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

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

3

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9

10 **Summary**

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

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

31

## 32 **Introduction**

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

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

57           Plants control stomatal conductance to prevent water loss but do so at the cost of slowing  
58 CO<sub>2</sub> diffusion into the leaf, leading to a subsequent reduction in carbon fixation. AMF have been  
59 shown to modify plant hormonal responses, leading to downstream effects that increase stomatal  
60 efficiency (Kaushal, 2019). In addition to water loss through poor stomatal control, many plants  
61 experience a decrease in photosynthesis under drought, which can cause physiological  
62 complications such as increasing photorespiration and reducing plant production (Reddy *et al.*,

63 2004). There is some evidence that mycorrhizal plants may be able to maintain a better  
64 photosynthetic rate than nonmycorrhizal plants under drought stress (Bakr *et al.*, 2018; Zhao *et*  
65 *al.*, 2015; Ruíz-Sánchez *et al.*, 2011). Finally, better nutrient uptake and balance may be a  
66 potentially important mechanism explaining drought response in mycorrhizal plants. Nutrient  
67 availability goes down in water-stressed soils because plants primarily absorb soluble nutrients  
68 (Rouphael *et al.*, 2012). Greater nutrient acquisition could explain why there is often increased  
69 growth in mycorrhizal plants, and recent research has shown that AMF phosphorous acquisition  
70 becomes increasingly important under drought stress (Püschel *et al.*, 2021). Overall, AMF  
71 potentially increase plant performance under drought via several mechanisms, ranging from the  
72 molecular to the whole-plant level.

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

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

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

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

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

122

## 123 **Materials and Methods**

### 124 **Experimental Design and Materials**

125 The experiment was conducted at University of Montana's Dietrich Greenhouse in Missoula,  
126 Montana using *C. canadensis* seeds collected from a population on MPG Ranch outside  
127 Florence, Montana (46°40'48.92" N, 114°1'40.73" W). Seeds were sown in common potting soil  
128 and watered as needed for 14 days. They were then transplanted into four-inch pots with a 1:1:1  
129 mixture of autoclaved local soil, sand, and Turface (pH 7.2, NO<sub>3</sub><sup>-</sup> 20.7 mg kg<sup>-1</sup>, P<sub>Merlich</sub> 20 mg kg<sup>-1</sup>).  
130 Half of all pots were inoculated at transplanting by placing 50 mL of AMF inoculum below  
131 the roots containing a mixture of eight species of AMF and 37 spores/mL in addition to hyphal  
132 fragments and colonized root pieces. Control plots were given heat treated (95°C for 12 hrs)  
133 AMF inoculum and 25 mL microbial wash made from an 1:10 (inoculum:water) sieved two  
134 times through a Fishman P8 filter paper (<20µm) to minimize differences in other soil biota  
135 among treatments. To allow for establishment, seedlings were watered as needed for an  
136 additional 21 days. Each plant was then exposed to one of three watering levels: control (no  
137 stress), moderate drought stress, and severe drought stress. The severity of drought stress was  
138 measured on a subset of pots as percent soil moisture. Each of the six treatments were replicated  
139 eight times, resulting in 48 pots total. All plants were harvested eight weeks after transplanting.

140

### 141 **Drought Treatments**

142 Water stress was implemented using a "wick" method (Toth *et al.*, 1988). Each pot had two felt  
143 strings with one end in the soil matrix and the other end in a basin of water (Fig. 1). Increasing  
144 the height the pots were raised from the basin of water reduced the amount of water delivered to  
145 the pots and thus increased the water stress (Fig. 1). Control, moderate, and severe watering  
146 treatments were kept at an average 18%, 8%, and 5% volumetric soil water content, respectively  
147 (Fig. S2). The control treatments were on average higher in the mycorrhizal pots (~20%)  
148 compared to non-mycorrhizal pots, due to unknown reasons (Fig. S2). However, this was not a  
149 concern as it only occurred in the control pots and the difference was relatively small.

150 Treatments were organized in blocks sharing a central container of water, with four of each  
151 watering treatment (control, moderate stress, and severe stress). AM and NM treatments were  
152 kept separate to eliminate the risk of contamination. The wicks were replaced three weeks after  
153 transplantation due to natural degradation and bacterial mats forming. After replacement,  
154 tetracycline at a concentration of 12.5 µg/ml were added to the water basins to prevent further  
155 bacterial mat formation.

156 **Measurements**

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

168

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

177

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

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

195

## 196 **Statistical Analysis**

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

202

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

207

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

210

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

213

## 214 **Results**

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

218 0.005) (Fig. 4) all had inoculation as a significant factor. Significant differences between  
219 watering treatments were found in five of the six plant performance variables (Table 1). Shoot  
220 dry weight ( $F_{(2,42)}= 32.703$ ,  $p= 2.73e-09$ ), root dry weight ( $F_{(2,42)}= 6.461$ ,  $p= 0.00358$ ), root water  
221 content ( $F_{(2,42)}= 6.603$ ,  $p= 0.00321$ ), leaf water potential ( $F_{(2,24)}= 100.05$ ,  $p= 2.28e-12$ ), and root  
222 shoot ratio ( $F_{(2,42)}= 5.226$ ,  $p= 0.0094$ ) all had significant difference between watering treatments.  
223 Additionally, interactive effects between inoculation and watering treatment were found in leaf  
224 water potential ( $F_{(2,24)}= 19.639$ ,  $p= 8.86e-06$ ). Overall, we find that the presence of AMF  
225 influences some aspects of plant performance, but that watering has a much higher influence on  
226 performance variables.

227 We found that three of the four proposed drought tolerance mechanisms differed between  
228 inoculation groups (question 2). Stomatal conductance ( $F_{(1,19)}= 9.483$ ,  $p= 0.00617$ ) (Fig. 5a),  
229 photosynthetic rate ( $F_{(1,19)}= 4.411$ ,  $p= 0.0493$ ) (Fig. 5b), and phosphorous concentrations ( $F_{(1,42)}=$   
230  $8.282$ ,  $p= 0.00627$ ) (Fig. 6b) all differed significantly between inoculation treatments. Significant  
231 differences between watering treatments were only found for nitrogen concentration ( $F_{(1,42)}=$   
232  $5.936$ ,  $p= 0.00536$ ) (Fig. 6a). Interactive effects between inoculation and watering treatments  
233 were found for both nitrogen ( $F_{(1,42)}= 4.146$ ,  $p= 0.02274$ ) and phosphorous ( $F_{(1,42)}= 5.879$ ,  $p=$   
234  $0.00561$ ) concentrations. In summary, inoculation influenced most of the proposed drought  
235 tolerance mechanisms, and the interaction between AMF and watering seemed to primarily  
236 influence plant nutrition.

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

240

## 241 **Discussion**

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

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

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

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

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

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

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

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

312

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317

### 318 **Literature Cited**

319

320 **Alpert P. 2006.** Constraints of tolerance: why are desiccation-tolerant organisms so small or  
321 rare? *Journal of Experimental Biology* **209**: 1575–1584.

322 **Anjum SA, Xie X, Wang L, Saleem MF, Man C, Lei W. 2011.** Morphological, physiological  
323 and biochemical responses of plants to drought stress. *African Journal of Agricultural Research*  
324 **6**: 2026–2032.

325 **Augé RM. 2001.** Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis.  
326 *Mycorrhiza* **11**: 3–42.

327 **Bahadur A, Batool A, Nasir F, Jiang S, Mingsen Q, Zhang Q, Pan J, Liu Y, Feng H. 2019.**  
328 Mechanistic Insights into Arbuscular Mycorrhizal Fungi-Mediated Drought Stress Tolerance in  
329 Plants. *International journal of molecular sciences* **20**: 4199.

330 **Bakr J, Pék Z, Helyes L, Posta K. 2018.** Mycorrhizal Inoculation Alleviates Water Deficit  
331 Impact on Field-Grown Processing Tomato. *Polish Journal of Environmental Studies* **27**: 1949–  
332 1958.

333 **Biosciences L-C. 2001.** *LI-6400 Portable Photosynthesis System*.

334 **Buhler DD, Owen MDK. 1997.** Emergence and survival of horseweed (*Coryza canadensis*).  
335 *Weed Science* **45**: 98–101.

336 **Dalgaard T, Hutchings NJ, Porter JR. 2003.** Agroecology, scaling and interdisciplinarity.  
337 *Agriculture, Ecosystems & Environment* **100**: 39–51.

338 **Delavaux CS, Smith-Ramesh LM, Kuebbing SE. 2017.** Beyond nutrients: a meta-analysis of  
339 the diverse effects of arbuscular mycorrhizal fungi on plants and soils. *Ecology* **98**: 2111–2119.

340 **Eziz A, Yan Z, Tian D, Han W, Tang Z, Fang J. 2017.** Drought effect on plant biomass  
341 allocation: A meta-analysis. *Ecology and Evolution* **7**: 11002–11010.

342 **Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA. 2009.** Plant drought stress: effects,  
343 mechanisms and management. *Agronomy for Sustainable Development* **29**: 185–212.

- 344 **Halvorson A, Reule C. 1994.** Nitrogen Fertilizer Requirements in an Annual Dryland Cropping  
345 System. *Agronomy Journal* **86**: 315–318.
- 346 **Hart MM, Reader RJ, Klironomos JN. 2003.** Plant coexistence mediated by arbuscular  
347 mycorrhizal fungi. *Trends in Ecology & Evolution* **18**: 418–423.
- 348 **Jarvis PG. 1976.** The Interpretation of the Variations in Leaf Water Potential and Stomatal  
349 Conductance Found in Canopies in the Field. *Philosophical Transactions of the Royal Society of*  
350 *London. Series B, Biological Sciences* **273**: 593–610.
- 351 **Johnson NC, Graham J-H, Smith FA. 1997.** Functioning of mycorrhizal associations along the  
352 mutualism–parasitism continuum\*. *New Phytologist* **135**: 575–585.
- 353 **Kathleen KT. 2013.** The extent of mycorrhizal colonization of roots and its influence on plant  
354 growth and phosphorus content. *Plant and soil* **371**: 1–13.
- 355 **Kaushal M. 2019.** Microbes in Cahoots with Plants: MIST to Hit the Jackpot of Agricultural  
356 Productivity during Drought. *International journal of molecular sciences* **20**: 1769.
- 357 **Lenoir I, Fontaine J, Sahraoui AL-H. 2016.** *Arbuscular mycorrhizal fungal responses to*  
358 *abiotic stresses: A review.*
- 359 **Mahajan S, Tuteja N. 2005.** Cold, salinity and drought stresses: An overview. *Archives of*  
360 *Biochemistry and Biophysics* **444**: 139–158.
- 361 **Martínez-Vilalta J, Sala A, Dolores A, Lucía G, Günter H, Sara P, Frida P, Francisco L.**  
362 **2016.** Dynamics of non-structural carbohydrates in terrestrial plants: a global synthesis.  
363 *Ecological Monographs* **86**: 495–516.
- 364 **McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA. 1990.** A new method which  
365 gives an objective measure of colonization of roots by vesicular—arbuscular mycorrhizal fungi.  
366 *New Phytologist* **115**: 495–501.
- 367 **Miransari M, Abrishamchi A, Khoshbakht K, Niknam V. 2014.** Plant hormones as signals in  
368 arbuscular mycorrhizal symbiosis. *Critical Reviews in Biotechnology* **34**: 123–133.
- 369 **Mohan JE, Cowden CC, Baas P, Dawadi A, Frankson PT, Helmick K, Hughes E, Khan S,**  
370 **Lang A, Machmuller M, et al. 2014.** Mycorrhizal fungi mediation of terrestrial ecosystem  
371 responses to global change: mini-review. *Fungal Ecology* **10**: 3–19.
- 372 **Moradi P. 2016.** Key plant products and common mechanisms utilized by plants in water deficit  
373 stress responses. *Botanical Sciences* **94**: 671.
- 374 **Petipas RH, Gonzalez JB, Palmer TM, Brody AK. 2017.** Habitat-specific AMF symbioses  
375 enhance drought tolerance of a native Kenyan grass. *Acta Oecologica-International Journal of*  
376 *Ecology* **78**: 71–78.

- 377 **Porto DL, de Santana Arauco AM, Boechat CL, Silva A de O, Moitinho MR, Gomes de**  
378 **Farias SG. 2020.** Arbuscular mycorrhizal fungi on the initial growth and nutrition of *Parkia*  
379 *platycephala* Benth. under water stress. *Cerne* **26**: 66–74.
- 380 **Püschel D, Bitterlich M, Rydlová J, Jansa J. 2021.** Drought accentuates the role of mycorrhiza  
381 in phosphorus uptake. *Soil Biology and Biochemistry* **157**: 108243.
- 382 **Reddy AR, Chaitanya KV, Vivekanandan M. 2004.** Drought-induced responses of  
383 photosynthesis and antioxidant metabolism in higher plants. *Journal of Plant Physiology* **161**:  
384 1189–1202.
- 385 **Rinaudo V, Bàrberi P, Giovannetti M, van der Heijden GA. 2010.** Mycorrhizal fungi  
386 suppress aggressive agricultural weeds. *Plant and soil* **333**: 7–20.
- 387 **Rodriguez D, Goudriaan J, Oyarzabal M, Pomar MC. 1996.** Phosphorus nutrition and water  
388 stress tolerance in wheat plants. *Journal of Plant Nutrition* **19**: 29–39.
- 389 **Rouphael Y, Cardarelli M, Schwarz D, Franken P, Colla G. 2012.** Plant Responses to  
390 Drought Stress. In: 171–198.
- 391 **Ruíz-Sánchez M, Armada E, Muñoz Y, García de Salamone IE, Aroca R, Ruíz-Lozano JM,**  
392 **Azcón R. 2011.** *Azospirillum* and arbuscular mycorrhizal colonization enhance rice growth and  
393 *physiological traits under well-watered and drought conditions.*
- 394 **Shah MA, Reshi Z, Rashid I. 2008.** *Mycorrhizal source and neighbour identity differently*  
395 *influence Anthemis cotula L. invasion in the Kashmir Himalaya, India.*
- 396 **Toth J, Nurthen EJ, Chan KY. 1988.** A simple wick method for watering potted plants which  
397 maintains a chosen moisture regime. *Australian Journal of Experimental Agriculture* **28**: 805–  
398 808.
- 399 **Van der Heijden MG, Sanders IR. 2002.** Mycorrhizal ecology: synthesis and perspectives. In:  
400 Mycorrhizal ecology. Springer, 441–456.
- 401 **Wright DP, Read DJ, Scholes JD. 1998.** Mycorrhizal sink strength influences whole plant  
402 carbon balance of *Trifolium repens* L. *Plant, Cell & Environment* **21**: 881–891.
- 403 **Wu Q-S, Xia R-X. 2006.** *Arbuscular mycorrhizal fungi influence growth, osmotic adjustment*  
404 *and photosynthesis of citrus under well-watered and water stress conditions.*
- 405 **Xu H, Cooke JEK, Zwiazek JJ. 2013.** Phylogenetic analysis of fungal aquaporins provides  
406 insight into their possible role in water transport of mycorrhizal associations. *Botany* **91**: 495–  
407 504.
- 408 **Zhao R, Guo W, Bi N, Guo J, Wang L, Zhao J, Zhang J. 2015.** *Arbuscular mycorrhizal fungi*  
409 *affect the growth, nutrient uptake and water status of maize (Zea mays L.) grown in two types of*  
410 *coal mine spoils under drought stress.*

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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<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> 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).

441 **Figure 10** Means and standard errors of percent arbuscules. The green color represents the  
442 control group (C), yellow the moderate stress group (M), and red the severe stress group (S).  
443 **Table 1.** The degrees of freedom (DF), F statistic, and p values (Inoculation, Watering, and  
444 Inoculation x Watering) for the six plant performance variable and four proposed drought  
445 tolerance mechanisms.

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448 **Figures**

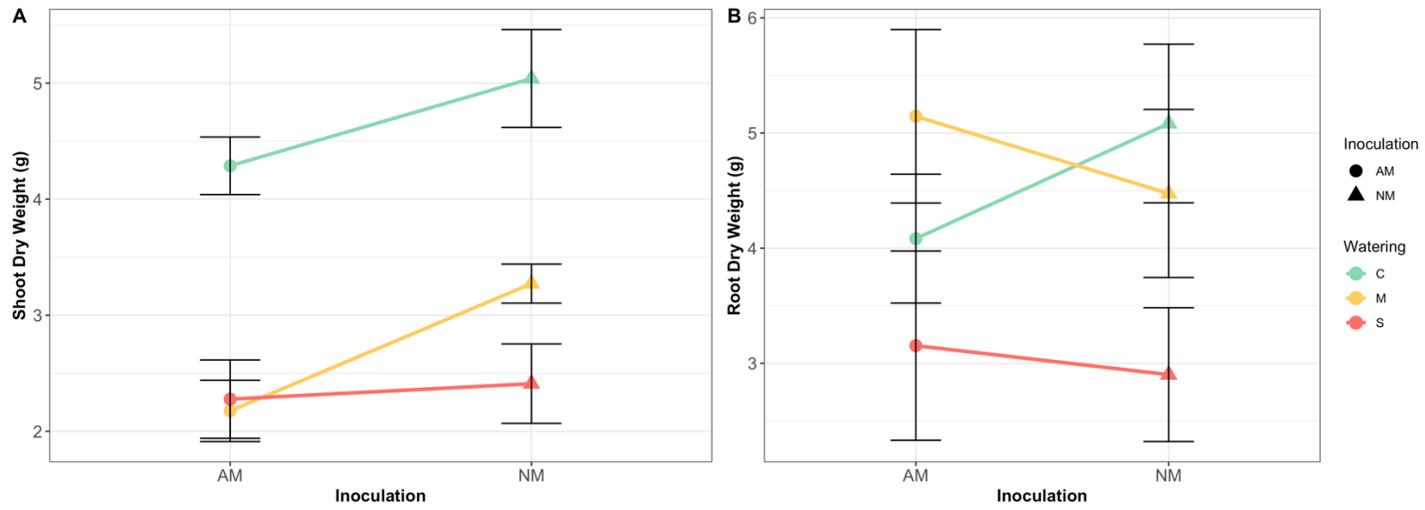
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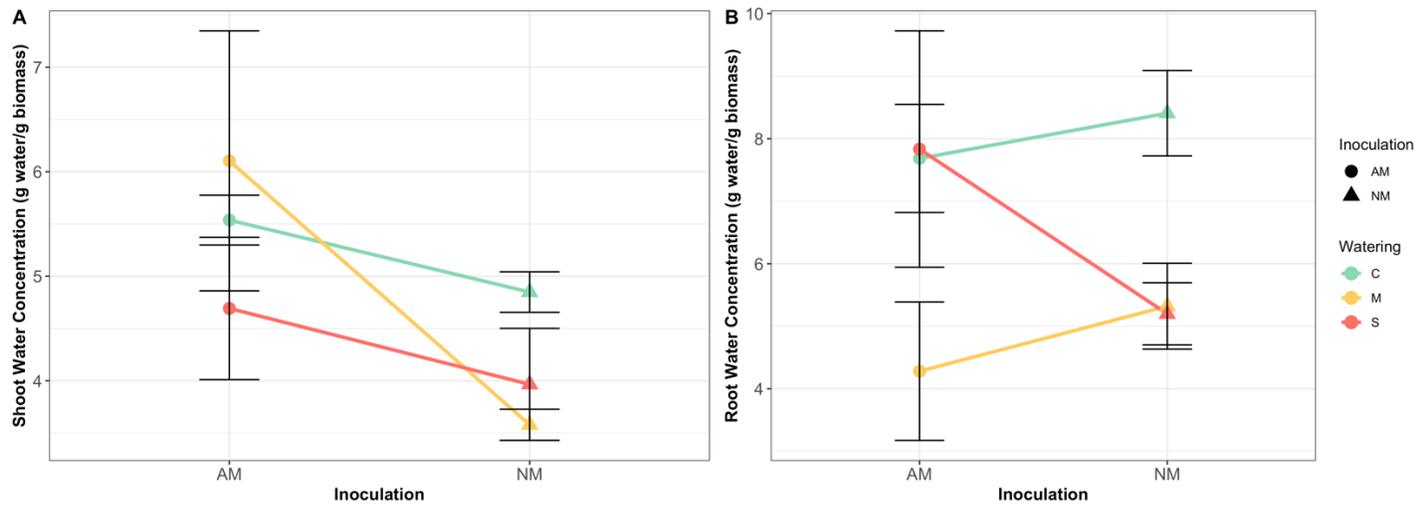
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451 **Figure 1**

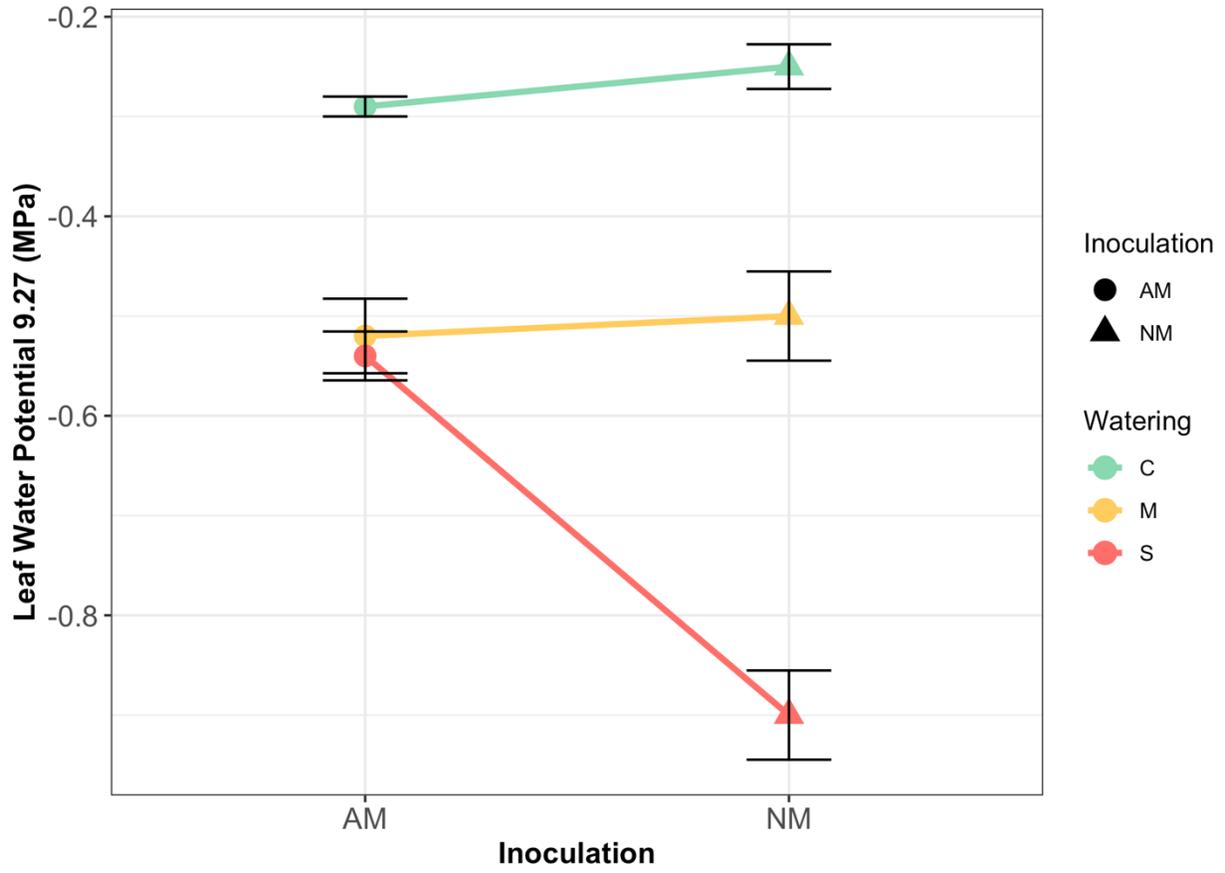
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454 **Figure 2**  
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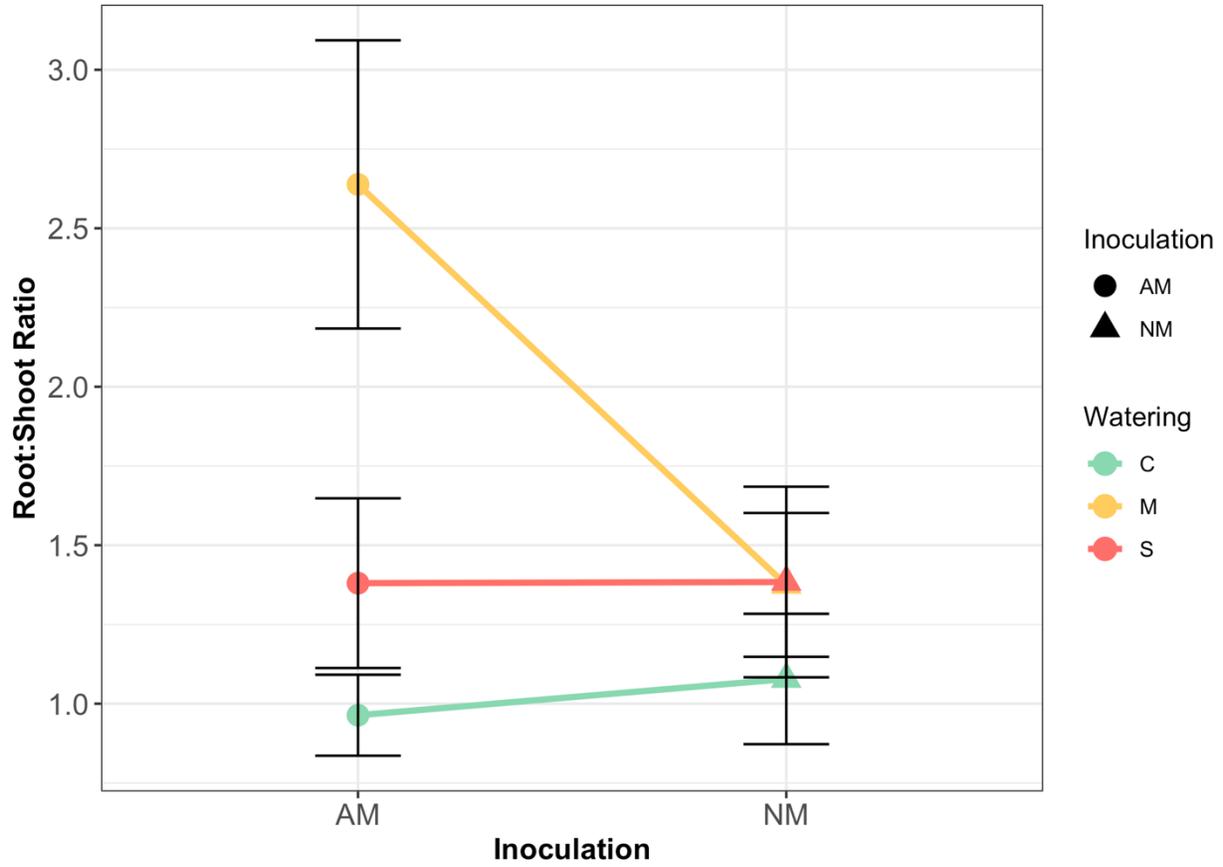


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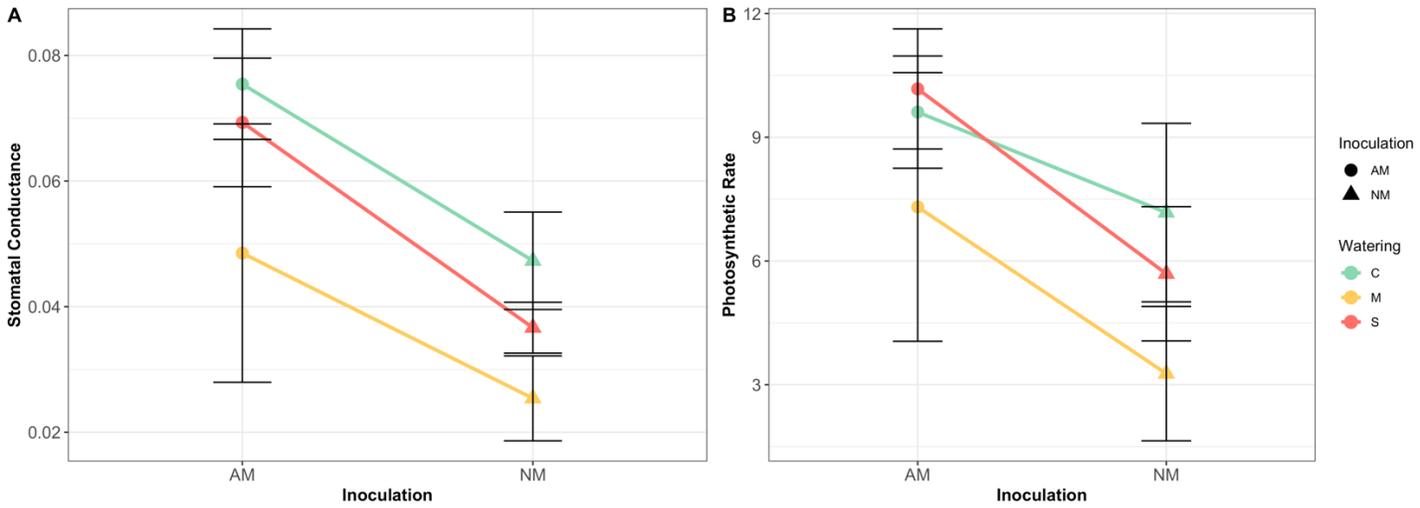


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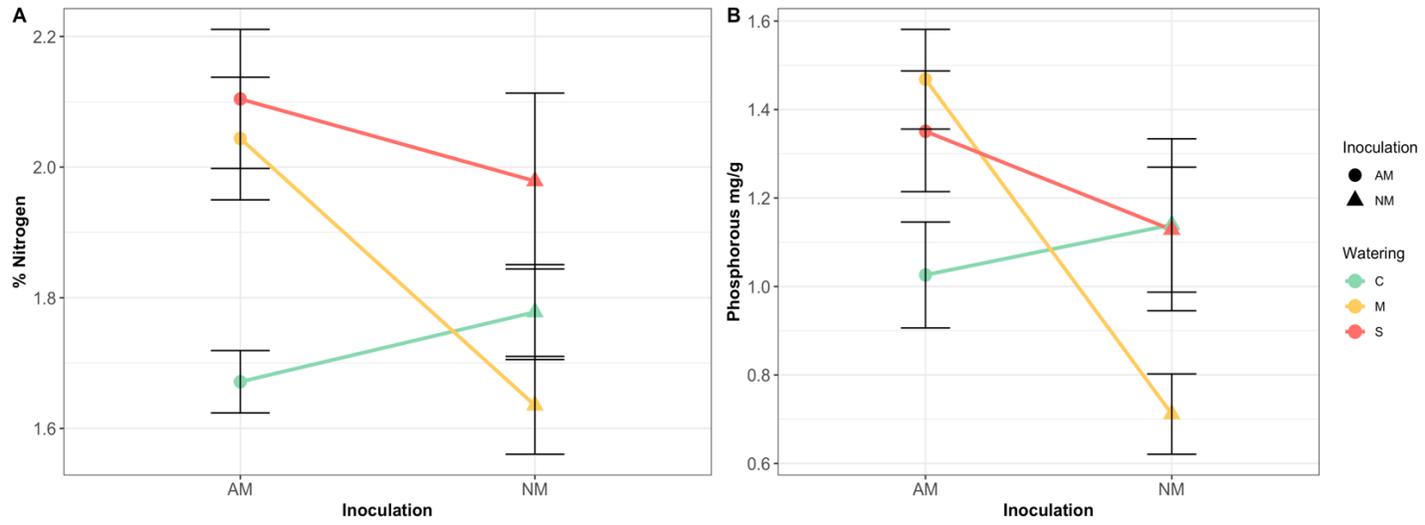
Figure 4



467 **Figure 5**  
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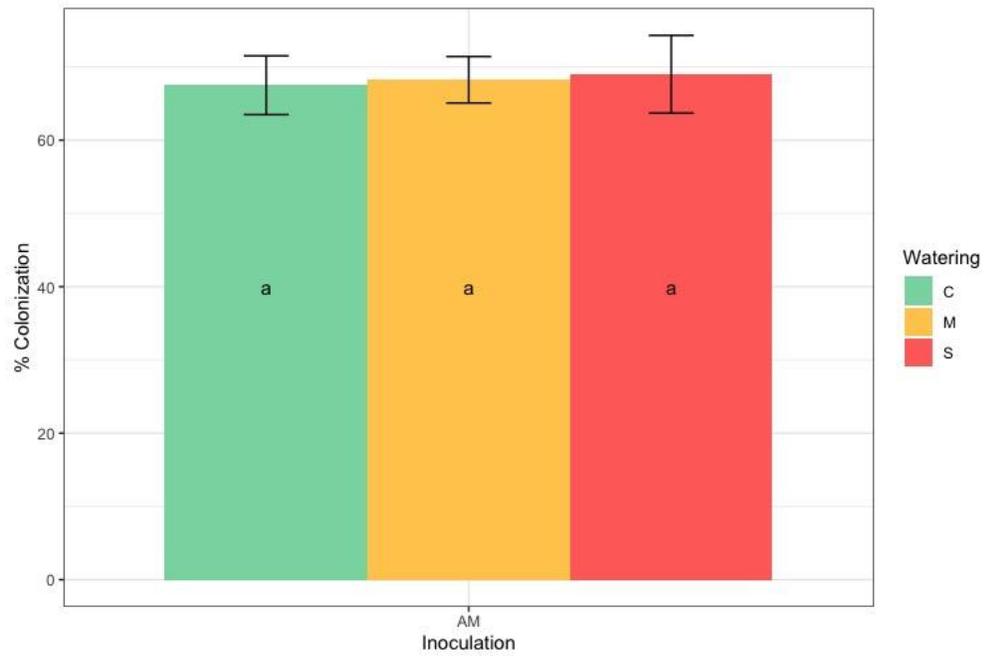


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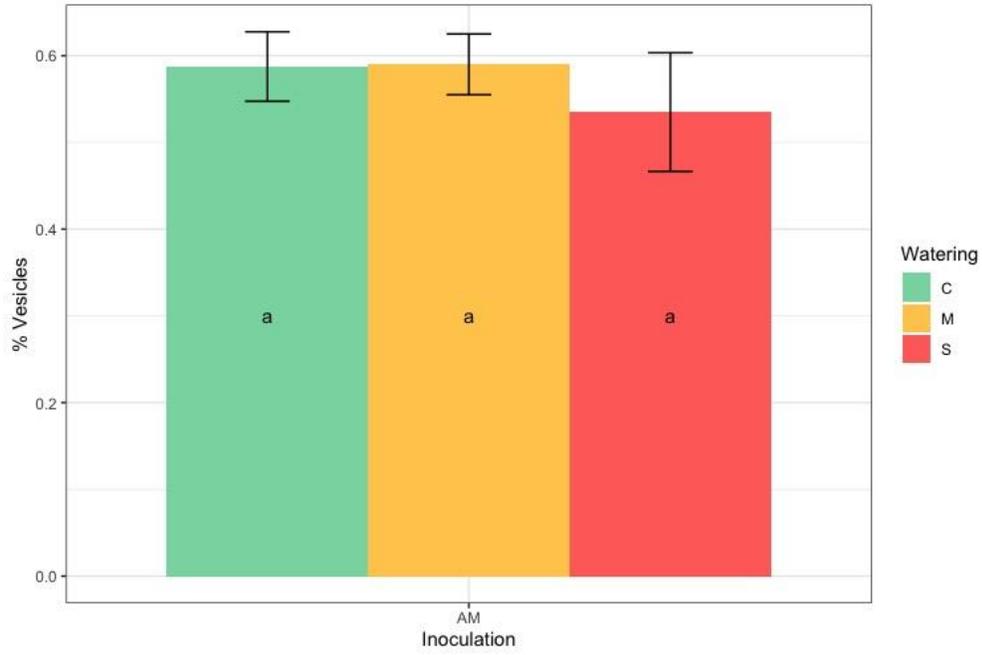
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**Figure 7**

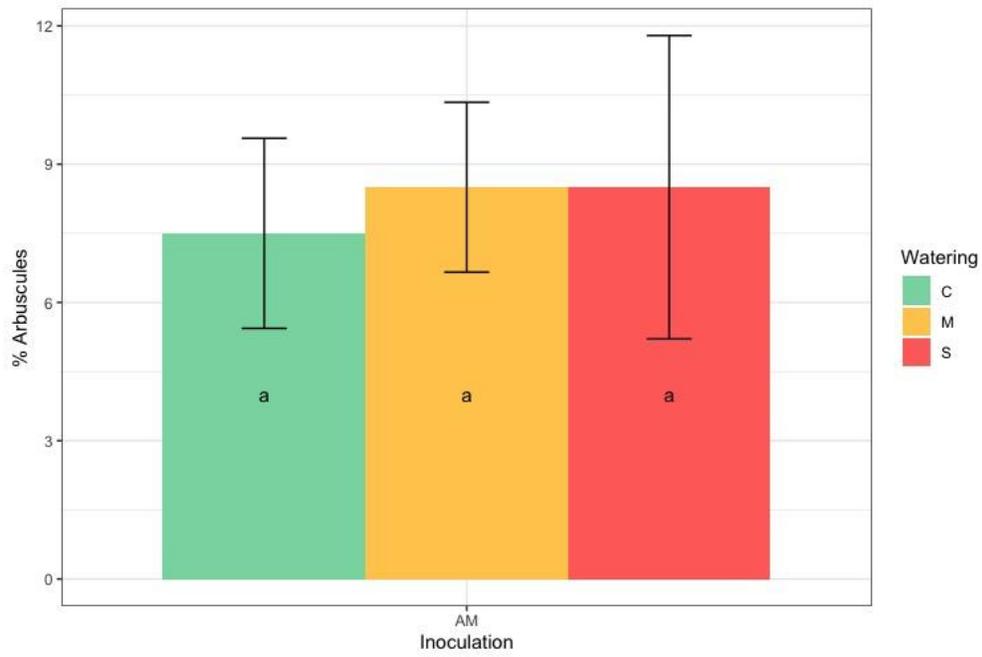


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**Figure 8**



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483 **Figure 9**  
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486 **Figure 10**

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

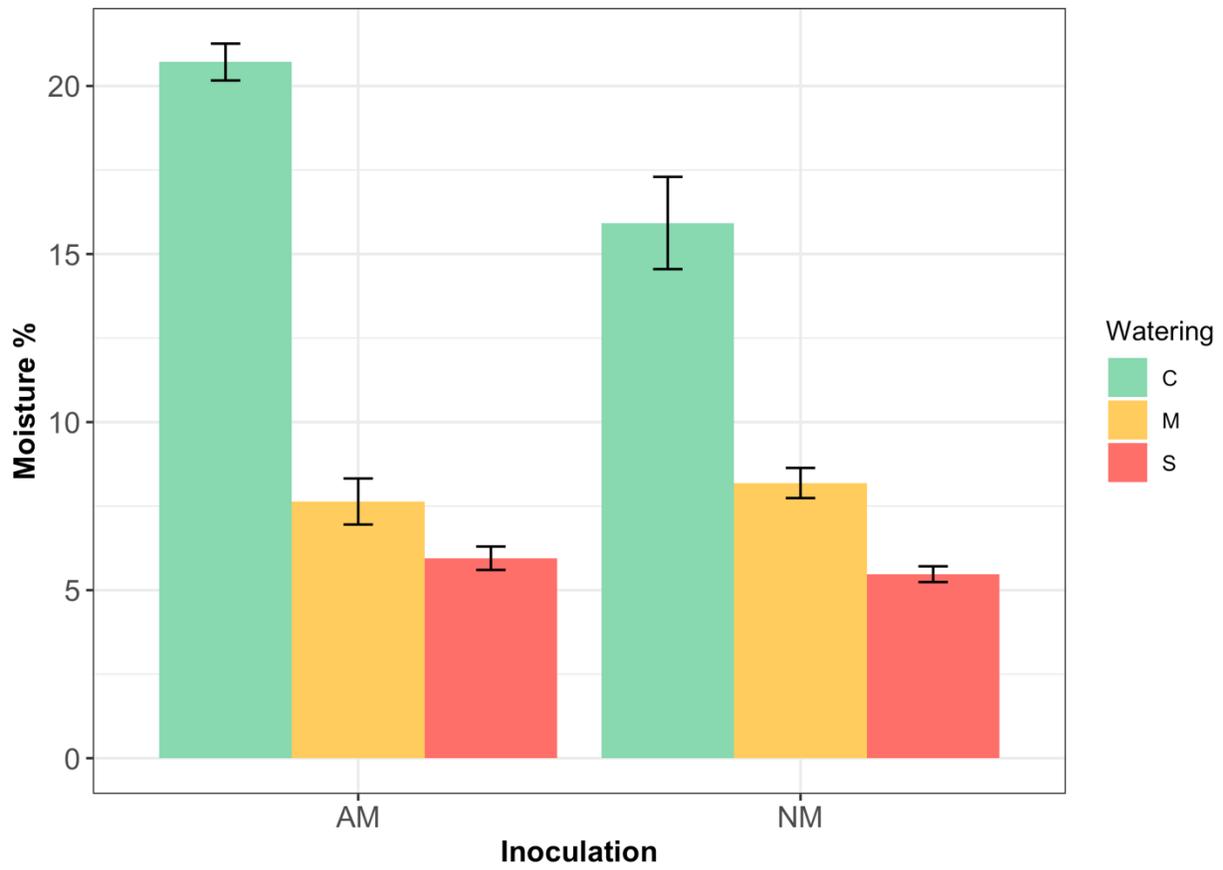
**Table 1.**

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493 Supplemental/Appendices

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495 Fig S2



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