Research Application Summary

Arbuscular mycorrhiza and water and nutrient supply differently impact seedling performance of acquisitive and conservative dry woodland species

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Abstract

Arbuscular mycorrhizal (AM) fungi are known to increase seedling survival and performance through enhancement of nutrient and water uptake. *Acacia etbaica*, *Acacia senegal* and *Boswellia papyrifera* dominate large areas in African drylands. We evaluated the effects of AM, soil and water availability on their seedling growth. The experiment was factorial and was set up in a greenhouse with and without AM, topsoil and subsoil, and four levels of water availability. AM symbiosis enhanced the acquisition of water and nutrients and increased gas exchange resulting in increased *Acacia* and *Boswellia* seedling biomass. The rapidly growing *Acacias* (acquisitive strategy) showed larger mycorrhizal benefit at higher water availability. The slowly growing *Boswellia* (conservative strategy), in contrast, showed larger mycorrhizal benefit at lower water availability: with its large coarse root and only few fine roots *Boswellia* benefits at low root density and resource availability as AM enlarges water and nutrient uptake capacity. This study suggests how acquisitive and conservative species of dry woodlands may benefit from AM in different ways and that inclusion of the mycorrhizal habit in trait-based approaches increases understanding of functional divergence of coexisting tree species.

Key words: *Acacia*, Arbuscular mycorrhiza, *Boswellia*, dry deciduous wood land, nutrient availability, plant strategy, trait-based ecology, water deficit

Résumé

Les champignons arbuscularmycorrhizal (AM) sont connus dans l'augmentation de la survie et de la performance des semis grâce à l'amélioration de l'absorption des éléments nutritifs et de l'eau. *Acacia etbaica*, *Acacia senegal* et *Boswelliapapyrifera* dominent de grandes surfaces dans les régions arides de l'Afrique. Nous avons évalué les effets de l'AM, le sol et la disponibilité de l'eau sur la croissance de leur plantule. L'expérience a été factorielle et effectuée dans une serre avec et sans l'AM, la couche arable et le sous-sol, et quatre niveaux de disponibilité de l'eau. La symbiose d'AM a amélioré l'acquisition de l'eau et des nutriments et a accru l'échange de gaz résultant de la biomasse accrue des semis d'*Acacia* et de *Boswellia*. Les *Acacias* à croissance rapide (stratégie acquisitive) ont montré des avantages des mycorhizes plus importants à une plus grande disponibilité de l'eau. Le *Boswellia* à croissance lente (stratégie conservatrice), au contraire, a montré des avantages des mycorhizes plus importants pour une plus faible disponibilité de l'eau: avec sa large racine grossière et seulement quelques racines fines, *Boswellia* bénéficie de la faible densité des racines et la disponibilité des ressources telles que AM agrandit la capacité d'absorption des éléments nutritifs et de l'eau. Cette étude suggère comment les espèces acquisitives et conservatrices des forêts sèches peuvent bénéficier d'AM dans différentes manières et que l'inclusion de l'habitude des mycorhizes dans les approches fondées sur les traits augmente la compréhension de la divergence fonctionnelle des espèces d'arbres coexistantes.

Mots clés: *Acacia*, Arbuscularmycorrhiza, *Boswellia*, terres arides de feuillus, disponibilité des nutriments, stratégie végétale, écologie basée sur le trait, déficit en eau

Background

Drought and low soil fertility are the two major rehabilitation constraints in arid areas, since they have a direct negative impact on plant physiological processes that affect establishment and survival of seedlings. They also have a negative effect on soil biodiversity (including beneficial root symbionts) that indirectly impact on seedling performance. Under these hostile conditions, improvement of nutrient and water acquisition increases establishment success (Engelbrecht *et al*., 2005). Land plants have evolved a range of strategies that enable them to effectively access nutrients and water under stress conditions. Plant strategy theory (Díaz *et al*., 2004) indicated a major axis to order two different strategies of resource acquisition, viz., a conservative versus an acquisitive strategy. Studies on plant strategies are usually limited to above-ground plant functional traits. However, due to coordination of above-ground and below-ground traits,it is likely that the same classification can be applied for nutrient and water acquisition by roots. Plant roots show a large diversity in morphological and physiological traits. A major root trait that is relevant for classifying plant strategies includes root morphology, ranging from thin, branched fine roots with a large number of long root hairs to coarse unbranched roots with few and shorter root hairs. Another set of traits relates to special modified organs for nutrient acquisition (cluster roots, dauciform roots) or the ability to associate with beneficial rhizosphere organisms such as N-fixing bacteria or mycorrhizal fungi (Hodge, 2009; Smith *et al*., 2010). However, root symbioses remain poorly integrated in plant strategy theory. In this study, we investigated in a fully factorial experiment the effects of AM, water stress, and soil fertility on carbon gain (growth), gas exchange and nutrient levels of seedlings.

Literature Summary

The most prevalent mycorrhizal association that plants form is with arbuscular mycorrhizal (AM) fungi (Brundrett, 2009). Plant species with different root (functional) traits generally differ in mycorrhizal responsiveness. Early research (Bayliss, 1975) indicated that plants with thick coarse unbranched roots with few short root hairs (magnolioid roots) would be more dependent on and responsive to mycorrhizal symbiosis than plants with fine branched roots with numerous long root hairs (graminoid roots). Mycorrhizal responsiveness and dependence could then be ordered along the axis of conservative versus acquisitive strategies. While plant strategy theory suggests that plant functional types segregate along both a light and a moisture resource axis in similar ways, studies provide evidence that a trait-based model better explains niche differentiation along a light gradient than along a moisture gradient. We are not aware of studies how different functional root traits (the magnolioid versus the graminoid root) interact with the mycorrhizal symbiosis to confer drought resistance. Such knowledge is not only relevant to improve understanding of plant functional types, but also for rehabilitation efforts, where degraded soils have often low levels of organic matter, nutrients and water.

Acacia species are cultivated for arabic gum while *Boswellia* provides frankincense; it is predicted that these will decline rapidly in abundance (Groenendijk *et al*., 2011). These species occur on poor and often eroded water-stressed soils and benefit from AM fungi as shown in greenhouse trials (Birhane *et al*., 2012). Species of *Acacia* possess an acquisitive strategy, while *Boswellia* possesses a conservative strategy.

Study Description

A greenhouse experiment with *Acacia senegal, A. etbaica*, and *Boswellia papyrifera* seedlings was conducted in the greenhouse during the rainy season in northern Ethiopia at

Mekelle University (13°292 N 39°282 E; altitude 2200 m a.s.l.) in 2009. The mean daily temperature of the greenhouse was 27 ^{0}C during the day and 22 ^{0}C during the night with mean daily average relative humidity of 62 % for the study period. The species will henceforth be referred to as *A. senegal*, *A. etbaica* and *Boswellia.*

Seeds of *A. etbaica* and *Boswellia* were collected in March 2007 from adult trees from the dry deciduous woodlands in Abergelle, north Ethiopia. Trees with single stem, healthy, and with uniform seed setting were considered during seed collection. Seeds were directly picked by hand from the tree branches either by climbing or standing on the ground depending on tree height. Seeds of *A. senegal* were obtained from the forestry research center, Addis Ababa, Ethiopia, collected from the lowlands of northwestern Ethiopia, in 2007. Seeds were treated before germination. Germination took place in plastic trays filled with autoclaved pure river sand under greenhouse conditions. All seeds germinated within 5-15 days. A total of 450 seedlings were individually transplanted to plastic pots, 8 cm diameter and 15 cm high. Potted seedlings were placed on metal mesh benches and watered regularly using micro-sprinkler irrigation every other day to field capacity until the plants were ready (one month) for the experiment. Two hundred eighty eight (288) seedlings of uniform size were transplanted to larger perforated 20 liter plastic containers, one seedling per container, filled with 15 kg autoclaved soil collected from the dry deciduous woodland.

Inoculum was collected during the dry season from the rhizosphere of dry deciduous woodland trees (mainly *Boswellia*) by the wet sieving and decanting method (Brundrett *et al*., 1996). In those soils, spores of *Glomus* species were dominant (Birhane *et al*., 2010). The fungal inoculum added to the experimental seedlings consisted of a mixture of soil, spore and root fragments, produced from rhizosphere soil and roots of pre-colonised *Sorghum bicolor* plants. About 50 g of fungal inoculum was added near the roots of each seedling at the center of the pot. In order to mimic the natural growth conditions for the seedlings the potting soil was excavated from Abergelle, from a similar habitat where both *Acacia* species and *Boswellia* trees naturally g

A three-factorial experimental design was used. The factors were the presence or absence of arbuscular mycorrhiza (AM+ and AM-), four water levels (field capacity, 75% of field capacity, 50% of field capacity, 25% of field capacity), and two soil types (topsoil and subsoil). The treatment units were arranged on greenhouse benchesin a completely randomised design. There were 6 replications which gave a total of 288 seedlings. Seedling biomass measurements, plant nutrient analysis and assessment of mycorrhizal colonisation, was done after harvest and gas exchange measurements at the end of the experiment.

Research Application

The presence of AM had significantly different effects on seedlings of the conservative *Boswellia* than on the more acquisitive *Acacia* species (Figs. 1 and 2). *Boswellia* allocated most resources to a coarse root, with few resources left for leaf area, while *Acacia* species produced a much larger leaf area and a smaller (in mass), but extensive, fine root system. Remarkably, these species differed much less in leaf level assimilation rates. We find support for the idea that the coarse roots allow *Boswellia* seedling to save resources for surviving the long dry period, and improving the positive impacts of AM on water and nutrient acquisition and in turn leaf assimilation, during dry spells in the rain season.

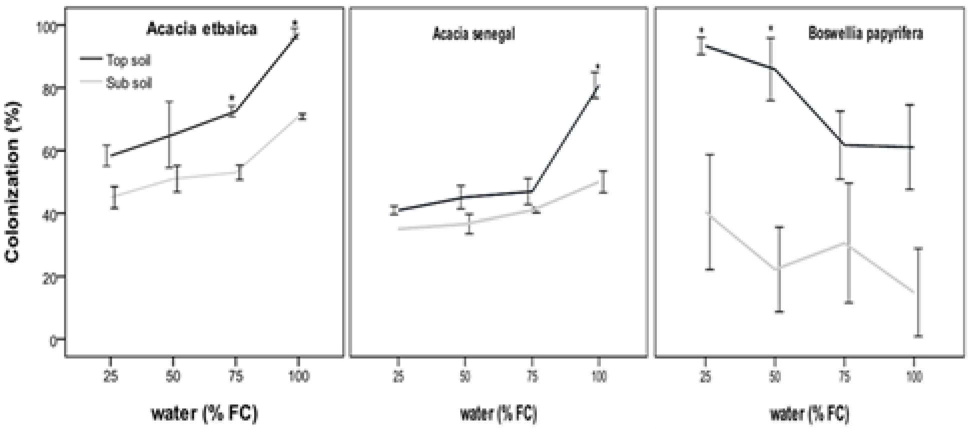

Figure 1. Fractional AM root colonization levels of seedlings (mean ± 1 SE) of *Acacia etbaica*, *A. senegal* and *Boswellia papyrifera* in topsoil (black line) and subsoil (gray line) under four water levels. Asterisk (*) indicates a significant difference between topsoil and subsoil at $p < 0.05$.

In contrast, *Acacia* seedlings, without coarse root system reserves, might not be able to maintain such a positive impact of AM during very dry periods, and only benefit at high water supply conditions when AM might enhance the acquisition of nutrients in these species. These findings strongly suggest how the mycorrhizal symbiosis contributes to the functional traits of root functioning along a species strategy axis from acquisitive to conservative strategies for the dry woodlands in the Horn of

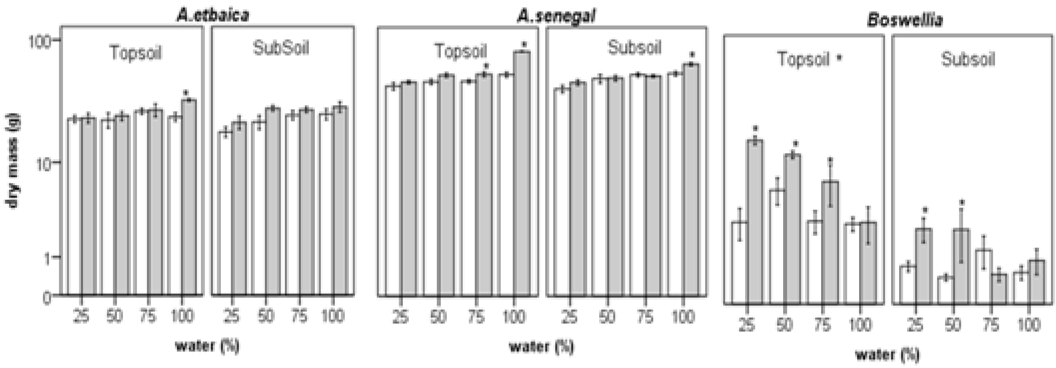

Figure 2. Dry mass response of seedlings (mean ± 1 SE) of *Acacia etbaica*, *A. senegal*, and *Boswellia papyrifera* without AM (open bars) and with AM (solid bars) under four water levels and in topsoil and subsoil. Note that dry mass on the y axis is on a log scale. Asterisk (*) above a solid bar indicates a significant difference between non-mycorrhizal and mycorrhizal treatment at $p < 0.05$. Asterisk (*) as superscript to topsoil indicates a significant difference between topsoil and subsoil at $p < 0.05$.

Africa. Larger species samples and a larger diversity of mycorrhizal fungi are needed to test generality of this species strategy axis in other dry forest areas across the world.

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References

Auge, R.M. 2001. Water relations, drought, and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* 11:3-42.

Baylis, G.T.S. 1975. The magnolioid mycorrhiza and mycotrophy in root systems derived from it. pp. 373-389. In: Sanders, F.E., Mosse, B. and Tinker, P.B. (Eds.). *Endomycorrhizas*. New York USA: Academic Press.

Birhane, E., Kuyper, T.W., Sterck, F. and Bongers, F. 2010. Arbuscular mycorrhizal associations in *Boswellia papyrifera* (frankincense-tree) dominated dry deciduous woodlands of Northern Ethiopia. *Forest Ecology and Management* 260:2160–2169.

Brundrett, M., Bougher, N., Dell, B., Giove, T. and Malajczuk, N. 1996. Working with mycorrhiza in forestry and agriculture. ACIAR Monograph 32. Canberra, Australia: Pirie Printers.

Diaz, S., Hodgson, J.G., Thompson, K., Cabido, M., Cornelissen J.H.C., Jalili, A., Montserrat-Marti, G., Grime, J.P., Zarrinkamar, F. and Astri, Y. 2004. The plant traits that drive ecosystems: Evidence from three continents. *Journal of Vegetation Science* 15:295–304.

Engelbrecht, B.M.J., Kursar, T.A. and Tyree MT. 2005. Drought effects on seedling survival in a tropical moist forest. *Trees* 19:312–321.

Giovannetti, M. and Mosse, B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection. *New Phytologist* 84:489–500.

Kaschuk, G., Kuyper, T.W., Leffelaar, P.A., Hungria, M. and Giller, K.E. 2009. Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and Arbuscular mycorrhizal symbioses? *Soil Biology & Biochemistry* 41: 1233–1244.

Querejeta, J.I., Egerton-Warburton, L.M. and Allen, M.F. 2003. Direct nocturnal water transfer from oaks to their mycorrhizal symbionts during severe soil drying. *Oecologia* 134:55-64.

Ruiz-Lozano, J.M. and Aroca, R. 2010. Host response to osmotic stresses: stomatal behaviour and water use efficiency of arbuscular mycorrhizal plants. pp. 239-256. In: Koltai, H. and Kapulnik, Y. (Eds.). *Arbuscular Mycorrhizas: Physiology and Function*. Springer Science +Business Media.

Smith, S.E., Facelli, E., Pope, S. and Smith, F.A. 2010. Plant performance in stressful environments: interpreting new and established knowledge of the roles of arbuscular mycorrhizas. *Plant Soil* 326:3–20.

Zangaro, W., Nishidate, F.R., Camargo, S.F.R., Romagnoli, G.G. and Vandressen, J. 2005. Relationships among arbuscular mycorrhizas, root morphology and seedling growth of tropical native woody species in southern Brazil. *Journal of Tropical Ecology* 21:529-540.