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Impact of arbuscular mycorrhizal fungi on Conyza canadensis drought responses and possible mechanisms

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Summary

- Arbuscular mycorrhizal fungi (AMF) are important plant mutualists that can facilitate plant responses to various environmental stressors, such as drought. A plant that may benefit from AMF-induced drought tolerance is *Conyza canadensis* due to its ability to thrive in dry conditions and its high colonization rate. However, no studies have researched *C. canadensis* in this context and the exact mechanisms of AMF-induced drought tolerance are still unknown.
- To better understand if and how AMF facilitate drought response in *C. canadensis*, we conducted a greenhouse experiment comparing the response of mycorrhizal and non-mycorrhizal plants to three watering levels. We measured dry biomass, water content, leaf water potential, photosynthetic rate (Pn), stomatal conductance, and shoot N and P concentrations.
- AMF improved plant performance under drought, and the magnitude of that improvement was modulated by the severity of drought imposed. We showed that AMF upregulate stomatal conductance, photosynthesis, and increase P uptake.
- In conclusion, we find that AMF protect *Conyza* from the most severe drought stress, and that this response is likely mediated by increased stomatal control and nutrient uptake. Colonization led to biomass reductions, which suggests AMF benefit *C. canadensis* more in the way of drought tolerance and nutrient uptake, rather than improving growth.

Key words: arbuscular mycorrhizal fungi, Conyza canadensis, drought tolerance, nutrient concentrations, photosynthetic rate, stomatal conductance.

Introduction

Increasing drought frequency due to climate change will negatively impact plant populations. Water stress can lead to limited nutrient uptake, a decline in photosynthesis, and internal damage caused by the accumulation of reactive oxygen species (ROS) (Mahajan & Tuteja, 2005; Farooq *et al.*, 2009; Anjum *et al.*, 2011). Plants have evolved a variety of mechanisms to deal with water stress, such as deep roots, succulent leaves, and thick cuticles (Moradi, 2016). Another strategy that some plants may utilize in addition to physiological and morphological adjustments are associations with mycorrhizal fungi (AMF) (Augé, 2001). AMF form symbiotic relationships with 80% of all land plants (Van der Heijden & Sanders, 2002) and provide plants with a plethora of services in exchange for photosynthetic carbon. The mechanisms behind many of these services are well-studied; however, there is still no definitive mechanism that explains AMF-induced drought tolerance, and several species-specific interactions are currently unexplored. Our research explores three likely mechanisms of AMF-induced drought tolerance in an herbaceous plant that does not display typical xeromorphic traits yet occurs in very dry conditions.

AMF may help improve plant tolerance to drought (Augé, 2001), and the benefits a plant receives from its symbiosis with AMF is likely context specific. Plant-AMF symbioses exist on a continuum of mutualism to parasitism and are dependent on the environmental conditions (Johnson *et al.*, 1997); therefore, there may be different plant responses to varying degrees of drought severity. There is some evidence that AMF may confer drought tolerance through biochemical and morphological mechanisms, such as increased hormonal response, gene regulation, or altered root structure (Wright *et al.*, 1998; Wu & Xia, 2006; Xu *et al.*, 2013; Kaushal, 2019; Bahadur *et al.*, 2019); however, other mechanisms may be equally if not more important. The three drought tolerance mechanisms we explore here are stomatal conductance, photosynthetic rate, and nitrogen and phosphorous concentrations.

Plants control stomatal conductance to prevent water loss but do so at the cost of slowing CO_2 diffusion into the leaf, leading to a subsequent reduction in carbon fixation. AMF have been shown to modify plant hormonal responses, leading to downstream effects that increase stomatal efficiency (Kaushal, 2019). In addition to water loss through poor stomatal control, many plants experience a decrease in photosynthesis under drought, which can cause physiological complications such as increasing photorespiration and reducing plant production (Reddy *et al.*,

2004). There is some evidence that mycorrhizal plants may be able to maintain a better photosynthetic rate than nonmycorrhizal plants under drought stress (Bakr *et al.*, 2018; Zhao *et al.*, 2015; Ruíz-Sánchez *et al.*, 2011). Finally, better nutrient uptake and balance may be a potentially important mechanism explaining drought response in mycorrhizal plants. Nutrient availability goes down in water-stressed soils because plants primarily absorb soluble nutrients (Rouphael *et al.*, 2012). Greater nutrient acquisition could explain why there is often increased growth in mycorrhizal plants, and recent research has shown that AMF phosphorous acquisition becomes increasingly important under drought stress (Püschel *et al.*, 2021). Overall, AMF potentially increase plant performance under drought via several mechanisms, ranging from the molecular to the whole-plant level.

Although AMF may help mediate drought, it is also important to consider that water stress affects the fungi as well. For instance, root colonization often decreases with increasing water stress (Mohan *et al.*, 2014), and under severe enough drought, the plant may become nonmycorrhizal (Lekberg, personal communication). Furthermore, drought stress can affect the ability of AMF to extend their hyphae into the soil matrix and may interrupt spore production in some species (Lenoir *et al.*, 2016). Some species of AMF have lower colonization rates under drought conditions, which suggests differences among AMF taxa in their ability to tolerate water stress (Porto *et al.*, 2020). How AMF respond to water stress is important for understanding AMF-plant dynamics under drought. Of course, the specific plant species and functional group also plays an important role in determining the symbiotic drought response.

Many studies pertaining to AMF-induced drought have been conducted with domesticated crop species (Delavaux *et al.*, 2017), which could limit inference about the AMF-plant symbiosis outside of agricultural ecosystems. Although the study of these plants may help inform future decisions regarding food-security, studies of agricultural systems may not scale up to natural systems (Dalgaard *et al.*, 2003). Here, we investigate *Conyza canadensis,* a ruderal forb in the Asteraceae family native to North America. Although generally a winter annual, *C. canadensis* has a flexible lifecycle that responds to soil and environmental conditions (Buhler & Owen, 1997). However, it is not well understood how *C. canadensis* tolerates drought stress during late season growth, especially given the fact that it does not possess many of the traits that are commonly associated with drought tolerance, such as succulent leaves or deep roots. Furthermore, *C. canadensis* is highly colonized by AMF. Although studies involving *C.*

canadensis and AMF are limited, work done by Shah et al. (2008) shows that *C. canadensis* has an average percent colonization as high as 70%. *C. canadensis* therefore provides an excellent research candidate for AMF-induced tolerant because it is uncharacteristically drought tolerant and highly colonized.

Here, we specifically look at plant performance variables that are indicative of increased or decreased drought response (biomass, shoot/root water content, leaf water potential, and root shoot ratio). Biomass is indicative of the plant's overall ability to grow and reflects plant water status due to the turgor pressure required for growth (Farooq *et al.*, 2009). Shoot and root water content is also indicative of the plant water balance, and the differences in these variables can suggest either changes in water use strategy or water acquisition. Leaf water potential is an indicator of drought stress because it correlates to xylem potential (Jarvis, 1976). Higher leaf water potential is indicative of milder perceived stress, and lower potential indicative of more severe perceived stress. Lastly, root shoot ratio is also an indicator of the current soil water environment and plant stress. Plants under drought tend to experience shift in biomass allocation, often with reduction in shoot biomass and increases in root biomass (Eziz *et al.*, 2017). Plants experiencing water stress will likely have a greater root to shoot ratio to exploit scant water resources. We also examine three likely drought tolerance mechanisms (stomatal conductance, photosynthetic rate, and nitrogen and phosphorous concentrations) that may explain why either mycorrhizal or non-mycorrhizal plants exhibit increased or decreased plant performance. The specific objectives of this research are to:

1) assess if inoculation with AM fungi affect plant performance (biomass, shoot/root water content, leaf water potential, and root shoot ratio) and if the differences depend on the level of drought stress (moderate or severe),
2) assess if there are differences in possible drought tolerance mechanisms (stomatal conductance, photosynthetic rate, or nitrogen and phosphorous concentrations) and if the differences depend on the level of drought stress,
3) determine if there are difference in % colonization among watering treatments, and if the differences depend on the level of drought stress

Materials and Methods

Experimental Design and Materials

The experiment was conducted at University of Montana's Dietrich Greenhouse in Missoula, Montana using *C. canadensis* seeds collected from a population on MPG Ranch outside Florence, Montana (46°40'48.92" N, 114°1'40.73" W). Seeds were sown in common potting soil and watered as needed for 14 days. They were then transplanted into four-inch pots with a 1:1:1 mixture of autoclaved local soil, sand, and Turface (pH 7.2, NO_3^- 20.7 mg kg^{-1}, $P_{Merlich}$ 20 mg kg^{-1}). Half of all pots were inoculated at transplanting by placing 50 mL of AMF inoculum below the roots containing a mixture of eight species of AMF and 37 spores/mL in addition to hyphal fragments and colonized root pieces. Control plots were given heat treated (95°C for 12 hrs) AMF inoculum and 25 mL microbial wash made from an 1:10 (inoculum:water) sieved two times through a Fishman P8 filter paper (<20μm) to minimize differences in other soil biota among treatments. To allow for establishment, seedlings were watered as needed for an additional 21 days. Each plant was then exposed to one of three watering levels: control (no stress), moderate drought stress, and severe drought stress. The severity of drought stress was measured on a subset of pots as percent soil moisture. Each of the six treatments were replicated eight times, resulting in 48 pots total. All plants were harvested eight weeks after transplanting.

Drought Treatments

Water stress was implemented using a "wick" method (Toth *et al.*, 1988). Each pot had two felt strings with one end in the soil matrix and the other end in a basin of water (Fig. **1**). Increasing the height the pots were raised from the basin of water reduced the amount of water delivered to the pots and thus increased the water stress (Fig. **1**). Control, moderate, and severe watering treatments were kept at an average 18%, 8%, and 5% volumetric soil water content, respectively (Fig. **S2**). The control treatments were on average higher in the mycorrhizal pots (~20%) compared to non-mycorrhizal pots, due to unknown reasons (Fig. **S2**). However, this was not a concern as it only occurred in the control pots and the difference was relatively small. Treatments were organized in blocks sharing a central container of water, with four of each watering treatment (control, moderate stress, and severe stress). AM and NM treatments were kept separate to eliminate the risk of contamination. The wicks were replaced three weeks after transplantation due to natural degradation and bacterial mats forming. After replacement, tetracycline at a concentration of 12.5 μg/ml were added to the water basins to prevent further bacterial mat formation.

Measurements

Preharvest measurements included stomatal conductance, photosynthetic rate (Pn), and leaf water potential. Pn and stomatal conductance were measured using a LI-COR portable photosynthesis system (Biosciences, 2001). Because *C. canadensis* leaves are thin, we measured the Pn and conductance on three leaves simultaneously per plant in order to completely fill the LI-COR leaf chamber. The average leaf area utilized in each measurement was therefore 3.5 cm. Leaf water potential was measured using a pressure bomb. One leaf per plant was cut at the base of the petiole with a razor and the body of the leaf was wrapped in plastic. Leaves were chosen near the base of the plant and were all roughly the same age. Samples were then placed in the pressure chamber with the petiole exposed. The chamber was sealed, and slowly pressurized until water was visibly coming from the leaf petiole under magnification. The pressure at which water was first visible was recorded as bars, then converted to millipascals (MPa).

Postharvest measurements included shoot and root biomass, shoot and root water content, and percent root colonization. Shoot biomass was measured by cutting the stem of the plant level with the soil level and immediately weighing the fresh weight. Any dead leaves were removed prior to weighing, as well. Root biomass was measured by first washing the soil off the roots and then squeezing excess water out of the roots with a paper towel. Roots were weighed once they were cleaned and dried of excess water. To obtain dry biomass, shoots and roots were oven dried in paper bags at 90º C for 48 hours and then weighed. Shoot and root water content were then calculated by subtracting dry biomass from fresh biomass.

To quantify if root colonization differed among the three moisture treatments, a representative sample of fine roots (≤ 1mm diameter) were taken from each plant, cleaned, stained with trypan dye, and mounted on microscope slides (McGonigle *et al.*, 1990). Eight, 2 cm long root segments were mounted on each half of the slide and arranged parallel to the long side of the slide for a total of 16 root segments per slide. The roots were examined under 100x magnification for the presence of arbuscules, vesicles, and hyphae, which show up as blue due to the trypan dye. Arbuscules are tree-like structures that serve as nutrient exchange sites between the fungi and the plant. Vesicles are oval structures that act as lipid storage compartments for the fungi, and hyphae are long, thin, fungal filaments. Arbuscules and vesicles were counted separately, and

hyphae were only counted if other mycorrhizal structures were not visible because the presence of arbuscules or vesicles implies there must be hyphae. If any fungal structures were present the intercept was marked as mycorrhizal and if no fungal structures were present the intercept was marked as non-mycorrhizal. This resulted in a total of 48 intercepts, and two more intercepts were chosen at random to reach 50 intercepts. Calculation of total percent colonization was done by dividing the number of mycorrhizal intercepts by the total number of intercepts (n=50). Percent vesicles and arbuscules was done by dividing the number of vesicle and arbuscule intercepts by the total number of intercepts.

Statistical Analysis

To assess whether AM and NM plants differed in plant performance under different levels of drought (question 1), we used two-way ANOVA models with inoculation treatment (AM and NM) and watering treatment (C, M, S) to test for overall effects on plant performance (biomass, shoot/root water content, leaf water potential, and root shoot ratio) and interactions. Separate ANOVA models were used for each plant performance variable.

To assess if the proposed drought tolerance mechanisms (stomatal conductance, photosynthetic rate, and nitrogen and phosphorous concentrations) differed between AM and NM plants (question 2), we used two-way ANOVA models with inoculation treatment and watering treatment to test for effects and interactions.

To assess whether there were among watering treatment differences in % root colonization (question 3), we used ANOVA models with watering treatment to test for effects.

All analyses were done in R (R Core Team 2019). All raw data and analyses are archived and available to the public in ScholarWorks at the University of Montana.

Results

We found significant differences between AM and NM plants in three of the six plant performance variables (question 1). Shoot dry weight ($F_{(1,42)}$= 6.917, p= 0.0119) (Fig. **2a**), shoot water content ($F_{(1,42)}$= 6.616, p= 0.0137) (Fig. **3a**), and leaf water potential ($F_{(1,42)}$= 9.376, p=

0.005) (Fig. **4**) all had inoculation as a significant factor. Significant differences between watering treatments were found in five of the six plant performance variables (Table 1). Shoot dry weight ($F_{(2,42)}$= 32.703, p= 2.73e-09), root dry weight ($F_{(2,42)}$= 6.461, p= 0.00358), root water content ($F_{(2,42)}$= 6.603, p= 0.00321), leaf water potential ($F_{(2,24)}$= 100.05, p= 2.28e-12), and root shoot ratio ($F_{(2,42)}$= 5.226, p= 0.0094) all had significant difference between watering treatments. Additionally, interactive effects between inoculation and watering treatment were found in leaf water potential ($F_{(2,24)}$= 19.639, p= 8.86e-06). Overall, we find that the presence of AMF influences some aspects of plant performance, but that watering has a much higher influence on performance variables.

We found that three of the four proposed drought tolerance mechanisms differed between inoculation groups (question 2). Stomatal conductance ($F_{(1,19)}$= 9.483, p= 0.00617) (Fig. **5a**), photosynthetic rate ($F_{(1,19)}$= 4.411, p= 0.0493) (Fig. **5b**), and phosphorous concentrations ($F_{(1,42)}$= 8.282, p= 0.00627) (Fig. **6b**) all differed significantly between inoculation treatments. Significant differences between watering treatments were only found for nitrogen concentration ($F_{(1,42)}$= 5.936, p= 0.00536) (Fig. **6a**). Interactive effects between inoculation and watering treatments were found for both nitrogen ($F_{(1,42)}$= 4.146, p= 0.02274) and phosphorous ($F_{(1,42)}$= 5.879, p= 0.00561) concentrations. In summary, inoculation influenced most of the proposed drought tolerance mechanisms, and the interaction between AMF and watering seemed to primarily influence plant nutrition.

We found no significant differences in percent colonization between the three watering treatments ($F_{(2,21)}$= 0.031, p = 0.93) (Fig **8**). Similar findings were found with percent vesicles ($F_{(2,21)}$= 0.385, p= 0.68) and percent arbuscules ($F_{(2,21)}$= 0.054, p= 0.94) (Figs. **9** and **10**).

Discussion

Due to the likelihood of increasing drought, it is becoming increasingly important to study symbiotic responses to drought, especially in currently understudied plants. Here, we show that the presence of AMF improved plant performance under drought, and that the magnitude of that improvement was modulated by the severity of drought imposed. Furthermore, we show that AMF upregulated stomatal conductance, photosynthesis, and increased phosphorous uptake. This suggests that the increase in plant performance is related to a combination of nutrient fertilization and increased stomatal efficiency. However, the presence of AMF suppressed shoot biomass but

not root biomass, suggesting that the benefits *C. canadensis* receives from its symbiosis with AMF is not growth related. It is more likely that *C. canadensis* receives the most benefit in terms of drought avoidance, which is suggested by higher leaf water potentials in the severely stressed plants. This suggests the AMF were somehow able to protect plants from the most severe stress.

The suppression of shoot biomass contradicts similar studies testing AMF-induced drought tolerance, which found AMF increased biomass (Wu & Xia, 2006; Bakr *et al.*, 2018). However, many of these studies are on agricultural crops. Ruderal species such as *C. canadensis* have different life histories than agricultural plants and therefore may respond differently to AMF colonization. In fact, studies on weeds and ruderal species have found that AMF decrease shoot biomass (Rinaudo *et al.*, 2010). The suppression of shoot biomass may be beneficial for *C. canadensis* under drought in the long run. By reducing biomass, the plant needs less water to maintain turgor pressure- a strategy which is common among plant populations in drier environments (Alpert, 2006). Overall, the suppression of growth in mycorrhizal *C. canadensis* reflects what other studies have found and suggests that AMF benefits *C. canadensis* mainly by increasing drought tolerance and nutrient acquisition.

Inoculation upregulated stomatal conductance, photosynthesis, and improved plant nutritional status, especially regarding phosphorous concentrations. Higher stomatal conductance suggests that the plant had more available water to transpire, and may suggest that AMF mediated more efficient use of this water via hormonal responses, such as abscisic acid (ABA) (Miransari *et al.*, 2014). Increased stomatal conductance and improved nutrition also led to an increase in photosynthesis. Although the upregulation of photosynthesis was apparently insufficient to prevent decreased shoot growth, it may be that much of that photosynthate was allocated instead as organic solutes. A potential increase in solutes, such as non-structural carbohydrates, would increase osmotic potential and improve plant water status (Martínez-Vilalta *et al.*, 2016). Finally, the increase in phosphorous accumulation reflects recent studies, which found that AMF improve phosphorous acquisition compared to non-mycorrhizal plants under drought conditions, but not necessarily in benign conditions (Püschel *et al.*, 2021). The accumulation of phosphorous may be a primary mechanism for improving drought tolerance (Halvorson & Reule, 1994; Rodriguez *et al.*, 1996). Overall, AMF improved *C. canadensis* drought tolerance through the mediation of several mechanisms.

We found that there were no significant differences in percent colonization across the three drought treatments, and that the AMF seemed unaffected by drought. However, root biomass was significantly different between watering treatments, and therefore fungal biomass was also likely different. Although colonization was similar, differences in fungal biomass may relate to differences in plant performance. However, we have no definitive way of knowing this without having measured fungal biomass. Furthermore, while increased percent colonization generally increases plant performance, there is variability among AMF and plant species (Kathleen, 2013). In summary, AMF were not influenced by drought, yet there may be differences in fungal biomass that could be affected by drought and influence plant drought response.

Despite increasing research and interest toward AMF-induced drought tolerance, many studies, including our own, are often limited by experimental design and scope. It is likely that the influence of AMF will shift under field conditions, due to factors such as competition and variations in nutrient availability. Furthermore, AMF communities will differ from the culture collections that we used here. Although we included five different AMF families in our inoculation, the effect of drought on mycorrhizal *C. canadensis* may largely depend on the soil community as a whole, and a different composition of AMF species may give different results (Hart *et al.*, 2003; Petipas *et al.*, 2017). Moreover, this study may have also been limited by the 'wick' method used for drought. Hyphae and roots may have disproportionally congregated around the wicks, which would influence local water availability. Additionally, constant soil moisture does not reflect what happens in most ecosystems, although it allowed for reduced variability and more control in our experiment. Although differences in plant performance variables adequately show that plants were responding to the drought stress imposed, it is difficult to ascertain if the AMF were experiencing similar drought conditions within the heterogeneous soil environment.

In summary, this study shows that AMF protect plants under severe stress, and that AMF benefit *C. canadensis* in ways unrelated to growth. The benefit of AMF is likely related to improved stomatal control, photosynthesis, and increased phosphorous accumulation, and the mechanisms driving better plant performance under drought is likely a combination of the three. Future research should focus on how AMF influence plant community dynamics under drought stress, as well as further gathering evidence and mechanistic insight into AMF-induced drought

tolerance. As well, future studies should utilize a more diverse array of plant and AMF species, as specific drought responses will vary based on species used.

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411

412 **Figure Captions**

413 **Fig 1.** The study design setup, with four replicated of each water treatment sharing one basin of
414 water. Each block consists of one inoculation treatment

415 **Fig 2.** Means and standard errors of Shoot Dry Weight (A), Root Dry Weight (B). AM is the
416 mycorrhizal treatment (circle) and NM is the non-mycorrhizal treatment (triangle). The green
417 color represents the control group (C), yellow the moderate stress group (M), and red the severe
418 stress group (S).

419 **Fig 3.** Means and standard error of Shoot Water Content (A) and Root Water Content (B). All
420 units are in grams water per gram biomass. AM is the mycorrhizal treatment (circle) and NM is
421 the non-mycorrhizal treatment (triangle). The green color represents the control group (C),
422 yellow the moderate stress group (M), and red the severe stress group (S).

423 **Fig. 4** Means and standard error of Lear Water Potential (MPa). AM is the mycorrhizal treatment
424 (circle) and NM is the non-mycorrhizal treatment (triangle). The green color represents the
425 control group (C), yellow the moderate stress group (M), and red the severe stress group (S).

426 **Fig. 5** Means and standard error of Root Shoot Ratio. AM is the mycorrhizal treatment and NM
427 is the non-mycorrhizal treatment. The green color represents the control group (C), yellow the
428 moderate stress group (M), and red the severe stress group (S).

429 **Fig. 6** Means and standard errors of stomatal conductance (A) and photosynthetic rate (B).
430 Stomatal conductance is measured as mol H_2O m-2 s-1 AM is the mycorrhizal treatment (circle)
431 and NM is the non-mycorrhizal treatment (triangle). The green color represents the control group
432 (C), yellow the moderate stress group (M), and red the severe stress group (S).

433 **Fig. 7** Means and standard errors of shoot % nitrogen (A) and phosphorous (B). Phosphorous is
434 measured mg/g. AM is the mycorrhizal treatment (circle) and NM is the non-mycorrhizal
435 treatment (triangle). The green color represents the control group (C), yellow the moderate stress
436 group (M), and red the severe stress group (S).

437 **Figure 8** Means and standard errors of percent colonization. The green color represents the
438 control group (C), yellow the moderate stress group (M), and red the severe stress group (S).

439 **Figure 9** Means and standard errors of percent vesicles. The green color represents the control
440 group (C), yellow the moderate stress group (M), and red the severe stress group (S).

Figure 10 Means and standard errors of percent arbuscules. The green color represents the control group (C), yellow the moderate stress group (M), and red the severe stress group (S).

Table 1. The degrees of freedom (DF), F statistic, and p values (Inoculation, Watering, and Inoculation x Watering) for the six plant performance variable and four proposed drought tolerance mechanisms.

Figures

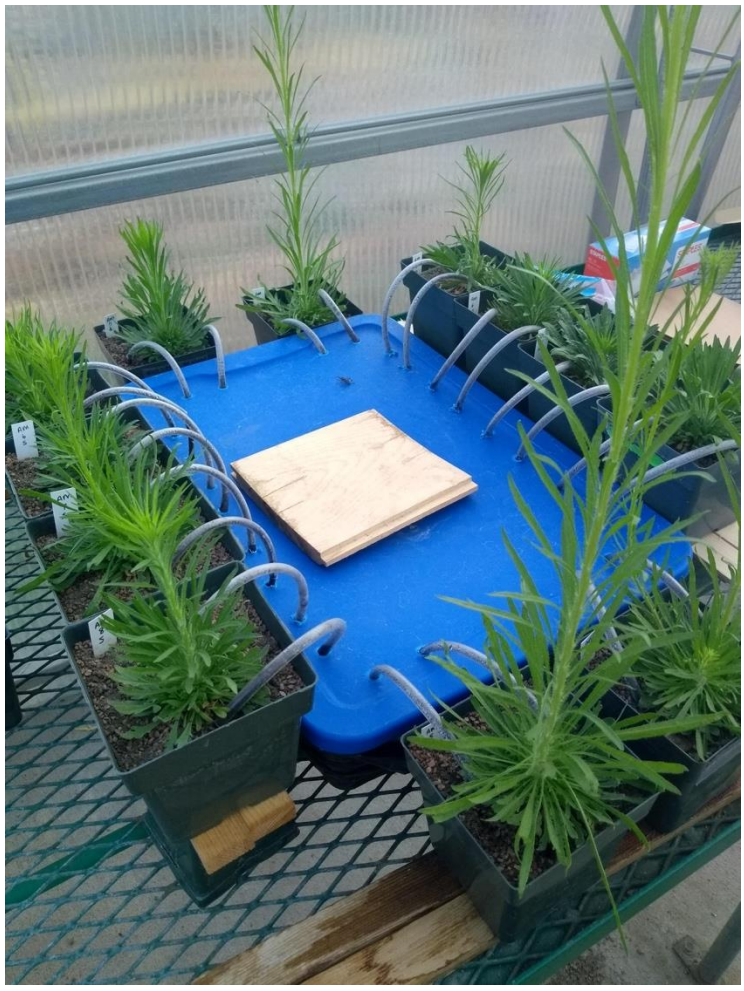

Figure 1

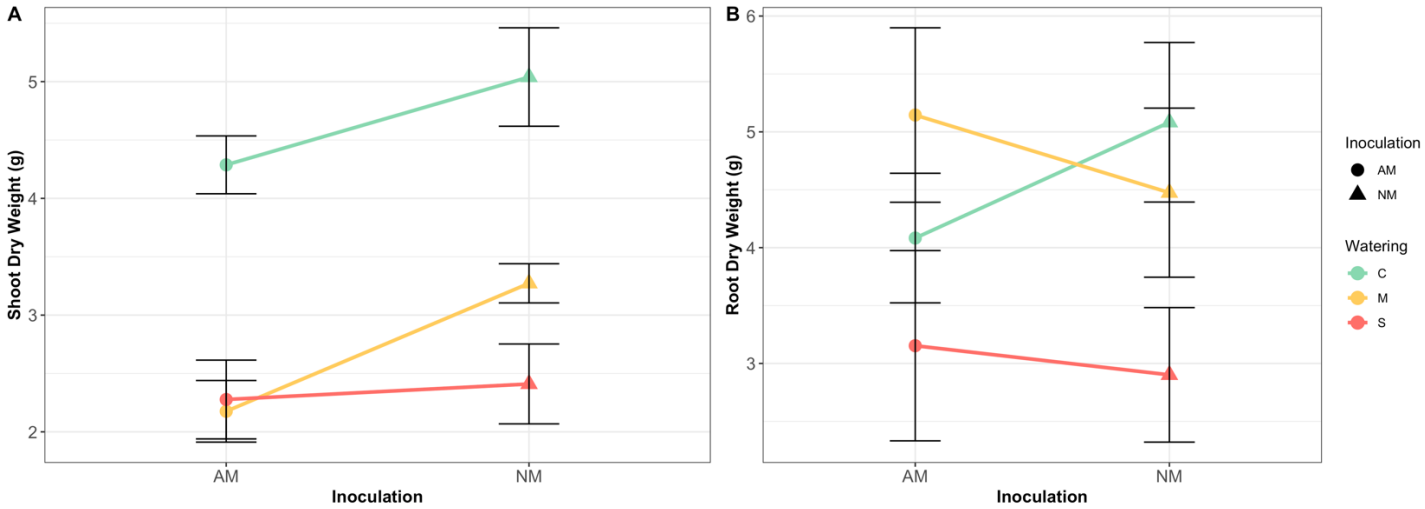

Figure 2

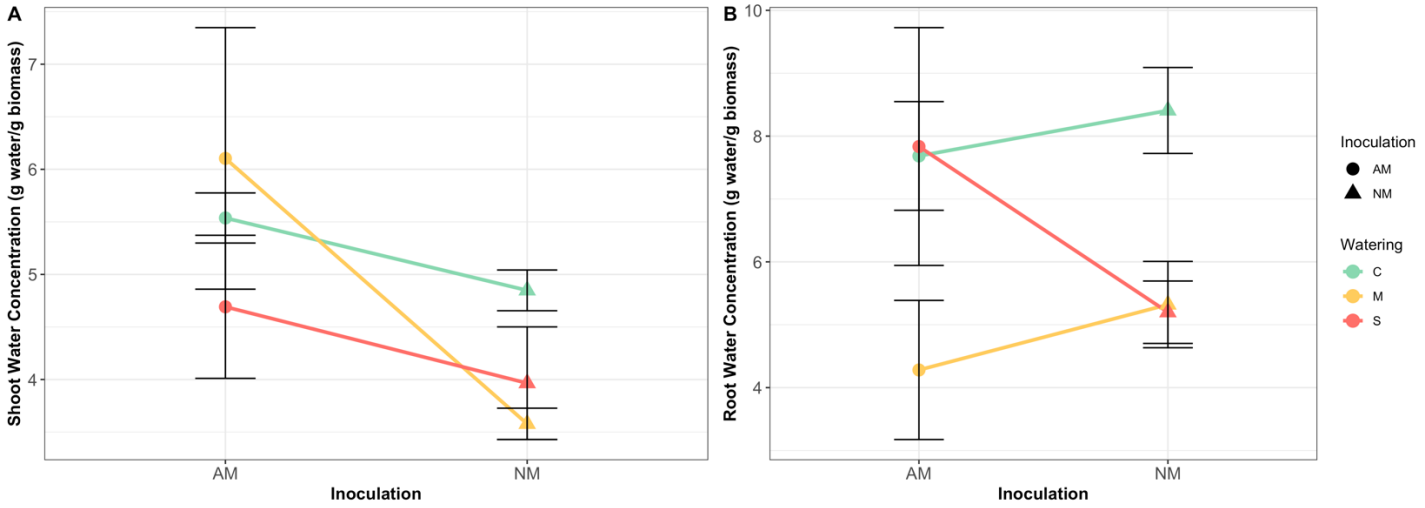

Figure 3

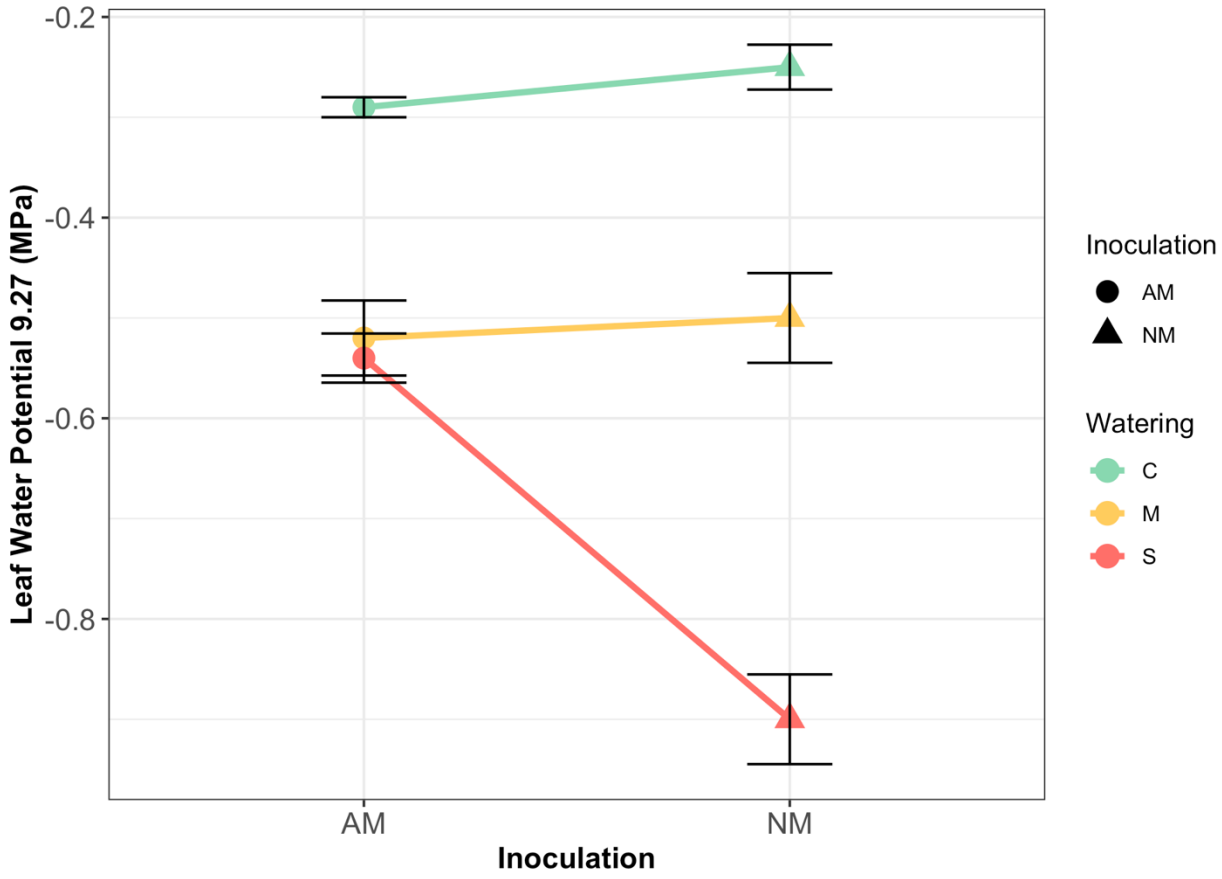
-0.2
-0.4
-0.6
-0.8
Leaf Water Potential 9.27 (MPa)
AM
NM
Inoculation
Inoculation
AM
NM
Watering
C
M
S

Figure 4

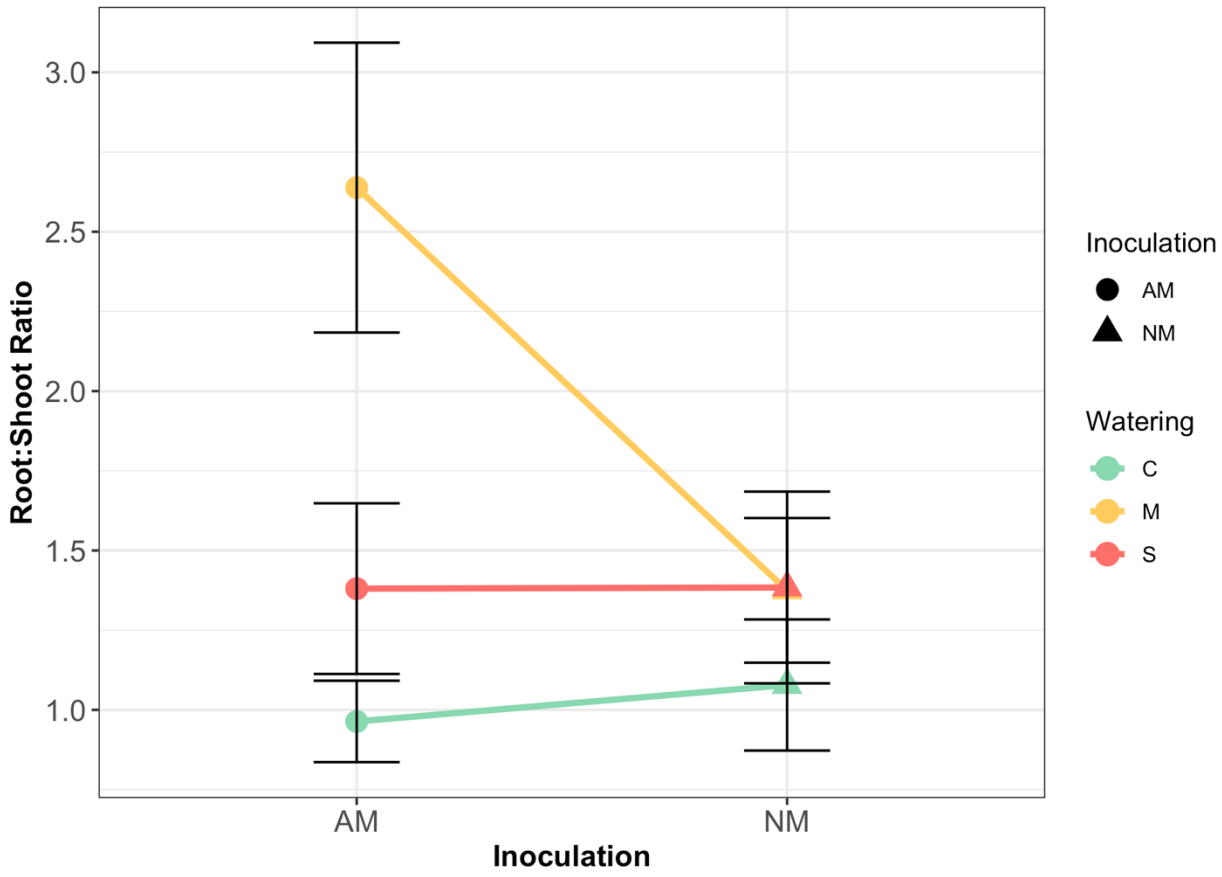

Figure 5

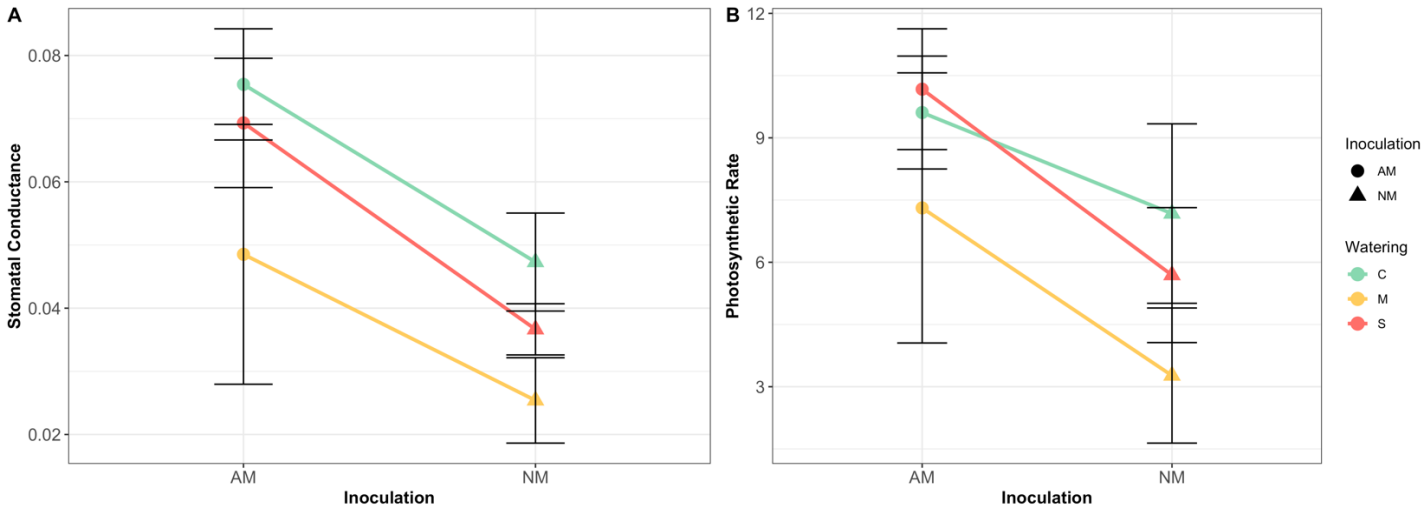

Figure 6

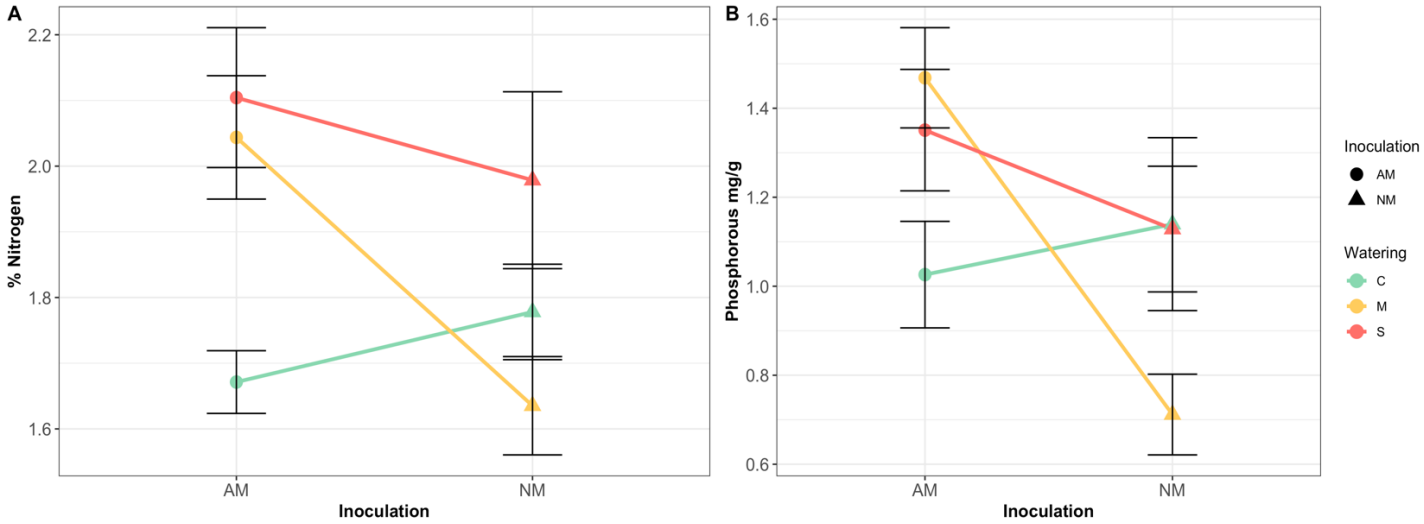

Figure 7

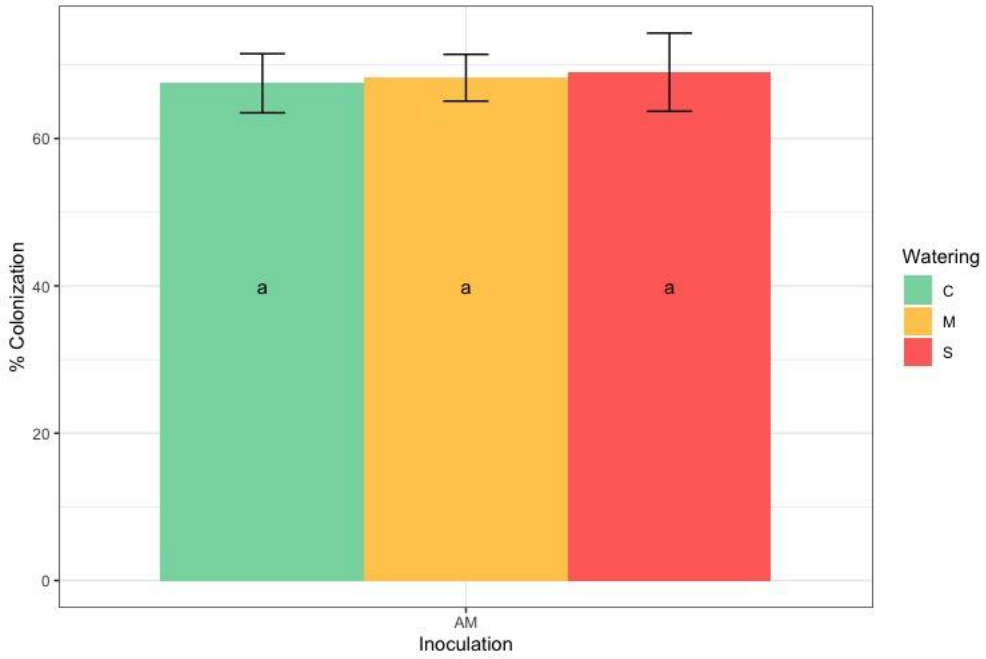

Figure 8

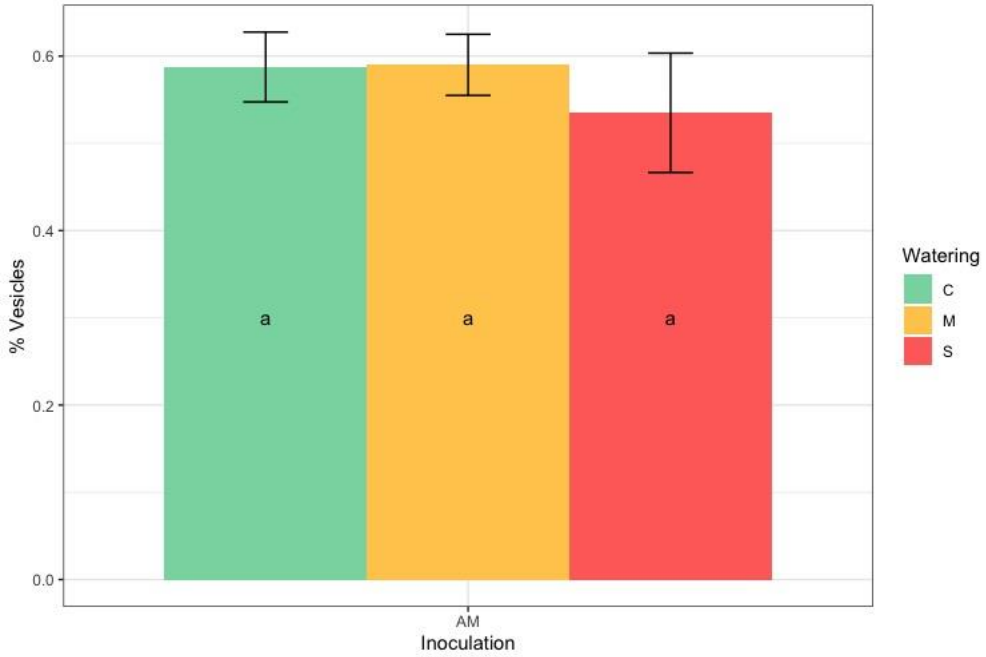

Figure 9

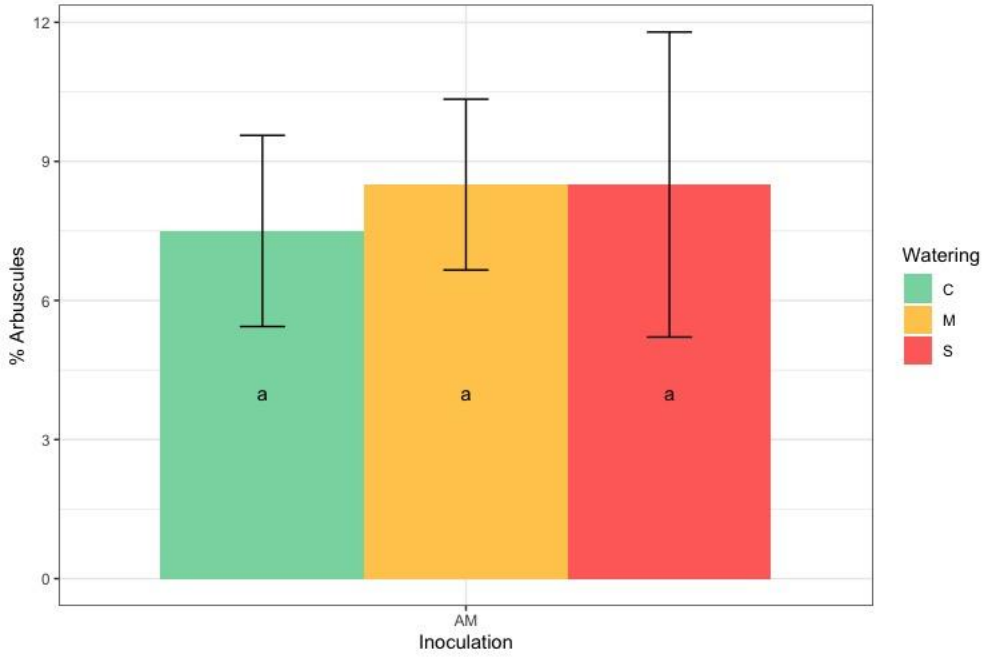

Figure 10

VARIABLE	FACTORS	DF	F statistic	P value
Shoot biomass				
	Inoculation	1	6.917	0.0119
	Watering	2	32.703	2.73E-09
	Inoculation:Watering	2	1.258	0.2948
Root biomass				
	Inoculation	1	0.05	0.8237
	Watering	2	6.461	0.00358
	Inoculation:Watering	2	0.578	0.5656
Shoot Water Content				
	Inoculation	1	6.616	0.0137
	Watering	2	2.535	0.0913
	Inoculation:Watering	2	1.22	0.3054
Root Water Content				
	Inoculation	1	0.315	0.5774
	Watering	2	6.603	0.00321
	Inoculation:Watering	2	1.743	0.1875
Leaf Water Potential				
	Inoculation	1	9.376	0.0054
	Watering	2	100.05	2.28E-12
	Inoculation:Watering	2	19.639	8.86E-06
Root shoot ratio				
	Inoculation	1	1.621	0.2099
	Watering	2	5.226	9.40E-03
	Inoculation:Watering	2	1.904	0.1616
Stomatal Conductance				
	Inoculation	1	9.483	0.0062
	Watering	2	2.649	0.0966
	Inoculation:Watering	2	0.094	0.9107
Photosynthetic Rate				
	Inoculation	1	4.411	0.0493
	Watering	2	1.359	0.2807
	Inoculation:Watering	2	0.142	0.8687
% Nitrogen				
	Inoculation	1	3.777	0.0587
	Watering	2	5.936	0.0054
	Inoculation:Watering	2	4.146	0.0227
Phosporous				
	Inoculation	1	8.282	0.0063
	Watering	2	1.134	0.3314
	Inoculation:Watering	2	5.879	0.0056

Table 1.

Supplemental/Appendices

Fig S2