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Arbuscular mycorrhizal fungi improve nutrient status of *Commiphora myrrha* seedlings under drought

Emiru Birhane [a,*], Frans Bongers [b], Abebe Damtew [a], Abadi Tesfay [a], Lindsey Norgrove [c,**], Thomas W. Kuyper [d]

[a] Department of Land Resources Management and Environmental Protection, Mekelle 23 University, Mekelle, PO Box 231, Ethiopia
[b] Forest Ecology and Forest Management Group, Wageningen University & Research, 6708 PB, Wageningen, Netherlands
[c] School of Agricultural, Forest and Food Sciences, Bern University of Applied Sciences, Länggasse 85, CH-3052 Zollikofen, Switzerland
[d] Soil Biology Group, Wageningen University & Research, 6708 PB, Wageningen, Netherlands

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ABSTRACT

In dryland ecosystems, tree and shrub seedling establishment, growth and survival are limited by access to water and nutrients. Arbuscular mycorrhizal fungi (AMF) increase seedling establishment and survival by enhancing nutrient and water acquisition. We executed a fully-factorial greenhouse experiment to determine the interactive effect of AMF (with and without), water deficit (four levels), and soil layer (topsoil and subsoil) on the biomass, growth, nutrient concentrations, and mycorrhizal root colonization of seedlings of *Commiphora myrrha*, a tree species that dominates large areas of dry forest and woodland in the Horn of Africa. Mycorrhizal seedlings had higher root and shoot biomass than non-mycorrhizal seedlings. They also had higher nutrient concentrations in root and shoot. Plant biomass was higher when plants were grown in topsoil at lower soil moisture levels. Mycorrhizal responsiveness was highest at lower soil moisture. The drought response index was higher for mycorrhizal than for non-mycorrhizal plants, indicating enhanced mycorrhizal benefits at lower water supply. Seedlings grew better in topsoil than in subsoil. Mycorrhizal colonization of roots of *C. myrrha* seedlings was higher with lower moisture and higher in topsoil than in subsoil. The increased performance of mycorrhizal *C. myrrha* indicates that mycorrhization is a major component of the adaptive strategy of seedlings of this species, similar to other species in these dryland deciduous ecosystems. We conclude that for restoration purposes with this species, nursery seedlings should be mycorrhized because of their enhanced growth performance.

1. Introduction

Species of the genus *Commiphora* (Burseraceae) occur mainly in north-eastern Africa and Arabia, but also species diversity is high in Madagascar and there is one neotropical species and also one species in India. The center of diversity is in the *Acacia-Commiphora* woodland of eastern Africa (Gostel et al., 2016). *Commiphora* species occur mainly in dry to arid forests and woodlands. *Commiphora myrrha* (T. Nees) Engl., the source of myrrh, is a small, thorny, resin-producing tree that can grow up to 4 m high and is predominantly found in *Acacia–Commiphora* bushland at 250–1300 m a.s.l., where mean annual rainfall is 230–300 mm (Bekele-Tesemma, 2007). Along with *Boswellia* and *Acacia* species, it is a prominent tree of these dry woodlands. The resin has been used as perfume, incense and medicine (Langenheim, 2003; Lemenih et al., 2014). *Commiphora* species are relatively more abundant in these drier areas than species of the two other genera.

In the dry tropics, low soil moisture, nutrient availability and microbial populations limit seedling establishment and natural regeneration (Khurana and Singh, 2001; Muthukumar and Udaiyan, 2006; Vieira and Scariot, 2006). Seedlings of woody plants in dry habitats show different responses to water deficit, such as reduced water potential, lower relative water content and reduced photosynthetic rate (Gindaba et al., 2005). Stomata play a pivotal role in controlling the balance between water loss and carbon gain (i.e., biomass production) (Augé et al., 2015). Plant respiration rates decrease during periods of drought, due to reduced photosynthate assimilation (Flexas et al., 2006). Additionally, drought can modify the partitioning of assimilates between above-ground and below-ground plant parts. Specifically, seedlings

* Corresponding author.
** Corresponding author.
E-mail addresses: emiru.birhane@mu.edu.et (E. Birhane), lindsey.norgrove@bfh.ch (L. Norgrove).

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invest more biomass in roots as a response to severe drought (Gindaba et al., 2005; Otieno et al., 2005). "Drought tolerance" refers to differential physiological mechanisms as a consequence of higher nutrient assimilation and better nutrition during drought periods and subsequent recovery permitting higher concentrations of soluble protein (Wu and Xia, 2006), and increased enzyme activity. Adaptation of plants to drought is further enhanced through interactions with and assistance by beneficial soil microorganisms, such as arbuscular mycorrhizal fungi (AMF) (Kuyper et al., 2021).

Arbuscular mycorrhizal fungi (AMF) enhance plant growth when phosphate or other immobile nutrients are in short supply (Fagbola et al., 2005; Lambers et al., 2008; Miransari, 2010; Ruiz-Lozano, 2003). The AM symbiosis increases concentrations of P, K, N, Zn, Mg, Cu and Ca in plant tissues under drought conditions (Huat et al., 2002), enhancing seedling growth thus acting as positive feedback (Fagbola et al., 2005). AMF increase drought tolerance, both through these nutritional effects and through changes in photosynthetic efficiency by maintaining stomatal conductance (Birhane et al., 2012; Augé et al., 2015) and the efficiency of photosystem II (Wang et al., 2010). AMF can improve tree seedling establishment by increasing acquisition of water and nutrients, resulting in enhanced potential for restoration (Wubet et al., 2003).

The effect of AMF on *C. myrrha* seedlings is poorly known. Understanding how mycorrhiza increase drought tolerance of *C. myrrha* seedlings would contribute to improved methods for regenerating this species on marginal arid lands. Therefore, a greenhouse experiment was conducted to evaluate the effect of AMF on the growth and biomass response of *C. myrrha* seedlings at different levels of water deficit and soil layer (considered a proxy for differences in soil quality). We grew *C. myrrha* seedlings to address the following research questions:

1. Do water availability, AMF, and soil layer affect the growth and biomass allocation of *C. myrrha* seedlings?
2. Do water availability and soil layer affect the level of AM colonization of roots of *C. myrrha* seedlings?

We hypothesized that: (1) mycorrhizal seedlings would accumulate more biomass and achieve higher nutrient concentrations than non-mycorrhizal seedlings; (2) the beneficial effect of AMF would be stronger under conditions of drought; and, (3) AMF colonization of roots would be higher and mycorrhizal benefits larger in the upper soil layer.

2. Materials and methods

We conducted a greenhouse experiment with *C. myrrha* seedlings in northern Ethiopia at Mekelle University (13°29′N, 39°28′E). The mean daily temperature of the greenhouse was 27 °C during the day and 22 °C during the night, with a mean daily average relative humidity of 62% for the study period. Light was at ambient conditions.

2.1. Seedling preparation and selection

Seeds of *C. myrrha* were obtained from the Central Ethiopia Environment and Forest Research Center (CEE-FRC) in Addis Ababa, Ethiopia, collected from the northwestern lowlands. Seeds were surface-sterilized (with 15% H_2O_2 for 20 min), soaked for 12 h in cold water, after which they readily germinated. Germination took place in plastic trays filled with autoclaved (60 min at 121 °C) pure river sand under greenhouse conditions. No nutrients were added during germination. All seeds germinated within 5–15 days. Four hundred and fifty germinated seeds were individually transplanted into plastic pots, 8 cm diameter and 15 cm high, filled with nursery soil. The nursery soil was sterilized before potting. The nursery soil had a 3:2:1 proportion of topsoil, manure and sand, respectively. Thereafter, the potted seedlings were placed on metal-mesh benches for a month and were watered regularly using micro-sprinkler irrigation to field capacity every other day, until the plants were ready for the experimental treatments. Ninety-six seedlings of uniform size were transplanted into larger perforated 20-L plastic containers, with one seedling per container, and the container filled with 15 kg of autoclaved field soil (see below).

2.2. AMF inoculum and potting soil

AMF inoculum was collected during the dry season in a natural stand of *C. myrrha* trees in the dry deciduous *Acacia-Commiphora* woodland in northwestern Ethiopia. Inoculum was collected from field soil after wet sieving and decanting (Brundrett et al., 1996). The inoculum from the *C. myrrha* rhizosphere was maintained and multiplied under sorghum, and that inoculum was used for inoculation. Visual inspection showed that it mainly consisted of members of the Glomeraceae, however, we did not identify the species of AMF. The fungal inoculum consisted of a mixture of spores, root fragments of sorghum and rhizosphere soil. About 50 g of inoculum was added near the roots of each seedling at the center of the pot. The inoculum contained about 76 spores 100 g^{-1} dry soil and sorghum root colonization was between 60 and 95%. No sterile inoculum was added to the control groups, however, the added inoculum represented <1% of organic carbon and nutrients that were in the pots. Microbial wash was not applied. To mimic the natural growth conditions for the seedlings, the potting soil was excavated from Abergelle Woreda in the Tigray Region of *Ethiopia*, where *C. myrrha* trees occur in *Commiphora-Acacia* woodland (Eshete et al., 2011). Prior to inoculation, the soils were sieved using a 2 mm sieve and sterilized in an autoclave at 121 °C for 2 h. Soil at the experimental site had the following chemical and physical properties, on average, at 0–30 cm depth: 26.1 g OC kg^{-1}; 31.1 mg available P (P-Olsen) kg^{-1}; 24.8 mg exchangeable K kg^{-1}; 2.9 g total N kg^{-1} and 2.62 cmol $^+$ CEC kg^{-1}. Soil texture was 47% sand, 32% silt and 21% clay. pH (H_2O; soil: water ratio 1:2.5) was 6.8; and EC (H_2O; soil: water ratio 1: 2.5) was 1.1 ds m^{-1} (Birhane et al., 2015).

2.3. Experimental design and treatments

We used a three-factorial experimental design for our study. The factors were arbuscular mycorrhiza (inoculated: AM+ and not inoculated: AM-), four water levels (field capacity = 100, 75% of field capacity = 75, 50% of field capacity = 50, 25% of field capacity = 25), and two soil layers (topsoil and subsoil). The topsoil was excavated in the upper 15 cm depth and subsoil below 15 cm depth. Daily deficit was estimated as the difference between pot weight with and without seedlings. The field capacity (FC) for the topsoil and subsoil were calculated and water was supplied to the seedlings as follows. For the topsoil: FC = 500 ml, 75% FC = 375 ml, 50% FC = 250 ml, 25% FC = 125 ml. For the subsoil: FC = 700 ml, 75% FC = 525 ml, 50% FC = 350 ml, 25% FC = 175 ml.

The amount of water to compensate for daily loss was estimated from measurements of pot weight. The mass of water required daily was then supplied to each pot individually. Due to differences in seedling specific water requirements, and the increase in water demand during active growth, treatment of the seedlings with the same amount of water was not considered appropriate (Gindaba, 2006). The treatment units were arranged on greenhouse benches in a randomized complete block design. There were 6 replications, so in all there were 96 pots. The experiment was run for six months.

2.4. Seedling response measurements

We measured several parameters of plant performance as seedling traits. Total shoot length (height) was measured using a graduated meter and diameter at root collar was measured using a digital caliper. The number of fully developed leaves was counted for each seedling. Leaf surface area was measured using an AM 100 Leaf area meter (ADC Bioscientific Ltd.). Harvested seedlings were divided into roots, stems, and leaves, and their dry biomass was determined after oven-drying the

samples at 80 °C until constant weight was achieved. We then calculated the root–shoot ratio for each seedling. Total root length was estimated using the grid line intersect method (Tennant, 1975).

2.5. Plant nutrient analysis

The mineral status of the plants was determined by elemental analysis of shoot and root tissue. After sun-drying, shoot and root samples were oven-dried at 80 °C for 48 h to constant mass. The samples were then ground and analyzed for N, P, and K. N concentration was determined by using the standard Kjeldahl method; P concentration was measured colorimetrically by spectrophotometer and K concentration was measured by Flame Photometry (Anderson and Ingram, 1993). Samples were analyzed at the National Soil Laboratory, Ethiopian Agricultural Research Institute, Addis Ababa, Ethiopia.

2.6. Assessment of mycorrhizal colonization

Mycorrhizal colonization was assessed for the presence or absence of arbuscules, vesicles, and hyphae, using the gridline intersect method (Giovannetti and Mosse, 1980). Subsamples of fine roots were collected, cleared with 10% KOH, rinsed with water, and stained with 0.05% trypan blue in lactoglycerol (Brundrett et al., 1996). Roots were divided into 1-cm pieces and mounted lengthwise on a microscope slide. Six slides per replicate, with 9 root pieces per slide, were examined by making three microscope observations (top, middle, and bottom) per 1 cm root piece at 400 × magnification. Colonization was expressed as percentage of the root colonized. The total mycorrhizal colonization, arbuscules, vesicles, and internal hyphae in the root cortex were recorded.

Relative mycorrhizal responsiveness (MR) and drought response index (DRI) were determined in accordance with Osonubi et al. (1991). MR was expressed as the ratio of total dry weight of the mycorrhizal plant and non-mycorrhizal plant. The DRI was calculated as the ratio of total dry weight of the (mycorrhizal or non-mycorrhizal) plant under conditions of water deficit to that of (mycorrhizal or non-mycorrhizal) plant under well-watered conditions.

2.7. Statistical analysis

Data were analyzed using PASW Statistics 17 software (SPSS Inc., Chicago, IL). Three-way analysis of variance (ANOVA), with AM, water deficit levels and soil layer as independent factors were used to test for differences in seedling size, biomass, and nutrient concentrations, and a two-way ANOVA (only water deficit and soil layer as the independent factors) was used to test for differences in root colonization among treatments. We tested for block effects in our randomized complete block design and, as we found no effects, we report the results excluding the block effect. MR and DRI could not be analyzed statistically as they are based on average values per treatment. Means were compared using the Tukey test if the F-test from ANOVA was significant ($P < 0.05$). To meet the assumptions of normal distribution and normality, data on root collar diameter, root biomass, fine-root dry biomass, and fine-root length were log transformed before analysis.

3. Results

3.1. Effect of AM, water, and soil layer on C. myrrha seedling performance

Plant dry biomass was significantly affected by AMF and soil, whereas the effect of water was not significant ($P = 0.063$, Table 1). Root and shoot biomass generally followed the same patterns, with soil and AMF being significant. Water was a significant source of variation for shoot biomass, but not for root biomass (Table 1). The water × soil interaction was also significant for shoot biomass, but not for root biomass. Root – shoot ratio did not respond significantly to variation in the three main factors, only the two-way interaction water × soil, and the three-way interaction AMF × water × soil were significant (Table 1). Seedling biomass was larger for mycorrhizal plants than for non-mycorrhizal plants, and larger when plants were growing in topsoil than in subsoil (Fig. 1). In topsoil seedlings performed best at 25% of field capacity (Tukey test, data not shown), whereas there was no consistent effect of water availability in subsoil. Fine-root length was only significantly affected by AMF (Table 1):mycorrhizal plants had higher fine-root length than non-mycorrhizal plants. Plants grown in topsoil produced more leaf area than plants in subsoil, whereas the significant AMF × water interaction term indicated that mycorrhizal effects were larger under the lowest field capacity. Mycorrhizal responsiveness (Table 2) was always larger than 1, indicating mycorrhizal benefit under all conditions, being highest at the lowest water availability. DRI was higher for mycorrhizal than for non-mycorrhizal plants and higher for seedlings growing in topsoil than those growing in subsoil. DRI in topsoil was usually larger than one, (Table 2).

Table 1
Results of a three-way ANOVA showing the effects of AMF, water level (% FC), and soil layer (topsoil and subsoil) on biomass (A), plant growth traits (B) and (shoot & root) N, P, and K concentration (C) of *C. myrrha* seedlings. Note:* Significant at the 0.05 probability level, ** Significant at the 0.01 probability level and *** Significant at the 0.001 probability level.

Parameter	AM		Water		Soil		AM * water		AM * soil		water * soil		AM * water * soil	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
A														
Shoot dry mass	4.690	*	3.355	*	21.178	***	.387	.762	.637	.427	4.734	**	.998	.398
Root dry mass	6.712	*	.490	.690	8.002	**	.965	.414	.662	.418	1.288	.284	.597	.619
Plant dry mass	5.409	*	2.530	.063	22.168	***	.816	.489	.138	.711	2.329	.081	.078	.972
Root–shoot	2.395	.126	.748	.527	1.623	.206	2.154	.100	.322	.572	3.116	*	4.770	**
B														
Root hair frequency	3.548	.063	.385	.764	3.206	.077	1.308	.278	.604	.439	.038	.990	.289	.833
Coarse root diameter	.039	.844	2.082	.109	3.174	.079	1.176	.324	.061	.806	.877	.456	.044	.988
Fine root length	4.073	*	.128	.943	1.462	.230	.243	.866	2.077	.153	.303	.823	.220	.882
Leaf area	.736	.394	1.102	.355	14.658	***	6.893	***	.076	.783	.515	.673	.899	.447
N-root	15.466	***	1.751	.163	28.720	***	1.549	.208	2.213	.141	.239	.869	.414	.743
N-shoot	10.438	*	.897	.446	92.760	***	1.757	.620	.986	.324	2.898	.*	.929	.431
P-root	40.843	***	4.286	**	2.391	.126	2.616	.057	4.580	.*	.845	.473	4.498	.**
P-shoot	50.688	***	3.472	*	3.465	.066	.866	.462	2.104	.151	.777	.510	3.980	.*
K-root	65.045	***	6.766	***	1.046	.309	2.574	.060	2.267	.136	1.009	.393	2.292	.084
K-shoot	61.538	***	6.766	***	.773	.382	2.584	.059	.778	.380	1.842	.146	2.257	.088

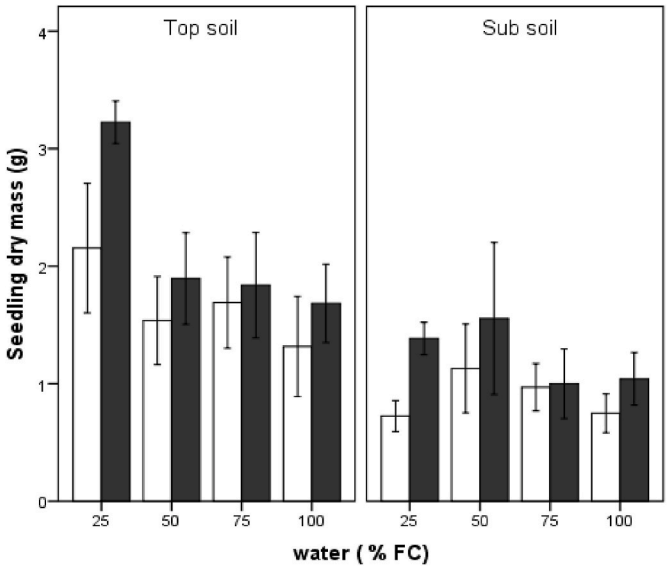

Fig. 1. *C. myrrha* seedling total biomass (mean ± 1 s.e.) under conditions of arbuscular mycorrhiza inoculation – inoculated (black bars) and not inoculated (white bars), soil layer (topsoil and subsoil), and water availability (25% field capacity (FC), 50% FC, 75% FC, and 100% FC) on.

Table 2
Mycorrhizal responsiveness (MR) and drought response index (DRI) of inoculated and non-inoculated *C. myrrha* seedlings at different water levels (25%FC, 50%FC, 75%FC and 100%FC), and soil depth (topsoil and subsoil).

Water levels (% FC)	Mycorrhizal responsiveness		DRI of mycorrhizal seedlings		DRI of non-mycorrhizal seedlings	
	Top soil	Sub soil	Top soil	Sub soil	Top soil	Sub soil
25	1.50	1.91	1.70	0.89	1.40	0.64
50	1.23	1.38	1.75	1.39	1.27	0.75
75	1.09	1.03	1.92	1.33	1.64	0.97
100	1.28	1.39				

3.2. Effect of AM, water level, and soil on nutrient concentration in roots and shoots

Three-way ANOVA showed that nitrogen (N) concentrations of shoots and roots were significantly affected by AMF and soil layer, but not by water (Table 1). Concentrations of phosphorus (P) and potassium (K) in both shoots and roots were significantly affected by AMF and water, but not different between soil layers. The two-way interactions and the three-way interaction were, in most cases, not significant sources of variation, except for shoot and root P, where the three-way interaction AMF × water × soil was significant (Table 1). Nitrogen concentrations in both shoots and roots were higher in mycorrhizal than in non-mycorrhizal seedlings, and higher in seedlings grown in topsoil than in subsoil (Fig. 2). Averaged over all treatments, shoots and roots of mycorrhizal seedlings had, respectively 15% and 43% higher concentrations of nitrogen. Mycorrhizal seedlings also had higher P concentrations (+19% in shoots, +17% in roots) and K concentrations (+15% in shoots, +21% in roots) than non-mycorrhizal seedlings. The effect of water varied with the different levels and did not exhibit a consistent pattern (Fig. 2). Seedlings growing in topsoil had 97% higher N concentrations in their shoots, and 55% higher N concentrations in their roots, a larger effect of soil on N concentrations than on biomass, suggesting that differential N availability between both soil layers was a major factor.

3.3. The effect of water and soil layer on mycorrhizal root colonization

Non-inoculated plants remained free of mycorrhizal root colonization. There were no significant differences in arbuscular, vesicular or hyphal colonization of *C. myrrha* seedlings at different water levels (Table 3). However, hyphal and vesicular colonization were significantly higher for seedlings grown in topsoil than subsoil (Table 3). A significant interaction of water * soil was additionally observed for vesicular colonization (Table 3). There was a significantly positive correlation between mycorrhizal root colonization of inoculated seedlings and seedling dry biomass in topsoil ($r = 0.51$, $p = 0.011$), but in subsoil these parameters were uncorrelated (Fig. 3).

4. Discussion

The impacts that AMF have on drought tolerance are complex, including both nutritional and non-nutritional effects (Wu et al., 2013; Kuyper et al., 2021). Wu et al. (2013) highlighted that extraradical hyphae can improve water and nutrient uptake because of access to smaller soil pores that are inaccessible to plants, however, the (very) small diameter of the fungal hyphae might be a limiting factor for water transport to roots because of physical constraints as flow rates through tubes scale with the fourth power of hyphal diameter (Kuyper et al., 2021). Enhanced uptake of nutrients such as P and K would also confer drought tolerance. AM fungi also modify the hormonal balance of the plant, resulting in increases in stomatal conductance and transpiration, which allow mycorrhizal plants a larger window of opportunity for growth during soil drying (Augé et al., 2015). These authors also noted that the mycorrhizal benefits are relatively larger under less favorable conditions such as drought. with a cascading impact on nutrient status. Changes in the hormonal status of the plant as a consequence of mycorrhization could also result in changes in root system architecture as demonstrated by Wu et al. (2013) for citrus plants. Nutritional and non-nutritional effects of mycorrhizal symbiosis in conferring drought tolerance to plants interact; separating both effects was beyond the aims of our study.

Our first hypothesis that mycorrhizal seedlings both gain biomass and have higher concentrations of the macronutrients N, P, K than non-mycorrhizal seedlings is supported by the results of our study and agrees with studies on other tree species (Birhane et al., 2012; Gholamhoseini et al., 2013; Turjaman et al., 2006; Wang et al., 2010; Xie et al., 2014). Inoculation with AMF did not result in changes in root - shoot ratios. Veresoglou et al. (2012) noted that mycorrhizal plants had a lower root – shoot ratio than non-mycorrhizal plants because plants outsource acquisition of nutrients and water to their fungal partner, reducing the need for investment in root biomass. However, as noted by Kuyper et al. (2021), mycorrhizal plants are usually larger than non-mycorrhizal plants, and such ontogenetic shifts could also result in changes in root – shoot ratio, and the shift may not be significant after allometric correction. Mycorrhizal plants had more root biomass and a higher fine-root length than non-mycorrhizal plants, suggesting that *absolute* increases in C gain due to the mycorrhizal symbiosis could outweigh *relative* allocation of this C gain between root and shoots.

Our second hypothesis was that the benefit of AMF is more pronounced under conditions of drought. This hypothesis was partly confirmed. Plant performance data did not show a significant AM × water interaction for root, shoot, and total biomass, however the interaction term was significant for leaf area, indicating that under drier conditions mycorrhizal plants were able to maintain a larger leaf area, which subsequently could feed back on photosynthesis and hence biomass performance over longer time periods. Evidence for an ecologically important AM × water interaction was also obtained through determination of mycorrhizal responsiveness, which was highest for seedlings growing at lowest water supply; and through determination of the drought response index, which was higher for mycorrhizal than for non-mycorrhizal seedlings. This drought response

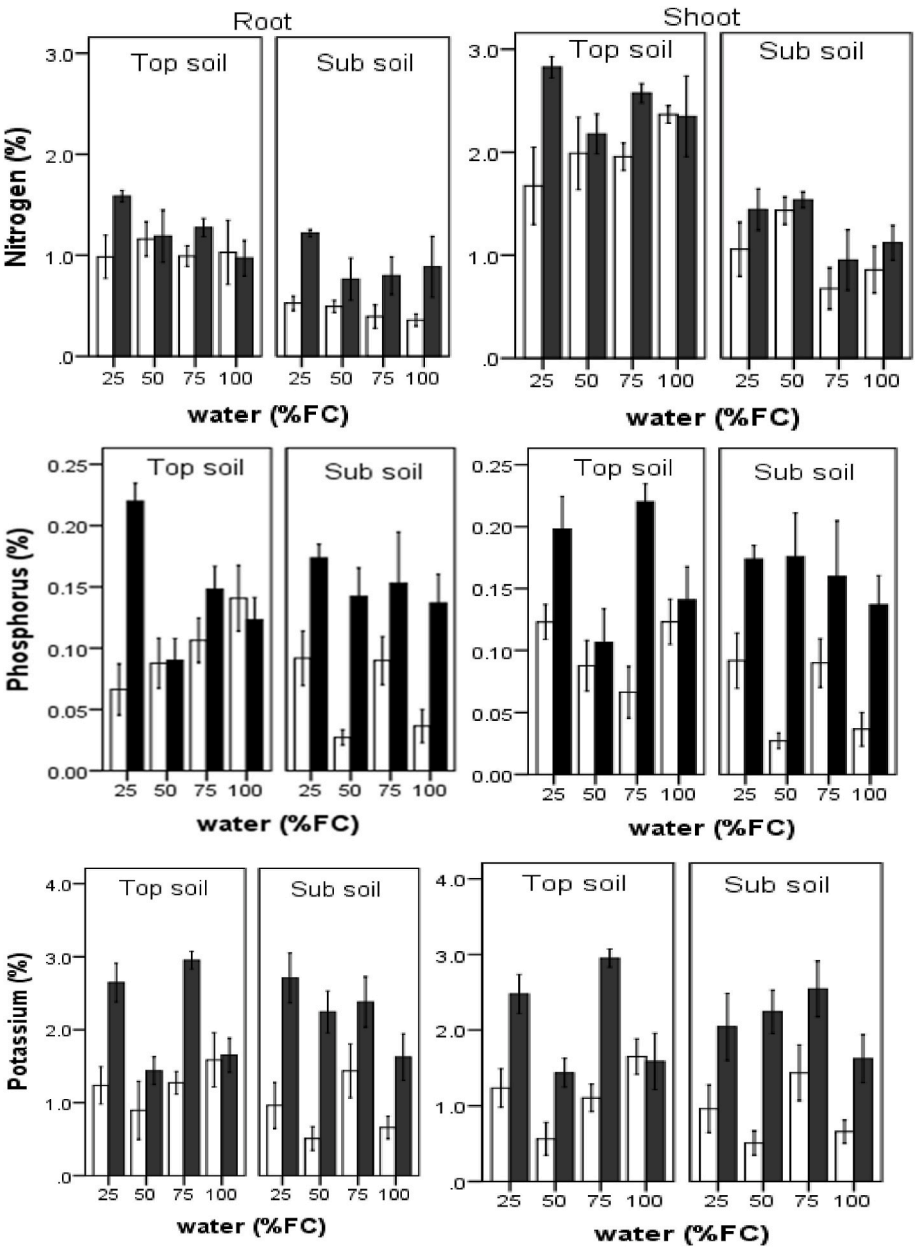

Fig. 2. Effect of water level (25% field capacity (FC), 50% FC, 75% FC, and 100%FC), mycorrhizal inoculation (inoculated – black bars and not inoculated – white bars), and soil layer (topsoil and subsoil) on shoot and root N, P, and K concentration of *C. myrrha* seedlings (mean±1 s.e.).

index was introduced by Osonubi et al. (1991) to quantify the mycorrhizal effect in conferring drought tolerance. They noted that mycorrhizal inoculation did generally increase DRI, but the effect was not significant. Their study with four legume trees showed that the index was always below 1, whereas it was almost consistently larger than 1 in our experiment. This observation then might cause doubts about the effectiveness of the drought treatment, as our seedlings, grew better in drier soil than in the soil at field capacity. This deviating behavior of DRI may be related to specific drought adaptation capabilities of members of the Burseraceae, as an earlier study on *Boswellia papyrifera* (Caill.) Hochst. equally showed reduced performance at the higher water availability, whereas two members of the Fabaceae (*Vachellia etbaica* (Schweinf.) Kyal. & Boatwr. (formerly *Acacia etbaica*) and *Senegalia senegal* (L.) Britton (formerly *Acacia senegal*)) showed increased performance at the higher water availability (Birhane et al., 2015). Hailemariam et al. (2018) equally noted that *Faidherbia albida* (Delile) A. Chev., another member of the Fabaceae, had higher growth performance at higher water supply. In another study on *B. papyrifera,* Birhane et al. (2012) also concluded that drought stress apparently benefits mycorrhizal seedlings. They referred to that strategy as *waiting in the underground*, where water and carbohydrate reserves can be stored in belowground coarse roots as an adaptation to long periods without rain.

The effect of a DRI larger than one was especially noteworthy when seedlings were grown in topsoil. We initially hypothesized that mycorrhizal colonization and plant benefits by mycorrhiza would be larger in topsoil than in subsoil, as soil from deeper layers, exposed from land degradation and soil erosion, could hamper tree regeneration. That hypothesis was confirmed, however, we did not predict that the effect of soil layer on plant biomass and shoot and root nitrogen content, and on various aspects of mycorrhizal colonization (vesicular and hyphal colonization) would be so large, often outweighing the effect of mycorrhizal inoculation and water. Species of *Commiphora* are reported to be shallow rooted (Breman and Kessler, 1997). Fan et al. (2017) provided data on rooting depth for eight species of *Commiphora*,

Table 3
Results of a two-way ANOVA analysis showing the effect of water level and soil layer, and their interaction on the percentages of arbuscular, vesicular, and hyphal colonization of *C. myrrha* seedlings.

Colonization	Water		Soil		Water * Soil	
	F	P	F	P	F	P
Arbuscular	0.522	0.669	0.159	0.692	1.220	0.315
Vesicular	0.365	0.779	6.366	*	3.607	*
Hyphal	0.575	0.634	8.801	**	0.536	0.660

Note:* Significant at the 0.05 probability level, ** Significant at the 0.01 probability level and *** Significant at the 0.001 probability level.

although unfortunately, *C. myrrha* was not included. They reported rooting depths ranging from 35 to 130 cm, with an average of 75 cm. While it may be possible that in some of these observations rooting depth is constrained by the shallowness of the soil profile, it could also be possible that chemical, physical or biological differences between topsoil and subsoil impact on the suitability of soil layers for roots.

In our study, we did not assess differences in soil chemical, physical or biological properties between layers, so we can only speculate on the causes of this large effect of soil layer. Topsoil and subsoil may differ in the quality of soil organic matter. Topsoil C:N was around 9, whereas that of subsoil was around 13, implying probably lower N mineralization during decomposition. Consistent with an hypothesis of enhanced N-limitation in subsoil, we observed a highly significant difference in shoot and root N concentrations between topsoil and subsoil, yet no differences in shoot and root P and K concentrations between seedlings grown in topsoil and subsoil. Consequently, leaf N:P ratios, which are considered an indication whether plant performance is N versus P-limited (Güsewell, 2004) were above 15 in topsoil (indicating P limitation) and 8 in subsoil (indicating N limitation). Likewise, Birhane et al. (2015), in an earlier study with topsoil and subsoil, noted a highly significant difference in biomass performance, mycorrhizal colonization, and shoot N concentration between topsoil and subsoil for *Boswellia papyrifera*. However, for two legume species, which were formerly classified in the genus *Acacia, Vachellia etbaica* and *Senegalia senegal* that both rely on N_2 fixation, there was no (*V. etbaica*) or only small (*S. senegal*) effect of soil layer on biomass performance. Leaf N:P ratios of these three tree species in topsoil ranged between 10 and 12, and in the subsoil for *S. senegal* and *B. papyrifera* between 6 and 8, consistent with N limitation in subsoil.

Next to potential differences in N cycling, topsoil and subsoil could differ in physical soil qualities such as bulk density, with higher bulk density in subsoils impeding root growth and potentially allowing anoxic conditions at the highest water availability. Too much water could also have negatively impacted on mycorrhizal fungal activity, and the significantly lower vesicular and hyphal colonization in subsoil than in topsoil could reflect some specific constraints on fungal growth. The significant relationship between mycorrhizal colonization and seedling performance in topsoil, but not in subsoil, could equally indicate limitations to mycorrhizal functioning. Irrespective of the specific cause for the differential performance of *C. myrrha* seedlings in topsoil and subsoil, our data suggest that land degradation and soil erosion could expose subsoil and this subsoil might subsequently hamper seedling growth and hence the regeneration of this species of significant cultural and economic value.

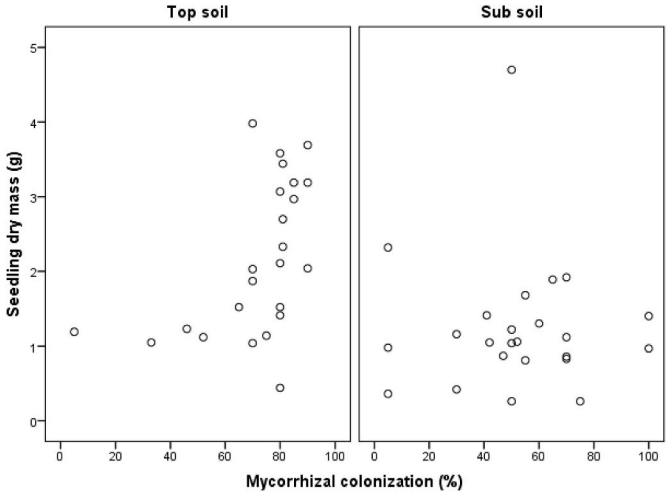

Fig. 3. Relationship of mycorrhizal root colonization and dry mass of *C. myrrha* seedlings in topsoil (r = 0.51, p = 0.011) and subsoil (r = 0.00, p = 0.98).

5. Conclusion

Seedlings of *C. myrrha* are responsive to AMF and the role of mycorrhiza is somewhat larger when seedlings are growing with less water. Better seedling performance in the drier soil possibly reflects specific adaptations of certain dryland tree seedlings to severe drought. Seedlings grow much better and have significantly higher mycorrhizal root colonization when growing in topsoil than when growing in subsoil, possibly associated with inadequate nitrogen nutrition in subsoils. Considering the beneficial effects of AMF on *C. myrrha* seedling performance, it is advisable to ensure adequate mycorrhization when managing regeneration of *C. myrrha*. Mycorrhizal management is especially important in cases of inoculum limitation, which is more of a problem in subsoils and hence in soils that have been eroded and where subsoil has become exposed. If adequate mycorrhization in the field cannot be ensured, due to inoculum limitation, inoculating seedlings with AMF inoculum in nurseries is recommended. Inoculation can be easily achieved by adding soil from field-grown *C. myrrha* to nursery plantations.

CRediT authorship contribution statement

Emiru Birhane: Formal analysis, Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft. **Frans Bongers:** Supervision, Conceptualization, Formal analysis, Funding acquisition, Methodology, Writing – review & editing. **Abebe Damtew:** Formal analysis, Data curation, Investigation, Validation, Writing – original draft, Writing – review & editing. **Abadi Tesfay:** Data curation, Investigation, Resources. **Lindsey Norgrove:** Formal analysis, Writing – review & editing. **Thomas W. Kuyper:** Conceptualization, Formal analysis, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jaridenv.2022.104877.

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