

An experimental setup to investigate the effect of mycorrhizal fungi inoculation on plant water uptake in unsaturated soils

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Abstract. The use of vegetation as a Nature Based Solution, NBS, is increasingly being used for climate adaptation and resilience for engineered and natural slopes. As such it is important to understand the biological processes associated with vegetation and how they could be beneficially implemented. Due to the large repetition required when experimenting with biological components it is impossible to carry out experiments that are highly instrumented. This paper looks at mycorrhizal fungi as a method to improve plant water uptake and presents an experimental setup to allow for a low level of instrumentation in triplicate, with a few being well instrumented. Through benchmarking against TDR-probes, it was found that the method of monitoring soil water content changes in a mini-lysimeter with a loadcell provides a reliable and convenient approach to carry out experiments in three replicates at least.

1 Introduction

Nature based solutions, NBS, are increasingly being used as engineering solutions [1-3]. They work with and enhance the positive benefits of natural systems to adapt and promote resilience to climate change, flood risk, and more. The use of vegetation to improve slope stability and reduce soil erosion is one engineering NBS. The hydrological ‘reinforcement’ of vegetation is due to suction generated by the removal of soil water via plant water uptake, i.e., transpiration. To improve upon this stabilizing technique, it is important to understand and possibly pilot the biological processes that already occur in the root zone.

One biological process that occurs in the root zone is the symbiotic association between plant roots and mycorrhizal fungi. When carrying out experiments with biological elements there are random components that require a minimum of three replicas to be carried out. There is also the need for experimental controls, for example bare soil and non-inoculated vegetation, and they have to be carried out at the same time to ensure the environmental conditions are the same. This results in a large number of experimental repetitions. The strategy pursued in this work is to have low instrumentation for most of the pots with a few with a high level of instrumentation to determine if the low-level measurements may be considered representative.

This paper first looks at what mycorrhizal fungi are and how they could improve plant water uptake. It then presents a methodology to determine the effect that

mycorrhizal inoculation has on plant water uptake by designing a mini-lysimeter that can be used to monitor soil water content, suction, and evapotranspiration. It finally presents some preliminary results from this set-up.

2 Background

2.1 Mycorrhizal fungi

Mycorrhizae, literally meaning ‘myco’ fungus and ‘rhiza’ root is symbiotic relationship that forms between mycorrhizal fungi and the roots of plants. The fungus is completely dependent on the plant for organic carbon and in exchange the mycorrhizal fungi improve the nutrient status, water absorption, growth and disease resistance of the host plant [4]. Mycorrhizal fungus consist of long filament or thread like structures called hyphae that make up the fungal mycelium. More than 90% of terrestrial plant species form a symbiotic relationship with these beneficial fungi. There are seven types of mycorrhizae that are classified by their fungal associations. Among them, arbuscular mycorrhiza and ectomycorrhiza are the most abundant and widespread.

The most common type of mycorrhizal fungi are arbuscular mycorrhizas (AM). They are virtually non-plant specific and can form relationships with 85% of plant species [5]. The hyphae penetrate the root cells and form arbuscules where they exchange nutrients with the plant. The hyphae are approximately 2 to 5 μm diameter [6]. Ectomycorrhiza create external sheaths around the roots called a Hartig net, and the hyphae are thicker than AM fungi at around 20 to 100 μm diameter. They are

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found in 10% of plant species, most commonly in the roots of woody plants such as trees and shrubs [4].

AM fungi colonization occurs by three sources of inoculum, called propagules, these are spores, infected root fragments, and hyphae. To colonize plant roots these propagules need to be present in the substrate and in close proximity to an active growing root. Once the root is colonized, the mycelium continues to grow with the plant root system, producing hyphae and additional spores. Propagules can be dispersed via water and wind and by dispersal in the soil via burrowing animals, but plants will often be colonized from hyphae when growing in soils with an established community of mycelium and plant roots of different species and ages [4]. In sites where the soil has been disturbed it can reduce the infectivity of the soil [7].

Mycorrhizal fungi are often used in agriculture as a biofertilizer to improve crop productivity as AM fungi can provide tolerance to drought, heat, salinity, metals, and disease [8]. Mycorrhizal fungi have also been studied for the use in ground engineering to improve the soil [9-11]; to re-vegetate soils for soil erosion, landslides, and desertification by enhancing vegetation growth [12,13], and to increase root growth [14].

The contribution of mycorrhizal fungi on plant water uptake is still being studied. It is believed that they can enhance plant water uptake as they can exploit a larger volume of the soil than the roots alone [15] and that the hyphae increase the water absorptive surface area [16,17]. The colonization of mycorrhizal fungi could also reduce the resistance of water flow into the roots [18]. It has also been found that inoculating with mycorrhizal fungi can reduce the effect of drought stress on plant growth [19,20] and increase transpiration rates [21].

2.2 Review of experimental procedures for fungal inoculation

2.2.1 Inoculum

As AM fungi cannot be grown in an axenic culture it must be increased and maintained by bulking in a stock culture by growing on potted plants. Studies often use the soil, spores, hyphae, and infected root fragments from these stock culture in experiments [17,19]. A further step has also been used to create a liquid culture by filtering water through inoculum and passing it through sieves to collect the spores [15,18]. Some studies also used spores collected from the field [16,20].

The inoculum is then either mixed into the soil [16], placed directly below or 1-5cm below the seedlings [15,17,19]. The amount of inoculum used provides approx. 500 – 900 spores per pot. There are also cases where the seeds were inoculated by soaking in a liquid inoculum before sowing [18].

2.2.2 Growing period

Experiments on the effect of mycorrhizal fungi on plant water relations are often carried out in greenhouses. To further control the environmental conditions a growth chamber can also be used [15,17].

The vegetation is initially grown for 2.5 – 5 weeks before carrying out any testing to allow time for the fungi to colonize and to influence water transport. After this time the plants are then harvested, or water stressed, for experimental tests. [15-19]

2.2.3 Nutrients

Mycorrhizal fungi can increase nutrient uptake in plants, but the nutrients have to be present in the growing medium. In experiments that use natural soils, there are often no additional nutrients needed for the growth of the plants as they rely on the nutrients already available in the soil [16,20,21]. To create control plants that are a similar size to the inoculated plants, the non-inoculated plants can be the only pots that are treated with nutrients [17].

A nutrient solution that is often used is the Hoagland nutrient solution [22]. It contains all essential elements for the growth of a wide range of plants and can be used for plants grown in artificial media, such as water culture or aggregate culture, that do not contain any nutrients. When using nutrient solutions to grow plants for mycorrhizal studies the phosphorus content is often removed or reduced as it can inhibit the growth of mycorrhizal fungi [23,24].

3 Materials and methods

A method was developed to explore the effect of mycorrhizal inoculation on soil water removal via transpiration.

3.1 Experimental setup

A mini-lysimeter was designed to monitor evapotranspiration and soil volumetric water content under laboratory conditions. The lysimeter was created using acrylic tube (H:20cm, D:12cm) attached to a base equipped with a drainage hole and valve [Fig. 1].

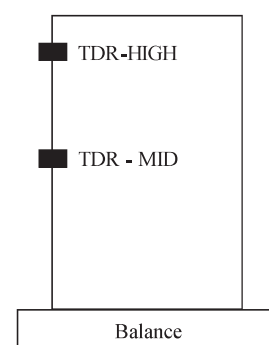


Fig.1. Mini-lysimeter used to monitor evapotranspiration and soil water content.

The containers were prepared with a 1cm gravel layer at the base to allow for one-dimensional water flow when saturating from the base. Sensor holes were created at two points along the height of the container to allow for Time Domain Reflectometry (TDR) moisture probes to be inserted into the soil at two levels. Each container was placed on single point load cell (BOSCHE GmbH & Co. KG, Damme, Germany) with an accuracy of $\pm 1g$.

To make a repeatable medium, an artificial soil was created by mixing 60% sand, 20% silt and 20% clay to create a well-graded soil. Composted woodchip was also added to provide 3% organic matter. The organic matter contributed to maintaining a low dry density upon wetting. When the medium was mixed without the organic matter the soil collapsed upon wetting from a bulk density of 1.42 g/cm^3 to 1.7 g/cm^3 . As a soil with this texture and a bulk density of $>1.6 \text{ g/cm}^3$ can affect root growth [25,26] therefore the density needed to be lower. By adding the organic matter, the bulk density after the volumetric collapse only increased to 1.55 g/cm^3 . The soil was autoclaved at 121°C for 20 min to sterilize. To compact into the containers the soil was mixed to 10% water content and compacted into the container in layers.

The mycorrhizal fungus inoculums for *Glomus intraradices* (AMF1) and *Glomus mosseae* (AMF2) were purchased from a commercial producer (Plantworks Ltd) as a single fungi species inoculum. It consisted of spores and infected root fragments in a mix of pumice and zeolite. 25g of the inoculum was mixed into the top 7cm of soil in the container (approx. 1000 spores).

Seeds of alfalfa (*Medicago sativa*) and perennial ryegrass (*Lolium perenne*) were surface sterilized in 10% hydrogen peroxide for 10 min and subsequently rinsed with deionised water. Approximately 100 seeds were sown per container. Plants were grown in a laboratory under 45W full-spectrum LED grow lights with a 16-hour photoperiod, and a 30-50% RH. Although the plants were grown in a temperature-controlled room, the grow lights created a day/night temperature cycle measured on the outer wall of the mini-lysimeter of $25/22^\circ\text{C}$. They were watered with 25ml Hoagland and Arnon Nutrient Solution 2 [19] with 25% reduced phosphorous three times weekly. Water was supplied to maintain a constant soil gravimetric water content of 20% during growth.

After 10 weeks the containers were partially saturated by connecting a reservoir to the base and raising the water level to 11cm below the soil surface until the container mass remained constant with time. The reservoir was then disconnected and the valve at the base of each container closed. The containers were then subjected to drying conditions until the plants showed signs of the onset of wilting. After drying the reservoir was reconnected and the water level set to 1 cm from the base of the soil column until the next drying cycle. The drying cycles were repeated at 14, 16, 30, 32 weeks.

3.2 Parameters measured

During the drying cycles the change in mass was monitored via the load cell balance. This was used to calculate the evapotranspiration rates in each container during the drying period and to cross-check the response of the replicates and controls. It was assumed that all change in mass was due to water removed via evapotranspiration. Daily water content measurements were also taken using the TDR probes.

After the final drying cycle, the containers were dismantled. Soil samples were taken at 6 points along the length of the column and oven dried to determine the final gravimetric water content. The roots were carefully washed in water to remove adhering soil particles and a small section of roots were then removed to determine the level of mycorrhizal colonization. The root fragments were cut into 0.5-1 cm fragments and cleared by boiling in 10% KOH solution for 3 minutes then rinsed several times in tap water. Cleared roots were boiled for 3 min in 5% ink-vinegar solution using black Sheffer ink and 5% acetic acid to stain [27]. The roots were de-stained by rinsing for 20 min in water with a few drops 5% acetic acid. The percentage of root colonization was determined by the line-intersect method [28]. The remaining roots and the shoots were oven-dried at 60°C for 48h and weighed to calculate the plant biomass.

3.3 Hydraulic characterisation of the bare soil

To determine the water retention characteristics of the artificial soil, the soil was compacted into the same container as illustrated before. Sensor holes were cut along the height of the container allowing the insertion of High Capacity Tensiometers (HCT) and Time Domain Reflectometry (TDR) probes into the soil at three levels, HIGH, MID and LOW. The TDR probes were inserted into the container after the soil had been partially saturated from the base.

The soil was fully saturated by connecting the reservoir to the base of the containers. The reservoir was then switched off and the soil allowed to dry by evaporation. After 9 days a fan was used to force ventilation and increase evaporation from the soil. The pore-water pressure and the water content were monitored during drying via HCT and TDR-probes. The anomalous behaviour of the TDR-probes at the start of drying period consisting of sudden drop in the measured volumetric water content (at days ~2-3) suggests the presence of a small air gap around the probes [Fig. 2a]. This is a problem that can occur due to the insertion of the rods into the soil. At the start of drying the soil is fully saturated and therefore the air gap is filled with water. This results in an overestimation of the soil volumetric water content due to the higher dielectric permittivity, K_a , of water. When the soil dries, and the air gap is emptied of water the gap is air-filled, resulting in an under estimation of the water content [29].

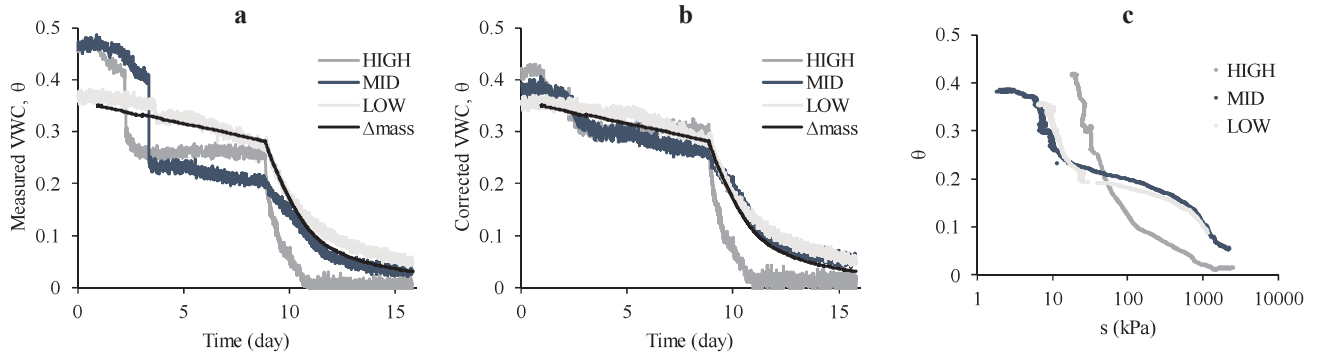


Fig. 2. The volumetric water content from TDR measurements during drying of the bare soil (a); the air gap corrected volumetric water content during drying (b) and the water retention curve determined from TDR and HCT measurements on the bare soil (c)

To correct for the presence of the gap, it was assumed that the gap is homothetic with the sampling volume around the TDR rods, and the measured K_a depended on the dielectric permittivity of the soil and the gap according to Equation 1:

$$\sqrt{K_a} = \frac{x_{gap}}{L} \sqrt{K_{a,gap}} + \left(1 - \frac{x_{gap}}{L}\right) \sqrt{K_{a,soil}} \quad (1)$$

where x_{gap}/L is the ratio between the size of the air gap compared to the size of the sampling volume, $K_{a,soil}$ and $K_{a,gap}$ are the values of dielectric permittivity of the soil and gap respectively.

To determine the size of the gap, the values of dielectric permittivity measured when the air gap was filled with water and, after when the gap is filled with air were compared (Equation 2)

$$\frac{x_{gap}}{L} = \frac{\sqrt{K_{a,before}} - \sqrt{K_{a,after}}}{\sqrt{K_{a,water}} - \sqrt{K_{a,air}}} \quad (2)$$

The corrected apparent dielectric permittivity was then be calculated using Equation 3,

$$K_{a,soil} = \left[\frac{\sqrt{K_a} - \frac{x_{gap}}{L} \sqrt{K_{a,gap}}}{\left(1 - \frac{x_{gap}}{L}\right)} \right]^2 \quad (3)$$

The volumetric water content, θ , was calculated using the equation suggested by Ledieu et al, [30] (Equation 4).

$$\theta = 0.1138 \sqrt{K_{a,soil}} - 0.1758 \quad (4)$$

Table 1. Ratio between the gap and sampling volume used for TDR correction.

Position	x_{gap}/L
HIGH	0.123
MID	0.181
LOW	0.028

The correction ratios used to correct the TDR-probe readings due to the air gap is show in Table 1. The soil volumetric water content corrected for the air gap is

shown in Fig. 2b. The volumetric water calculated from the change in mass of the container now shows a similar trend to the TDR measurements. The comparison between the water content measurements, shown in Fig. 3, demonstrates that the average volumetric water content calculated from the change in mass in comparable to the TDR measurements. The TDR positioned in the top of the container does not show as good a correlation. This is because the evaporation occurred in the top of the pot and so should be considerably lower than the average water content.

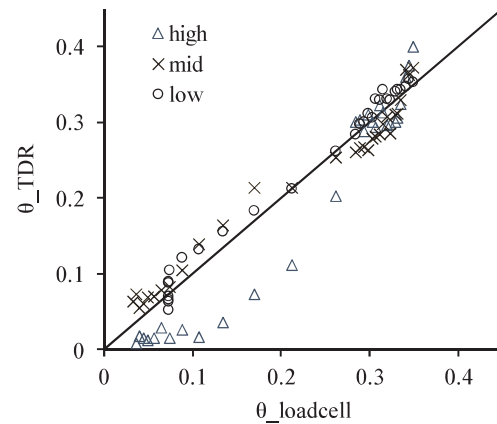


Fig. 3. Comparison between the soil volumetric water content measured with TDR and average volumetric water content calculated from the change in mass in the test with bare soil.

The water content and the suction, measured using the HCT, was then used to determine the water retention curve of the soil [Fig. 2c]. Positions MID and LOW show a very similar curve. This suggests that the correction carried out for the TDR air gap was appropriate. It also indicates that the soil sample preparation created a homogeneous soil, and that the equipment was working well. These soil-water retention curves exhibit bimodal characteristics, suggesting that soil has dual-porosity that could be due to soil aggregates or different grain size fractions within the soil.

The soil-water retention curve for the TOP of the container is monomodal and differs from the MID and LOW positions. Before drying the container was fully

saturated by water infiltrating from the base, by connecting to a reservoir. It was observed that the fines in the soil were washed up through the soil to the surface. This is likely why the water retention at the surface is monomodal as the larger pores were filled with the fines.

4 Preliminary experiment to monitor water uptake in mycorrhizal plants

An initial experiment was set up where two plant species (*Medicago sativa* and *Lolium perenne*) were tested in triplicate by considering no fungal inoculation and inoculation with *Glomus intraradices* (AMF1) and *Glomus mosseae* (AMF2) respectively. In addition, bare soil (BS) was also tested in triplicate for a total of 21 containers. The evapotranspiration rates, ET, were calculated for each container from the change in mass over time. It was assumed that all change in mass was due to the removal of soil water via evapotranspiration.

The full dataset for this experiment is to be published elsewhere. As an example, the results for three containers containing *M. sativa*, non-inoculated and inoculated with AMF1 and AMF2 are shown herein. Fig. 4 shows the evapotranspiration, ET, rates during a drying cycle. At the start of the test, when water is readily available in the soil, ET is occurring at its maximum rate i.e., potential evapotranspiration, PET. The PET for the container inoculated with AMF1 is 14 mm/day, inoculation with AMF2 give at PET of 18 mm/day whereas the non-inoculated container has a PET of approx. 10 mm/day.

When the water in the soil decreases the soil-root hydraulic system can no longer support the evaporative demand of the atmosphere and evapotranspiration becomes limited by the soil water availability, therefore termed water-limited evapotranspiration. This transition in the water-limited regime of evapotranspiration occurred sooner when inoculated with mycorrhizal fungi.

The average soil volumetric water content was calculated from the measured final water content and the change in mass over the drying cycle, Fig. 5. The initial water content was found to be the same in the containers, demonstrating that the saturation using the connected reservoir at the start of the drying test created the same initial conditions in all the containers. The reduction in soil volumetric water content for the non-inoculated container was less than when inoculating with mycorrhizal fungi. This suggests that the inoculation of mycorrhizal fungi has allowed the vegetation to remove more water from the soil.

The comparison between the TDR measurements and the volumetric water content calculated from the change in mass and the final water content is shown in Fig. 6. As the TDR measurements throughout this test were taken daily, it is not possible to carry out an air gap correction with the TDR measurements as this requires measurements recorded immediately before and after the gap is emptied of water. Even without carrying out the correction, there is one-to-one correlation, demonstrating

that the measurements made with the loadcells are representative of the TDR measurements. This suggests that the strategy of having low instrumentation is suitable when all containers cannot be highly instrumented.

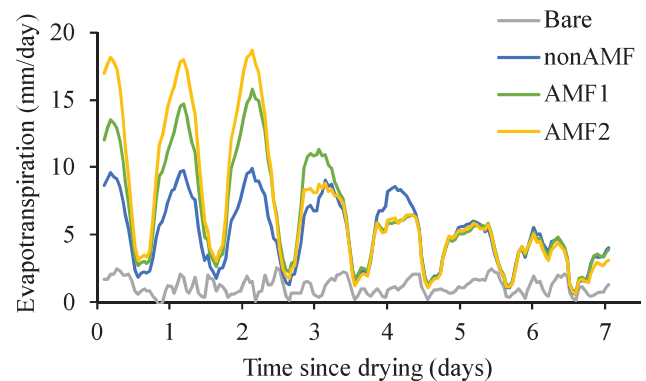


Fig. 4. Evapotranspiration rates calculated for the change in mass per container for a non-inoculated *M. sativa* and inoculated with mycorrhizal fungi during Drying Cycle at 32 weeks.

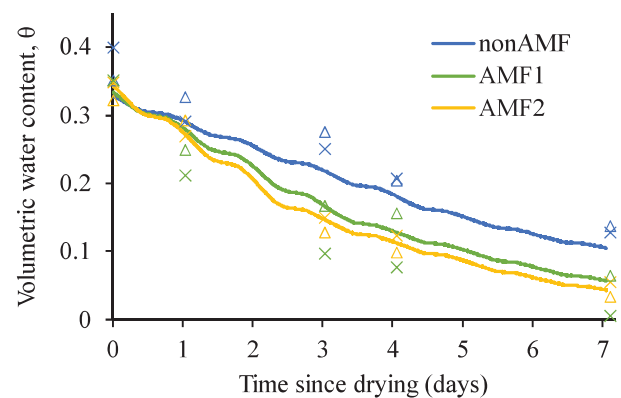


Fig. 5. Total volumetric water content of soil during drying for vegetation inoculated with mycorrhizal fungi and non-inoculated calculated from final water content with TDR measurement from the top, Δ , and middle of the pot, \times .

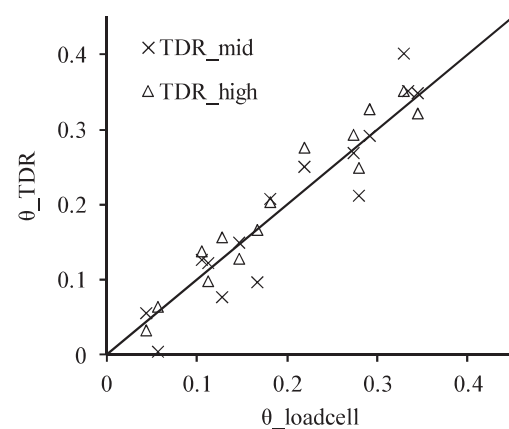


Fig. 6. Comparison between soil volumetric water content measured with TDR and calculated from the loadcell.

The colonization rates from the container inoculated with AMF1 and AMF2 was 29% and 21% respectively.

There was a 1% root colonization rate of the non-inoculated plants. This suggests that the soil was not fully sterilized and fungi spores were already present in the soil. It could also indicate that there was a transfer of spores from the inoculated containers to the non-inoculated containers. This could be due to air dispersal of some spores.

Table 2. Effect on mycorrhizal inoculation on *M. sativa* biomass

	Dry shoot mass [g]	Dry root mass [g]	Colonization rate [%]
nonAMF	8.81	7.89	1.0
AMF1	13.54	7.03	29.4
AMF2	13.37	5.81	21.4

The containers inoculated with AM fungi had a higher shoot mass than the non-inoculated container, suggesting the colonization of mycorrhizal fungi effected the biomass of vegetation (Table 2). Whilst the inoculation with AMF increased the shoot mass there was a decrease in the root biomass.

5 Conclusions

The mini-lysimeter is a useful technique to determine the effect of mycorrhizal inoculation on the soil water removal and any changes in the hydraulic of the root zone. It is moderately instrumented but allows testing in triplicate which is the minimum requirement when testing natural biological systems. It could be used to understand the result of using different fungal species, vegetation species and soil properties on the hydraulic and hydrological properties of the root zone. This could be a useful tool to select mycorrhizal fungi species to use in a laboratory mock-up or field scale experiment.

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