

Effect of Arbuscular Mycorrhiza Fungi on Transpiration and Leaf Water Potential in Drought-Stressed Maize

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Abstract

Stomata behavior has profound implications for water fluxes between soil and the atmosphere. It remains unknown what exactly triggers stomata closure when there is a soil water deficit. Meanwhile, it is understood that arbuscular mycorrhizae fungi (AMF) improve the water retention quality of soils and root water uptake. The objective of this study was to demonstrate a link between the influence of AMF on the water status of maize plants when exposed to drought. It was hypothesized that soils in the rhizosphere of plants inoculated with AMF have a less severe drop in soil matric potential, allowing for increased and extended water uptake at the soil-root interface during soil drying, and subsequently, as soil dried, an enhanced plant water status would be observed for inoculated plants. To test these hypotheses, measurements were taken of transpiration rates, soil water content, leaf water potential, above- and belowground biomass, and root morphology of maize grown with and without AMF inoculation and exposed to drought conditions. The results demonstrated that, as soil dried, AMF symbiosis allowed for a more gradual decline of leaf water potential, facilitating both higher and sustained transpiration rates when compared with plants grown without AMF inoculation. It is concluded that AMF supported maintaining the hydraulic continuity between maize roots and drying soils, reducing the drop in matric potential at the root-soil interface. These findings express the importance in linking AMF colonization with plant hydraulics and stomatal conductance to improve plant resistance to drought, as well as supporting more precise predictions of plant response to soil drying, especially in the face of climate change.

Introduction

The combustion of fossil fuels and the subsequent increased concentration of atmospheric CO₂ has led to a positive radiative forcing in the Earth's climate system. Among other effects, this anthropogenic forcing has increased climate variability and the frequency of extreme natural events, including drought (Field & Barros, 2014). Drought affects more people than any other extreme event (Chiang et al., 2021) and is increasing in frequency, duration, and intensity in many locations across the globe (Carrão et al., 2016; John et al., 2018).

Drought is the primary threat to agricultural production, causing declines in yield and increasing plant mortality (Sperry & Love, 2015, p. 14). Of particular concern are arid and semi-arid environments where crop yields are already sensitive to water-limitations (Field & Barros, 2014; Hillel, D., & C. Rosenzweig). Compounding these challenges are growing competition for arable land, increasing affluence, and intensified consumption patterns (Gerbens-Leenes et al.; Smith et al., 2010). Human population growth amplifies existing pressures, with the global population projected to reach 9.7 billion by the year 2050 (United Nations et al., 2019). To meet rising demands, forecasts for global crop production call for increases of 100%–110% from 2005 to 2050 (Tilman et al., 2011). Remarkably, agricultural production and distribution is already failing to meet global needs. The Food & Agriculture Organization of the United Nations (2020) reported that, as of 2014, one billion people were undernourished, and drought-induced food shortages had led to migrations, massive famines, civil unrest, and worsening social tensions.

Relying on additional freshwater or more efficient irrigation technologies to counter the effects of drought on crop production are not adequate solutions. The agricultural sector already accounts for approximately 70% of fresh water usage (Siebert et al., 2010). As competition for fresh water resources increases, there will be growing pressure on the agriculture sector to give up some of its share, requiring it to actually produce more food with less water (Vadez et al., 2014).

The function and effects of food production extend beyond food security and dependence on freshwater resources. Agricultural land represents five billion hectares, or 38%, of the global land surface, of which approximately one-third is cropland (the remaining two-thirds being meadow and pasture for grazing livestock) (Food & Agriculture Organization of the United Nations, 2020). Clearing additional land for agriculture causes habitat fragmentation, threatens biodiversity, and in many cases, increases greenhouse gas emissions, thus exacerbating the effects of climate change (Tilman et al., 2011). Furthermore, converting additional land for food production may not be an option. Smith et al. (2010) indicated that only one-fifth of the cropland required to meet the rising demand for food until 2050 is available through land

conversion, and it's been posited that converting additional land for agriculture may destabilize the regulatory capacities of the Earth system (Rockström et al., 2007).

Clearly, adequate, reliable, global food production is in jeopardy and how that challenge is approached has major social and ecological implications. By understanding in detail how major food crops respond to water-limited conditions, there is an opportunity to develop agricultural products and practices that improve crop resilience to drought. Doing so will also allow for increased food security, as well as better prediction of the biogeochemical feedbacks between cropland, water and carbon cycles, and the climate, thus supporting an overall improved response to climate change (Sperry & Love, 2015).

Theoretical Framework

Soil-Plant-Air-Continuum

The soil-plant-air continuum (SPAC) describes the pathway of water movement from soil into plant roots, through the plant vascular system into the leaves, and through the stomata, where water evaporates into the atmosphere per the cohesion tension theory (Sperry et al., 1998). The flow of water through the SPAC is passive and driven by a gradient in water potential. This gradient depends on soil hydraulic conductivity, xylem conductance, stomata conductance, and the hydraulic capacities of the leaf and stem. These factors interact with boundary conditions including soil water potential (ψ_{soil}), vapor pressure deficit (VPD), and the aerodynamic conductance of the atmosphere into which the plant water vapor evaporates (Manzoni et al., 2014).

Stomata are the gatekeepers, regulating the exchange of water vapor from the plant to the atmosphere. Conductance of water vapor through the stomata is vitally important to agronomy and ecology, partially because greater than 90% of water taken up by roots is lost through transpiration (Jasechko et al., 2013), which, together with evaporation, accounts for approximately 35% of terrestrial precipitation (van der Ent et al., 2014). These enormous water fluxes exert major influences across scales, from plant growth and productivity to global carbon and water cycling (Hetherington & Woodward, 2003; Lin et al., 2015). In water limited conditions, e.g. drought, stomata must open and close within a particular margin to avoid embolism and possible plant mortality (Manzoni et al., 2014).

It has been found that hormonal signals, such as those from abscisic acid, trigger stomatal closure (Brodribb & McAdam, 2017; Buckley, 2017). Hydraulic prompts have also been found to trigger closure. For example, stomata close in order to avoid excessive drops in leaf water potential (ψ_{leaf}) by responding to non-linearities in the relationship between transpiration rate (E) and ψ_{leaf} (Sperry et al., 2016; 2015). Still, despite decades of fruitful study, what triggers stomata closure remains a controversial matter (Comstock

& Mencuccini, 1998). There are competing theories on above-ground triggers, including stomata closure being closely coordinated with xylem cavitation (Sperry & Love, 2015), driven by signals resulting from changes in the leaf turgor pressure (Huber et al., 2019), or a mediated response of the water status in the leaves (Ripullone et al., 2007). In contrast to these studies, Corso et al. (2020) concluded that neither xylem vulnerability nor leaf status is the cause of stomata closure in wheat.

Concurrently, inquiries have been made into belowground triggers, the findings of which repeatedly point to the limits in belowground hydraulic properties as the initial catalyst for stomata closure during water deficits. For example, losses in root hydraulic conductance in olive trees was found to induce stomata closure (Rodriguez-Dominguez & Brodribb, 2020). Similarly, increased resistance in soil-root hydraulics drove stomata closure in tomato plants under water stress (Abdalla et al., 2021). With maize, Hayat et al. (2019) found that decreases in soil-plant hydraulic conductance was the primary origin of stomata closure, and a recent meta-analysis and modeling study asserts soil hydraulic properties (and not xylem conductance) triggers stomatal closure (Carminati & Javaux, 2020).

Following the logic that it is soil hydraulic properties that trigger stomata response to water stress, one must consider the conditions of the rhizosphere. The rhizosphere is the region of soil in the vicinity of plant roots which differs from surrounding soil in part from the input of root exudates and the high diversity of bacterial and fungal communities. Here, exchanges between the plant, microorganisms and fungal communities occur which are central to ecosystem functioning and biogeochemical cycles, including, but not limited to, the formation of soil organic matter and carbon sequestration, two examples with profound implications for agriculture and climate change. It has been stated that it is impossible to solve ecological problems without a thorough understanding of the processes operating in the soils of the rhizosphere (Sokolova, 2015). Still, the effects of the rhizosphere on root water uptake are commonly neglected (Carminati et al., 2010, p. 163).

Amongst the vast remaining unknowns in the rhizosphere, it is understood that by changing the structure of a soil, arbuscular mycorrhizal fungi (AMF) can influence soil hydraulic properties (Augé, 2001, 2004; Bitterlich, M. et al., 2018). Clarity on the effect of AMF symbiosis in root water uptake, and associated plant responses to water limitation, offer prospects for supporting crops in meeting the evaporative demand required for survival when soils are persistently dry or there is a continued high-pressure deficit.

Arbuscular mycorrhizae fungi

AMF have co-evolved with vascular plants for hundreds of millions of years and today share interdependent connections with more than 71% of angiosperms (Brundrett & Tedersoo, 2019), including major crop species (Rouphael et al., 2015; Turrini et al., 2018). There is a multitude of beneficial effects AMF symbiosis can have on crops such as improving nutrient status and quality (Begum et al., 2019; Bona et al.,

2017; Castellanos-Morales et al., 2010; Hart et al., 2015). AMF symbiosis can also buffer plant responses to both biotic and abiotic stress (Gianinazzi et al., 2010), in part by increasing nutrient supplies (e.g. Cu, Fe, N, P, and Zn) in exchange for plant carbon (Begum et al., 2019), and supporting the formation of soil aggregates by binding small soil particles together, thus increasing the water-holding capacity of soil (Bedini et al., 2009; Gianinazzi et al., 2010; Leifheit et al., 2014; Rillig et al., 2010).

Additionally, AMF symbiosis effects hydraulic conductivity within plant roots through the extension of root soil contact via a hyphal network, which helps maintain higher stomatal conductance and E when there is a soil water deficit (Bitterlich et al., 2018; Quiroga et al., 2019). Still, there remains a need to understand the influence of AMF on ψ_{leaf} in conjunction with E rates during soil drying to better comprehend the mechanism by which AMF influence plant water status during water deficits.

Aims

This paper aims to further substantiate the observation that AMF symbiosis significantly influences the overall hydraulic coordination through the SPAC. To do so, a comparison is made of E and ψ_{leaf} between AMF inoculated and non-inoculated maize grown in a climate chamber and exposed to drought treatment. It is hypothesized that, in increasingly water deficit conditions, plants inoculated with AMF will have a less severe drop in soil matric potential in the rhizosphere, thereby reducing water fluxes at the root-soil interface (Fig 1a). Subsequently, as soil dries, AMF inoculated plants will present an enhanced plant water status, as exhibited by less negative ψ_{leaf} rates, and maintain higher E rates during soil drying than those plants grown without AMF inoculation (Fig. 1b). This indicates a sustained gradient of pressure through the vascular tissue, influencing stomata behavior, delaying potential xylem embolism and preventing plant mortality.

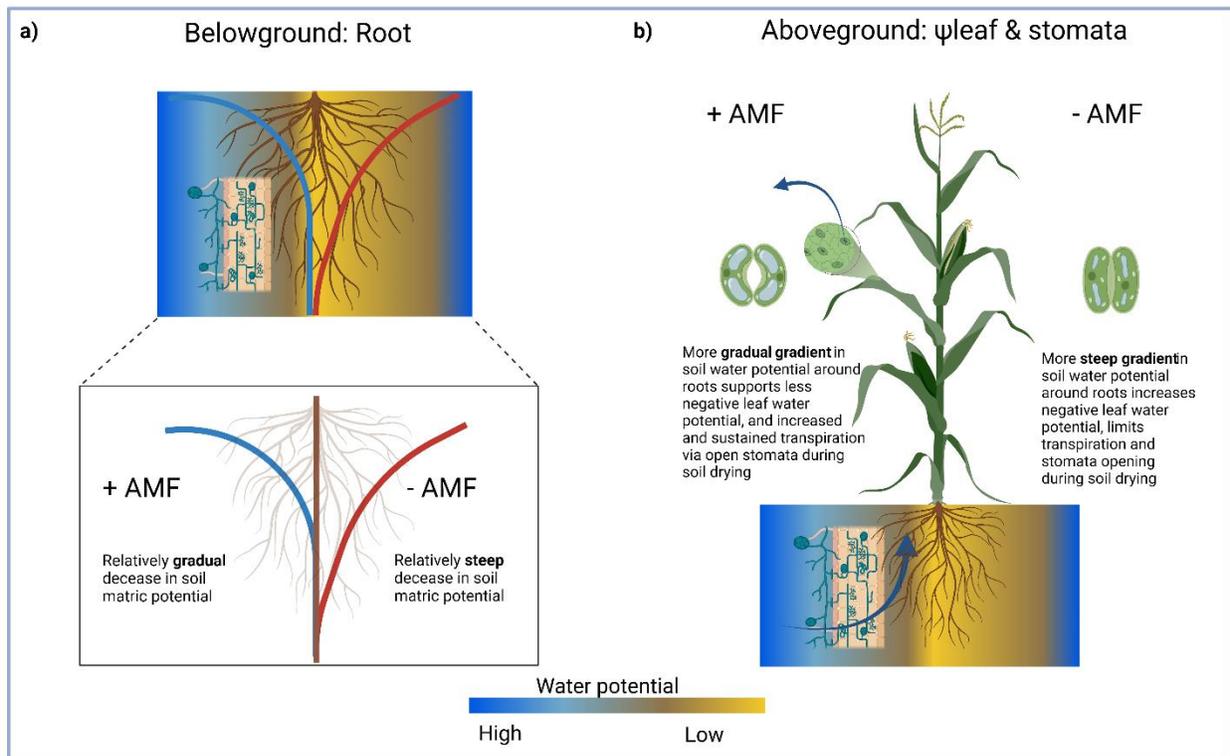


Figure 1. Graphical abstract: **a)** Hypothesized influence of AMF inoculation (+AMF) on soil matric potential. AMF symbiosis is understood to support radial movement of water toward plant roots, extend contact of colonized roots to otherwise inaccessible soil water resources, and to store water resources, thus buffering the decline in soil matric potential during soil drying. This study aims to show that by supporting a more gradual decrease in soil matric potential, **b)** leaf water potential in AMF inoculated plants remains less negative, transpiration rates are sustained longer, and corresponding stomata closure during soil drying is concretely linked with belowground hydraulic conductance. Created with BioRender.com.

Materials & Methodology

Soil & plant preparation

Sandy soil for this study was sourced in 2019 from the Helmholtz Center for Environmental Research SPP2089 experimental study site in Bad Lauchstädt, Germany. The sand was air dried and manually sifted through a 2 mm sieve. PVC pots with a 15 cm inner diameter and 40 cm height were washed with a soft detergent, rinsed and air dried. The pots have a 1.5 mm diameter outlet valve roughly 2 cm from the base allowing for release of excess water following irrigation. Glass wool was fixed over these outlets to ensure substrate was not lost through the valves.

AMF treated pots were prepared with approximately 1000 spores of sterile *Rhizophagus irregularis* DAOM197198 (Agronutrition, Labege, France). First, the spores were rinsed from their storage buffer six times using tap water and a 40 μm sieve. Washed spores were then resuspended in 5 mL of distilled water. The spore solution was hand mixed, with an additional 1 L of distilled water, into 8.26 kg unsterilized soil

and filled directly into pots. Individual batches were mixed for each pot to ensure consistency in spore allocation and homogeneity of mixture. Mixing containers were rinsed between batches. For those pots without AMF inoculation, 8.26 kg of dried soil was hand mixed with 1012.14 g distilled water in separate batches and filled directly into the pots. The result was that all pots contained 8.26 kg of soil and 1012.14 g liquid (including spore solution for inoculated pots), resulting in a volumetric water content (VWC) of 18% and bulk density of 1.4%. VWC of soil was calculated according to the following equation, where V_l is the volume of the liquid phase within the columns [L^3] contained in a total volume V_t [L^3].

$$VWC = \frac{V_l}{V_t} * 100$$

B73 maize (*Zea mays* L.) seeds were sterilized in a 10% H_2O_2 solution for ten minutes and then rinsed with distilled water. This process was repeated four times, after which the seeds were left to soak in distilled water for five minutes. Seeds were then submerged in a $CaSO_4$ solution for three hours, then placed on moist filter paper in Petri dishes to germinate.

Once germinated, two seeds were planted in each pot at a depth of 1 cm. Seedlings were planted such that they were equally spaced from each other and the edge of the pot. Once epicotyls were expressed above the soil surface, 0.2 kg of polyolefin pellets were added to the top of each pot, creating a layer of roughly 2 cm, to prevent splash effects during irrigation and soil surface evaporation.

In total, 76 pots were prepared, including six replicates for ten combinations of three maize genotypes. The remaining 16 were unplanted and/or test pots. In this study, only twelve of the pots are investigated. These are the pots with two B73 maize plants, six of which were inoculated and six un-inoculated.

Experiment set-up

Planted pots were randomly spaced in rows across laboratory tables in a climate-controlled chamber for eight weeks (Fig. 2). During this time, the day/night temperature was 28/18 °C, and the day/night relative humidity (RH) was 62/67%. The photoperiod was 14-hours and daytime light intensity was set to 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Lascar Electronics EasyLog USB Version 7.6; Whiteparish, Salisbury, UK). Seedlings were watered daily with 50 mL water per pot. As the plants grew, irrigation increased to 130 mL water every second day, at which time an additional 50 mL of adjusted Hoagland solution (Hoagland, 1950) (Supplementary Table 1) was applied.



Figure 2. *Experiment set-up: a) Two plants were planted in each pot; b) Once epicotyls were expressed above the soil surface, polyolefin pellets were added to reduce splashing during irrigation and prevent soil surface evaporation. Potted plants were randomly spaced in rows across two tables in a climate-controlled chamber: c) Mature plants on balances during drought treatment.*

Soil & plant measurements during soil drying

On both the 57th and 58th days after planting, pots were heavily irrigated such that excess water poured out the lower overflow valve. Pots were then relocated to a second chamber, randomized, and placed onto balances across two tables in the center of the chamber. At this time, the temperature and humidity conditions were adjusted to increase the VPD throughout the daytime photo period. Daytime conditions were set in two phases of 6-hours each: 29°C at 50% RH (VPD1) and 33°C at 40% RH (VPD2). Nighttime conditions were 18°C with 78% RH for 11 hours, and there was a one-hour transition period between day and night conditions (Fig. 3a, 3b).

Soil and plant measurements began on the 59th day after planting and spanned 11 days. Mid-day volumetric soil water content (θ [$\text{cm}^3 \text{cm}^{-3}$]) was measured daily using a time-domain refractometer (TDR) (E-Test, Lublin, Poland). Three TDR measurements were taken from different areas in the top of each pot and averaged for a representative daily soil water content value (Fig. 3c). The hydraulic properties of the soil mixture were determined through the evaporation method via a Hyprop system (UMS, Munich, Germany). The Peters Durner Iden (PDI) model was used to determine the soil retention curve and unsaturated hydraulic conductivity curve (Supplementary Fig. 1) (Peters et al., 2015). The soil hydraulic properties

were estimated by fitting the measured soil matric potential and solving the Richards equation (Abdalla et al., 2021; Cai et al., 2020).

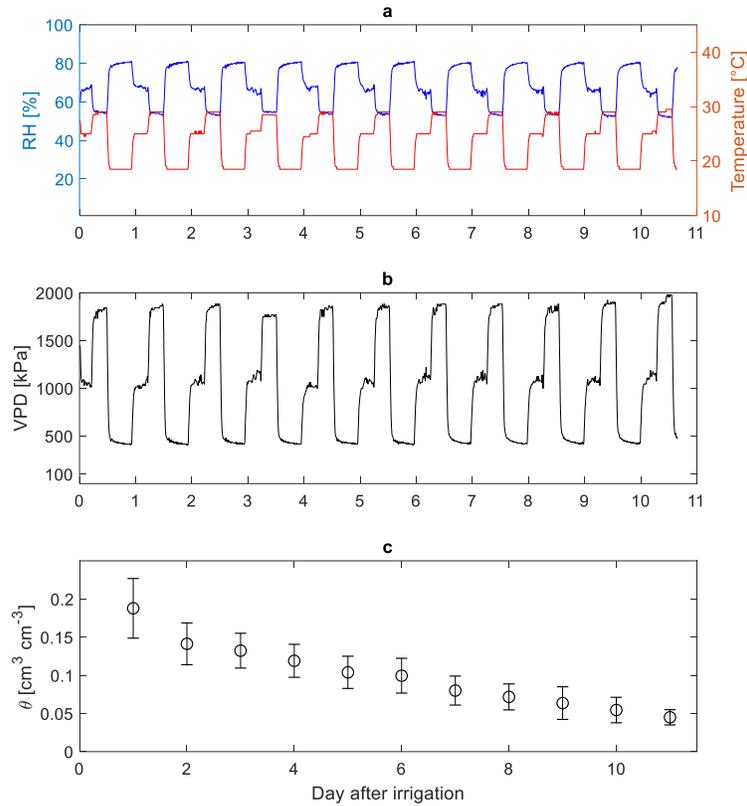


Figure 3. Plants were subjected to drought conditions including **a)** increasing temperature and reduced relative humidity during the daytime which resulted in **b)** an increasing vapor pressure deficit (VPD); **c)** TDR measurements tracked soil water content (θ [$\text{cm}^3 \text{cm}^{-3}$]) decline through the duration of drought treatment.

Transpiration was measured according to the weight loss of the pots which were positioned on balances that recorded weight every 10 minutes. Transpiration values were produced by calculating the difference in weight divided by the corresponding time. Average E values were calculated for each pot during predawn (no light, low VPD), VPD1, and VPD2.

Water potential of the leaf was determined using a pressure chamber (Scholander et al., 1965). Three leaf samples were excised from diverse mature leaves on each plant. Three plants were sampled for each measurement sequence. Excised leaf samples were immediately sealed in a plastic bag and processed in the pressure chamber (Model 3115, Soil Moisture Equipment Corp, Santa Barbara, CA, Unites States), where pressure was increased at a rate of 0.1 bars s^{-1} until the meniscus of xylem sap appeared at the cut surface. This sampling and measuring were conducted three times daily across the 11-day campaign, at predawn, during VPD1, and again during VPD2.

Stomatal conductance (g_s) and photosynthetic rate were intended to be measured daily and initially attempted via a Li-Cor LI-6400XT Portable Photosynthesis System (Li-Cor Biosciences, Lincoln, NE). The Li-Cor proved unreliable, and a porometer was employed for g_s measurements in its place. The porometer was calibrated by allowing moist filter paper to acclimate to the chamber's climate 24-hours prior to use. The resulting g_s measurements from the porometer were limited to three of the 11-day measurement campaign and so excluded from the experiment analysis.

Leaf area was measured daily for each plant throughout the 11-day campaign. Leaves were measured from the point where the proximal sheath separated from the stem, and to the distal end of blade. Leaf area was obtained by multiplying the leaf length and width by a factor of 0.7 (van Oosterom, Carberryb, & O'Leary, 2001) to derive leaf area (cm^2). Measurements for ψ_{leaf} were taken following the predawn leaf sampling, before VPD1 and VPD2 leaf sampling.

Post-harvest measurements

Following the 11-day campaign, the height of each plant was measured as the distance in centimeters from the soil line of the plant to the furthest point on the plant tassel. Since there were two plants in each pot, aboveground biomass for the two plants were weighed together. Leaves and stems were weighed separately. Leaves and stems were then dried in a laboratory oven at 60°C for 24 hours and weighed again for dry weight. Roots were removed from the stem at harvest, gently rinsed with tap water, suspended in water, and scanned (EPSON Perfection V800 Photo Scanner, Seiko Epson K.K., Suwa, Japan). The scans were analyzed for total root length and root length in distinct diameter classes using WinRHIZO software (Regent Instruments Inc., Quebec, Canada). Following scanning, roots were dried in the same conditions as aboveground biomass and measured for dry weight.

Root samples were also collected for ink staining to determine mycorrhization (Vierheilig & Piché, 1998). For staining, randomly selected roots were cut into 1 cm pieces with a maximum thickness of 1 mm. Samples were washed with double-distilled water (ddH_2O) and incubated in an ink solution with a heating block for 5 minutes at 95°C . Samples were then de-stained using a 20% acetic acid solution for 15 minutes. This process was repeated three times, after which the samples were washed, prepared into slides, and 150 views were observed at 200x magnification. Each view was classified into six categories: no colonization, extraradical hyphae, intraradical hyphae, arbuscule, arbuscule and vesicle, and only vesicle.

Data analysis

Soil moisture, plant traits, AMF root colonization, and water vapor exchange measurements were analyzed to gain understanding of how AMF inoculation effects soil hydraulic properties and related plant responses during soil water deficit. A median filter was applied to correct transpiration data for pots where the

balances were disturbed during manual measurements. Transpiration and soil water content were not available per plant, as there were two plants in each pot. Dry above and below-ground biomass measurements were used to calculate root to shoot ratios for each treatment.

Normality of data was confirmed via Wilkens-Shapiro test using R version 3.6.1. T-tests were applied to evaluate significance of differences in biomass and root morphological traits. ANOVA was used to evaluate differences in transpiration rates, soil water content, and leaf water potential between treatments, VPD1, and VPD2. T-tests and ANOVA analysis were executed using MATLAB (R2021a). P-values < 0.05 were taken as statistically significant.

Results

AMF effect on biomass and root colonization

There was no significant difference found between inoculated and non-inoculated plants for fresh and dry weights, stem length, and root/shoot ratio (Fig. 4a-d), root diameter, root dry weight, and root length (Supplementary Figures 3a & 3b, 4a & 4b), nor dry and fresh weights of stems (Supplementary Fig. 5a & 5b).

The percentage of root length colonized with AMF was > 90% for inoculated plants, whereas non-inoculated plant roots were significantly less colonized (*p-value* << 0.001), between 40-60% (Fig. 5a). In non-inoculated plants, the distribution of AMF colonization was found to be inhomogeneous, with AMF colonized roots observed amidst others with no colonization. It was also observed that the density of AMF structures was lower in non-inoculated plants when compared to inoculated plants.

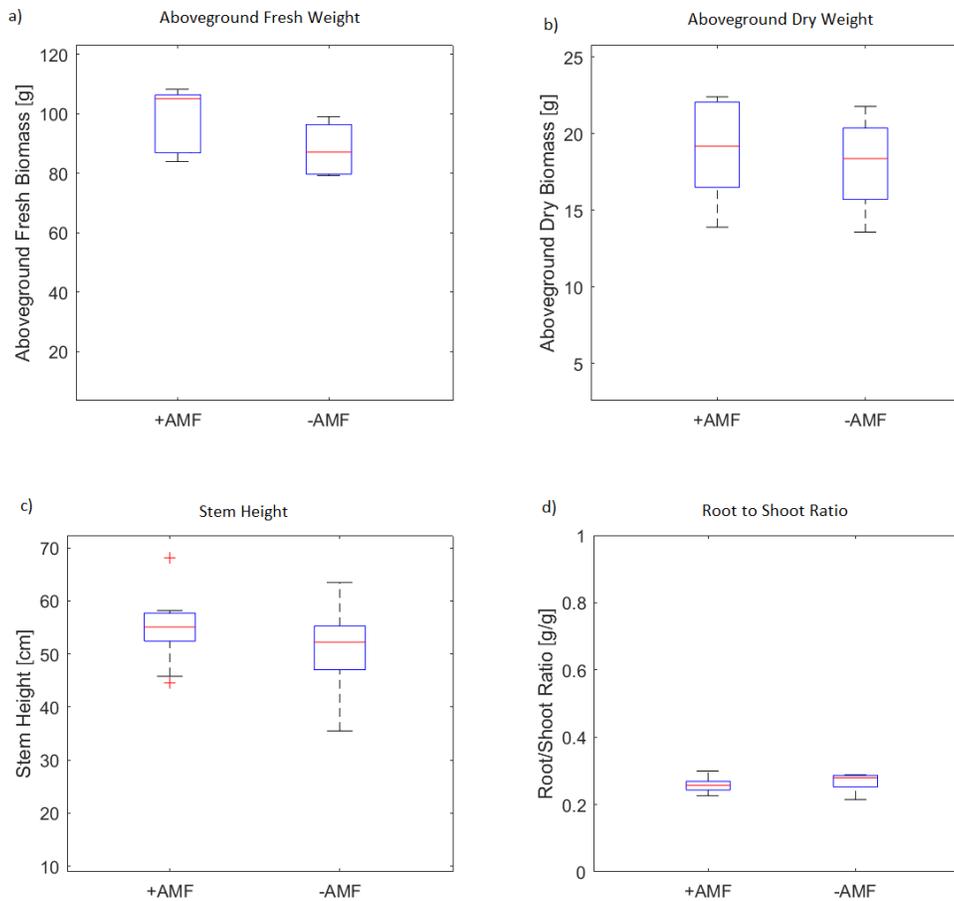


Figure 4. No significant difference found between inoculated (+AMF) and non-inoculated (-AMF) plants for **a)** aboveground fresh weight, **b)** aboveground dry weight, **c)** stem height, and **d)** root to shoot ratio ($p < 0.05$).

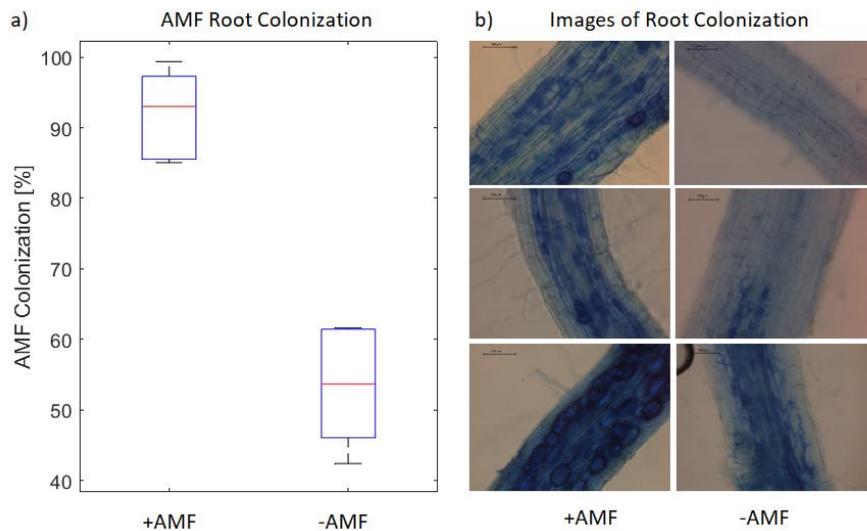


Figure 5. a) Greater than 90% root colonization found in AMF inoculated plants (+AMF), whereas < 60% root colonization found in non-inoculated plants (-AMF); **b)** Bright field microscopic images of root samples from inoculated and non-inoculated plants taken with light microscopy (Leica DM 1000 LED) at 200× magnification. (Photos courtesy of Isabelle Metzner).

AMF effect on transpiration as a function of soil water content & soil matric potential

When plotted as a function of θ and ψ_{soil} , AMF inoculated plants maintained higher E rates during soil drying (θ ; $\text{cm}^3 \text{cm}^{-3}$) than non-inoculated plants (Fig. 6). Under ambient soil water content conditions, both inoculated and non-inoculated plants expressed comparable E rates. When θ began to drop below $0.10 \text{ cm}^3 \text{cm}^{-3}$, inoculated plants maintained higher E rates. When $\theta \leq 0.08$, AMF inoculated plants exhibited higher E rates up to $3.3 \times 10^{-3} \text{ cm}^3 \text{s}^{-1}$, while E was reduced by a factor of 2 in non-inoculated plants (mostly below $1.5 \times 10^{-3} \text{ cm}^3 \text{s}^{-1}$) (Fig. 6a, 6c). Likewise, as ψ_{soil} declined, AMF inoculated plants exhibited a more gradual decline in E than non-inoculated plants (Fig. 6b & 6d).

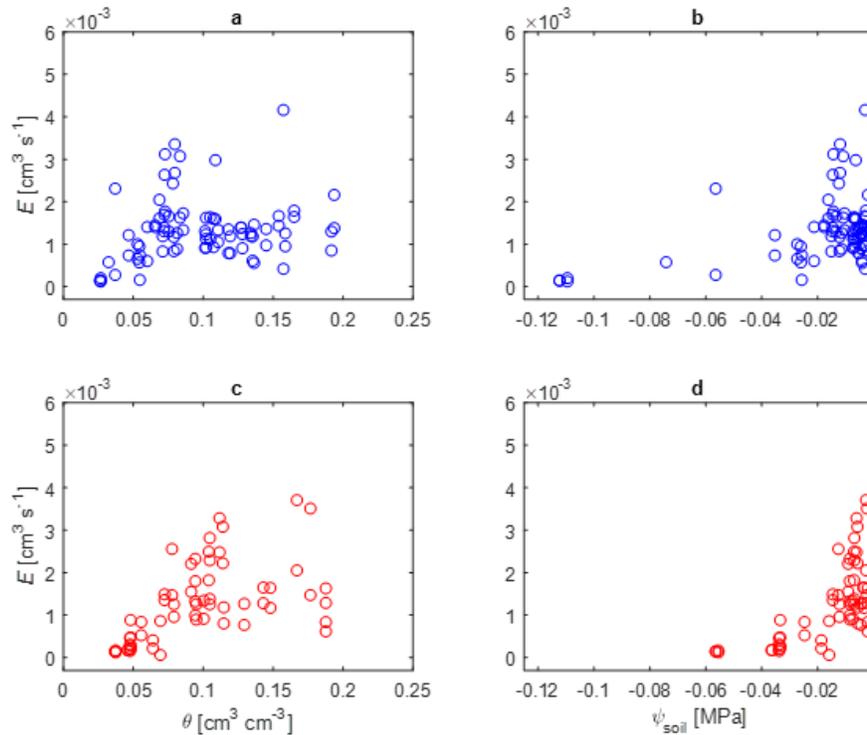


Figure 6. Transpiration rate (E) of AMF inoculated plants (**a, b**) and non-inoculated plants (**c, d**) as a function of soil water content (θ ; **a, c**) and soil water potential (ψ_{soil}); **c, d**) Plants with AMF inoculation sustain higher E as θ drops below $0.10 \text{ cm}^3 \text{s}^{-1}$ compared to non-inoculated plants that show a steep decline in E as θ decreases below $0.10 \text{ cm}^3 \text{s}^{-1}$ ($N = 18$). (Figure courtesy of Mohanned Abdalla).

AMF effect on transpiration as a function of leaf water potential

When analysed as a function of ψ_{leaf} , E rates during soil drying for AMF inoculated plants exhibited a more gradual decline than non-inoculated plants. The former also exhibited an overall less-negative value in ψ_{leaf} during soil drying when compared to non-inoculated plants (Fig. 7a, 7b). When ψ_{leaf} declined to $< -1.5 \text{ MPa}$, inoculated plants had an E rate 4 times greater than non-inoculated plants.

Fitting E as a function of ψ_{leaf} showed that near a given ψ_{leaf} , AMF inoculated plants achieved higher E rates, demonstrating AMF inoculated plants have a more buffered E response to increasingly negative ψ_{leaf} during soil drying (Fig. 7c).

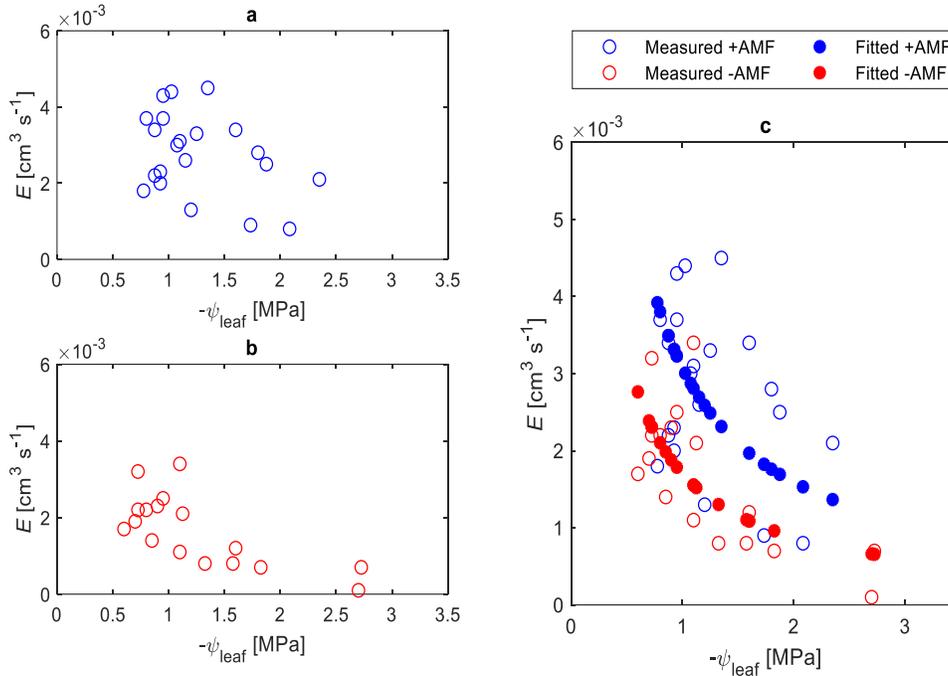


Figure 7. Transpiration (E [$\text{cm}^3 \text{cm}^{-3}$]) plotted as a function of leaf water potential (ψ_{leaf}) for **a)** AMF inoculated and **b)** non-inoculated plants; **c)** The relationship between E and ψ_{leaf} when fitted using the Weibull function shows inoculated plants maintained overall higher E rates and slightly less negative ψ_{leaf} rates as soil dried ($N = 18$). (Figure courtesy of Mohanned Abdalla)

Discussion

AMF effect on soil matric potential

The outcome of this study supports the hypothesis that AMF inoculation influences the gradient of matric potential in the rhizosphere, as is reflected by the higher and more gradually decreasing E rates of inoculated plants as soils dried. The delayed and attenuated decline in soil matric potential could be due to several reasons. AMF extend the overall effective root radius (Ahmed and Abdalla 2021), and AMF hyphae extend to water-filled pores in bulk soils (Begum et al., 2019; Gianinazzi et al., 2010; Gutjahr & Paszkowski, 2013; Orfanoudakis et al., 2010). Doing so provides inoculated plants access to additional soil resources. AMF have also been shown to deplete soil water resources beyond the rhizosphere, in the bulk soil (Bitterlich, M. et al., 2018; Pauwels et al., 2020), possibly redirecting it to plant-root zones, while easing radial water fluxes toward plant roots in order to restore soil hydraulic conductance in the root zone.

There are additional ways in which AMF symbiosis may affect root available moisture which, while not confirmed here, complement the results. For example, AMF symbiosis has been shown to enhance root hydraulic conductance under abiotic stress through regulating aquaporin activity (Aroca et al., 2007), improving soil water retention through supporting the formation of aggregates (Bitterlich, M. et al., 2018), and fostering beneficial microbes which enhance abiotic stress tolerance (Kapoor et al., 2013). Additionally, the concept that AMF effect hydraulic continuity in the rhizosphere is complimentary to work published by Carminati et al. (2011), which showed that water potential gradients in the rhizosphere were considerably smaller than those found in the bulk soil, describing the rhizosphere as a hydraulic conductor and water reservoir that can be accessed by plant roots during water deficits. It could also be that AMF perform a similar function as Carminati et al. (2017) observed with root hairs, softening gradients in water pressure that are expected when E is high and the soil is becoming dry and less hydraulically conductive.

However, finding that AMF inoculation supports maintaining E and ψ_{leaf} during water deficits in this experiment is not meant to suggest the same would be true in all circumstances. It should be noted that the experiment was conducted in pots, thus excluding the influence of water and nutrient resources which could come from the periphery, as would be the case in a field setting. AMF symbiosis in pots can lead to higher resource acquisition because the mass flow in the soil declines later than in non-inoculated pots, and may also increase the rate of resource depletion (Bitterlich, M. et al., 2018).

AMF effect on transpiration and leaf water potential

The results of this study indicate that the combined effect of AMF in the soil provided increased water availability to the plant and therefore supported aboveground hydraulic continuity during the onset of water deficits. This was observed in higher mid-day and more gradually decreasing E rates of inoculated plants when compared with those of non-inoculated plants during water deficits. The same trend in E was observed in other studies with maize (*Zea mays* L.), soybean (*Glycine max* L. cv. Williams), and barley (*Hordeum vulgare* cv. Pallas) when subjected to water deficits (Li et al., 2014; Porcel & Ruiz-Lozano, 2004; Subramanian et al., 1997).

Hayat et al. (2019) found that when the soil-plant hydraulic conductance declined in maize, stomata regulation reduced E to prevent non-linearity between ψ_{leaf} and E . While the present study did not measure soil-plant hydraulic conductivity, it could be that the higher mid-day and more gradually decreasing E rates observed with AMF inoculated plants indicate sustained soil-plant hydraulic conductivity. This remains an opportunity for further research. Ahmed and Abdalla found tomato plants with AMF symbiosis had higher soil-plant conductivity during soil drying than non-inoculated plants, and it was previously reported a loss in soil hydraulic conductance triggers stomata closure (Carminati & Javaux, 2020). Stomata are the gatekeepers to water vapor fluxes between vegetation and the atmosphere, and their behavior has profound

effects in global biogeochemical cycling. Sharpening the focus on the precise ways in which AMF inoculation influence ψ_{leaf} and E is valuable for both adaptation of agricultural practices amidst increasing water deficits as well as improving the ability to more accurately model fluxes in the SPAC.

Work by Henry et al. (2019) investigates a so-called safety-efficiency tradeoff, where species with greater g_s under high water availability (g_{max}) demonstrate a greater sensitivity to closure during leaf dehydration, also understood as having a higher ψ_{leaf} when g_s is reduced by 50% (ψ_{gs50}). This tradeoff is proposed to be influenced by plant stomatal sizes and density, hydraulic design, leaf economics and/or life history. The present study showed with the application of a nutrient solution, no significant difference in above ground biomass occurred within the measured genotype, suggesting similar leaf economics. What would be interesting is to compliment the safety-efficiency trade-off theory with consideration of rhizosphere conditions in stomata closure. The current study suggests plants of the same genotype and similar phenomics may exhibit a different threshold for the safety-efficiency tradeoff due to AMF root colonization. Here is an opportunity for further investigation.

AMF effect on root colonization and biomass

AMF root colonization for inoculated plants was > 90% whereas non-inoculated roots were between 40-60%. This higher-than-expected colonization rate in non-inoculated plants is presumably the consequence of having used unsterilized soil. While this may have diminished the contrast in water relations between the two treatments, it does speak to Augé's (2001) remarks that there is undue emphasis on non-inoculated, or non-mycorrhizae controls, because such plants are anomalous in nature. He goes on to suggest future research be more productive by excluding controls to emphasize the examination of the behavior of AMF colonized plants in different soils, at different P levels, and at varying levels of colonization. Indeed, the latter is accomplished in this study and offers insight into the contrasting influence of significantly different colonization rates.

It would be expected that non-inoculated plants have a comparatively stunted growth than inoculated counterparts, which have increased access to nutrients and water resources (Bardgett & Wardle, 2010; Bolandnazar et al., 2007). It is understood here that the absence of variation in biomass between inoculated and non-inoculated plants is a combined effect of relatively high root colonization in non-inoculated plants as well as the regular fertilization and irrigation both treatments received during the growing period. The lack of statistical difference in plant biomass between the two treatments strengthens the observations of the influence of AMF on plant water relations by reducing the impact of biomass variation, e.g., leaf area on transpiration rates. Statistical analysis indicated that soil moisture content was a more significant indicator for transpiration than VPD. Indeed, VPD would be expected to be less significant if transpiration

is similar due to similar biomass. This finding is confirmed by the work of Liu et al. (2020), who found that soil moisture content, and not VPD, is the dominant driver of dryness stress in terrestrial vegetation.

Experiment design and execution

It is visible in Fig. 2 that, in addition to having two plants per pot, there is also a mesh hyphal root compartment. While neither of these design aspects supported the aims of this study, it is worth noting the experiment design was representative of a collaborative effort of several groups with varied interests. This study did not include the majority of pots and treatments which were prepared (76 in total), but rather focused on those pots which had two B73 plants in each pot. Focus was on the interplay between mycorrhiza colonization, E and ψ_{leaf} under drought conditions. If executed independently, it would be preferable for these aims to have one plant per pot for plant specific E rates, root biomass, and to eliminate any possible influence of the mesh hyphal compartment.

Outlook

The influence of AMF symbiosis varies greatly depending on environment and the genotype of both plant and fungi (Ryan & Graham, 2018). While studies on AMF symbiosis are extensive and span disciplines, to fully utilize the functional complementarity of AMF symbiosis in agriculture, further research is required in determining which environmental conditions, alongside which of the diverse combinations of fungi and crops, result in preferred outcomes.

This will require transcending existing divisions amidst disciplines to assess the spatio-temporal and environmental conditions which effect the interplay between AMF and plants, and the underlying mechanistic explanations. Understanding the rhizosphere as a self-organizing system offers opportunities to understand and thus leverage resilience in soil-root-plant systems (Vetterlein et al., 2021).

In tandem with this effort is an opportunity to bridge the gap between science and the formulation of innovative products to support the sustainable, or regenerative, intensification of food production systems as amplification of drought conditions are anticipated (Turrini et al., 2018).

Conclusion

This study aimed to substantiate the link between effects of AMF inoculation in the rhizosphere and aboveground water relations, specifically leaf water potential and evaporation as it relates to stomata regulation under water deficits. It was expected that plants grown with AMF inoculation would have a less severe drop in soil matric potential, allowing for increased and extended root water uptake and that subsequently, as the soil dried, AMF inoculated plants would exhibit less negative leaf water potential rates and maintain higher transpiration rates than non-inoculated plants. The results supported this hypothesis, indicating an inextricable link between the effect of AMF in soils and plant-water status under drought conditions. Specifically, by improving root access to soil moisture, AMF symbiosis buffered the decline in leaf water potential as soil dried, allowing for sustained opening of stomata as was indicated through higher and maintained transpiration in inoculated plants when compared with non-inoculated plants.

These results are significant in the sense that conditions in the rhizosphere are often overlooked in understanding plant water relations. By tracing the effect of AMF on soil matric potential and root water uptake through the leaf, and out through the plant via transpiration, a direct outline of the link between belowground conditions and stomata response to soil water deficits is illustrated. Identifying what conditions influence stomata closure and the mechanisms behind it are vital in understanding massive gas exchanges between vegetation and the atmosphere, with implications not only for resilient food production but also for modelling and predicting the effects of climate change. While there remains vast opportunity to advance understanding of this topic, this study does suggest AMF symbiosis could serve as a valuable method for increasing drought resilience for major crops amidst climate change.

Supplementary table & figures

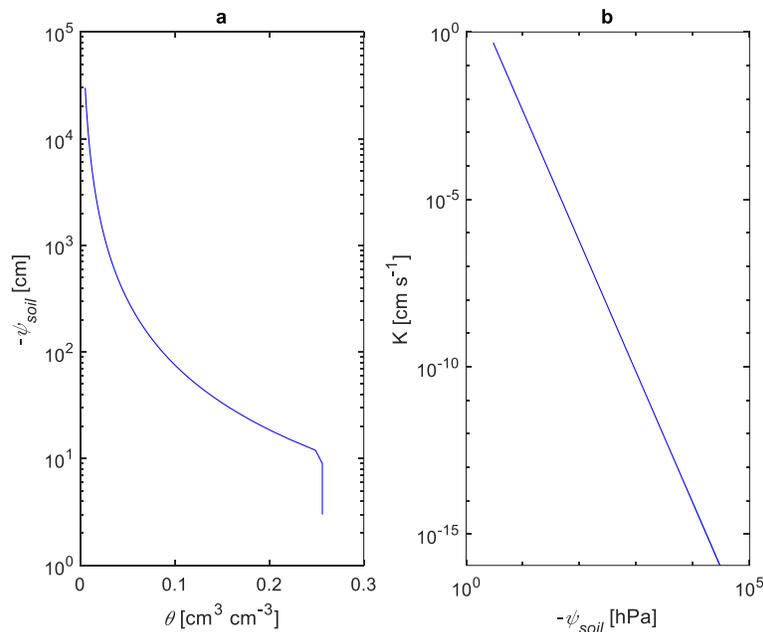
Supplementary Table 1. Nutrient solution used for fertilization. Once seeds had germinated, 50 mL of adjusted Hoagland solution was added in addition to distilled water irrigation every second day until 57th day after planting.

Adjusted 5x Hoagland Solution

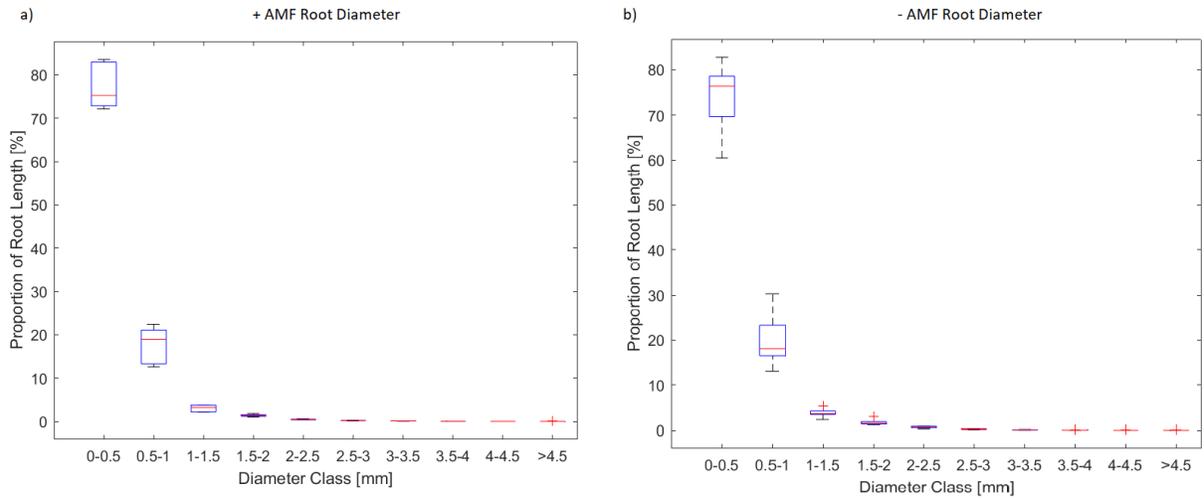
Input	Quantity
1M H ₂ O	1840 mL
1M KNO ₃	60 mL
1M Ca(NO ₃) x 4H ₂ O	40 mL
1M MgSO ₄	40 mL
Microelements	10 mL
0.5% Fe-Sequestrene	20 mL
1M NH ₄ Cl	10 mL
1M NH ₄ H ₂ PO ₄	100 μ L

Microelements

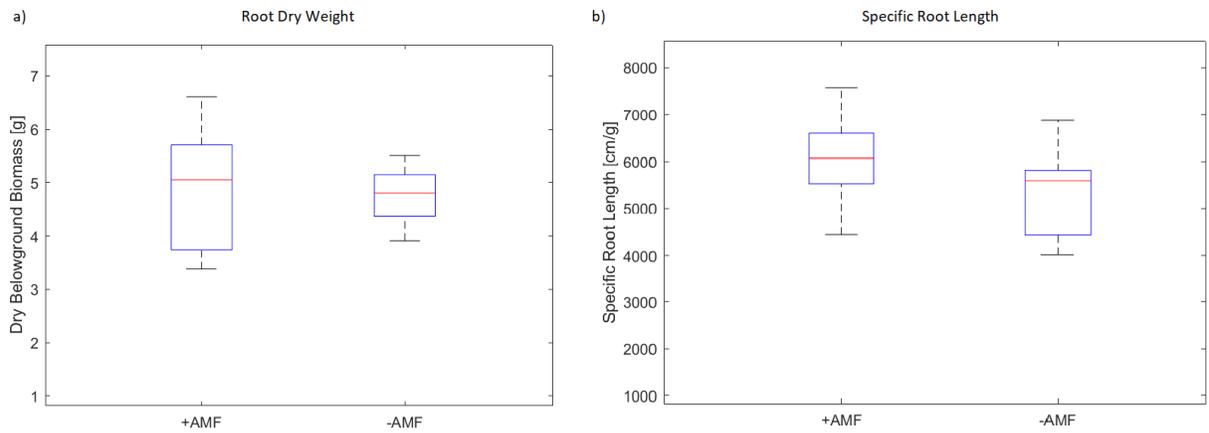
H ₃ BO ₃	2.86 g/L
MnCl ₂	1.15 g/L
ZnCl ₂	0.11 g/L
CuCl ₂	0.05 g/L
Na ₂ MoO ₄ x 2H ₂ O	0.025 g/l



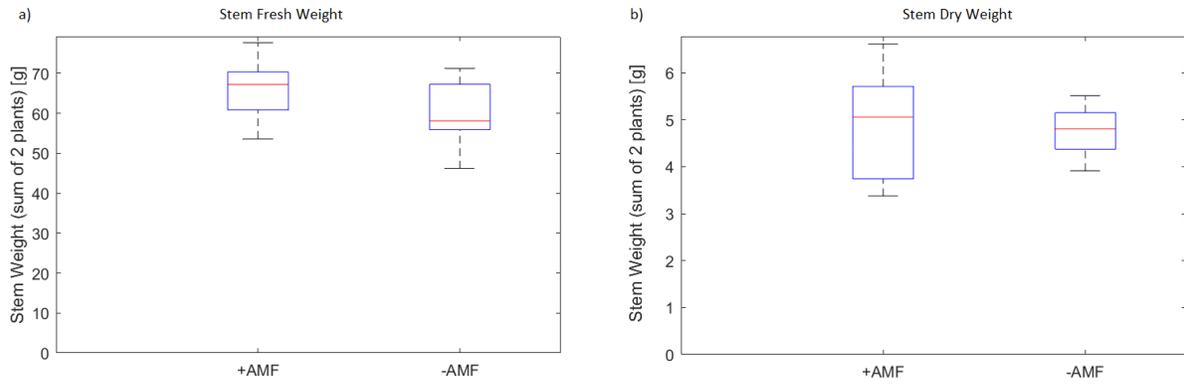
Supplementary Figure 2. **a)** Soil water retention curve of the soil estimated from the evaporation method (Hyprop) and fitted with the Peters-Durner-Iden (PDI) parameterization; **b)** Relationship between soil hydraulic conductivity (K [cm s^{-1}]) and soil matric potential fitted with the PDI parameterization. (Figure courtesy of Mohammed Abdalla).



Supplementary Figure 3. Root diameters for **a)** AMF inoculated (+AMF) and **b)** non-inoculated (-AMF) plants. No significance difference was found between the two treatments ($p < 0.05$).



Supplementary Figure 4. No significant difference was found between inoculated (+AMF) plants and non-inoculated plants (-AMF) for **a)** dry weight of roots, and **b)** specific root length.



Supplementary Figure 5. No significant difference was found between inoculated (+AMF) and non-inoculated (-AMF) plants for **a)** stem fresh weight and **b)** stem dry weight.

Declaration of Originality

The research work contained in this thesis was conducted between February 2020 and October 2021. It is original work except where due reference is made. It has not been and shall not be submitted for the award of any degree or diploma to any other institution of higher learning.

Date: 13 October 2021

Location: Bayreuth, Germany

Signature: 

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