and Graham, 2018), given the rapid response to drought that we observed in this experiment, it is important for future research to understand both the roles of AMF in sustainable bioenergy feedstock production and how this may change with increasing variability in precipitation (Hawkes et al., 2011).

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**Table 1.** Results from three-factor general linear models (sample date and plot location as block terms) examining effects of drought, fertilizer, and switchgrass cultivar on AMF operational taxonomic unit (OTU) richness, AMF root colonization and AMF extraradical hyphal (ERH) length. Significant effects (p<0.05) are in bold.

| | | **AMF OTU richness (chao1)** | | **AMF % root colonization** | | **AMF ERH length** | |
|---|---|---|---|---|---|---|---|
| **Effect** | **df** | **F** | **p** | **F** | **p** | **F** | **p** |
| Drought | 1 | 1.16 | 0.28 | **4.21** | **0.04** | 0.02 | 0.90 |
| Fertilizer | 1 | 0.81 | 0.37 | 0.29 | 0.59 | 0.10 | 0.76 |
| Cultivar | 1 | 0.42 | 0.52 | 1.34 | 0.25 | 0.48 | 0.49 |
| Drought*Fert | 1 | **6.18** | **0.02** | 0.02 | 0.88 | 2.49 | 0.12 |
| Drought*Cultivar | 1 | **4.40** | **0.04** | 0.10 | 0.76 | 0.06 | 0.81 |
| Fert*Cultivar | 1 | 0.05 | 0.82 | 0.24 | 0.62 | 2.77 | 0.10 |
| Drought*Cultivar*Fert | 1 | 0.85 | 0.36 | 0.23 | 0.63 | 0.64 | 0.43 |
| Block | 3 | 1.90 | 0.14 | 0.48 | 0.69 | 1.84 | 0.15 |
| Sample Date | 1 | **66.94** | **<0.001** | **74.14** | **<0.001** | **70.82** | **<0.001** |

**Table. 2.** Results from Indicator Species Analysis for AMF OTUs associated with drought or fertilizer treatment. P-values are calculated based on 999 randomizations in a Monte Carlo simulation. Only significant indicator taxa are reported.

| **OTU** | **Genus** | **Indicator Group** | **IV statistic (p-value)** |
|---|---|---|---|
| OTU64 | *Rhizophagus* | Cave-in-Rock, Drought | 0.38 (0.001) |
| OTU87 | *Rhizophagus* | Cave-in-Rock, Drought | 0.33 (0.007) |
| OTU236 | *Rhizophagus* | Cave-in-Rock, Drought | 0.32 (0.01) |
| OTU253 | *Rhizophagus* | Cave-in-Rock, Drought | 0.29 (0.03) |
| OTU308 | *Rhizophagus* | Cave-in-Rock, Drought | 0.28 (0.04) |
| OTU174 | *Rhizophagus* | Cave-in-Rock, Drought | 0.28 (0.04) |
| OTU401 | *Rhizophagus* | Cave-in-Rock, Drought | 0.28 (0.03) |
| OTU244 | *Rhizophagus* | Cave-in-Rock, Drought | 0.28 (0.04) |
| OTU367 | *Rhizophagus* | Cave-in-Rock, Drought | 0.27 (0.04) |
| OTU240 | Unk. Glomeraceae | Cave-in-Rock, Drought | 0.33 (0.008) |
| OTU65 | *Septoglomus* | Cave-in-Rock, Ambient | 0.26 (0.05) |
| OTU373 | Unk. Glomeraceae | Cave-in-Rock, Ambient | 0.32 (0.01) |
| OTU77 | Unk. Glomeraceae | Southlow, Ambient | 0.35 (0.003) |
| OTU387 | Unk. Glomeraceae | Southlow, Ambient | 0.36 (0.002) |
| OTU446 | Unk. Glomeraceae | Southlow, Ambient | 0.31 (0.008) |
| OTU460 | Unk. Glomeraceae | Southlow, Ambient | 0.32 (0.007) |
| OTU519 | *Rhizophagus* | Southlow, Ambient | 0.28 (0.03) |
| OTU80 | *Glomus* | Fertilized | 0.24 (0.02) |
| OTU202 | *Glomus* | Fertilized | 0.22 (0.03) |
| OTU161 | *Rhizophagus* | Fertilized | 0.23 (0.03) |
| OTU353 | *Rhizophagus* | Fertilized | 0.02 (0.05) |
| OTU65 | *Septoglomus* | Fertilized | 0.20 (0.05) |
| OTU210 | Unk. Glomeraceae | Fertilized | 0.26 (0.01) |
| OTU155 | Unk. Glomeraceae | Fertilized | 0.23 (0.02) |
| OTU47 | *Claroideoglomus* | Unfertilized | 0.27 (0.008) |
| OTU280 | *Rhizophagus* | Unfertilized | 0.21 (0.04) |
| OTU425 | *Septoglomus* | Unfertilized | 0.22 (0.03) |
| OTU13 | Unk. Glomeraceae | Unfertilized | 0.30 (0.002) |
| OTU387 | Unk. Glomeraceae | Unfertilized | 0.28 (0.004) |
| OTU436 | Unk. Glomeraceae | Unfertilized | 0.26 (0.008) |
| OTU11 | Unk. Glomeraceae | Unfertilized | 0.22 (0.04) |
| OTU18 | Unk. Glomeraceae | Unfertilized | 0.21 (0.04) |

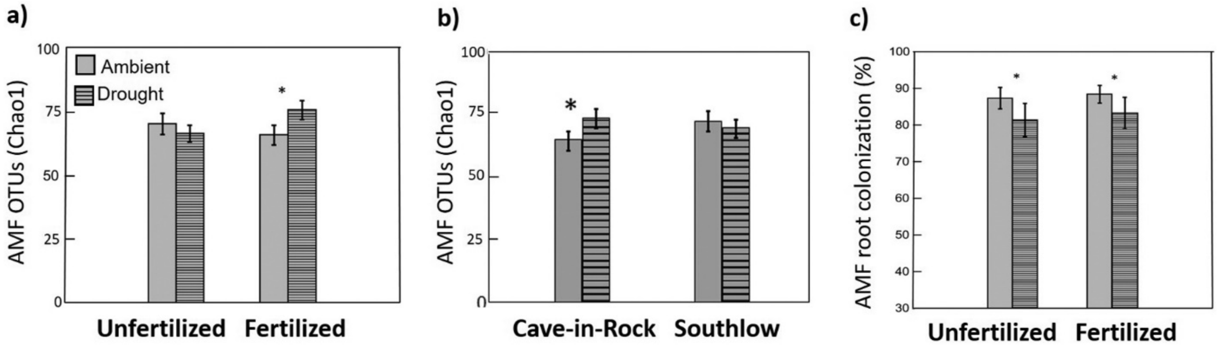
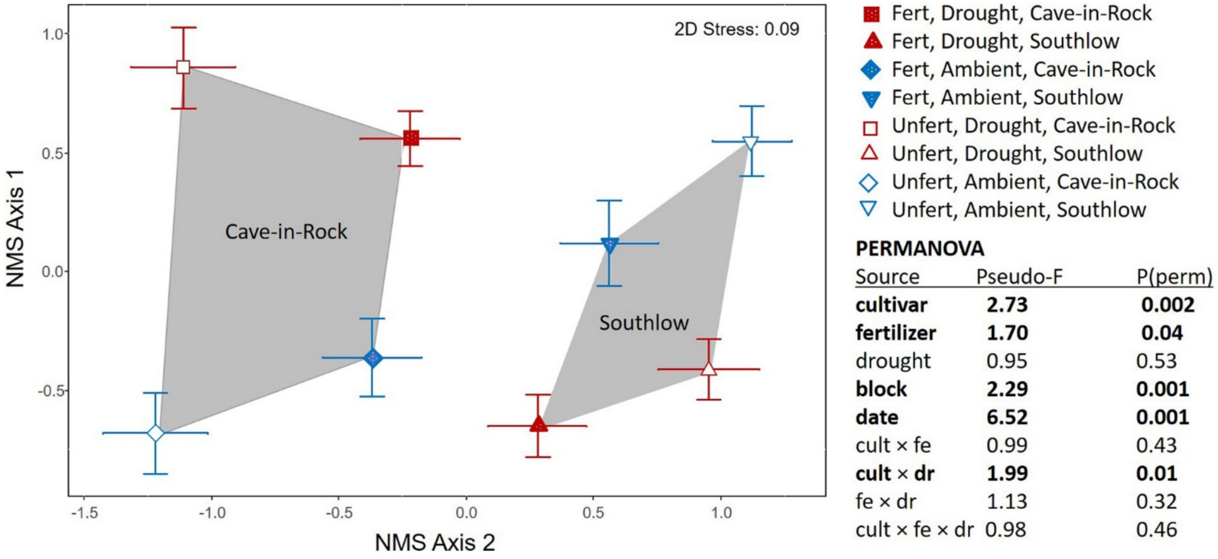


**Figure 1.** Significant effects of drought, fertilizer, and cultivar treatments on (a-b) AMF OTU richness and (c) AMF root colonization. Bars are means ±1 SE. Asterisks indicate significant pairwise differences (p<0.05) in Fishers LSD post-hoc comparisons.



**PERMANOVA**

| Source | Pseudo-F | P(perm) |
|---|---|---|
| **cultivar** | **2.73** | **0.002** |
| **fertilizer** | **1.70** | **0.04** |
| drought | 0.95 | 0.53 |
| **block** | **2.29** | **0.001** |
| **date** | **6.52** | **0.001** |
| cult × fe | 0.99 | 0.43 |
| **cult × dr** | **1.99** | **0.01** |
| fe × dr | 1.13 | 0.32 |
| cult × fe × dr | 0.98 | 0.46 |

**Figure 2.** PERMANOVA and Non-metric Multidimensional Scaling plot of the treatment means and standard errors across sampling dates and blocks. Drought treatments are in red, while ambient treatments are in blue. Unfertilized treatments are indicated with open shapes, while fertilized treatments are filled.