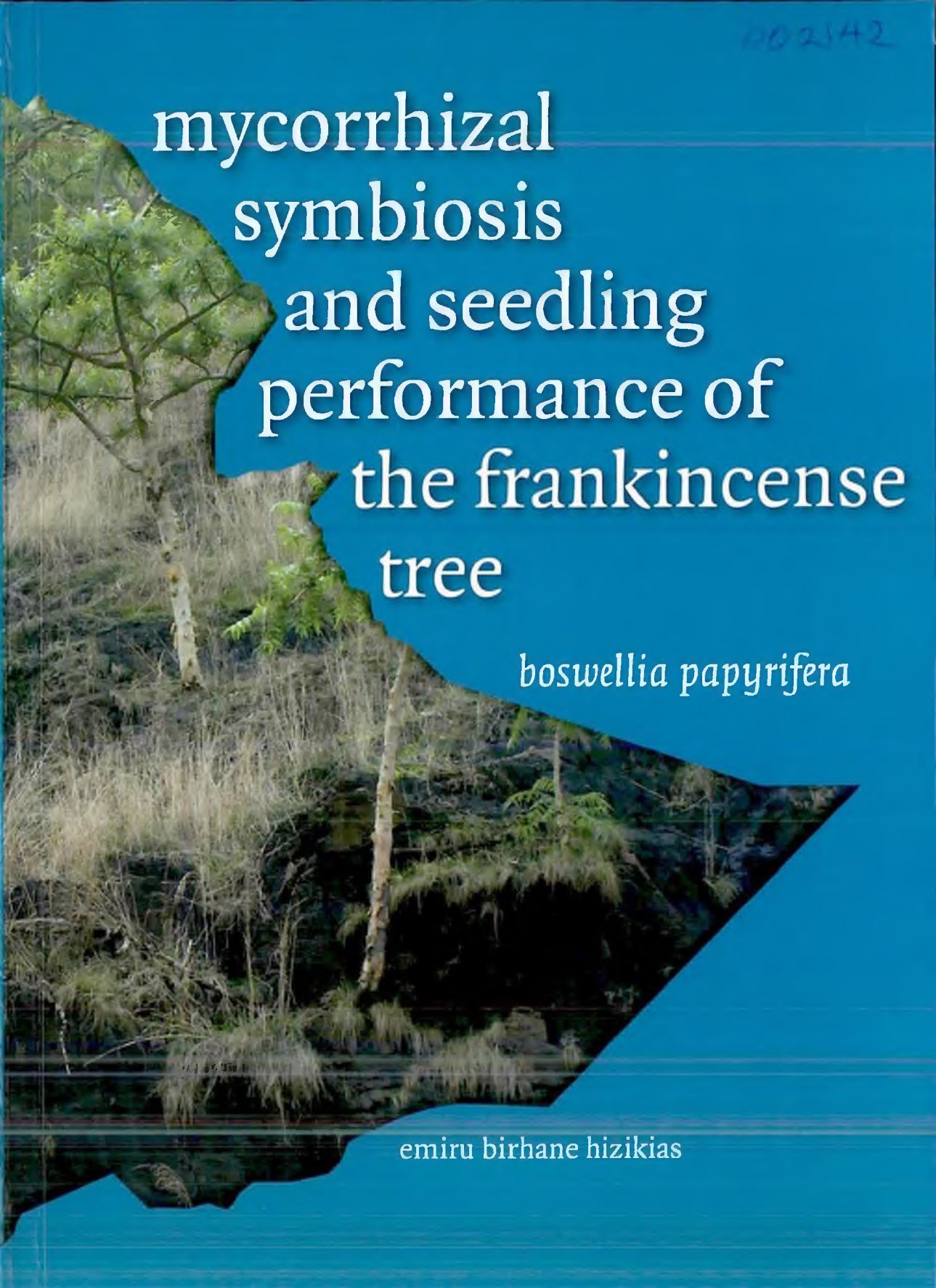


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**MYCORRHIZAL SYMBIOSIS AND SEEDLING PERFORMANCE OF
THE FRANKINCENSE TREE (*BOSWELLIA PAPYRIFERA*)**

Emiru Birhane Hizikias

2011

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**MYCORRHIZAL SYMBIOSIS AND SEEDLING PERFORMANCE OF
THE FRANKINCENSE TREE (*BOSWELLIA PAPYRIFERA*)**

Emiru Birhane Hizikias

Thesis

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Emiru Birhane Hizikias

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To **Abay Hizikias**, you took me out from the darkness of ignorance to the light of education

To *Gidey Gebreyohannes, Thomas and Fanuel* you are always my strength.

To **Birhane Hizikias** who wanted to see the end, but left early.

To my parents, brothers, sisters and friends

THANK YOU!!!



Abstract

Arid areas are characterized by a seasonal climate with a long dry period. In such stressful environment, resource availability is driven by longterm and shortterm rainfall pulses. Arbuscular Mycorrhizal (AM) fungi enhance access to moisture and nutrients and thereby influence plant performance. In this dissertation I applied field observations and greenhouse experiments to address four questions: 1) What are the major environmental factors influencing AM incidence in the *Boswellia*-dominated dry deciduous woodlands? 2) How do *Boswellia* seedlings respond when they are exposed to AM fungi and water pulses? 3) How do AM fungi, water deficit and soil fertility influence the growth and gas exchange of *Boswellia* and *Acacia* seedlings? 4) Does the AM symbiosis influence competition between *Acacia* and *Boswellia* seedlings at different water pulse levels?

The present study showed that almost all woodland plants in northern Ethiopia are colonized by AM fungi. Root colonization levels in dry and wet seasons demonstrated that in the sites with the harshest conditions, AM plants and fungi respond to pulsed resource availability by temporally disconnecting carbon gain by the plant and carbon expenditure by the fungus. Consequently, we studied below-ground processes in conferring adaptation to highly pulsed resources in *Boswellia* seedlings. The strong interactive AM fungi and water pulse showed that mycorrhizal *Boswellia* benefits from drought pulses during the short rainy season. *Boswellia* acquires carbon and water after rain events and store probably carbon and water in coarse roots, suggesting conservative strategy. From this observation we carried out an experiment to test whether other trees (*Acacias*) than *Boswellia* in this habitat also show this conservative acquisition strategy, or whether more acquisitive strategies may also be beneficial under such climates.

My study show that acquisitive and conservative species both benefit from the AM symbiosis, but that the acquisitive *Acacias* mainly benefit at higher water availability, whereas the conservative *Boswellia* benefits at water or nutrient-stressed conditions. I also investigate on how mycorrhiza and water availability affect competition between plants with different resource acquisition strategies in these drylands. Seedlings of *Boswellia* are competitively inferior to seedlings of *Acacia*, and neither the presence of AM fungi nor a stronger water limitation (through pulsing) affected this outcome.



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Chapter 1

Introduction



The *Boswellia* woodland habitat: coping with an extreme and pulsed environment

Globally about one third of the terrestrial environment is covered by dryland ecosystems (Ffolliott et al., 1995), and nearly over 50% of the African continent is covered by drylands (FAO, 2010). Over 65% (620,000 km²) of the landmass of Ethiopia (NCSS, 1993) consists of dry or semi-dry lowlands (500-1500 m a.s.l.) in which 10% of the population of the country lives (Gebreegizabher, 2006). In these areas where conditions for agriculture are often harsh and unpredictable, trees and shrubs are vital assets of non-timber forest products for farmers (Sunninchan, 2005). Woodlands in these areas produce economically important forest products such as frankincense from *Boswellia papyrifera* (*Boswellia*) and gum and resin from *Acacia* trees.

The dry deciduous woodlands dominated by *Boswellia* are rapidly degrading. The possible causes that have been reported for this degradation include the spread of sedentary farming, incidence of uncontrolled fires, poor incense harvesting, the growth of pastoralist populations, attendant increases in livestock, the increasing urban demand for fuel wood and charcoal, insect outbreaks, and re-settlement (Abyiu et al., 2010, Gebrehiwot, 2003, Bongers et al., 2006, Ogbazgi, 2001, Ogbazgi et al., 2006, TFAP, 1996). Fire and intensive grazing at the beginning of the rainy season seriously hamper regeneration, particularly when seedlings re-sprout (Teketay, 1997, Amente et al., 2006). The seedlings of many dry woodland species are succulent, palatable and grow slowly which make them vulnerable to browsing and trampling (Ogbazgi, 2001). It has been suggested that exclosures improve natural regeneration and seedling establishment (Ogbazgi, 2001, Gebrehiwot, 2003). The current trend and intensity of tapping employed in the harvesting of frankincense has a negative impact on the natural regeneration, viability of seeds, germination, reproduction, survival and growth of *Boswellia* seedlings (Rijkers et al., 2006). In addition to these anthropogenic factors, the natural growing conditions that include drought and low soil nutrient availability may limit seedling recruitment.

Boswellia is found in *Acacia-Commiphora* woodland and wooded grassland in the dry lowlands (500-1500 m a.s.l., temperature 20-25°C, and rainfall less than 900 mm per annum (Azene Bekele-Tessema, 2007) with a growing period of 45-100 days (Ogbazgi et al., 2006). The distribution and abundance of the species is determined by altitude, land use intensity and soil fertility, and the species is restricted to hilly areas and top summits of eroded shallow soil of low fertility (Ogbazgi et al., 2006). In these arid environments, high temperatures and erratic moisture inputs impose a pulsed pattern of water and soil nutrient availability between dry and wet seasons and within the wet season (Collins et al., 2008,

Schwinning and Sala, 2004, Chesson et al., 2004). The frankincense tree grows in extremely dry conditions where water stress lasts for the 8-10 months-long dry season. Water stress also occurs because of irregular rains during the 2-4-months-long wet season, which results in shorter soil moisture pulses. These short dry spells in the wet season influence seedling survivorship and establishment of saplings (Engelbrecht et al., 2006).

The effect of precipitation pulses on plants in arid ecosystems are often explained by the pulse-reserve model framework (Collins et al., 2008, Reynolds et al., 2004, Schwinning and Sala, 2004). According to this framework, episodic precipitation events stimulate biological activity that generates reserves of biomass, propagules and organic matter that prime the ecosystem to respond rapidly to subsequent precipitation events (Collins et al., 2008). The pulse reserve model predicts that a rain event triggers a production response some of which is diverted to reserve as seed for annuals and perennials, or storage in roots, stems or as nonstructural carbohydrates for perennials (Ogle and Reynolds, 2004). Plants have evolved efficient strategies how to cope with pulses of precipitation. In the short growing season plants in the dry deciduous woodlands experience intervals of dry periods and resource-rich pulses. In the inter-pulse dry periods these plants would not be able to take up resources due to low water availability. During the resource-rich pulse phase most resources are acquired and growth takes place. Plants grow and accumulate resources actively during the moisture-rich pulse phase to survive the inter-pulse dry periods (Goldberg and Novoplansky, 1997). Díaz et al. (2004) noted that on a global scale plants can be ordered along an axis of evolutionary specialization ranging from conservative to acquisitive strategies. While these strategies are based on above-ground properties, it is likely that above-ground and below-ground traits are co-ordinated (Liu et al., 2010) and that root traits (including their relationship with soil biota such as mycorrhizal fungi) would also fit into that axis of conservative to acquisitive strategies.

Boswellia appears to be ecologically adapted to such pulsed growth conditions as trees (Abiyu et al., 2010, Ogbazgi et al., 2006, Gebrehiwot et al., 2005), but no information exists on how *Boswellia* seedlings utilize this pulsed resource availability. The strategies how dry deciduous woodland species cope with such pulsed resource availability are equally poorly known for other species of these woodlands. Such pulsed conditions in combination with the exposure to various stresses such as tapping, overgrazing, erosion and fire may contribute to seedling die-back and possibly the lack of regeneration (Figure 1-1).

Plant adaptive strategies to drought-pulsed environments

Drought influences a wide variety of morphological and physiological processes (Tyree et al., 2003, Otieno et al., 2001). Plants in the *Boswellia*-dominated dry deciduous woodlands may shed leaves to avoid dry conditions during the dry season. Seedlings also dieback completely aboveground by which they avoid drought, grazing and fire during the dry season (Gindaba et al., 2004). Shoot die-back at the seedling stage and leaf shedding of water-stressed plants are regarded as beneficial morphological adaptations that reduce water loss and prolong survival (Kozlowski et al., 1991). Deep tap roots and extensive shallow feeder roots enable plants to take up water (Markesteyn and Poorter, 2009, Gebrehiwot et al., 2005, Smith and Huston, 1989). Large-diameter root structures enable plants in the drylands to store moisture during long dry periods. Another mechanism of acclimatization to drought is through adjustment in allocation pattern (Lambers et al., 2008).

When they support leaves, seedlings may respond differently to the dry spells during the rainy season. The different physiological responses to water stress include reduction in water potential, lower relative water content, reduced whole plant water use efficiency and reduced photosynthetic rate (Kursar et al., 2009, Tyree et al., 2002, Flexas et al., 2006, Gindaba et al., 2005). During drought they close their stomata which hinders the entrance of CO₂ that plays a pivotal role in controlling the balance between water loss and carbon gain, *i.e.* biomass production (Engelbrecht et al., 2002). They show a decrease in respiration rate during water stress, due to reduced photosynthesis (Flexas et al., 2006). Their ability to regulate the stomata and also structural adjustments in leaf area help them to minimize water loss, while deep root systems and the development of a large gradient between soil and leaf water potential allows them to maximize the potential for water uptake (Serrano and Penuelas, 2005). Under water stress seedlings invest more biomass in roots (Otieno et al., 2005, Smith and Huston, 1989). Plants in the drylands generally have drought-resistance strategies (both drought avoidance and drought tolerance) that enable them to postpone dehydration through adjustment of water loss or water acquisition, thus optimally utilizing water resources. Moreover the roots of plants in such environments establish a mutualistic association with mycorrhizal fungi which results in reduced moisture and nutrient stress (Ruiz-Lozano and Aroca, 2010, Wang, 2004, Augé, 2001, Michelsen, 1993, Nobel and Cui, 1992) (Figure 1-1).

Mycorrhizal associations and mycorrhizal functioning in dry deciduous tropical woodlands

Arbuscular Mycorrhiza (AM) is the ancestral and predominant type of mycorrhiza in land plants (Wang and Qui, 2006; Brundrett, 2009). Around 80% of all plant species are colonized by AM fungi (Muthukumar et al., 2003). AM frequently occurs in herbaceous plants but also in trees, especially in tropical forest (Li et al., 2006, Lambers et al., 2008, Quilambo, 2003). Surveys on tropical species have shown that most of the woody species are colonized by AM fungi (Zhao et al., 2001, Onguene and Kuypers, 2001, Tao et al., 2004). The development of AM varies with season, soil depth and management (Jasper et al., 1993, Abbot and Robson, 1991). High spore abundance is found during the dry season, related to low carbon availability to the fungus as a consequence of plant phenology (Moreira-Souza et al., 2003). Disturbance affects the composition and functioning of AM fungal communities (Alarcon and Cuenca, 2005, Silva et al., 2005) by reducing the infectivity of hyphae, spores and root fragments (Jasper et al., 1991). Soil disturbance reduces the density of spores, species richness and the length of extra-radical mycelium of AM fungi (Boddington and Dodd, 2000, Fagbola et al., 2001). Mycorrhizal woody plants can extend their roots deep into the soil (Powers et al., 2005, Wang et al., 2004, Ingleby et al., 1997) as the soil physical and chemical characteristics at different depths vary. The distribution and level of colonization by AM fungi vary with depth (Dalpé et al., 2000). In order to understand the functioning of AM symbioses in natural ecosystems, it is vital to study the factors that affect inoculum potential of AM fungi and colonization of woody plants by AM fungi (Figure 1-1).

Role of AM in plant growth, adaptation and competition

Seedling establishment is often limited by the lack of moisture in dry areas (Vieria, 2006, Khurana and Singh, 2001). The establishment and regeneration of seedlings can be improved by the AM symbiosis through improvement of the acquisition of water and nutrients (Smith et al., 2010).

AM fungi increase water acquisition or improve drought resistance of plants. Some of the mechanisms are a direct consequence of the improved nutritional status of mycorrhizal plants compared to non-mycorrhizal plants, but non-nutritional mechanisms have also been proposed. In many conditions the interaction between nutritional and non-nutritional mechanisms results in improved water relations (Del-Amico et al., 2002, Augé, 2001, Augé et al. 1992, Michelsen and Rosendahl, 1990). AM plants maintain or often

even increase stomatal conductance and transpiration, increase photosynthesis (through a process called sink stimulation of photosynthesis by root symbionts (Kaschuk et al., 2009)), which results in increased growth and plant size. When plant size increases more water moves into the plant roots that could lower tissue dehydration (Smith and Read, 2008). Hyphae themselves can directly take up and transport water to mycorrhizal plants (Kyllo et al., 2003). AM fungi improve soil structure through the production of glomalin, a glue that holds soil particles together, which improves aggregate stability (Wright and Upadhyaya, 1998, Rillig and Mummey, 2006), and in turn increases the water-holding capacity of the soil. A study by Augé (2004) suggested that increases in stomatal conductance of mycorrhizal plants were equally due to root colonization and to soil colonization by AM fungal hyphae. Hyphae bridge gaps between the soil and roots as well as binding soil particles to each other and roots (Estrada-Luna et al., 2000). They also reduce the hydraulic resistance to water uptake by the roots (Smith and Read, 2008). AM fungi enhance accumulation of more solutes in plants that increase the osmotic potential of the host which enables it to take up more water from the soil under water stress conditions (Qiangsheng et al., 2006). Enhanced dehydration tolerance was also indicated by the tendency for AM plants to sustain turgor and stomatal conductance at lower relative water content than non-mycorrhizal plants (Augé, 2001, Ruiz-Lozano, 2003, Ruiz-Lozano et al., 1995).

The AM symbiosis increases plant tissue P, K, N, Zn, Mg, Cu and Ca mass fractions under drought conditions (Huat et al., 2002, Tarafdar and Kumar, 1996, Ruiz-Lozano et al., 1995, Reena and Bagyaraj, 1990). Increased nutrient absorption rate in turn enhances seedling growth (Habte and Fox, 1989, Michelsen, 1993, Fagbola et al., 2001). Tropical dryland species positively respond to inoculation with different AM fungal species by increased biomass, plant height, stem girth, number of leaves, and root length (Fagbola et al., 2005, Caravaca et al., 2003a, Reena and Bagyaraj, 1990). The typical (and therefore most dependent and responsive) mycorrhizal plant has poorly developed roots with few and short root hairs that limit the volume of soil explored by them. The outgrowth of mycorrhizal hyphae into soil far beyond the root or root hair zone considerably increases the volume of soil being used as the fungus absorbs the poorly mobile nutrients and translocates it to the root (Joner et al., 2000, Haselwandter and Bowen, 1996). The larger soil volume explored and exploited increases the nutrient uptake by shortening the distance that nutrients have to diffuse from the soil to the roots (Kungu, 2006). In addition, mycorrhizal hyphae can penetrate soil pores inaccessible to roots and may compete more

effectively with saprotrophic microorganisms for recently mineralized nutrients than plants themselves (Smith and Read, 2008).

AM plants often show higher photosynthetic rates than non-mycorrhizal counterparts, which is consistent with positive AM effects on stomatal conductance (Augé, 2001, Ruiz-Lozano et al., 1995). This increase could be related to reduction in CO₂ resistance at the gas and liquid phase transport, increased photosynthetic storage and export, and increased photosynthetic P use efficiency (Nobel and Cui, 1992). Influence of AM fungi on photosynthetic use efficiency of plants results in a larger leaf area, that results in a more vigorous root system and more rapid growth.

The AM symbiosis affects competition between plant species and thereby the community structure of ecosystems. When mycorrhizal plant density increases there will be an increasing overlap in phosphorus depletion zones by the roots of competing plants that decreases the usefulness of hyphae as a means of phosphate acquisition (Perez and Urcelay, 2009, Koide and Dickie, 2002). The effect of AM on plant competition has mainly been studied for grasses and herbaceous plants, and studies on the role of mycorrhiza on competition between tree seedlings are very scarce (Danieli-Silva et al., 2010). Analyzing competing AM woody seedlings having different resource uptake strategies (conservative and acquisitive) would increase our understanding of the role of AM in competition.

Though *Boswellia* is economically and ecologically important, *Boswellia* woodlands are degrading and *Boswellia* populations are decreasing (Abiyu et al., 2010, Ogbazgi, 2001, Ogbazgi et al., 2006, TFAP, 1996). Lack of regeneration is evident in stands that have been studied so far (Eshete et al., 2005, Gebrehiwot et al., 2003, Ogbazgi, 2001). Harvesting activities exploit the natural populations of *Boswellia* trees, since no plantations have yet been established. To sustainably maintain the production system, solving the lack of regeneration is mandatory. Different approaches have been employed to restore *Boswellia* forests. Research has been done on use, distribution, population structure, chemistry of gum and impact of interference, vegetative propagation, mycorrhizal status and productivity of *Boswellia-Commiphora* woodland (Eshete et al., 2011, Birhane et al., 2010, Negussie et al., 2008, Rijkers et al., 2006, Eshete et al., 2005, Gebrehiwot et al., 2003, Lemeneh and Teketay, 2003, Ogbazgi, 2001), but none of these solved the bottleneck, *i.e.* the lack of regeneration. Most of these studies focused on the tree, regardless of the soil and soil biota. They didn't investigate the roles of moisture and nutrient availability for seedling growth.

In this thesis I analyze how AM influences the establishment and growth of seedlings and how this is influenced by pulsed resource availability and competition (Figure 1-1). Seedlings in the dry deciduous woodlands, specifically *Boswellia*, have no problem of germination. During the short rainy season, it is common to find large numbers of seedlings (Negussie et al., 2008). In the dry season, all seedlings die-back to the ground. Seedlings of *Boswellia* stay below-ground during the dry period as a means of escaping stress or grazing; and re-sprout during the next rainy season to continue growth (*waiting in the underground*). This waiting in the underground strategy may take several years or even decades for *Boswellia* seedlings. It is still unknown when these seedlings have accumulated enough resources to grow to a height in one season that allows them to escape from grazing and fire. Exclosures may improve microsite conditions and the substrate for seedling regeneration and survival through reducing grazing damage. Exclosures also enhance herbaceous cover that stops topsoil erosion. Protection of the topsoil helps not only to conserve *in-situ* nutrient losses but could also increase AM fungal inoculum. Higher mycorrhizal inoculum makes colonization of roots of dry deciduous seedlings more easy. Enhanced mycorrhizal abundance improves resource availability to seedlings during the short rainy season. Under pulsed moisture conditions efficient and quick resource uptake and storage is vital. This storage might enable mycorrhizal seedlings to accumulate enough resources at a faster rate than non-mycorrhizal seedlings (Figure 1-1). My general hypothesis for this study is that the coupled effect of nutrient and moisture availability via the AM symbiosis will increase the performance of seedlings in a resource pulsed environment (Figure 1-1).

Research objective and questions

This thesis aims at unraveling the role of AM fungi on the growth and development of *Boswellia* seedlings in a highly pulsed environment. It focuses on the study of the AM status of trees and shrubs of the *Boswellia*-dominated dry deciduous woodlands in relation to season, soil depth and management. I tested the effect of AM and water pulsing, water deficit, soil fertility and competition on the performance of *Boswellia* and *Acacia* seedlings in the greenhouse. In this thesis the following major questions are addressed:

1. What are the major environmental factors affecting mycorrhizal inoculum (spore abundance and root colonization) in the *Boswellia*-dominated dry deciduous woodlands? This question is tackled in chapter 2, where a field study was conducted on AM status in relation to season, management and soil depth.

2. How do *Boswellia* seedlings respond to AM and water pulsing treatments? This question is studied in chapter 3, where a greenhouse experiment was conducted to assess the response of mycorrhizal and non-mycorrhizal *Boswellia* seedlings to water pulses.
3. How do AM, water deficit and soil fertility influence the growth, allocation and gas exchange of *Boswellia* and *Acacia* seedlings? This question is addressed in chapter 4 by conducting a greenhouse experiment, in which the roles of AM, water deficit and soil fertility on the growth, gas exchange, and nutrient levels of *Boswellia* and *Acacia* species were assessed.
4. Does AM influence competition between *Acacia etbaica* and *Boswellia* seedlings at different water pulse levels? This question is studied in chapter 5, where the competitive performance of these seedlings was assessed in the greenhouse by exposing them to water pulse levels. Both intra- and interspecific competition was studied.

Study site

The field study was conducted in three *Boswellia*-dominated dry deciduous woodlands of northern Ethiopia, namely Abergelle (13°14 to 13°42 N, 38°38 to 39°02 E), Humera (13°42 to 14°28 N, 36°20 to 37°31 E) and Metema (12°30 to 12°48 N, 36°17 to 36°55 E). These sites are the main areas in the country where incense and gum are collected. These sites have an erratic rainfall most of which occurs between mid-June and August. They have a unimodal rainfall that ranges between 647 to 965 mm annually. The topography is flat to rugged, surrounded by hills and mountains. The altitude ranges from 537 m to 1640 m a.s.l. The soils are shallow and have a high degree of stoniness. The vegetation is categorized as *Combretum-Terminalia* and *Acacia-Commiphora* woodland (NBSAP, 2005), dry forest dominated by *Boswellia* and *Acacia* species.

The greenhouse experiments on water pulsing and competition (Chapters 3 and 5) were conducted in northern Ethiopia at Illala plant tissue culture greenhouse (13°3'N 39°E; altitude 2000 m a.s.l.), Tigray Agricultural Research Institute, from June 01 2008 to October 30 2009. The mean daily temperature of the greenhouse was 25 °C during the day and 22 °C during the night with mean daily average relative humidity of 51% for the study period. The water deficit experiment (Chapter 4) was conducted at Mekelle university greenhouse (13°29'N 39°28'E; altitude 2200 m a.s.l.) from May 01 2009 to October 30

2009. The mean daily temperature of the greenhouse was 27 °C during the day and 22 °C during the night with mean daily average relative humidity of 62 % for the study period.

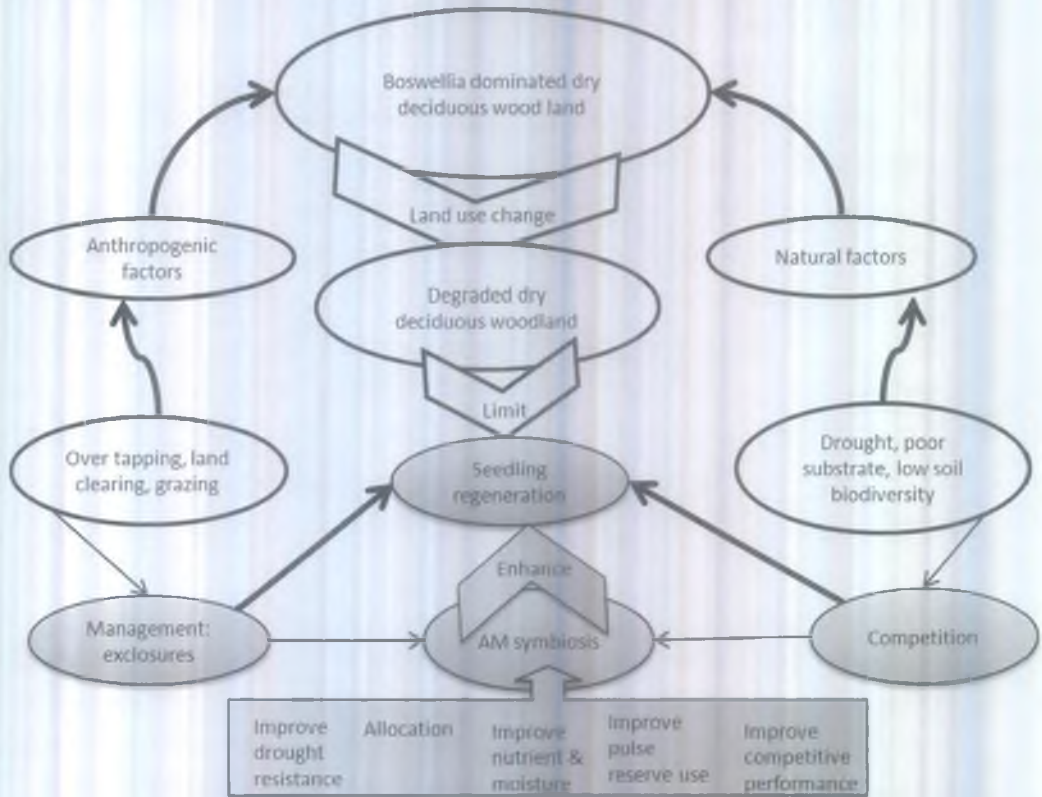


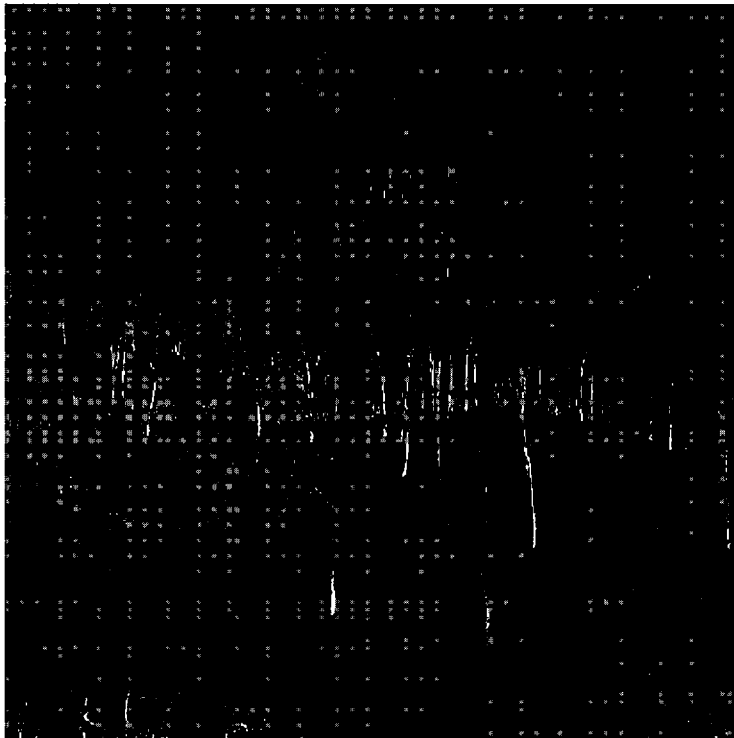
Figure 1-1. Schematic representation on how arbuscular mycorrhizal (AM) fungi coupled with management affect seedling regeneration of *Boswellia* in dry deciduous woodlands in northern Ethiopia.

Chapter 2

*Arbuscular mycorrhizal associations in Boswellia papyrifera (frankincense-tree)
dominated dry deciduous woodlands of Northern Ethiopia*

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Abstract

This study assessed the arbuscular mycorrhizal (AM) status of *Boswellia papyrifera* (frankincense-tree) dominated dry deciduous woodlands in relation to season, management and soil depth in Ethiopia. We studied 43 woody species in 52 plots in three areas. All woody species were colonized by AM fungi, with average root colonization being relatively low (16.6% - ranging from 0 to 95%). Mean spore abundance ranged from 8 to 69 spores 100 g⁻¹ of dry soil. *Glomus* was the dominant genus in all study sites. Season had a strong effect on root colonization and spore abundance. While spore abundance was higher ($P < 0.001$) in the dry season in all three study sites, root colonization showed a more variable response. Root colonization was reduced in the dry season in the site that was least subject to stress, but increased in the dry season in the harshest sites. Management in the form of exclosures (that exclude grazing) had a positive effect on spore abundance in one of the two sites considered. Spore abundance did not significantly differ ($P=0.17$) between the two soil depths. Our results show that in this arid region all trees are mycorrhizal. This has profound consequences for rehabilitation efforts of such dry deciduous woodlands: underground processes are vital for understanding species adaptation to pulsed resource availability and deserve increasing attention.

Key-words: AM fungi; dry tropical woodland; Exclosure; Management

Introduction

More than one third of the earth's land surface is arid or semi-arid, supporting more than 1.2 billion people (Wickens, 1998). In these areas where conditions for agriculture are harsh and unpredictable, trees and shrubs are vital assets of non-wood forest products for farmers (Sunninchan et al., 2005) and for the conservation of dryland biological diversity (Ogbazghi et al., 2006). Low moisture availability is a major stress for the establishment, survival and growth of woody species in dry areas. One of the mechanisms that enable plants to survive these conditions is their ability to form a mutualistic association with fungi (Caravaca et al., 2003; Yamato et al., 2009).

Arbuscular mycorrhiza (AM) is the most widespread and common root-fungus association in land plants (Wang and Qiu, 2006; Brundrett, 2009). Brundrett (2009) estimated that out of 280,000 plant species, over 200,000 form AM. AM associations occur in many stressful environments and enhance water and nutrient uptake in dry conditions (Nobel and Cui, 1992; Michelsen, 1993; Augé, 2001). Most of the tropical woody species form AM (Onguene and Kuyper, 2001; Wang and Qiu, 2006; Brundrett, 2009), both in the humid and semi-arid tropics. In these dryland ecosystems, levels of colonization by and spore density of AM fungi (AMF) show distinct seasonal patterns (Abbott and Robson, 1991). Soil properties (soil depth, pH, organic matter levels) and management also generate variability in these parameters. Spore abundance is high during the dry season, which is related to plant phenology (Jasper et al., 1989; Guadarrama and Alvarez-Sanchez, 1999; Moreira-Souza et al., 2003). Most plants are not or less photosynthetically active as a result of leaf fall or stomata closure during the dry season, and the combination of an interrupted carbon flow to the roots with drying out of soils induces spore formation (Cardoso et al., 2003). In the dry period AMF mostly occur as soil spore banks.

AMF spore abundance can be affected by management (Alarcon and Cuenca, 2005; Silva et al., 2005). Among management regimes, exclosures are effective in increasing herbaceous cover and decreasing surface erosion, which in turn could increase spore density. Vegetation with a higher degree of cover showed higher propagule densities than degraded bushland and cultivated areas (Michelsen and Rosendahl, 1989; Carpenter et al., 2001). Hence we also compared spore density between exclosures and open area.

The distribution and density of AMF spores differs with depth (Dalpé et al., 2000). Generally spore density, like root length density, declines with soil depth. Other factors that determine the distribution of moisture and soil nutrients over the profile also play a role in the distribution and density of AMF spores (Cardoso et al., 2003). Several studies

noted spores at deeper soil layers (below 40 cm) because some woody plants extended their roots deep into the soil (Ingleby et al., 1997; Powers et al., 2005). Inferences based only on surface soil and root examination may then lead to incorrect generalizations about root symbionts when deeper layers support more AMF (Virginia et al., 1986). In areas where intensive grazing is coupled with erosion and shallow soil, it is likely that more spores will occur in the deeper layers as a result of loss of top soil and deeper distribution of roots.

This paper investigates the AM status of trees and shrubs in dry deciduous woodlands of Ethiopia that are dominated by the economically important frankincense tree (*Boswellia papyrifera*). Considering the strong seasonality in the area, we hypothesized higher spore density during the dry season but higher root colonization during the rainy season. The installment of exclosures as a management system to increase herbaceous cover led to the hypothesis of higher spore density in exclosures than in open areas. Finally we hypothesized a significant difference in spore density and levels of colonization between the upper and lower soil layers. To test these hypotheses we analyzed the effect of season, management and soil depth on the density of AMF spores and levels of root colonization in three different dry deciduous woodlands of Ethiopia.

Materials and Methods

Description of the study sites

The study was conducted in Abergelle, Humera and Metema (Figure 2-1; Table 2-1), three *Boswellia*-dominated dry deciduous forest areas of north-west Ethiopia. These sites are the main areas in the country where gum and incense are collected. The main species for incense production is *Boswellia papyrifera*, while *Acacia senegal* and *A. seyal* are used for gum production.

Abergelle, which is the driest site, has erratic rainfall, most of which occurs between mid-June and August (Figure 2-2). The topography is flat, surrounded by hills and mountains. The soils are shallow and have a high degree of stoniness, which limits the water-holding capacity and root space. The vegetation of Abergelle is categorized as *Combretum-Terminalia* and *Acacia-Commiphora* woodland (NBSAP, 2005), dry forest dominated by *Boswellia papyrifera*, *Acacia etbaica*, *Terminalia brownii* and *Lannea fruticosa*. They are also categorized as Ethiopian undifferentiated woodland under Sudanian regional centre of endemism (Gebrehiwot, 2003). They are also categorized as

Ethiopian undifferentiated woodland under Sudanian regional centre of endemism (Gebrehiwot, 2003).

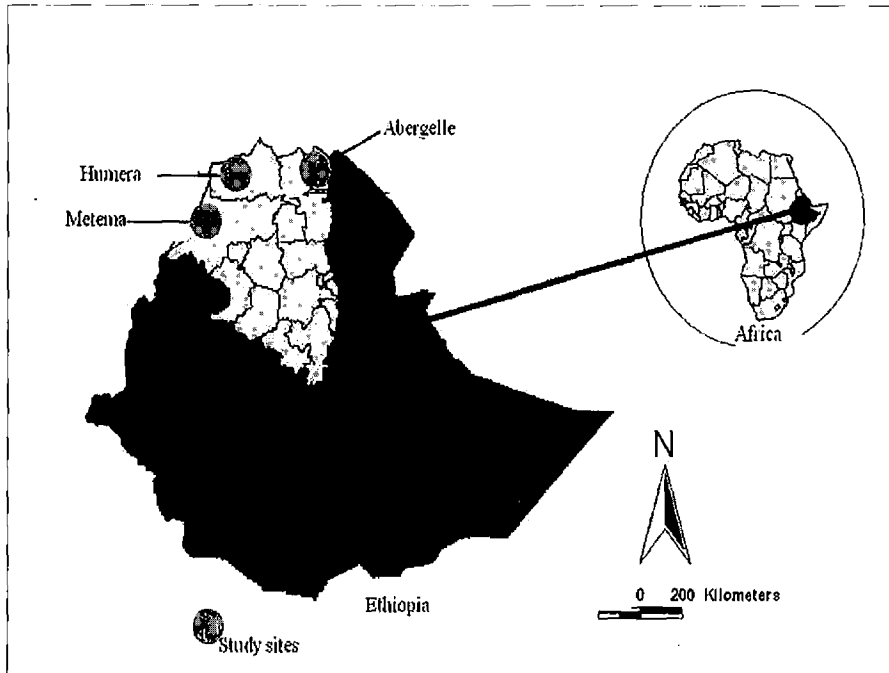


Figure 2-1. Map showing the three study sites.

Humera has a slightly less harsh climate with a dry season that lasts 8 months; rainfall is concentrated between June and September (Figure 2-2). The study area has a rugged topography with flat plains surrounded by mountain tops. The vegetation cover is categorized as *Combretum-Terminalia* woodland, *Acacia-Commiphora* woodland, and *Acacia-Boswellia* woodland (NBSAP, 2005). Metema receives the highest rainfall in a unimodal pattern (Figure 2-2) from June until the end of September, but with most of the rainfall during July and August. Nearly all of the land in the study area is in the lowlands except some mountain tops that surround the flat plains. The soils in the area are deep and predominantly black with vertic properties. The vegetation is categorized as *Combretum-Terminalia* woodland (NBSAP, 2005) where *Acacia spp.*, *Balanites aegyptiaca*, *Boswellia papyrifera*, *Combretum spp.*, *Stereospermum kunthianum* and *Terminalia brownii* are the dominant species.

Study plots and plant selection

In a reconnaissance survey to identify the study plots and species in the areas with *Boswellia*-dominated dry deciduous forests (Table 2-1), the specific sites were delineated with a GPS. 16, 16, and 20 sample plots were selected from Abergelle, Humera and Metema, respectively.

Table2-1. Location, elevation, soil types and characteristics of the study sites.

Locality	Abergelle	Humera	Metema
Region	Tigray	Tigray	Amhara
Specific area	Serabite/Jijike & Siye	Adigoshu/Tekeze	Lemlem Terara & Masho Terara
Area of study site (ha)	51	295	64
Location	13°14' to 13°42'N, 38°38' to 39°02'E	13°42' to 14°28'N, 36°20' to 37°31' E	12°30'N to 12°48'N, 36°17' to 36°55'E,
Altitude (m)	1500 -1640	537-913	549-600
Geology	Limestone; mixture of schist & limestone with quartz	Metamorphic terraine, phyllite & quartzite	Pre-cambrian: chlorite schists; quartzites
Soil types	Cambic Arenosols, Chromic Cambisols & Leptosols	Vertisols, Leptosols, Eutric cambisols, Vertic Luvisols	Haplic Luvisols, Humic Nitisols, Eutric Vertisols
Slope position	Middle	Middle	Middle
Disturbance	High	Medium	Medium
Erosion type	Sheet & rill	Gully	Rill

To conduct vegetation sampling we used sample quadrants measuring 20 m x 20 m plot size. After we delineated the sites with GPS, we developed a map for each specific study site and we distributed the samples randomly. The same sample plots were also used for the collection of soil samples and characterization of study plots. In each plot all individual trees were identified and enumerated for diameter at breast height (DBH), height, crown diameter, number, origin, quality, growth form, type of damage and crown position.

In addition to vegetation characteristics, soil chemical and physical properties, soil depth, stone cover, slope, herbaceous cover, altitude, erosion type and location were assessed (Table 2-2). Two management regimes were considered in Abergelle and Humera and only one management regime (open area) in Metema. These management regimes were exclosures and open areas (= control). In exclosures grazing and cutting of trees is prohibited while grass harvest is allowed through cut and carry system after the grasses set

their seeds. In open areas there is continuous grazing; cutting of trees is also prohibited. Exclosures were closed for 22 years in Abergelle and for 12 years in Humera to enhance vegetation restoration and reduce soil erosion. These management systems are well recognized in Abergelle and Humera but not in Metema study site.

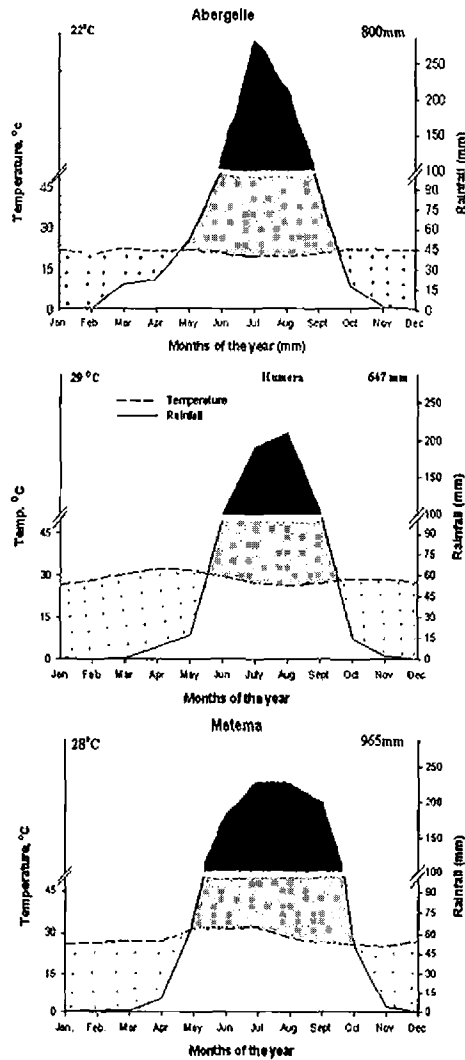


Figure 2-2 Mean monthly Rainfall(mm), minimum and maximum temperature per month (°C) (NMA, 2009). (a): Annual average of temperature and annual precipitation sum. The length of observation period were 15 to 27 years . Abergelle is represented by Abyadi, 20 km.

Soil and root samples were collected to determine AMF spore abundance, mycorrhizal colonization of roots, and soil physical and chemical properties for at least three individuals of all woody species in each plot. Plant nomenclature follows Friis (1992) and the Flora of Ethiopia (Edwards et al., 1995; Hedberg et al., 1995).

Collection of rhizosphere soil

Soil sampling was done during the dry and the rainy season. Soil samples were excavated from two depths, 0-15 cm and 15-30 cm using simple digging and measuring the depths with a ruler. Samples were only taken where the soil was at least 30 cm deep. Soil samples were taken at the four directions at the crown edge of each selected individual tree or shrub. The four rooting-zone soil samples with fine roots were mixed thoroughly to form a composite soil sample. Three sets of soil samples were collected from each sampling spot. The first set of soil samples was used for spore analysis. The second and third set of soil samples were used for physical and chemical soil analysis respectively. In total 1208 soil samples from 43 species weighing about 1 kg each were transported to the laboratory using sealed plastic bags. The soil samples were air-dried, passed through a 2 mm sieve and stored at 4 °C until analysis.

Physical and chemical analysis of soil samples

Soil pH, electrolytic conductivity, bulk density, texture, organic matter, available phosphorus, available potassium, and total nitrogen were analyzed. Organic matter was determined using Walkley-Black and total nitrogen was determined using Kjeldahl (Bremmer and Mulvaney, 1982). Available phosphorus was analyzed using the Olsen method (Olsen and Sommers, 1982). pH and EC were calculated using a suspension of 1:5 soil: water ratio. Particle size analyses were done using the Hydrometer method (Gee and Bauder, 1982). For bulk density the core method was used (Blake and Hartge, 1982). A total of 184 composite soil samples; 48, 60 and 76 for Abergelle, Humera and Metema respectively, were analyzed.

Assessment of AM colonization

Live fine roots were collected from each plant for assessment of mycorrhizal colonization. Live fine roots were distinguished from dead roots based on visual judgment using brittleness, color and resilience. Root samples were collected from three individuals of a total of 43 species. A total of 276 root samples were excavated. Roots were traced back to

the stem of the target tree. The fine roots were excavated starting from the trunk and working out towards to the fine roots. Roots were excised in amounts of about 1-5 g for each species and placed in sealed plastic to prevent desiccation.

Table 2-2. Mean plot characteristics (\pm SE) in each study site.

Site characteristics	Unit	Abergelle	Humera	Metema	F	P
pH		7.42 ^a \pm 0.05	6.91 ^b \pm 0.09	6.44 ^c \pm 0.07	47.641	0.000
EC	dSm ⁻¹	1.88 ^b \pm 0.08	2.26 ^a \pm 0.23	1.17 ^c \pm 0.08	19.536	0.000
OM	%	4.50 ^a \pm 0.24	2.61 ^b \pm 0.20	4.85 ^a \pm 0.22	24.044	0.000
Available P	μ gg ⁻¹	30.78 \pm 1.50	29.83 \pm 1.87	27.48 \pm 2.54	0.672	0.512
Available K	cmol ⁺ kg ⁻¹	0.16 \pm 0.00	0.14 \pm 0.01	0.17 \pm 0.00	2.840	0.061
Total N	%	0.21 \pm 0.01	0.18 \pm 0.01	0.18 \pm 0.00	1.859	0.159
Sand	%	45.25 \pm 2.22	44.60 \pm 3.67	46.10 \pm 1.13	0.121	0.886
Silt	%	33.50 ^b \pm 1.70	25.80 ^b \pm 3.13	23.00 ^b \pm 1.27	9.828	0.000
Clay	%	21.25 ^b \pm 1.31	29.60 ^a \pm 2.25	30.90 ^a \pm 1.11	14.326	0.000
BD	g cm ⁻³	1.21 ^a \pm 0.035	1.26 ^a \pm 0.025	1.04 ^b \pm 0.06	7.921	0.000
Herbaceous cover	%	31.88 ^c \pm 0.60	43.64 ^b \pm 1.06	77.57 ^a \pm 1.10	562.141	0.000
Stone cover	%	78.63 ^a \pm 0.20	60.77 ^b \pm 0.83	30.24 ^c \pm 1.61	1163.510	0.000
Soil depth	cm	21.35 ^b \pm 0.20	22.45 ^b \pm 0.42	114.39 ^a \pm 2.13	4088.121	0.000
Erosion	%	19.72 ^c \pm 0.70	39.57 ^b \pm 1.25	50.04 ^a \pm 1.17	239.265	0.000

Roots were chopped into 2-4 cm long segments before clearing. Fresh roots were gently cleansed using tap water and were fixed in 50 % ethanol in tightly sealed plastic bags and stored at room temperature until they were transported to the laboratory. The preserved roots were stained using the procedure in Brundrett et al. (1996). Roots were treated with 10 % KOH for 15-25 min. at 121°C in an autoclave. Clear pieces of roots were rinsed with tap water to remove KOH. Dark roots were further bleached with 10 % H₂O₂ for 3 min. at room temperature. Then the roots were acidified with 2 % HCl (v/v) for 1 hour at room temperature and stained overnight with 0.01 % Trypan blue in lactoglycerol (Lactic acid: glycerol: de-ionized water, in 5:1:1 ratio). Excess stain was removed with 50 % glycerol for 1-2 hours. Samples were then stored for microscopic analysis. Five slides, each with six to nine randomly selected stained roots (0.5-1 cm long root) were prepared from every individual plant sample. A total of 30-45 roots per species were examined for the presence of structures characteristic of AMF (arbuscules, vesicles, hyphal coils, intraradical and extraradical aseptate hyphae, auxiliary cells, and spores). Fractional root

colonization by AMF structures was determined using the gridline intersection method (Giovannetti and Mosse, 1980). In the tables fractional mycorrhizal colonization is given as one of the five percentage classes where 1=1-5 %, 2=6-25 %, 3=26-50 %, 4=51-75 % and class 5=76-100 % (Kormanik and McGraw, 1982).

Assessment of AMF spores

Spores were extracted by sieving followed by flotation-centrifugation in 60% sucrose (Brundrett et al., 1966; Onguene and Kuyper, 2001). A sieved soil sub-sample of 25 g was taken for the assessment of AMF spores. It was soaked in 100 ml tap water and left to stand for five min. to allow sedimentation of coarse sand. The samples were then vigorously stirred and suspended for two min. and allowed to settle for 30 seconds. The suspension was then decanted over a series of four sieves with a mesh size of 850 μm , 500 μm , 250 μm , and 100 μm . The suspension from each mesh was then transferred to its respective beaker separately. Suspending and decanting were repeated three times with 25 ml tap water. Equal aliquots were prepared and transferred by weighing into 100 ml centrifuge tubes. The transferred aliquots then were centrifuged at 2000 rpm for five min. The supernatant was discarded and the pellet was suspended again in 60 % sucrose and centrifuged at 2000 rpm for two min. The spores found in the supernatant were poured over a 40 μm sieve, and quickly washed with abundant water to remove the sucrose. The spores were transferred from the sieves on to a filter paper fitted in a funnel placed in an Erlenmeyer flask with a gentle flow of water using a wash bottle. Then the water was filtered using gravity. The filter paper with spores was placed in inverted Petri dishes. The number of spores was expressed as the mean of three replicates. Spore abundance (total number of spores in 100 g soil) was determined by counting all spores with a normal appearance under a stereo-microscope. For observation and identification of spore characters, spores were mounted on glass slides and identified to genus level, whenever possible, using a compound microscope at 100 – 400x magnification, based on descriptions in Brundrett et al. (1996) and information from the INVAM website (<http://www.invam.caf.wvu.edu>).

Establishment of trap culture

AMF spores from the rhizosphere of *Boswellia papyrifera* were multiplied using Sudan grass (*Sorghum bicolor*) under greenhouse conditions to determine the colonization potential of the spores. The inoculum consisted of a mixture of soil and root samples

collected from the rhizosphere of *Boswellia papyrifera* trees from Abergelle study site. The roots separated from these trap plants were washed, cleared and stained to assess the degree of AM colonization

Statistical analysis

AMF spore abundance and AM root colonization data were statistically analyzed using SPSS (PASW statistics 17) software. Analysis of variance (ANOVA) was used to test for significant sources of variation in AMF spore density and root colonization among soil depth, management, season and site. Means were compared using Tukey and Gabriel post-hoc test when F-test from ANOVA was significant at $P < 0.05$. In order to meet the assumption of normal distribution, data on fractional mycorrhizal colonization were arcsine square root transformed and spore numbers log transformed [$^{10}\log(n+0.5)$] before statistical analysis. The relationship between AM colonization levels and spore numbers was tested by Pearson correlation test.

Results

Soil properties and species composition

The three sites differed in their mean plot characteristics (Table 2-2). Vegetation cover was lower, soil depths were shallower and more stony in Abergelle, followed by Humera and Metema. Textural differences between the sites were small. Gully erosion was more common in Humera while sheet and rill erosion were more common in Abergelle and Metema. The highest percentage of erosion was noted for Metema, despite its higher herbaceous cover, followed by Humera and Abergelle. The pH values, which ranged from 4.9 to 8.1, were lowest in Metema and highest in Abergelle. EC values indicated that the soils were not saline. Sites did not differ in N, P or K ($P > 0.05$).

A total of 43 species belonging to 18 families were found in the three sites, with Fabaceae (12 species), Combretaceae (9) and Anacardiaceae (4) as dominant families (Table 2-3). The Burseraceae had only two species, *Boswellia papyrifera* and *Commiphora africana*. *Boswellia papyrifera* was the dominant species in all three sites followed by *Ipomea* spp, *Acacia etbaica* and *Senna singueana* (Abergelle); *Combretum hartmannianum* and *Dichrostachys cinerea* (Humera); and *Pterocarpus leucens* and *Lannea fruticosa* (Metema). In addition to *Boswellia papyrifera* only *Combretum molle*, *Dichrostachys cinerea* and *Lannea fruticosa* were common to the three sites (Table 2-3).

Chapter 2 – Mycorrhiza of the dry deciduous woodlands

Table 2-3. Study species density, AM spore counts, degree of colonization and AM spore genus presence and absence.

Study species	Family	Tree density (ha ⁻¹) ^a			Spore counts (100 g ⁻¹ soil) ^b			Degree of colonization (class) ^c			AM Spore genus ^d				
		Abergelle	Humera	Metema	Abergelle	Humera	Metema	Abergelle	Humera	Metema	Glomus	Acaul ospora	Giga spora	Scute llospora	NI ^e
<i>Acacia abyssinica</i>	Fabaceae	-	5.0	-	-	11.0±2.8	-	-	3	-	+	+	-	-	-
<i>Acacia asak</i>	Fabaceae	20.3	-	-	-	-	-	1	-	-	-	-	-	-	-
<i>Acacia etbaica</i>	Fabaceae	187.5	5.0	-	23.1±2.0	13.3±3.8	-	3	2	-	+	+	+	+	-
<i>Acacia oerfota</i>	Fabaceae	5.0	-	-	28.3±5.5	-	-	2	-	-	+	+	-	+	-
<i>Acacia polyacantha</i>	Fabaceae	-	-	3.8	-	-	-	-	-	1	-	-	-	-	-
<i>Acacia tortilis</i>	Fabaceae	5.0	-	-	32.0±7.1	-	-	2	-	-	+	+	-	-	-
<i>Albizia amara</i>	Fabaceae	5.0	-	-	36.0±11.8	-	-	3	-	-	+	+	+	-	-
<i>Anogeissus leiocarpus</i>	Combretaceae	-	5.0	1.3	-	15.3±3.4	23.3±2.9	-	2	2	+	+	-	-	-
<i>Balanites aegyptiaca</i>	Balanitaceae	-	5.0	-	-	16.0±3.9	-	-	2	-	+	+	-	+	-
<i>Boscia angustifolia</i>	Capparidaceae	1.6	-	-	-	-	-	4	-	-	-	-	-	-	-
<i>Boswellia papyrifera</i>	Burseraceae	387.5	365.6	142.5	20.6±1.3	45.7±3.2	29.0±6.1	3	2	2	+	+	+	+	-
<i>Combretum adenogonium</i>	Combretaceae	5.0	-	-	36.0±6.0	-	-	3	-	-	+	+	-	-	-
<i>Combretum collinum</i>	Combretaceae	-	-	5.0	-	-	37.0±7.3	-	-	2	+	+	-	-	-
<i>Combretum fragrans</i>	Combretaceae	-	6.3	-	-	-	-	-	4	-	-	-	-	-	-
<i>Combretum hartmannianum</i>	Combretaceae	-	81.3	-	-	38.3±3.9	-	+	2	+	+	-	-	-	-
<i>Combretum molle</i>	Combretaceae	7.8	1.6	5.0	41.6±6.2	22.6±5.6	33.0±5.4	2	2	2	+	+	-	-	-
<i>Combretum spp.</i>	Combretaceae	-	5.0	-	-	32.0±11.0	-	-	2	-	+	-	+	-	-
<i>Commiphora africana</i>	Burseraceae	5.0	-	-	34.6±7.0	-	-	2	-	-	+	+	+	-	-
<i>Cordia africana</i>	Boraginaceae	-	-	1.3	-	-	-	-	-	2	-	-	-	-	-
<i>Dalbergia melanoxylon</i>	Fabaceae	-	1.6	2.5	-	33.0±12.2	18.3±3.0	-	2	2	+	+	-	-	-
<i>Dichrostachys cinerea</i>	Fabaceae	4.7	43.8	1.3	31.3±6.1	24.7±7.6	18.3±3.7	2	2	2	+	+	+	+	-
<i>Dimeto¹</i>	Fabaceae	-	-	3.8	-	-	20.3±3.2	-	-	2	+	+	-	-	-
<i>Ficus thonningii</i>	Moraceae	1.6	-	-	-	-	-	5	-	-	-	-	-	-	+
<i>Fluggea virosa</i>	Euphorbiaceae	-	-	3.8	-	-	24.0±4.0	-	-	2	+	+	-	-	-
<i>Ipomea spp.</i>	Convolvulaceae	284.4	-	-	33.0±5.9	-	-	2	-	-	+	+	+	-	-
<i>Lannea fruticosa</i>	Anacardiaceae	3.1	4.7	2.0	37.3±5.9	28.5±7.4	19.6±3.4	2	2	2	+	+	+	-	-
<i>Lannea triphylla</i>	Anacardiaceae	5.0	-	-	24.0±4.8	-	-	2	-	-	+	+	-	-	-

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Study species	Family	Tree density (ha ⁻¹) ^a			Spore counts (100 g ⁻¹ soil) ^b			Degree of colonization (class) ^c			AM Spore genus ^d				
		Abergelle	Humera	Metema	Abergelle	Humera	Metema	Abergelle	Humera	Metema	Glomus	Acaul ospora	Giga spora	Scute llospora	Ni ^e
<i>Lonchocarpus laxiflorus</i>	Celastraceae	-	-	5.0	-	-	27.6±5.4	-	-	2	+	+	-	+	-
<i>Maerua angolensis</i>	Capparidaceae	6.3	-	-	-	-	-	1	-	-	-	-	-	-	+
<i>Maytenus senegalensis</i>	Celastraceae	-	-	5.0	-	-	-	-	-	1	-	-	-	-	+
<i>Ochna leucophloeos</i>	Ochnaceae	-	-	2.5	-	-	-	-	-	4	-	-	-	-	-
<i>Ptilostigma thonningii</i>	Fabaceae	-	-	2.5	-	-	-	-	-	3	-	-	-	-	+
<i>Pterocarpus leucens</i>	Fabaceae	-	-	36.3	-	-	40.0±6.1	-	-	2	+	+	-	-	-
<i>Rhus natalensis</i>	Anacardiaceae	5.0	-	-	46.3±6.8	-	-	3	-	-	+	+	+	-	-
<i>Sclerocarya birrea</i>	Anacardiaceae	4.7	-	-	-	-	-	5	-	-	-	-	-	-	-
<i>Senna singueana</i>	Fabaceae	154.7	-	-	22.2±3.4	-	-	2	-	-	+	+	+	+	-
<i>Sterculia setigera</i>	Sterculiaceae	-	5.0	2.5	-	8.0±1.6	38.6±11.2	-	2	2	+	+	-	-	-
<i>Stereospermum kunthianum</i>	Bignoniaceae	3.1	-	5.0	35.0±7.3	-	25.6±6.8	2	-	2	+	+	+	+	-
<i>Strychos innocua</i>	Loganiaceae	-	-	3.8	-	-	28.6±4.9	-	-	2	+	+	-	-	-
<i>Terminalia brownii</i>	Combretaceae	-	-	1.3	-	-	27.6±5.5	-	-	2	+	+	-	-	-
<i>Terminalia laxiflora</i>	Combretaceae	-	5.0	6.3	-	68.9±20.9	28.6±7.0	-	2	2	+	+	+	-	-
<i>Ximenia americana</i>	Oleaceae	-	-	2.5	-	-	41.6±9.4	-	-	2	+	+	-	-	-
<i>Ziziphus abyssinica</i>	Rhamnaceae	-	-	3.8	-	-	25.6±3.7	-	-	2	+	+	-	-	-

^a Mean values ± SE; (-): the species not found in the study area, (Trees); woody species with a height of greater than 1.5m were considered as trees.

^b Mean value ± SE of spore counts

^c Class: mycorrhizal root colonization class; referring to the following classes of mycorrhizal colonization of fine roots(-): not present in the study area; 1: 1-5%; 2: 6-25%; 3: 26-50%; 4: 51-75%; 5: 76-100%

^d AM fungi spore genera per species (+): AM spore genus present; (-): AM spore genus not present

^e Ni: AM spore genera not identified (+): AM spore identified to a genus level; (-): AM Spore not identified to a genus level.

^f vernacular name (Amharic language). It was not identified to a species level.

Mycorrhizal status of sites and species

All 43 plant species were colonized by AMF (Table 2-3). Root length colonization ranged from 0 to 95% and was on average 16.6%. The majority of the species showed AM structures in their roots: vesicles (32 species), hyphal coils (32) and arbuscules (30). Colonization levels of the same species did not differ much between the various sites (Table 2-3). Mycorrhizal fungal spores were observed in the rhizosphere of 38 species (Table 2-3). Mean spore density ranged from 8 to 69 spores 100 g⁻¹ dry soil (Table 2-3). *Glomus* was the most abundant and most frequently found genus followed by *Acaulospora*, *Gigaspora* and *Scutellospora* (Table 2-3). More than one genus of AMF was identified in the rhizosphere of most species. Five species, among which *Boswellia papyrifera*, harboured four genera of AMF in the rhizosphere. Six plant species had spores that for various reasons could not be assigned to genus. No spores were observed in the rhizosphere of five species (Table 2-3), even though the roots of these species were colonized by AMF.

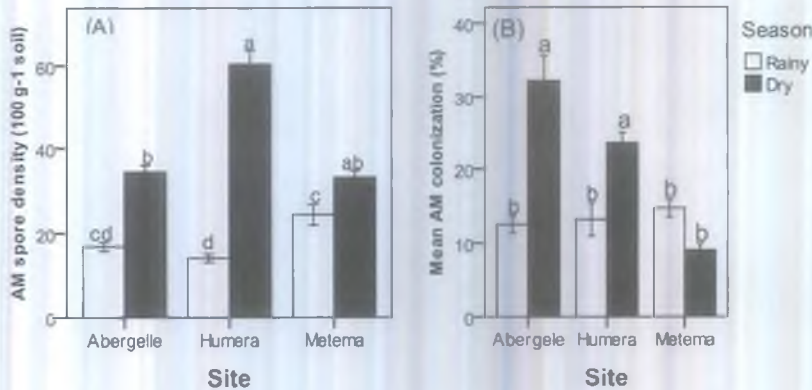


Figure 2-3. Arbuscular mycorrhizal (AM) fungal spore numbers (A) and AM fungal colonization (B) in dry (black bars) and rainy (white bars) season in three dry deciduous forests in northern Ethiopia. Bars indicate Mean ± SE.

Spore density and mycorrhizal colonization were not significantly correlated ($r^2 = 0.013$, $P = 0.38$) for the species common to the three sites (*Boswellia papyrifera*, *Dichrostachys cinerea* and *Lannea fruticosa*). Similarly no correlation was found between either spore number or AM colonization and chemical and physical soil properties (data not shown).

Influence of site, season and depth on AMF root colonization and spore abundance

Fractional colonization was lower in Metema than Abergelle and Humera ($F = 20.692$, $P < 0.001$). Season and the interaction site x season were also significant sources of variation (Table 2-4). While in Metema colonization levels were not different between both seasons, there was a strong seasonal effect in the two other sites. Colonization was significantly higher in the dry season in Abergelle and Humera (Figure 2-3b). Because site differences could be affected by differences in plant species composition, we reanalyzed the data for the three species that occurred in all three sites (*Boswellia papyrifera*, *Dichrostachys cinerea* and *Lannea fruticosa*). This analysis confirmed higher root colonization in the dry season in Abergelle and Humera, whereas in Metema colonization tended to be lower in the dry season (Table 2-6; Figure 2-5).

Table 2-4. Analysis of variance for AM spore density and AM colonization (effect of site, season and depth). Interaction was tested for all but only presented for the significant ones.

Source	AM spore density		AM root colonization	
	F	Sig.	F	Sig.
Site	4.170	0.016	20.692	0.000
Season	214.822	0.000	28.026	0.000
Site x Season	14.559	0.000	30.229	0.000

Table 2-5. Analysis of Variance for AM spore density in enclosure and open area.

Interaction was tested for all but only presented for the significant ones.

Source	F	Sig.
Site	11.040	0.001
Management	4.380	0.037
Season	235.704	0.000
Site x Management	19.345	0.000

Spore density was higher in Metema than in Abergelle and Humera ($F = 4.170$, $P < 0.05$). Overall spore density was higher in the dry season for all three sites ($F = 214.8$, $P < 0.001$). The significant site x season interaction indicated that the difference was much larger for Humera and lower for Metema (Table 2-4; Figure 2-3a). Because site differences could be affected by differences in plant species composition, we reanalyzed the data for the three species that occurred in all three sites. The analysis confirmed higher spore

densities in dry season than in the rainy season, with the largest difference in Humera (Table 2-6; Figure 2-5).

Soil depth was not a significant source of variation in AMF spore abundance for the three species that occurred in all sites ($P = 0.17$; Table 2-6).

Effect of management on AMF spore abundance

Exclosures had a positive effect on the spore abundance at Humera, but not in Abergelle (Table 2-5; Figure 2-4). Spore abundance in exclosures in Humera ranged between 29 to 71 spores 100 g^{-1} dry soil while in open areas it ranged between 8 to 45. Spore abundance in exclosures in Abergelle ranged between 20 to 24 spores 100 g^{-1} dry soil while it ranged between 21 to 46 in open areas. This site effect may have been due to differences in species composition between the sites, because management did not show any significant effect on spore abundance for the three species common to the three sites, but the site \times management and management \times season interactions were significant sources of variation (Table 2-6).

Glomus was the dominant genus both in the exclosures and open areas. Four genera were recorded in exclosures and 3 in open areas at Abergelle. *Glomus* was the only genus observed in the exclosures and open areas in Humera.

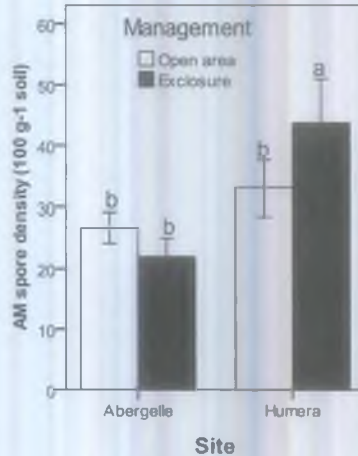


Figure 2-4. AM fungal spore density in dry deciduous forests under area exclosed for cattle grazing and human interference (black bars) and open areas (white bars). Bars indicate Mean \pm 1 SE

Discussion

AMF colonization and spore abundance among sites and species

All species studied were mycorrhizal. Our observations confirm other surveys in Africa that showed most or all of the woody species being mycorrhizal and a majority being AM. Forty of the forty seven indigenous tree and shrub species from dry woodland areas of Tanzania were AM (Högberg, 1982), the remaining seven species (all caesalps) forming ectomycorrhiza. Onguene and Kuyper (2001) assessed the mycorrhizal status of 100 tree species in the rain forest of south Cameroon. All species were mycorrhizal, with 26 species forming ectomycorrhiza and the remaining species being AM. Our study is also in agreement with all the species studied for mycorrhizal status in the dry afro-montane forests (Wubet et al., 2003), coffee forests of the south west (Muleta et al., 2007) and *Acacia* spp. in the nurseries of Ethiopia (Michelsen, 1992).

Based on literature data (Wang and Qiu, 2006), the following genera have not been mentioned before as forming AM: *Boswellia* (*B. papyrifera*, Burseraceae), *Piliostigma* (*P. thonningii*, Fabaceae), *Stereospermum* (*S. kunthianum*, Bignoniaceae) and *Ximenia* (*X. americana*, Olacaceae). The other member of the Burseraceae, *Commiphora africana* was already reported as AM by Högberg (1982). Our observations that *Ximenia americana* forms mycorrhiza is intriguing in the light of suggestions by Brundrett (2009) that the parasitic species of the Olacaceae, like other species of the Santalales, do not form AM. However, Orwa et al. (2009) noted for *Ximenia americana* that the species is only sometimes semi-parasitic with haustoria on roots. Other members of Olacaceae have also been described as forming AM. For a rainforest in Cameroon Onguene and Kuyper (2001) recorded AM in three species, viz. *Coula edulis*, *Ongokea gore* (also hemiparasitic) and *Strombosia grandifolia*.

While the mycorrhizal status of most genera investigated was already reported before, many species have never been reported before to form AM. To the best of our knowledge the following species can be added to the list of AM plants: *Acacia asak*, *A. etbaica*, *A. oerfota*, and *Pterocarpus leucens* (Fabaceae); *Combretum adenogonium*, *C. collinum*, *C. fragrans*, *C. hartmannianum*, *Terminalia brownii* and *T. laxiflora* (Combretaceae); *Ficus thonningii* (Moraceae); *Fluggea virosa* (Euphorbiaceae); *Lannea fruticosa* and *L. triphylla* (Anacardiaceae); *Lonchocarpus laxiflorus* (Celastraceae); *Maerua angolensis* (Capparidaceae); and *Sterculia setigera* (Sterculiaceae).

Levels of colonization were generally consistent for the same species in different sites, for different species of the same genus, and different genera of the same family. It had been proposed that woody species with a sparse and coarse rooting system with few poorly developed root hairs are both very responsive to mycorrhizal colonization and also show high levels of colonization (Janos, 1980; Haselwandter and Bowen, 1996). However, our data do not support that suggestion as far as levels of colonization are concerned. *Boswellia papyrifera*, dominant in the dry woodland, is susceptible to wind damage as their fine roots with sparse root hairs do not grow deep into the soil (Gebrehiwot, 2003). Most of the plants in the study area had coarse roots but they didn't show high levels of colonization, the average level being 16.6%. Similar observations were also made by Onguene and Kuyper (2001) and Zangaro et al. (2007) that trees with coarse roots showed less AM colonization.

Table 2-6. Analysis of variance for AM spore density (effect of site, season, management and depth) and AM colonization (effect of site and season) of the three common species (*Boswellia papyrifera*, *Dichrostachys cinerea* and *Lannea fruticosa*) found in the three study sites. Interaction was tested for all but only presented for the significant ones.

Source	AM spore density		AM root colonization	
	F	Sig.	F	Sig.
Site	27.633	.000	4.568	0.015
Season	143.538	.000	2.832	0.098
Site x Season	25.082	.000	7.551	0.001
Management	.198	.656		
Depth	1.893	.169		
Site x Management	4.427	.036		
Management x Season	4.547	.033		

Root colonization consisted of internal hyphae (in all samples), vesicles, arbuscules and coils. Vesicles were about as frequent as arbuscules. The presence of arbuscules or coils in roots is generally used to designate plants with functional AM (Brundrett, 2009). We did not observe arbuscules in the roots of *Pterocarpus leucens* and *Senna singueana*. The dominance of the genus *Glomus* followed by *Acaulospora* in most samples was based on the frequent occurrence of vesicles of different shapes. Most root samples had ellipsoid to round shapes typical for the genus *Glomus* while those with irregular and rectangular shapes indicating *Acaulospora* were less frequent. Dominance of *Glomus* species has been

reported in earlier studies on Ethiopian trees (Wubet et al., 2003; Muleta et al., 2007; Wubet et al., 2009). These studies also noted that *Gigaspora* and *Scutellospora* species were rare. Muleta et al. (2008) noted that in coffee systems *Acaulospora* was the second most common genus of AMF after *Glomus*. Wubet et al. (2003) did not record any *Acaulospora* sequences from three tree species from the afro-montane forest close to Addis Ababa. Most (70 %) of the studied tree species showed association with two or more AMF genera. *Boswellia papyrifera*, *Combretum molle*, *Senna singuena*, *Stereospermum kunthianum*, and *Dichrostachys cinerea* revealed an association with four AMF genera.

Spore abundance in the present study was low. A survey of tropical systems showed a mean spore abundance of 476 (55 to 1908 100 g⁻¹ soil) (Zhao et al., 2001) or 5 to 6400 100 g⁻¹ soil in a valley savanna of the dry tropics (Tao et al., 2004). Spore abundance was comparable to coffee systems in Ethiopia (Muleta et al., 2007: 4-67 per 100 g soil; Muleta et al., 2008: 38-119 per 100 g soil). Low spore abundance seems to be common in degraded, (semi-)arid regions (Requena et al., 1996; Ingleby et al., 1997). Soil disturbance, degradation and erosion can reduce levels of AMF propagules (Brundrett et al., 1996; Carpenter et al., 2001). Low spore density does not always imply inoculum limitation as the present study showed that all plants were colonized (Table 2-2). Inoculum sufficiency was further demonstrated by trap cultures using *Sorghum bicolor* for four months. The grass had a range of 70 to 100% colonization after inoculating with the soil brought from the study sites. Similarly Requena et al. (1996) noted that the legume *Anthyllis cytisoides* harboured low spore numbers but achieved high levels of mycorrhizal colonization. Interestingly, mycorrhizal inoculum potential was highest in the dry season, suggesting that mycorrhizal potential is not mainly determined by spore abundance (see below).

Effect of season and depth

In the study sites, spore density increased during the dry season. Higher surface soil temperatures and drier conditions could stimulate spore production (Ingleby et al., 1997; Cardoso et al., 2003; Tao et al., 2004).

There was a conspicuous contrast in the colonization pattern in our study sites. While all sites showed seasonality in colonization levels (Fig. 2-3), there was a conspicuous contrast between Metema on the one hand, and Humera and Abergelle on the other. Colonization levels in Metema declined in the dry season, consistent with our hypothesis. However, colonization levels in Humera and Abergelle were higher in the dry season, when most of the trees were leafless and little (in case of photosynthetic stems) or

no carbon was fixed. Both sites are characterized by more harsh environmental conditions than Metema, such as less rainfall or a shorter rainy season, and shallower soils with higher stone cover. In a site with less harsh climate (rainfall > 1000 mm annually; dry season less than 4 months), Michelsen et al. (1993) observed that mycorrhizal colonization tended to be higher in the rainy season.

This contrast suggests different mycorrhizal strategies. We hypothesize that under harsher conditions a temporal uncoupling of carbon fixation by the plant (rainy season) and carbon expenditure by the fungus (dry season) could take place. While it has often been stated that there is a close association between carbon fixation by the plant and carbon use by the fungus, as shown by pulse labeling (Johnson et al., 2002), two other studies have suggested temporal uncoupling. For a bulbous vernal herb, *Hyacinthoides non-scripta*, Merryweather and Fitter (1995) demonstrated largest mycorrhizal activity and nutrient inflow to the plant when the plant was dormant. For another vernal herb, *Erythronium americanum* a similar process was shown by Lapointe and Molard (1997). Interestingly, these later authors claimed that such temporal uncoupling is most relevant as a strategy of drought tolerance. The observations by Requena et al. (1996) that in the dry period in the Mediterranean when grasses and shrubs die off above-ground, the mycorrhizal network is more important for root colonization than spore inoculum fits with this hypothesis of uncoupling. Such temporal uncoupling could also result in a changed mycorrhizal phenology, viz., from a system where drought induces spore formation which leads to recolonization in the rainy season in Metema, to a system where the short rainy period is so short that plants cannot afford such an annual mycorrhizal strategy.

Considering that the pattern of increased root colonization during the dry season at Abergelle and Humera was observed in all three plant species that occurred at the three sites (Figure 2-5), we suggest that this strategy may be characteristic for strongly pulsed environments. These data also suggest that the strategy is not driven by plant but by fungal properties. Local adaptation of AMF in arid and semi-arid environments has been reported from the Ethiopian nurseries with *Acacia* species (Michelsen, 1993) and various shrubs in the Mediterranean area (Caravaca et al., 2003; Caravaca et al., 2005). Further research is needed to understand the below-ground dynamics of plants in relation to their mutualistic associates, specifically AMF.

The hypothesis that spore density varies with depth is rejected in the present study. Spore density didn't differ for the two depths considered. In shallow soils differences in spore density may not be expected. In shallow soils roots are concentrated in the upper

surface and more spores are found at the surface soil layer (Ingleby et al., 1997; Wang et al., 2004). Roots are distributed horizontally at the surface of the soil and root biomass declines with depth (Powers et al., 2005).

Effect of management on spore abundance

The hypothesis that vegetation in exclosures supports higher spore abundance is partly supported in this study. Disturbance has a negative impact on the establishment and growth of plant species, and thereby negatively affects also mutualistic root symbioses. In particular, degradation reduces the inoculum potential of AMF (Michelsen, 1992; Lovera and Cuenca, 1996, Guadarrama and Alvarez-Sanchez, 1999, Gai et al., 2006). In Humera exclosure there was a higher herbaceous cover compared to the open area, indicating the site potential to support relatively higher biological activity and the role of exclosures for recovery of mycorrhizal inoculum potential.

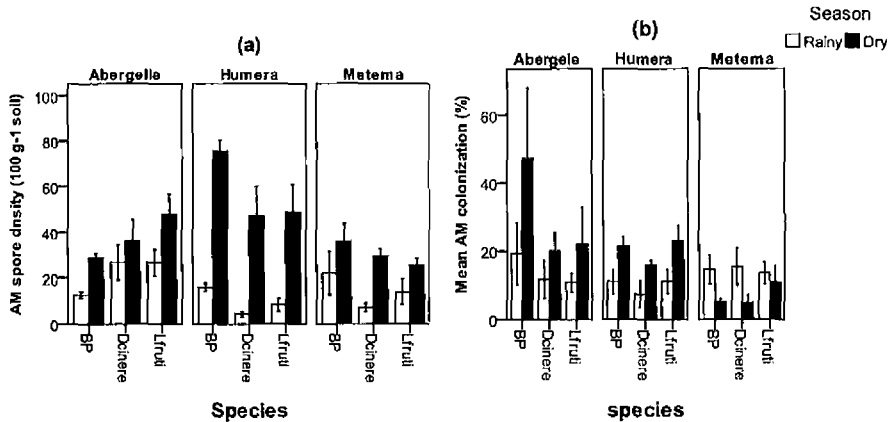


Figure 2-5. Arbuscular mycorrhizal (AM) fungal spore numbers (a) and AM fungal colonization (b) in dry (black bars) and rainy (white bars) season for the three common species found in the dry deciduous woodlands of northern Ethiopia. Species (BP: *Boswellia papyrifera*; Dcinere: *Dicrostachys cinerea* & Lfruti: *Lanena fruticosa*). Bars indicate Mean ± SE.

However, there was no significant difference in spore density between exclosure and open area in Abergelle ($P = 0.452$). This finding seems surprising considering that the exclosure in Humera was established 12 years ago, while that in Abergelle was established already 22 years ago. However, the effectiveness of exclosures should be taken into

consideration. Exclosures in Humera were more controlled and far from settlement areas, while in Abergelle they were near habitation. Consequently the possibility for encroachment is high in Abergelle. Grass was continuously harvested from the exclosures in Abergelle but less commonly so in Humera. A more controlled experiment on the effectiveness of exclosures to influence AM spore density and colonization could be vital to demonstrate the role of exclosures in the restoration and maintenance of plant cover and soil biological diversity.

Conclusion

The present study confirmed that in arid and semi-arid regions (almost) all woodland plants are colonized by AMF. Data on root colonization levels in dry and wet seasons demonstrated that in the sites with the harshest conditions, AM plants and fungi apparently adapt to pulsed resource availability by temporally disconnecting carbon gain by the plant and carbon expenditure by the fungus. This observation suggests that AMF can play an ecologically significant role in the adaptation, establishment, growth and survival of woodland species. This knowledge is essential for rehabilitation efforts of the dry deciduous woodland. For this it will be imperative to experimentally study below-ground processes in conferring adaptation to highly pulsed resources.

Chapter 3

*Arbuscular mycorrhizal fungi enhance growth, photosynthesis and water use efficiency of *Boswellia papyrifera* seedlings in a drought pulse environment*

Emiru Birhane, Frank J. Sterck, Masresha Fetene, Frans Bongers, Thomas W. Kuyper



Abstract

Under drought conditions arbuscular mycorrhizal (AM) fungi alter water relations of plants and improve their resistance to drought. In this study we tested the effect of AM inoculation and water pulsing on the performance (growth, gas exchange, nutrient status and mycorrhizal responsiveness) of *Boswellia papyrifera* seedlings. A greenhouse factorial experiment with AM inoculation and two water pulse levels were used. The watering treatments were continuous watering (WC) where plants were watered to field capacity every other day during 4 months; and short water pulse (SP) where in these 4 months switches to drought occurred, with 15 days of watering followed by 15 days of no watering. There were significantly higher levels of AM colonization under water pulsed conditions than under continuous watering. Mycorrhizal seedlings achieved higher plant dry mass than control seedlings. Stomatal conductance and the phosphorus mass fraction in shoot and root were also significantly higher for mycorrhizal seedlings. Mycorrhizal seedlings that received water pulse (SP) had the highest plant dry mass. Both a larger leaf area and higher assimilation rates contributed to higher plant dry mass. Water pulsing increased water use efficiency in non-mycorrhizal seedlings through a reduction in transpiration, while in mycorrhizal seedlings water pulsing increased water use efficiency, assimilation and transpiration. *Boswellia* seedlings allocated most carbon to the storage root. *Boswellia* seedlings had higher mass fractions of N, P and K in roots than in shoots. Water stress apparently benefits *Boswellia* seedlings when they are mycorrhizal.

Key-words: Water pulse; AM fungi; *Boswellia papyrifera*; seedling

Introduction

Prolonged seasonal drought severely affects the regeneration and survival of tree seedlings and saplings in the drylands (Gindaba et al., 2004, Gebrehiwot et al., 2005, Gebrekirstos et al., 2006). Arbuscular mycorrhizal (AM) fungi can alter water relations of plants such that they improve the resistance of plants to drought (Ruiz-Lozano and Aroca, 2010, Zhang et al., 2010, Apple, 2010, Augé, 2001, Smith and Read, 2008, Lambers et al., 2008). Various mechanisms have been proposed through which the AM symbiosis improves drought resistance. Augé (2001) subdivided drought resistance (survival at low water availability) into drought avoidance (where plants maintain a high internal water potential) and drought tolerance (survival at low internal water potential). In both mechanisms the AM symbiosis could be involved. The underlying mechanisms through which the AM symbiosis improves water relations of plants are partly nutritional (enhanced uptake of P, but also of K, N, Ca, Mg, Zn, Cu), partly non-nutritional. These non-nutritional mechanisms include hormonal effects (through abscisic acid) due to mycorrhizal colonization, improved soil–hyphal contact (especially important during soil drying), more effective scavenging for water in micropores, direct water uptake by hyphae, and increased photosynthesis through sink stimulation. As Augé (2001) noted, field effects of AM fungi on increased plant performance under drought stress are usually a combination of these nutritional and non-nutritional effects. While mycorrhizal plants thus acquire more water and nutrients, they inevitably could transpire more water too. However, several studies have also reported a mycorrhiza-induced increase in plant water use efficiency (WUE) (Ruiz-Lozano and Aroca, 2010).

In this study we focused on the role of the AM symbiosis in the water relations of the frankincense tree *Boswellia papyrifera* (Burseraceae – henceforth *Boswellia*). The species grows under extreme dry conditions where water shortage likely lasts for 8-9 months during the long dry season. In this dry season seedlings naturally die-back above-ground. Next to that period of 8-9 months of drought, water deficiency also occurs during the 3-4 months of the wet season because of irregular rains. Both types of water pulses may select for specific plant adaptations, both morphologically and physiologically (Schwinning and Ehleringer, 2001, Schwinning and Sala, 2004). *Boswellia* is ecologically adapted to such pulsed growth conditions (Abiyu et al., 2010, Ogbazgi et al., 2006, Gebrehiwot et al., 2005). In an earlier study we concluded that the AM symbiosis contributes to this adaptation and hence makes a major contribution to establishment, growth and survival of this woodland species under the prevailing harsh climate. Root

colonization levels were in fact higher during the dry than during the wet season. As the plants were leafless during the dry period (although *Boswellia* possesses photosynthetic stems), it seems plausible that carbon gain by the plant and carbon expenditure by the fungus are temporally disconnected. Such a temporal disconnect may be even more important for seedlings that are dormant above-ground for a period of 8-9 months (*waiting in the underground*). Such temporal disconnect could be a specific adaptation to pulsed resource availability (Birhane et al., 2010). A temporal disconnect driven by pulsed light availability has been described before (Lapointe 2001, Lapointe and Mollard, 1997). Querejeta et al. (2007) have described how hydraulic lift by (ectomycorrhizal) tree roots results in water exudation from roots that allow mycorrhizal hyphae to remain active during periods of severe soil drying.

In this study we tested the effect of the AM symbiosis in combination with water availability (pulsed or not) for three *Boswellia* seedling age groups to determine seedling performance and gas exchange. We hypothesized that:

- Mycorrhizal plants show larger seedling growth than non-mycorrhizal plants;
- Mycorrhizal *Boswellia* seedlings show increased gas exchange, leaf water potential and relative water content than seedlings without AM;
- The mycorrhizal benefit is larger under conditions of a water pulse (addition of a high amount of water followed by a dry period) compared to conditions of regular watering
- Higher biomass in water pulsed mycorrhizal seedlings is a result of increased assimilation rate and water use efficiency.

Material and Methods

A greenhouse experiment with *Boswellia* seedlings was conducted in northern Ethiopia at Illala plant tissue culture greenhouse, Tigray Agricultural Research Institute, Mekelle (13°3'N 39°E; altitude 2000 m a.s.l.) from June 1st, 2008 to October 30th, 2009. Mean daily temperature of the greenhouse was 25 °C during the day and 22 °C during the night with mean daily average relative humidity of 51% for the study period.

Seedling preparation and selection

Seeds from adult *Boswellia* trees from the dry deciduous woodlands in Abergelle, northern Ethiopia were collected in March 2007. Healthy trees with a single stem and with uniform seed setting were selected for seed collection. Seeds were directly picked by hand from

tree branches either by climbing or standing on the ground depending on tree height. Seeds were soaked in cold water for 12 hours to accelerate germination. Germination took place in plastic trays filled with autoclaved pure river sand under greenhouse conditions. All seeds germinated within 5-10 days. 160 germinated seeds were individually transplanted to plastic pots, 8cm diameter and 15cm high. Potted seedlings were placed on metal mesh benches and were watered regularly using micro-sprinkler irrigation every other day to field capacity until the plants were ready for the experiment that exposed them to the water pulse experiment. Dimethoate was sprayed to ward off ants and aphids which were observed on leaves. 132 seedlings of uniform size were transplanted to larger perforated 20 litre plastic containers, one seedling per container, filled with 15 kg autoclaved soil.

Preparation of inoculum of AM Fungi

Spores of AM Fungi were collected during the dry season from the rhizosphere of the same *Boswellia* trees by the wet sieving and decanting method (Brundrett et al., 1996). Most spores belonged to the genus *Glomus* (Birhane et al., 2010) and these were not further identified to species. Spore cultures were maintained on plants of *Sorghum bicolor*. The fungal inoculum added to the seedlings consisted of a mixture of soil, spores and root fragments, produced from the rhizosphere 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 also excavated from Abergelle, in a similar habitat where *Boswellia* trees naturally grow. Before inoculation, the potting soils were sieved and sterilised by an autoclave at 121°C for two-hours. Control seedlings were planted in sterilised soils.

Experimental design and treatments

The experiment consisted of a three-factorial design: AM Fungi (present or absent), water supply (continuous watering versus pulsed watering), and seedling age (seedlings grown for 4 months and harvested; seedlings grown for 12 months and harvested; and seedlings grown for 16 months and harvested). We mimicked seasonality by supplying water for 4 months followed by 8 months without any water. After one year we mimicked the second rainy season through supplying water for another 4 months (Figure 3-1). Seedlings harvested at the end of the first 4 months were given water to field capacity; seedlings grown for 12 months were given water to field capacity for 4 months and were then kept without water for 8 months; and seedlings that were grown for 16 months were given water

to field capacity for the two rainy seasons, four months each, and left without water for 8 months. In order to simulate rainfall pulses during the wet season, we supplied the seedlings either with water for 15 days followed by 15 days of drought (water pulse, SP) or every other day (continuous water, WC). All pots therefore received the same amount of water. This experimental factor represents dry spells within a normal rainy season. The treatment units were arranged in the greenhouse bench in a completely randomized design. There were 11 replications which gave a total of 144 seedlings (132 seedlings used during the experiment; twelve additional seedlings were harvested at the start of the experiment).

Measurement of seedling traits

We harvested seedlings four times (at the start of the experiment, and after 4, 12 and 16 months) (Figure 3-1). We determined plant size, biomass and growth rate. Total shoot length (plant height) was measured using a graduated meter and root collar diameter was measured using a digital caliper. The number of fully developed leaves was assessed for each seedling. Leaf surface area was measured using AM 100 Leaf area meter (ADC Bioscientific Ltd.). Harvested seedlings were divided into coarse roots, fine roots, stems and leaves and their dry mass was determined after oven drying at 80 °C until constant weight was achieved. We then calculated leaf, stem, fine root and coarse root mass fractions [dry mass per unit dry plant mass; g g^{-1}], leaf area ratio [leaf area (cm^2)/plant dry mass (g)], specific leaf area [leaf area (cm^2)/leaf dry mass (g)], and root: shoot ratio (Hunt, 1990). Total root length was estimated using the grid line intersect method (Tennant, 1975). In addition specific root length (root length per unit dry root mass, mm g^{-1}), root length per unit plant mass and root length per unit leaf area were calculated as root traits. The number and length of primary roots per plant were assessed and determined. Relative growth rate was calculated according to Hunt (1990) and Chiariello et al. (1989).

Plant nutrient analysis

Mineral status of the plants was determined by conducting shoot and root tissue elemental analysis. After sun drying, shoot and root samples were oven-dried at 80 °C for 48 hours. Samples were then wet-digested and were analyzed for N, P, and K. Total N was determined using the standard Kjeldahl method, P colorimetrically by spectrophotometer and K by Flame Photometry (Anderson and Ingram 1993).

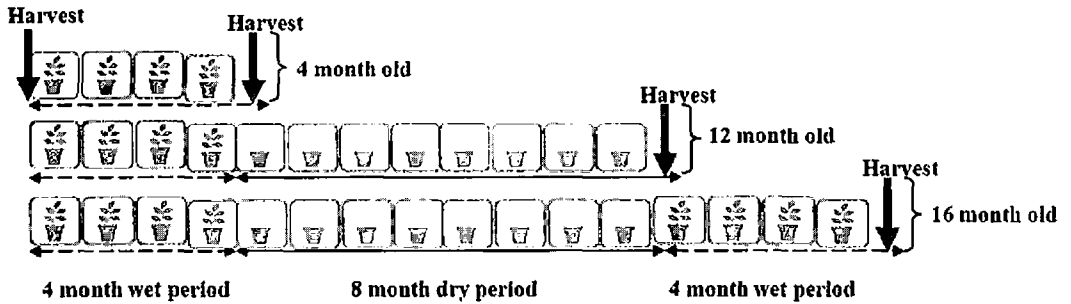


Figure 3-1. Schematic presentation of the water pulse experiment. The experiment included three ‘seasons’: a wet period of 4 months, a dry period of 8 months (when seedlings die back), and another wet period of 4 months. After each period 11 plants were harvested per treatment. Treatments included the water pulse, with constant water supply or with water pulses every two weeks, and inoculation, comparing mycorrhizal inoculation with no inoculation.

Mycorrhizal colonisation

Mycorrhizal colonisation was assessed using the grid line intersection method (Giovannetti and Mosse, 1980). Subsamples of (non-suberized) roots were collected, cleared with 10% KOH, and stained with 0.01% trypan blue in lactoglycerol (Brundrett et al., 1996). Roots were then divided into 1 cm pieces and mounted lengthwise on a microscope slide. Eleven slides per treatment per harvest containing 9 root pieces per slide were examined by making 3 microscope observations (top, middle and bottom) per 1 cm root piece at 400x magnification (n=891). Colonisation was expressed as percentage of the root length colonised. Total fractional colonisation, and those of arbuscules, vesicles, and internal hyphae in the root cortex were recorded.

Mycorrhizal responsiveness (MR) was expressed as the ratio of total dry weight of mycorrhizal plant and non-mycorrhizal plant. Drought response index (DRI) was calculated as the ratio of total dry weight of plants under drought stress (SP) to plants under well-watered conditions (WC).

Gas exchange measurements

Gas exchange was measured for the 16-month-old seedlings. Measurements were made between 0900 and 1200 h from five mature fully expanded leaves two times per leaf (n=11) under full sunlight using a LCP-002 portable photosynthesis system (LC Pro ADC

Bioscientific Ltd.). Measurements included net photosynthesis rate, stomatal conductance, transpiration rate and dark respiration rate. Photosynthetic water use efficiency was calculated as the ratio between the photosynthesis rate and transpiration rate. Predawn (0300–0600 h) and midday (1200–1400 h) leaf water pressure potential were measured using a pressure chamber apparatus (Scholander et al., 1965) using well-expanded leaves. Relative water content of leaves were measured according to Koide et al. (1989). Water pressure potential and relative water content were determined twice in the course of the experiment.

Statistical analysis

A three-way analysis of variance (ANOVA) was used to test for significant sources of variation in differences in seedling size, mass and nutrient levels; a two-way ANOVA was applied for root colonisation (water pulse; seedling age) and gas exchange (water pulse, mycorrhiza). Repeated-measures ANOVA was used to analyse treatment effects on predawn and midday relative water content and leaf water potential of seedlings. Because of the large number of parameters assessed, a sequential Bonferroni correction was applied at $P < \alpha/(1+k-i)$ significance level, with $\alpha=0.05$; k = number of parameters (40); i = the sequential value after sorting in ascending order. when F-test from ANOVA was significant. Gabriel post-hoc test for unequal sample size and LSD for main effect comparison were performed. In order to meet the assumptions of normal distribution and homogeneity of variances, data on leaf number were arcsine square root transformed; data on root collar diameter, shoot length, stem dry mass, coarse root dry mass, fine root dry mass, shoot dry mass, total plant dry mass, leaf relative growth rate, root length per leaf area, net photosynthetic rate and dark respiration rate were log transformed [$^{10}\log(n+0.5)$] before statistical analysis. Treatment effects were statistically analyzed using SPSS (PASW statistics 17) software.

Results

Plant size and growth

Most plant traits were significantly affected by seedling age, and some (especially root biomass) by mycorrhiza, while water pulse was in most cases not a significant source of variation. The interaction water pulse \times mycorrhiza was also significant for several plant traits, again those related to below-ground performance (Table 3-1). *Boswellia* seedlings were mycorrhiza-responsive. Because DRI was smaller than 1 in non-mycorrhizal

seedlings but larger than 1 in mycorrhizal seedlings, MR was much higher after water pulsing than with continuous water supply (Figure 3-2). For the three age classes, mycorrhizal responsiveness was around 300% for seedlings exposed to water pulsing, whereas it was around 150% for seedling in the water control treatment. Mycorrhizal responsiveness increased with the duration of the experiment. Both a larger leaf area (Table 3-1, Figure 3-3a; significant interaction water pulse × mycorrhiza) and higher assimilation rates per unit leaf area (Table 3-2, Figure 3-3c; water pulse, mycorrhiza and interaction water pulse × mycorrhiza all significant sources of variation) contributed to this increased plant dry mass. Higher assimilation per unit leaf area coincided with higher P mass fractions in mycorrhizal seedlings.

Boswellia seedlings allocated more carbon to the roots regardless of treatment and age groups. The seedling mass fraction was 90% for the coarse root. For coarse root biomass the interaction water pulse × mycorrhiza was significant. The coarse root mass fraction of AM *Boswellia* seedlings was higher by 63% than the non-AM ones.

Plant nutrients

Nutrient mass fractions in shoot and root were significantly affected by seedling age (for N, P, K), mycorrhiza (for P), but not by water pulse (Table 3-1). Phosphorus mass fractions in shoot and roots were significantly higher for AM seedlings. The interaction AM inoculation × age was significant for shoot N, and shoot and root P while the interaction age × water pulse was significant for root N and K (Table 3-1). *Boswellia* seedlings had significantly higher mass fractions of N, and K in their roots than in the shoots ($p < 0.001$).

AM colonisation

All control *Boswellia* seedlings at all harvests remained free of mycorrhiza. Water pulse was a significant source of variation for fractional colonisation (Table 3-2). Average fractional colonisation for the water control seedlings was 64 %, compared to 89% for the seedlings that received a water pulse. Arbuscular, vesicular, and hyphal colonisation and mycorrhizal root length were all significantly higher for seedlings in the water pulse treatment than in the regular water treatment (Table 3-2).

Gas exchange

Seedling age, mycorrhiza and water pulse were all significant sources of variation for stomatal conductance, whereas none of the interactions were significant (Table 3-1).

Chapter 3 – Drought pulsing

Table 3-1. ANOVA table showing the effect of age, mycorrhiza, and water pulse on plant traits of *Boswellia* seedlings. Except for gas exchange, all traits were measured and/or calculated after the harvest. A three-way ANOVA was used to test the effect of age, mycorrhiza and water

Parameters	Units	Age		AM		Water		Age x AM		Age x water		AM x water		Age x AM x water	
		F	P	F	p	F	p	F	p	F	p	F	p	F	p
Root collar diameter	mm	4.336	0.016	6.354	0.013	0.372	0.544	0.449	0.640	1.933	0.150	2.357	0.128	1.698	0.189
Height	mm	43.221	0.000*	8.861	0.004	0.174	0.678	1.339	0.267	1.157	0.319	0.567	0.453	0.530	0.590
Leaf number	number	260.006	0.000*	36.341	0.000*	0.105	0.747	10.297	0.000*	0.254	0.776	0.006	0.940	0.426	0.654
Leaf area	cm ²	18.553	0.000*	3.891	0.052	1.977	0.163	3.595	0.032	1.476	0.234	9.817	0.001*	1.694	0.190
Coarse root length	mm	70.279	0.000*	0.619	0.433	0.107	0.744	0.367	0.693	0.501	0.607	0.423	0.517	0.296	0.744
Coarse root diameter	mm	45.546	0.000*	0.057	0.812	0.006	0.937	0.227	0.797	0.205	0.815	0.652	0.421	0.057	0.944
Fine root length	mm	7.487	0.001*	0.004	0.952	1.258	0.264	0.876	0.419	0.887	0.414	0.241	0.625	0.984	0.377
Root branch number	number	3.961	0.022	0.395	0.531	0.864	0.355	0.113	0.893	0.355	0.702	0.019	0.892	0.942	0.393
Leaf dry mass	g	11.014	0.000*	2.066	0.154	0.730	0.395	5.206	0.007	0.853	0.430	0.009	0.926	0.698	0.500
Stem dry mass	g	18.873	0.000*	0.605	0.439	0.000	0.989	4.562	0.013	3.025	0.053	5.714	0.019	0.611	0.545
Coarse root dry mass	g	23.355	0.000*	110.464	0.000*	9.342	0.003	0.105	0.901	1.148	0.321	31.221	0.000*	0.000	1.000
Fine root dry mass	g	14.795	0.000*	4.128	0.044	0.000	0.999	0.768	0.466	0.320	0.727	0.164	0.686	5.950	0.003
Shoot dry mass	g	12.585	0.000*	8.309	0.005	0.095	0.759	3.426	0.036	3.687	0.028	5.085	0.026	0.391	0.677
Root dry mass	g	24.297	0.000*	107.133	0.000*	9.404	0.003	0.077	0.926	1.011	0.367	31.084	0.000*	0.016	0.984
Plant dry mass	g	26.998	0.000*	98.902	0.000*	7.599	0.007	0.271	0.763	1.209	0.302	30.021	0.000*	0.176	0.839
Biomass increase	%	11.270	0.000*	45.520	0.000*	0.384	0.537	1.912	0.152	0.596	0.553	8.421	0.004	0.657	0.520
Plant relative growth rate	%.month ⁻¹	23.606	0.000*	94.144	0.000*	6.200	0.014	0.102	0.903	1.166	0.315	27.247	0.000*	0.246	0.783
Shoot relative growth rate	%.month ⁻¹	5.517	0.005*	5.194	0.025	0.179	0.673	1.249	0.291	5.347	0.006	6.903	0.010	1.087	0.341
root relative growth rate	%.month ⁻¹	20.571	0.000*	101.055	0.000*	7.624	0.007	0.069	0.933	0.936	0.395	27.514	0.000*	0.030	0.971
Leaf relative growth rate	%.month ⁻¹	1.854	0.165	0.066	0.798	0.466	0.497	1.585	0.213	1.410	0.252	0.001	0.979	0.059	0.943
Stem relative growth rate	%.month ⁻¹	9.836	0.000*	0.259	0.612	0.016	0.901	5.244	0.007	7.806	0.001*	4.815	0.031	0.558	0.574
Coarse root relative growth rate	%.month ⁻¹	18.933	0.000*	104.123	0.000*	7.532	0.007	0.066	0.937	1.078	0.343	27.583	0.000*	0.004	0.996
Fine root relative growth rate	%.month ⁻¹	12.221	0.000*	9.805	0.002	0.007	0.934	0.102	0.904	0.027	0.973	1.907	0.170	8.960	0.000*
Leaf area ratio	cm ² g ⁻¹	7.388	0.001*	7.763	0.006	0.340	0.561	5.885	0.004	0.307	0.736	0.705	0.403	0.681	0.508
Specific leaf area	cm ² g ⁻¹	7.458	0.001*	0.045	0.833	0.262	0.610	0.602	0.550	1.069	0.347	1.080	0.302	0.854	0.429
Root to shoot ratio	gg ⁻¹	0.894	0.412	7.603	0.007	3.281	0.073	4.272	0.016	6.673	0.002	0.688	0.408	0.956	0.387

Chapter 3 – Drought pulsing

Parameters	Units	Age		AM		Water		Age x AM		Age x water		AM x water		Age x AM x water	
		F	P	F	p	F	p	F	p	F	p	F	p	F	p
Specific root length	mm g ⁻¹	10.437	0.000*	17.994	0.000*	1.531	0.218	5.958	0.003	1.326	0.269	0.473	0.493	1.611	0.204
Root length per plant mass	mm g ⁻¹	10.567	0.000*	17.113	0.000*	0.730	0.395	5.803	0.004	0.499	0.609	0.751	0.388	0.639	0.530
Root length per leaf area	mm cm ⁻²	9.124	0.000*	0.026	0.872	0.022	0.882	0.949	0.391	1.377	0.258	0.422	0.518	2.856	0.063
Pre-down relative water content	%	244.871	0.000*	0.008	0.930	2.345	0.130	5.150	0.026	0.000	1.000	2.368	0.128	1.746	0.190
Mid-day relative water content	%	184.994	0.000*	2.223	0.140	0.309	0.580	0.026	0.872	0.181	0.671	1.782	0.186	1.667	0.200
Pre-down leaf water potential	bars	23.202	0.000*	1.535	0.219	0.155	0.694	3.343	0.071	5.807	0.018	1.187	0.279	0.897	0.346
Mid-day leaf water potential	bars	18.827	0.000*	0.003	0.960	1.042	0.310	9.056	0.004	4.205	0.044	0.603	0.440	4.051	0.048
Stomatal conductance	mmol m ⁻² s ⁻¹	15.072	0.000*	13.777	0.000*	25.929	0.000*	0.276	0.600	9.964	0.002	0.684	0.409	5.018	0.026
Shoot Nitrogen	%	6.207	0.003*	0.930	0.337	0.100	0.752	10.495	0.000*	5.717	0.005	0.333	0.565	6.120	0.003
Root Nitrogen	%	45.861	0.000*	5.862	0.017	4.329	0.040	0.529	0.591	10.509	0.000*	0.320	0.573	2.367	0.099
Shoot Phosphorus	%	32.617	0.000*	13.527	0.000*	9.614	0.003	12.928	0.000*	5.463	0.006	1.973	0.163	5.086	0.008
Root Phosphorus	%	64.023	0.000*	13.888	0.000*	7.697	0.007	9.703	0.000*	1.847	0.163	0.019	0.889	0.242	0.785
Shoot Potassium	%	156.679	0.000*	0.850	0.359	5.591	0.020	7.805	0.001*	2.006	0.140	0.056	0.814	13.358	0.000*
Root Potassium	%	262.489	0.000*	2.425	0.122	8.366	0.005	0.810	0.448	8.105	0.001*	0.220	0.640	0.517	0.598

*Significant after Bonferroni correction

Assimilation and water use efficiency of the oldest seedlings were significantly affected by mycorrhiza, water pulse and the interaction (Table 3-3). Transpiration rate was not affected by mycorrhiza or water pulse, but the interaction was highly significant. Mycorrhizal plants in the water pulse treatment had significantly higher assimilation rates than the other treatments (Figure 3-3c). Transpiration rates were significantly higher in non-mycorrhizal plants with regular water and in mycorrhizal plants under pulsed conditions than in the other treatments (Figure 3-3b). Water use efficiency was higher for mycorrhizal than for non-mycorrhizal plants and higher for water-pulsed plants than for plants that received water regularly (Figure 3-3d). Stomatal conductance was higher for mycorrhizal plants than for non-mycorrhizal plants and higher for regularly watered plants than for plants in the water pulse treatment (Figure 3-3f). Remarkably, stomatal conductance and water use efficiency were not correlated. No significant differences were observed in relative water content and leaf water potential among treatments within the same age class (Table 3-1).

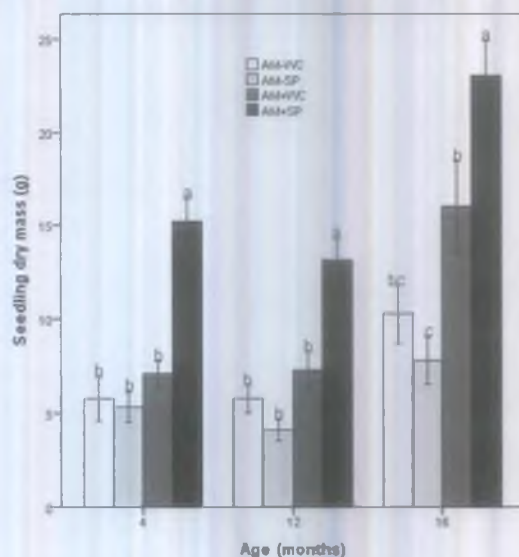


Figure 3-2. The effects of arbuscular mycorrhiza and water regime on seedling mass (mean \pm 1 SE) of *Boswellia*. Arbuscular mycorrhiza treatment (AM+) is compared with a control without inoculation (AM-), and water pulse every two weeks (SP) during the wet seasons is compared with a control with permanent water supply during the wet season (WC). Seedlings in the 12 month category died back during the 8 month dry period, as a consequence of which there were no differences in biomass between the 4 and 12 months old seedlings. Different letters (per age class) indicate significant differences between treatments ($P < 0.05$).

Discussion

In this study we observed a positive effect of AM fungi on the growth and biomass of *Boswellia* seedlings. The high mycorrhizal responsiveness of *Boswellia*, especially under conditions of water pulsing, confirmed the crucial role that the mycorrhizal symbiosis plays in these harsh environments. The positive mycorrhizal effect is attributed to the improvement of phosphorus nutrition, which was evident by the significantly higher P mass fractions in shoots and roots of mycorrhizal plants compared to non-mycorrhizal plants. The beneficial effect of the mycorrhizal symbiosis was much more clear after water pulsing. This combination had a very large effect on root properties and also on leaf area

Water pulsing had a negative effect in non-mycorrhizal *Boswellia* seedlings. Interestingly, water pulsing improved performance of mycorrhizal seedlings compared to regular watering, and this effect became stronger over time. This contrast confirms the essential role of the mycorrhizal symbiosis in case of unpredictable water availability. This effect could be attributed to the increase in leaf area and the increase in assimilation rate per unit leaf area. The AM fungi helped to increase the root relative growth rate in terms of root mass in order to store more water so that they could transpire more water to maintain their stomata open (Figure 3-3b and c). The large diameter coarse root showed a significantly higher relative growth rate. Coarse root dry mass is used as a proxy for water storage and the higher root mass contributed to the higher availability of moisture when the seedlings were water stressed. Mycorrhizal fungi have the potential to fundamentally alter the relationship between leaf area and plant mass that increases the relative investment of fixed carbon in leaf (Wright et al.,1998, Daughtride et al.,1986). The greater biomass during water pulse conditions with the mycorrhizal than non-mycorrhizal seedlings indicated greater total plant acquisition of P (Estrada-Luna et al., 2000). The consistent higher plant dry mass of seedlings under drought pulse when they are mycorrhizal indicated not only larger mycorrhizal benefits but also their efficiency to increase growth under drought pulse conditions.

In conjunction with larger plant size and an improved phosphorus status of mycorrhizal plants, we noted an increase in assimilation (in combination with water pulsing), water use efficiency and stomatal conductance (both independent of water pulsing; Figure 3-3). An increase in stomatal conductance in mycorrhizal plants has been

Table 3-2. Fractional mycorrhizal colonization and mycorrhizal root length (mean \pm S.E.) for inoculated seedlings of *Boswellia*. A two-way ANOVA is used to test for the effects of age and water and their interaction.

Colonization percentage	Age					Water				Age x water	
	4	12	16	F	p	WC	SP	F	P	F	p
Arbuscular colonization	0.404 \pm 0.03	0.464 \pm 0.03	0.577 \pm 0.03	1.953	0.151	0.344 \pm 0.02	0.62 \pm 0.02	14.815	0.000	0.315	0.731
Vesicular colonization	0.631 \pm 0.03	0.637 \pm 0.03	0.657 \pm 0.03	0.054	0.948	0.448 \pm 0.02	0.814 \pm 0.02	29.148	0.000	4.795	0.012
Hyphal colonization	0.649 \pm 0.04b	0.725 \pm 0.04b	0.915 \pm 0.04a	7.443	0.001	0.641 \pm 0.03	0.885 \pm 0.03	15.596	0.000	0.094	0.911
Mycorrhizal root length	13.091 \pm 1.333	21.281 \pm 1.333	32.35 \pm 1.333	2.470	0.093	13.489 \pm 1.264	32.137 \pm 1.333	6.790	0.012	0.957	0.390

Table 3-3. Gas exchange (mean \pm S.E.) in 16 month old *Boswellia* seedlings. A two-way ANOVA is used to test for the effects of mycorrhiza and water and their interaction.

Traits	Units	AM inoculation				Water				AM x water	
		AM-	AM+	F	p	WC	SP	F	p	F	p
Assimilation	$\mu\text{mol C m}^{-2} \text{s}^{-1}$	2.88 \pm 1.057	3.72 \pm 1.069	8.466	0.004	2.95 \pm 1.073	3.66 \pm 1.049	5.539	0.020	8.116	0.005
Transpiration	$\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$	4.046 \pm 0.093	4.214 \pm 0.111	1.345	0.248	4.182 \pm 0.120	4.077 \pm 0.081	0.519	0.472	82.019	0.000
Respiration	$\mu\text{mol C m}^{-2} \text{s}^{-1}$	-0.73 \pm 1.230	-1.285 \pm 1.294	2.837	0.097	-0.75 \pm 1.23	-1.26 \pm 1.291	2.490	0.120	4.074	0.048
Water use efficiency	$\mu\text{mol mmol}^{-1}$	0.842 \pm 0.044	0.990 \pm 0.053	4.663	0.032	0.801 \pm 0.057	1.031 \pm 0.039	11.193	0.001	7.380	0.000

repeatedly observed. Such increases were noted both for comparisons of mycorrhizal and non-mycorrhizal plants of the same size and P status, and for cases where mycorrhizal plants were significantly larger and with higher leaf nutrient mass fractions (Augé, 2001). Subsequent studies have confirmed this pattern, while also showing the importance of plant identity of functional type. Querejeta et al. (2003) observed that AM fungi enhanced stomatal conductance much more in the slow-growing *Olea europaea* subsp. *sylvestris* than in the more fast-growing *Rhamnus lycioides*. Possibly, plant strategy (along the axis from a conservative to acquisitive strategy for both above-ground and below-ground resources, cf. Díaz et al. (2004)) is a major determinant how AM fungi affect stomatal conductance. In a subsequent study, Querejeta et al. (2007) compared seedlings of the dryland shrub *Pistacia lentiscus* and *Retama sphaerocarpa*, a leafless legume with photosynthesizing stems. *Retama* was less responsive to mycorrhizal inoculation than *Pistacia* and showed no increase in stomatal conductance (as assessed from the $\delta^{18}\text{O}$ signal, a proxy measure for stomatal conductance). Querejeta et al. (2006) compared native, drought-adapted fungi AM fungi with non-native strains and noted that the native species more strongly increased stomatal conductance than non-native species. Larger increases in stomatal conductance in native AM fungal species coincided with larger improvements in nutrient uptake by the native species. A major effect of AM fungal species provenance was also noted by Ruiz-Lozano et al. (1995) and Marulanda et al. (2006).

Increased stomatal conductance of mycorrhizal plants compared to non-mycorrhizal plants would normally translate into increased photosynthesis. However, this response was not observed when plants were adequately watered (WC). Instead, transpiration rates of mycorrhizal plants were significantly lower than those of non-mycorrhizal plants, and as a consequence the mycorrhizal symbiosis did improve water use efficiency. Under water pulse (SP) increased stomatal conductance resulted in increased assimilation rates. Simultaneously, transpiration rates also increased, although the fractional increase was somewhat less. Consequently, water use efficiency was also higher for mycorrhizal plants compared to non-mycorrhizal plants under conditions of drought stress. Even though *Boswellia* seedlings always allocated most carbon to the coarse root system, mycorrhizal seedlings had 63% more C allocated to the roots than non-mycorrhizal seedlings. The significantly higher biomass and nutrients in the roots indicated that AM *Boswellia* seedlings store the resources acquired during the growing season in the roots when they die-back as a resource conservation strategy. Considering the structure of the

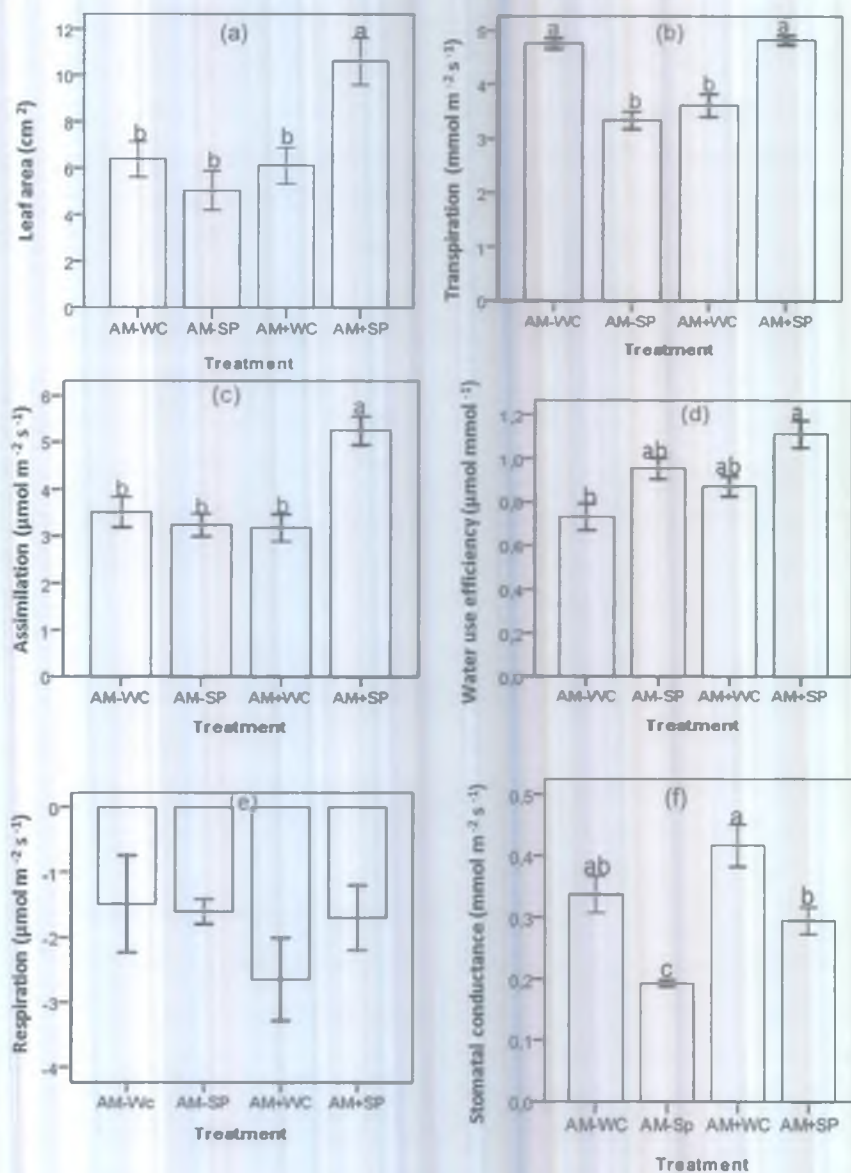


Figure 3-3. The effects of arbuscular mycorrhiza and water regime on gas exchange (mean ± 1 SE) of 16-month old *Boswellia* seedlings. Arbuscular mycorrhiza treatment (AM+) is compared with a control without inoculation (AM-), and water pulse every two weeks (SP) during the wet seasons is compared with a control with permanent water supply during the wet season (WC). Different letters indicate significant differences between treatments ($P < 0.05$).

coarse root, it is likely that this organ also serves as a water storage organ from which mycorrhizal fungi might benefit under conditions of drought stress. Querejeta et al. (2007) studied the effect of severe soil drying on the functioning of the mycorrhizal network in an oak savanna in California. The authors concluded that in this ectomycorrhizal symbiosis the trees accesses water from groundwater, and that this water is exuded in soil from which the ectomycorrhizal fungal mycelium subsequently benefits. In our greenhouse the seedlings did not have access to additional water sources. However, one may be tempted to hypothesize that the coarse root does not only provide stored carbohydrates to the AM fungus, but also water. Such provision of water through exudation explains why mycorrhizal plants did actually have access to more water during the water pulses, because transpiration rates of water-pulsed mycorrhizal plants was significantly higher than that of plants provided with regular water. Provision of water to hyphae through the coarse roots could also explain how mature trees could maintain (and even increase) levels of mycorrhizal colonization during dry periods, as observed in our previous field study (Birhane et al., 2010).

A better water status of mycorrhizal seedlings compared to non-mycorrhizal seedlings may result in seedlings that are better able to capture resources during the next rainy season. After a period of 8 months of not-watering, when seedlings had died back above-ground, an increase in atmospheric humidity resulted in renewed above-ground emergence of seedlings. Similar behavior to increased atmospheric humidity was observed in miombo trees (Chidumayo and Frost, 1996). Seedling emergence, before the next round of watering started, was higher for mycorrhizal seedlings (53%) compared to non-mycorrhizal seedlings (37%), even though the difference was not significant. Faster emergence expands the window of opportunity for *Boswellia* seedlings to further achieve a positive carbon and nutrient balance and may ultimately shorten the period (of several years to decades) that the tree shows annual cycles of die-back, before it finally achieves the height growth that allows it to escape from grazers and fire.

Conclusion

Our results show that AM colonization changed plant growth, biomass (especially below-ground biomass), phosphorus mass fraction in leaves and roots and photosynthetic performance of *Boswellia* seedlings. The strong interaction between the presence of AM and the water pulse treatment showed that mycorrhizal *Boswellia* actually benefits from

drought pulses during the short rainy season. Water pulsing increased leaf area and phosphorus mass fractions in root and shoots, resulting in increased assimilation rates. Even though transpiration rates were also higher, water use efficiency was highest in mycorrhizal seedlings under conditions of water pulses. The conservative acquisition strategy of *Boswellia*, where carbon acquired and possibly also water acquired after rain events are stored in coarse roots, may be beneficial in this harsh climate. By this strategy (*waiting in the underground*) seedlings may disconnect carbon gain from nutrient gain, and may store reserves below-ground till they are able to produce shoots that grow sufficiently high to escape the impacts of grazing and fire. A major question for further research is whether other trees than *Boswellia* in this habitat show this conservative acquisition strategy, or whether more acquisitive strategies may also be beneficial under such climates. A further question is how mycorrhiza and water availability would affect competition between plants with different resource acquisition strategies in these drylands. Such knowledge is essential for sustainable management of these economically and ecologically important species.



Chapter 4

*Arbuscular mycorrhiza as a support system to rehabilitate Boswellia and Acacia
seedlings in dry deciduous woodlands*

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Abstract

In dry ecosystems seedling growth and survival are limited by access to moisture and nutrients. Arbuscular mycorrhizal (AM) fungi are known to increase seedling establishment and survival through enhancement of nutrient and moisture availability. We used a greenhouse experiment to determine the interactive effect of AM, water deficit and soil fertility on the biomass, gas exchange, water relations, and root colonization of seedlings of *Acacia etbaica*, *Acacia senegal* and *Boswellia papyrifera*, tree species that dominate large areas of dry forest in the Horn of Africa. A full factorial design was used: with and without AM, top and subsoil, and four levels of water deficit. AM increased the biomass of *Boswellia papyrifera* and *Acacia senegal* seedlings. AM increased allocation to shoots in *Acacia*, but to roots in *Boswellia* seedlings. All three species had higher assimilation, transpiration, stomatal conductance, midday water potential and nutrient levels when mycorrhizal. *Acacia* seedlings responded positively to increased moisture while *Boswellia* seedlings responded better to water deficit levels of less than 50% field capacity. Rich soil condition favored all three species, although the response was more visible in increasing the biomass of *Boswellia* seedlings. Colonization increased with moisture in the *Acacias* while it decreased in *Boswellia* seedlings. Rich soil positively affected colonization of all three species. AM increased biomass growth of *Acacia* and *Boswellia* seedlings through increased gas exchange, water relations, nutrient levels and colonization. The increased performance of mycorrhizal plants indicates that mycorrhiza is a major component of the adaptive strategy of seedlings in the dry deciduous ecosystem. For successful rehabilitation efforts seedlings need to be mycorrhizal.

Key-words: arbuscular mycorrhiza; dry deciduous wood land; water deficit; soil fertility; *Acacia*; *Boswellia*

Introduction

Drought and low soil fertility are the two major rehabilitation constraints in arid areas, since they have a negative impact on soil biodiversity (including beneficial root symbionts), plant physiological processes that affect establishment and survival of seedlings (Cardoso and Kuyper, 2006, Engelbrecht et al., 2005, Otieno et al., 2001, Huston and Smith, 1989). Under such hostile conditions improvement of soil nutrient availability and water may increase establishment success. Application of nutrients such as Nitrogen, Phosphorus or Potassium improved the growth of dry tropical seedlings of various species (Khurana and Singh, 2001).

In nature land plants have developed a range of strategies that enable them to effectively access nutrients and moisture in stress conditions. Plant strategy theory (Díaz et al., 2004) has indicated a major axis to order two different strategies of resource acquisition, viz., a conservative versus an acquisitive strategy. In that study the plant traits that allowed that subdivision were above-ground plant traits. However, due to coordination of above-ground and below-ground traits (Liu et al., 2010), it is likely that the same classification can be applied for the organs of nutrient and water acquisition, the 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 (thin, much branched fine roots with a large number of long root hairs versus coarse unbranched roots with fewer and shorter root hairs). Another set of traits relates to special 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). The most prevalent mycorrhizal association that plants form is with arbuscular mycorrhizal (AM) fungi (Brundrett, 2009). The AM symbiosis generally improves acquisition of water and nutrients (Fagbola et al., 2005, Ruiz-Lozano et al., 2005).

The role of the AM symbiosis in improving water relations of plants has been reviewed by Augé (2001) and Ruiz-Lozano and Aroca (2010). These reviews concluded that AM plants show greater depletion of soil water, higher stomatal conductance and transpiration, better supply of nutrients in dry soil and higher drought tolerance when they grow under water limitation. Improved water status is both a direct consequence of improved plant nutrition (especially P), but non-nutritional factors of the AM symbiosis (hormonal effects, sink stimulation of photosynthesis) also improve photosynthesis and stomatal conductance under conditions of drought stress. Nutritional and non-nutritional

effects usually occur together. Under water-limiting conditions the uptake of immobile nutrients such as P is diffusion limited, and improved access to water improves diffusion. Nutrient diffusion to roots is directly determined by volumetric soil water content (Smith et al., 2010) and by the tortuosity factor, which is also dependent on soil water content (Neumann and George, 2004). The lower hydraulic conductivity during P deficiency in roots can be increased by the AM symbiosis through ensuring continuous P supply to enhance water uptake (Smith et al., 2010). Hyphae of AM fungi bridge the soil-root gap created during drought through binding roots to the soil and maintain liquid flow that avoids the loss of hydraulic conductivity caused by air gaps (Augé, 2004, Augé, 2001). AM plants accumulate more solutes and then increase the osmotic potential of the host plant that enables it to take up more water from the soil (Qiangsheng et al., 2006). Enhanced dehydration tolerance was also indicated by the tendency for AM plants to sustain turgor and stomatal conductance at lower relative water content (Ruiz-Lozano, 2003, Ruiz-Lozano, 1995). Low soil water content is strongly correlated with low nutrient availability, so that the mycorrhizal symbiosis plays a significant role in alleviation of both water and nutrient deficiency (Smith et al., 2010).

Plant species with different root traits generally differ in mycorrhizal responsiveness. Early research (Bayliss, 1975, St. John 1980, Janos 1980, Hetrick, 1991) indicated that plants with thick coarse unbranched roots with few short root hairs (magnolioid roots) would be more dependent on and responsive to the mycorrhizal symbiosis than plants with fine branched roots with numerous long root hairs (graminoid roots). However, in a comparison of 78 seedlings of subtropical forest trees, Zangaro et al. (2005) noted the opposite pattern, with plants having graminoid roots showing a much higher mycorrhizal responsiveness than plants with magnolioid roots. One possible explanation for this counterintuitive result is that Zangaro et al. (2005) tested seedlings in their earliest stage, where differences in seed carbon and nutrient reserves (correlated with seed mass) affected growth rate and mycorrhizal responsiveness. Less research has been done how different root strategies (the magnolioid strategy as an example of a conservative strategy versus the graminoid strategy as an example of an acquisitive strategy) interact with the mycorrhizal symbiosis to confer drought resistance. Such knowledge is not only relevant to improve classifications of plant functional types, but also for rehabilitation efforts, where degraded soils that are often poor in organic matter show low levels of water and nutrients.

Water potential, relative water content, and gas exchange are commonly used parameters to express water stress and the adaptation of a species to drought (Serrano and Penuelas, 2005, Moriana et al., 2002, Gindaba et al., 2005, Hsiao, 1973). A species could be adapted to drought through enhancing water uptake or through efficient control of water loss (Tyree et al., 2003). High biomass investment in roots and increased root surface area through increased specific root length (Slot and Poorter, 2007) increase moisture and nutrient uptake. Lowering leaf surface area to reduce transpiration loss (Engelbrecht et al., 2003), stomatal closure, osmotic regulation and withstanding a low leaf water potential are strategies that help the plant to survive low water levels (Poorter and Markesteijn, 2008, Tyree et al., 2003). Transpiration, gas exchange and biomass allocation are highly interrelated, thus monitoring these parameters during the development of water stress provides reliable information about species performance and ecological potentials (Hao et al., 2009, Otieno et al., 2005).

In this study we grew seedlings of the economically and ecologically important tree species *Acacia etbaica* and *A. senegal* (Fabaceae) and *Boswellia papyrifera* (Burseraceae) in greenhouse conditions. These species occur on poor and often eroded water-stressed soils and benefit from AM fungi as shown in greenhouse trials (Birhane et al., Chapter 3; Michelsen, 1992). Species of *Acacia* show an acquisitive strategy, while *Boswellia* shows a conservative strategy (Table 4-1). We investigated the effects of AM, soil water deficit and soil fertility and their interactions on carbon gain (growth), gas exchange, water relations, nutrient levels, and root colonization of seedlings. We hypothesized that:

1. AM plants will grow more rapidly than non-mycorrhizal plants, and this effect will be stronger under water or nutrient-stressed conditions.
2. The acquisitive *Acacia* species will be less mycorrhiza responsive than the conservative *Boswellia*.
3. Higher growth in mycorrhizal plants is achieved by more effective nutrient acquisition, and the maintenance of stomatal conductance, transpiration and assimilation because plants remained better hydrated. Again, these effects will be larger for *Boswellia* with its conservative acquisition strategy.
4. Colonization of roots by AM increases with increasing soil and moisture stress.

Methodology

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

greenhouse (13°29'N 39°28'E; altitude 2200 m a.s.l.) from May 01 2009 to October 30 2009. The mean daily temperature of the greenhouse was 27 °C during the day and 22 °C during the night with mean daily average relative humidity of 62 % for the study period. General characteristics of the three species are given in Table 4-1. The species will henceforth be referred to as *A. senegal*, *A. etbaica* and *Boswellia*.

Seedling preparation and selection

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 at Addis Ababa, Ethiopia, collected from the lowlands of northwestern Ethiopia, in 2007. Both *A. etbaica* and *A. senegal* grow in the same ecosystem and co-occur with *Boswellia* in dry deciduous woodlands. In the dry northern areas *Boswellia* is found together with *A. etbaica* and in the northwestern somewhat less dry areas it grows together with *A. senegal*. Seeds were treated before germination (Table 4-1). Germination took place in plastic trays filled with autoclaved pure river sand under greenhouse conditions. All seeds germinated within 5-15 days. 450 germinants were individually transplanted to plastic pots, 8cm diameter and 15cm high. Potted seedlings were placed on metal mesh benches and were watered regularly using micro-sprinkler irrigation every other day to field capacity until the plants were ready (one month) for the experiment that exposed them to the treatment combinations. 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.

AM inoculum and potting soil

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 that are referable to *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-colonized *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 grow. Two different soil profiles, namely topsoil (0-30 cm) and subsoil (30-60 cm) were collected. Top soil and subsoil characteristics were respectively: 47% and 42% sand; 32% and 36% silt; 21% and 22% clay; pH(H₂O) 6.9 and 6.8; EC(ds/m) 1.3 and 1.1; OC(%) 2.61 and 2.20; P(mg/kg) 31.14 and 25.20; K (mg/kg) 24.75 and 21.55; N (%) 0.29 and 0.17 and CEC (cmol⁺ kg⁻¹) 2.62 and 2.18. Before the inoculation procedure, the soils were sieved and sterilized by an autoclave at 121⁰C for two hours. Control seedlings were planted with sterilized soils.

Experimental design and Treatments

A factorial experimental design was used. The factors were Arbuscular Mycorrhiza (inoculated=AM+ and not inoculated=AM-), four water levels (Field capacity, 75% of field capacity, 50% of field capacity, 25% of field capacity), and two soil levels (topsoil and subsoil). The treatment units were arranged in the greenhouse bench in a completely randomized design. There were 6 replications which gave a total of 288 seedlings. The amount of water required to meet the daily transpiration requirement was estimated from measurements of pot weight to create the water deficit levels. The mass of water deficit required daily was then supplied to each treatment. Due to differences in initial seedling sizes, species-specific water requirements, and the increase in water demand during active growth, treatment of the different species with the same amount of water was not considered appropriate (Gindba et al., 2005, Sack and Grub, 2002).

Seedling biomass measurements

Seedlings were harvested after 180 days to determine plant mass. Harvested seedlings were divided into root and shoot and their dry mass was determined after oven-drying the samples at 80 °C until constant weight was achieved. We then calculated root to shoot ratio of each seedling.

Plant nutrient analysis

Mineral status of the plants was assessed by conducting shoot and root tissue elemental analysis for all replicates. After sun drying, shoot and root samples were oven dried at 80 °C for 48 hours. Samples were then ground and analyzed for N, P, and K. Total N was determined using the standard Kjeldahl method; P was measured colorimetrically by spectrophotometer; and K was measured by Flame Photometry (Anderson and Ingram

1993). Samples were analyzed at the National Soil Laboratory, Ethiopian Agricultural Research Institute, Addis Ababa, Ethiopia.

Assessment of mycorrhizal colonization

Mycorrhizal colonization was assessed as the presence or absence of arbuscules, vesicles and hyphae using the grid line intersection method (Giovannetti and Mosse, 1980). Subsamples of non-suberized roots were collected from each replicate, cleared with 10% KOH, and stained with 0.01 trypan blue in lactoglycerol (Brundrett et al., 1996). Roots were then divided into 1 cm pieces and mounted lengthwise on a microscope slide. Six slides containing nine root pieces per replicate were prepared and examined. 3 microscope observations (top, middle and bottom) were made at 400x magnification. Fractional colonization was expressed as percentage of the root colonized. The total mycorrhizal colonization, arbuscules, vesicles, and internal hyphae in the root cortex were recorded per plant.

Gas exchange measurements

Gas exchange was measured at the end of the experiment, before plants were harvested. Measurements were made between 0900 and 1200 h from five mature fully expanded leaves two times per leaf under full sunlight using a LCP-002 portable photosynthesis system (LC Pro ADC Bioscientific Ltd.). Measurements included net photosynthesis rate, stomatal conductance, transpiration rate and dark respiration rate. Photosynthetic water use efficiency was calculated as the ratio between the photosynthetic rate and transpiration rate.

Pre-dawn (0300–0600 h) and midday (1200–1400 h) leaf water pressure potential was measured using a pressure chamber apparatus (Scholander et al., 1965) using one well-expanded leaf per plant. Relative water content of leaves was measured according to Koide et al. (1989). Water pressure potential and relative water content were determined twice, at pre-dawn and mid-day.

Statistical analysis

Analysis of variance was used to test for the differences in seedling mass, nutrient levels, gas exchange, and root colonization among soil fertility, water deficit levels, AM inoculation and their interaction. Repeated-measures ANOVA was used to analyze treatment effects on predawn and midday relative water content and leaf water potential of

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Table 4-1. General description of the tree/shrub species investigated (Orwa et al., 2009, Azene Bekelle-Tessema and Tengneas, 2007, Bein et al., 1996)

	<i>Acacia etbaica</i>	<i>Acacia senegal</i>	<i>Boswellia papyrifera</i>
Size	Deciduous shrub 2.5-12 m tall	Deciduous shrub 15 m tall	Deciduous tree 4-12 m tall
Form	Usually branched from the ground, the crown often flattened	Usually branched from the ground. Branches fork repeatedly, and in mature trees form a rounded, flat-topped crown.	Thick branch tipped with clusters of leaves, rounded crown
Habitat	Occurs in dry bush land, thickets, semi-desert scrub and wooded grasslands	Found in <i>Acacia-Commiphora</i> woodland and wooded grassland	Found in <i>Acacia-Commiphora</i> woodland and wooded grassland, on steep rocky slopes, lava flows or in sandy river valleys
Altitude (m)	100-1 800 m	100-1700 m	500-1 800 m
Mean annual rainfall	200-1400 mm	300-1200 mm	300-950 mm
Soil type	Varies from coarse-textured, deep sandy soils to dry, rocky soils	Varies from coarse-textured, deep sandy soils to dry, rocky soils, slightly acidic to moderately alkaline, intolerant to water logging	Varies from coarse-textured, deep sandy soils to dry, rocky soils, slightly acidic to moderately alkaline
Seed treatment and Propagation	Immerse in boiling water, allow to cool and soak for 24 hours; Direct sowing at site.	Soaking seed in water for 12 hours; Seedlings, direct sowing.	Soaking seeds in cold water for 12 hours; Seedlings, Large branch cuttings
Management	Pollarding, coppicing.	Weeding and protection at early stages, lopping, coppicing	3-5 years rest before the second harvest of resin. Exclosures for seedlings. Overexploitation damages the trees.
Human uses	Firewood, timber, flavouring (smoke from wood for flavouring milk), medicine (leaves), fodder, bee forage, tannin	Firewood, construction, food (seed), medicine, soil improvement, gum, dye (seeds), fish net (root fibers). Beverage, employment	Live fence, incense (resin), employment, essential oil, medicinal, beverage

seedlings. Means were compared using Tukey test when F-test from ANOVA was significant. Gabriel post-hoc test for unequal sample comparison was performed. In order to meet the assumptions of normal distribution and homogeneity of variances, data on stomatal conductance, water use efficiency and leaf water potential were log transformed before statistical analysis. Treatment effects were statistically analyzed using SPSS (PASW statistics 17) software.

Results

Plant biomass

Analysis of variance showed significant effects for AM in *A. senegal* (shoot weight) and *Boswellia* (root weight), but not for *A. etbaica*. Moisture was a significant source of variation for *A. etbaica* (shoot), *A. senegal* (shoot and root) and *Boswellia* (root), while soil was especially a significant source of variation for *Boswellia* (both shoot and root). Significant interactions included AM×water (for *A. senegal* and *Boswellia*), AM×soil (for *Boswellia*) and water×soil (for *A. senegal* and *Boswellia*) (Table 4-2). Mycorrhizal responsiveness was largest for *Boswellia*, followed by *A. senegal*. AM and topsoil increased biomass (Figure 4-1). The significant interaction AM×water was due to the fact that in the mycorrhizal condition both species of *Acacia* performed better at higher water availability, while *Boswellia* performed better at low water availability (Figure 4-1).

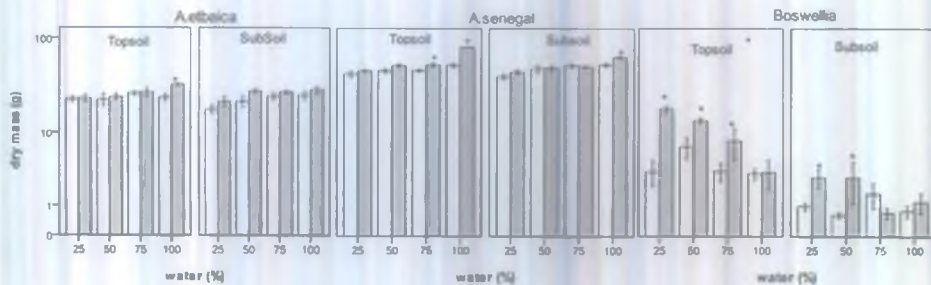


Figure 4-1: Dry mass response of seedlings (mean \pm 1 SE) of *Acacia etbaica*, *A. senegal*, and *Boswellia papyrifera* without AM (open bars) and with AM (solid bars) under four water levels and in topsoil and subsoil. Note that dry mass on the y axis is on a log scale. Asterisk (*) above a solid bar 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 $p < 0.05$.

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Table 4-2. Results of three-way ANOVA of the effects of AM inoculation, water deficit levels and soil fertility and their interactions on shoot, root and total plant dry biomass (g) and root to shoot ratio of seedlings of *Acacia etbaica*, *A. senegal* and *Boswellia papyrifera*. P-value < 0.05 indicates significant source of variation between treatments.

Species	variable	AM		Water		Soil		AM x water		AM x soil		water x soil		AM x water x soil	
		F	p	F	p	F	p	F	p	F	P	F	P	F	P
<i>Acacia etbaica</i>	Shoot	2.454	.121	4.359	.007	1.732	.192	1.214	.310	.051	.822	.851	.470	1.793	.155
	Root	.068	.795	1.926	.132	1.140	.289	.771	.513	.025	.876	1.740	.166	.620	.604
	Plant	.786	.378	4.448	.006	.081	.776	1.203	.314	.004	.950	.922	.434	1.323	.273
	Root: shoot	2.780	.099	1.231	.304	2.940	.090	.930	.430	.242	.624	1.272	.290	1.433	.239
<i>Acacia senegal</i>	Shoot	14.335	.000	29.942	.000	4.282	.042	8.407	.000	.353	.554	.429	.733	2.388	.075
	Root	.370	.545	13.605	.000	.141	.708	10.992	.000	1.408	.239	5.153	.003	3.663	.016
	Plant	8.827	.004	53.168	.000	2.536	.115	23.358	.000	.104	.747	3.794	.013	7.603	.000
	Root: shoot	8.210	.005	3.471	.020	2.508	.117	1.900	.136	.931	.338	1.527	.214	.135	.939
<i>Boswellia papyrifera</i>	Shoot	2.779	.099	2.030	.116	20.043	.000	3.520	.019	.306	.582	1.198	.316	2.036	.115
	Root	38.000	.000	11.369	.000	91.249	.000	6.989	.000	26.340	.000	7.792	.000	5.090	.003
	Plant	34.770	.000	10.146	.000	91.334	.000	7.706	.000	20.051	.000	6.199	.001	4.536	.005
	Root: shoot	2.148	.150	2.228	.098	.933	.339	.809	.495	.367	.548	4.836	.005	4.679	.006

Gas exchange

Analysis of variance showed significant effects of AM on assimilation, transpiration and stomatal conductance for all three species, while water use efficiency was not a significant source of variation for all three species. Moisture was generally a significant source of variation for assimilation, transpiration, stomatal conductance and water use efficiency in all three species. Soil was also a major source of variation on photosynthesis parameters for all three species (Table 4-3). The two acacias showed similar trend in assimilation rate, transpiration and stomatal conductance. Mycorrhizal plants of all three species had higher assimilation and transpiration rates and higher stomatal conductance than non-mycorrhizal plants. Assimilation and transpiration rates and stomatal conductance were higher on topsoil than on subsoil. Assimilation rates and stomatal conductance increased at higher water levels for both *Acacia* species, but decreased for *Boswellia* (Figure 4-2). Water use efficiency was higher under subsoil conditions for *A. etbaica*, while was higher under topsoil conditions for *A. senegal* (Table 4-3).

Water relations

Analysis of variance on water content and water potential showed significant effects for AM (especially *Boswellia*, also *A. senegal*), moisture (*Boswellia*) and soil (*Boswellia* and *A. senegal*). For *A. etbaica*, these main factors were generally not a significant source of variation (Table 4-4). In the mycorrhizal condition the seedlings of the three species showed dehydration tolerance. The AM symbiosis increased the midday water potential (Table 4-4). Moreover, the AM symbiosis improved the pre-dawn relative water content of *A. senegal* and both the pre-dawn and mid-day relative water content and pre-dawn water potential of *Boswellia* seedlings (Table 4-4). Rich soil conditions increased the pre-dawn relative water content and water potential of *A. etbaica*, mid-day relative water content and water potential and pre-dawn water potential of *A. senegal*, and both pre-dawn and mid-day water potential of *Boswellia* seedlings (Table 4-4). Moisture stress significantly increased both the pre-dawn and mid-day relative water content and water potential of *Boswellia* seedlings.

Plant nutrients

Analysis of variance showed highly significant effects of mycorrhiza for the three macronutrient mass fractions in shoots and roots for all three species. The effect of moisture was significant for both *Acacia* species, but generally not for *Boswellia*. Soil was

a major source of variation for all macronutrient mass fractions in *A. senegal*. Significant interactions included AM×water (for *A. etbaica* and *Boswellia*), while the AM×soil and water×soil were only significant sources of variation in a few cases (Table 4-5).

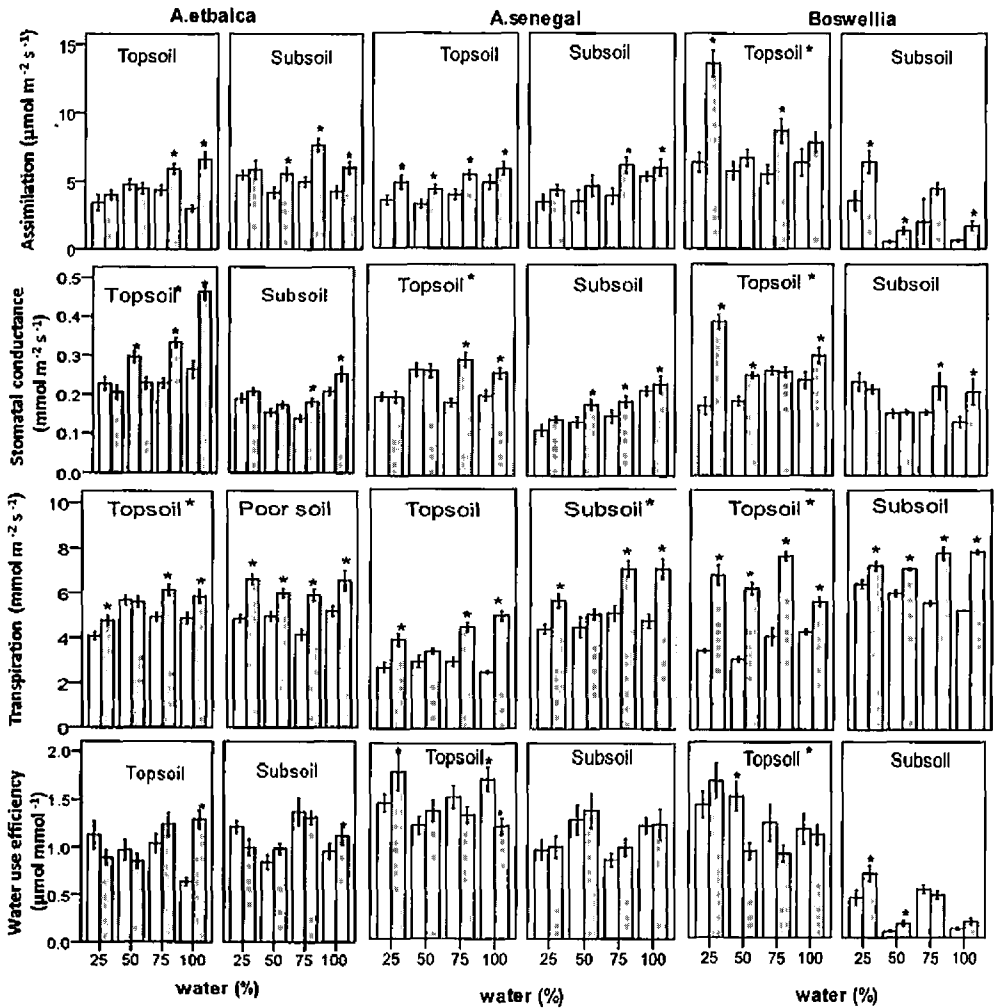


Figure 4-2: Gas exchange response of seedlings (mean \pm 1 SE) of *Acacia etbaica*, *A. senegal* and *Boswellia papyrifera* without AM (open bars) and with AM (solid bars) under four water levels and in topsoil and subsoil. Asterisk (*) above a solid bar indicates a significant difference between non-mycorrhizal and mycorrhizal treatment at $p < 0.05$. Asterisk (*) as superscript to topsoil indicates a significant difference between topsoil and subsoil at $p < 0.05$.

Mycorrhizal plants had higher mass fractions of all three nutrients in shoots and roots than non-mycorrhizal plants in all three species. Higher water levels enhanced nutrient levels of *A. etbaica* and the N levels of *A. senegal* seedlings (Table 4-5). Seedlings planted in topsoil had higher nutrient mass fractions in *A. senegal* seedlings. For seedlings of *Boswellia*, nutrient fractions were generally higher when plants were grown at lower water availability, whereas for both *Acacia* species nutrient levels increased with water availability (Figure 4-3).

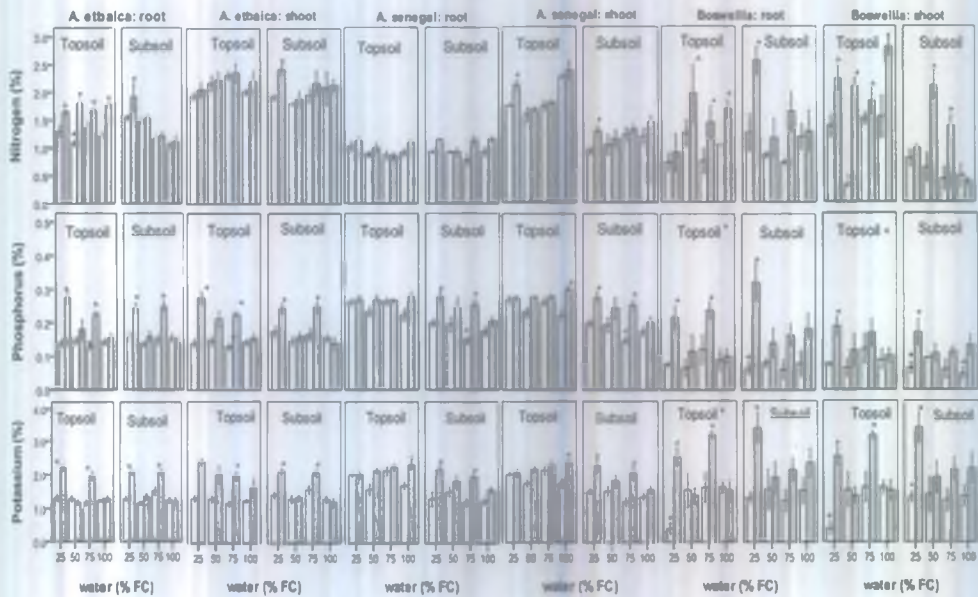


Figure 4-3: Nutrient mass fractions of seedlings (mean \pm 1 SE) of *Acacia etbaica*, *A. senegal* and *Boswellia papyrifera* seedlings without AM (open bars) and with AM (solid bars) under four water levels and in topsoil and subsoil. Asterisk (*) above a solid bar indicates a significant difference between non-mycorrhizal and mycorrhizal treatment at $p < 0.05$. Asterisk (*) as superscript to topsoil indicates a significant difference between topsoil and subsoil at $p < 0.05$.

Root colonization

Root colonization of seedlings planted in topsoil and in subsoil increased with increased moisture levels for *A. etbaica* and *A. senegal* seedlings (Figure 4-4). Root colonization in all three species was higher in topsoil than in subsoil (Table 4-6).

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Table 4-3. Results of three-way ANOVA of the effects of AM inoculation, water deficit levels and soil fertility and their interactions on net assimilation rate ($\mu\text{mol C m}^{-2} \text{s}^{-1}$), transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$), stomatal conductance ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$), water use efficiency ($\mu\text{mol C mmol}^{-1} \text{H}_2\text{O}$) and respiration rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$) of seedlings of *Acacia etbaica*, *A. senegal* and *Boswellia papyrifera*. P-value < 0.05 indicates significant sources of variation between treatments.

Species	variable	AM		Water		Soil		AM x water		AM x soil		water x soil		AM x water x soil	
		F	p	F	p	F	p	F	P	F	P	F	P	F	P
<i>Acacia etbaica</i>	Assimilation	28.957	.000	6.011	.000	26.395	.000	.546	.651	.398	.528	8.227	.000	6.445	.000
	Transpiration	39.852	.000	5.878	.001	20.732	.000	3.759	.011	.979	.323	7.362	.000	7.907	.000
	Stomatal conductance	39.412	.000	15.626	.000	142.817	.000	7.201	.000	.071	.790	39.899	.000	9.397	.000
	Water use efficiency	.550	.458	3.981	.008	6.254	.013	3.891	.009	.026	.873	5.849	.001	2.115	.097
	Respiration	.818	.366	2.102	.100	.137	.711	1.239	.295	.082	.774	1.061	.366	3.605	.014
<i>Acacia senegal</i>	Assimilation	25.157	.000	2.265	.079	.016	.899	1.345	.258	.010	.920	2.849	.037	2.331	.073
	Transpiration	82.874	.000	12.536	.000	268.682	.000	1.137	.333	1.613	.204	5.916	.001	8.727	.000
	Stomatal conductance	33.441	.000	7.313	.000	116.459	.000	1.014	.386	1.400	.237	21.473	.000	5.136	.002
	Water use efficiency	.910	.340	.474	.701	95.173	.000	.457	.712	4.286	.039	1.538	.203	.919	.431
	Respiration	8.645	.003	2.195	.088	10.615	.001	2.276	.079	5.356	.021	.493	.688	.651	.583
<i>Boswellia papyrifera</i>	Assimilation	24.406	.000	11.392	.000	97.420	.000	3.407	.018	1.931	.166	.693	.557	.988	.399
	Transpiration	159.075	.000	2.873	.037	69.184	.000	1.421	.237	10.407	.001	1.875	.135	5.283	.002
	Stomatal conductance	36.761	.000	6.864	.000	52.118	.000	2.119	.099	4.610	.033	2.639	.050	9.907	.000
	Water use efficiency	1.227	.269	23.945	.000	218.210	.000	1.020	.384	7.433	.007	15.306	.000	1.856	.138
	Respiration	1.603	.207	2.385	.070	.380	.538	10.558	.000	3.382	.067	1.306	.254	.001	.975

Table 4-4. Results of three-way ANOVA of the effects of AM inoculation, water deficit levels and soil fertility and their interactions on pre-dawn and mid-day relative water content (RWC,%) and water potential (WP, bars) of seedlings of *Acacia etbaica*, *A. senegal* and *Boswellia papyrifera*. P-value < 0.05 indicates significant source of variation between treatments.

Species	Variable	AM		Moisture		Soil		AM x water		AM x soil		water x soil		AM x water x soil	
		F	p	F	p	F	p	F	p	F	P	F	P	F	P
<i>Acacia etbaica</i>	Predawn RWC	1.680	.199	2.035	.116	4.733	.033	.596	.620	1.220	.273	1.666	.181	.296	.828
	Midday RWC	1.723	.193	.946	.422	.405	.526	.427	.734	.022	.882	1.058	.372	.245	.864
	Predawn WP	.996	.321	.825	.484	8.761	.004	.807	.494	.158	.692	.455	.714	.213	.887
	Midday WP	13.829	.000	.972	.410	.729	.396	.863	.464	1.414	.238	.575	.633	.780	.508
<i>Acacia senegal</i>	Predawn RWC	6.970	.010	.695	.558	.999	.321	.999	.398	2.785	.099	.183	.908	.255	.857
	Midday RWC	1.663	.201	.842	.475	8.241	.005	.738	.532	.464	.497	.374	.772	.330	.803
	Predawn WP	3.668	.059	1.257	.295	20.935	.000	.213	.887	.005	.945	1.150	.334	.304	.822
	Midday WP	7.378	.008	.292	.831	5.554	.021	.365	.779	.841	.362	.671	.572	1.435	.239
<i>Boswellia papyrifera</i>	Predawn RWC	89.566	.000	12.967	.000	.805	.373	14.389	.000	2.268	.137	1.536	.214	2.781	.048
	Midday RWC	23.388	.000	30.905	.000	.205	.652	1.333	.272	.834	.365	16.901	.000	4.011	.011
	Predawn WP	38.488	.000	4.529	.006	35.494	.000	2.854	.044	10.028	.002	4.281	.008	5.710	.002
	Midday WP	30.604	.000	23.481	.000	12.577	.001	5.865	.001	1.058	.308	5.093	.003	.839	.478

Chapter 4 – Water deficit

Table 4-5. Results of three-way ANOVA of the effects of AM inoculation, water deficit levels and soil fertility and their interactions on root and shoot nitrogen (N), phosphorus (P) and potassium (K) mass fraction of seedlings of *Acacia etbaica*, *A. senegal* and *Boswellia papyrifera*. P-value < 0.05 indicates significant source of variation between treatments.

Species		AM		Water		Soil		AM x water		AM x soil		water x soil		AM x water x soil	
		F	p	F	P	F	P	F	p	F	P	F	P	F	P
<i>Acacia etbaica</i>	N root	44.980	.000	9.011	.000	5.344	.023	.977	.408	14.817	.000	11.976	.000	2.660	.054
	N shoot	5.084	.027	1.088	.359	2.604	.111	.507	.679	.620	.433	3.016	.035	.591	.622
	P root	64.172	.000	13.015	.000	.031	.860	13.501	.000	2.145	.147	1.104	.352	.592	.622
	p shoot	63.509	.000	13.625	.000	.000	.995	10.801	.000	7.909	.006	2.503	.065	.679	.568
	K root	82.705	.000	36.786	.000	.355	.553	24.687	.000	.199	.657	2.489	.066	1.663	.182
	K shoot	66.556	.000	10.623	.000	1.999	.161	6.261	.001	12.481	.001	3.773	.014	.426	.735
<i>Acacia senegal</i>	N root	22.739	.000	5.554	.002	.403	.527	.992	.401	3.012	.086	1.011	.392	2.739	.049
	N shoot	11.445	.001	13.036	.000	173.396	.000	1.566	.204	.141	.708	4.915	.003	.091	.965
	P root	35.778	.000	3.096	.031	36.827	.000	.186	.906	7.269	.009	1.576	.202	3.373	.022
	P shoot	48.930	.000	2.834	.043	54.058	.000	.336	.800	4.094	.046	2.116	.105	4.651	.005
	K root	47.606	.000	1.960	.127	44.634	.000	.025	.995	3.700	.058	2.927	.039	5.347	.002
	K shoot	46.052	.000	2.361	.078	43.912	.000	.183	.908	3.587	.062	3.163	.029	5.417	.002
<i>Boswellia papyrifera</i>	N root	19.349	.000	.481	.696	.715	.400	.518	.671	.119	.731	6.683	.000	1.924	.132
	N shoot	73.490	.000	.078	.972	68.111	.000	7.152	.000	4.696	.033	15.938	.000	4.569	.005
	P root	37.422	.000	3.295	.025	.250	.619	3.759	.014	2.142	.147	2.284	.085	.829	.482
	P shoot	18.059	.000	1.207	.313	1.395	.241	1.414	.245	.100	.753	1.064	.369	.700	.555
	K root	28.443	.000	1.194	.317	1.337	.251	6.537	.001	.293	.590	3.563	.018	.914	.438
	K shoot	31.460	.000	1.671	.180	.684	.411	6.537	.001	.460	.500	3.649	.016	1.057	.372

Discussion

The species responded differently to the treatments and treatment combinations. AM increased the total dry mass of *A. senegal* and *Boswellia*, but not significantly for *A. etbaica* seedlings. All three species had higher assimilation and transpiration rates, stomatal conductance, mid-day water potential and root and shoot NPK mass fractions when mycorrhizal compared to the non-mycorrhizal condition. Mycorrhizal seedlings of both *A. etbaica* and *A. senegal* responded positively to increased moisture, whereas mycorrhizal seedlings of *Boswellia* responded positively to water stress. While soil properties had minor effects on most physiological traits, they favored biomass growth of the tree species. In the following section we discuss each hypothesis, and link the behavior of the individual species to their acquisition strategy of resources.

AM plants grow more rapidly than non-mycorrhizal plants, and this effect will be stronger under water or nutrient stressed conditions. This hypothesis was partly confirmed. Mycorrhizal responsiveness was largest for *Boswellia* and smallest (no significant effect of mycorrhiza on biomass) for *A. etbaica*. The biomass increment of *A. etbaica* and *A. senegal* seedlings decreased with increasing soil water stress. The effect of nutrient stress was weak for both *Acacia* species. In contrast, the biomass growth of *Boswellia* seedlings

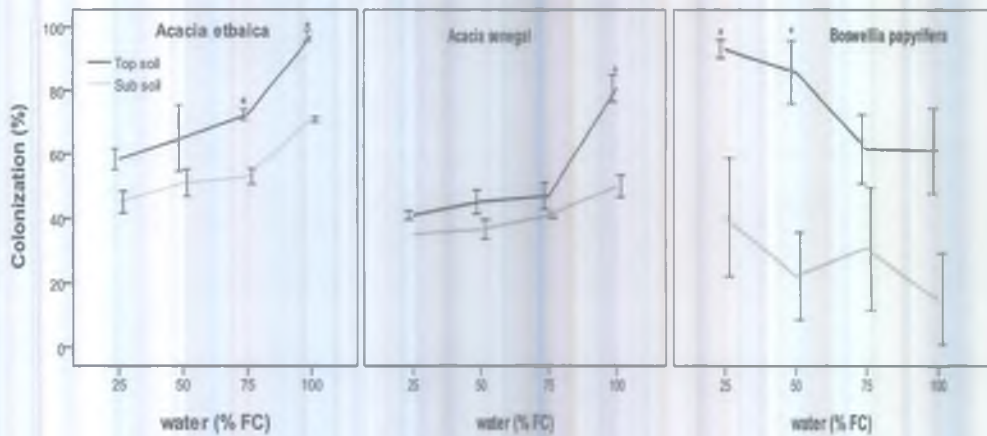


Figure 4-4: Fractional AM root colonization levels of seedlings (mean \pm 1 SE) of *Acacia etbaica*, *A. senegal* and *Boswellia papyrifera* in topsoil (black line) and subsoil (gray line) under four water levels. Asterisk (*) indicates a significant difference between topsoil and subsoil at $p < 0.05$.

increased with soil water stress. In this experiment *Boswellia* responded strongly to soil provenance (topsoil versus subsoil), even though the soils did not differ much in their soil fertility status. The two species of *Acacia* that showed a lower mycorrhizal responsiveness also showed a much weaker reaction to soil fertility. Taken together these data suggest that relatively small differences in soil fertility status may be important for plants that are dependent on and responsive to mycorrhiza. If so, soil degradation would likely have a large negative impact on the regeneration of *Boswellia*. Both species of *Acacia* accumulated much more biomass during the experiment than seedlings of *Boswellia*. Our data provide therefore support for the original hypothesis by Bayliss (1975) and Janos (1980) that plants with a coarse root system are more responsive. Our data also suggest that species with a conservative strategy are more responsive to mycorrhiza than species with an acquisitive strategy, and that mycorrhizal benefit is especially important under conditions where water is strongly limiting. The positive response of mycorrhizal seedlings of *Boswellia* to water stress confirms earlier results of a water pulsing experiment (Birhane et al., Chapter 3).

Table 4-6. Results of two-way ANOVA of the effects of water deficit levels and soil fertility and their interactions on arbuscular, vesicular and hyphal colonization percentage of seedlings of *Acacia etbaica*, *A. senegal* and *Boswellia papyrifera*. P-value < 0.05 indicates significant source of variation between treatments.

Species	Colonization	Water		Soil		Water x soil	
		F	p	F	p	F	P
<i>Acacia etbaica</i>	Arbuscular	6.326	.001	25.479	.000	2.630	.063
	Vesicular	4.916	.005	13.172	.001	.832	.484
	Hyphal	19.407	.000	33.124	.000	.912	.444
<i>Acacia senegal</i>	Arbuscular	1.065	.375	12.865	.001	1.877	.149
	Vesicular	1.780	.167	4.292	.045	1.486	.233
	Hyphal	34.781	.000	36.426	.000	8.204	.000
<i>Boswellia papyrifera</i>	Arbuscular	2.778	.054	41.505	.000	4.472	.008
	Vesicular	2.377	.084	27.474	.000	3.814	.017
	Hyphal	1.642	.195	25.446	.000	.503	.682

Biomass responses were partly reflected by gas exchange parameters. Mycorrhizal symbiosis improved stomatal conductance of seedlings of all three species. Higher stomatal conductance of mycorrhizal compared to non-mycorrhizal plants has been often reported (Augé, 2001, Ruiz-Lozano and Aroca, 2010, Birhane et al., Chapter 3). In agreement with the data on biomass, seedlings of *Boswellia* had higher assimilation and

transpiration rates, and higher stomatal conductance at lower water levels. Seedlings of both *Acacia* species had higher gas exchange at higher soil water availability. Querejeta et al. (2007) compared mycorrhizal responsiveness and stomatal conductance of two Mediterranean shrubs under drought stress conditions. They noted that the relatively slow-growing *Pistacia lentiscus* was both more responsive to mycorrhiza and showed larger increases in stomatal conductance (as assessed from $\delta^{18}\text{O}$) than *Retama sphaerocarpa*. Our results fits with this pattern. An earlier study by Querejeta et al. (2003) in which the slow-growing *Olea europaea* and the faster-growing *Rhamnus lycioides* were compared, indicated a lower stomatal conductance of the slow-growing plants with the conservative strategy, both under conditions of drought stress and at sufficient water availability. However, despite lower stomatal conductance, *Olea* maintained higher assimilation rates under drought stress than *Rhamnus*, and this latter result is consistent with our findings.

The strategy of *Boswellia* seedlings is also characterized by die-back of the above-ground shoot during the long dry season. This above-ground die-back, while the plant remains active below-ground, allows the plant to conserve or even acquire resources in the dry period. The very large carbon allocation below-ground (see also Birhane et al., Chapter 3) is a major element of this conservative strategy. *Boswellia* seedlings allocated 97% of the biomass to the root whereas the *Acacias* allocated more to the shoot (60% for *A.senegal* and 52% for *A.etbaica*). Similar results were reported for species found in the Mediterranean area (Garcia et al., 2011). Mycorrhizal *Arbutus unedo* seedlings had lower leaf area, while the water deficit increased root dry weight and the root : shoot ratio. The lower biomass of *Boswellia* seedlings is likely a consequence of this carbon allocation below-ground because too little carbon may be left for leaf area production and carbon gain at whole plant level. Both species of *Acacia* with their more acquisitive strategies invested more in leaf area and achieved higher biomass at the end of the experiment (note log scale in Figure 4-1).

All three species had lower growth in subsoil compared to topsoil, but this effect was only significant for *Boswellia* seedlings. Removal of topsoil through erosion could therefore have a larger negative impact on *Boswellia* than on the *Acacia* species and may result in competitive replacement by the latter species (see Birhane et al., Chapter 5). When growing in subsoil these seedlings showed a higher water potential as a consequence of which they conserve more moisture.

We observed nodules consistently throughout the experiment in *A. senegal* seedlings while nodules were prevalent only at the start of the experiment for *A.etbaica*

seedlings. However, leaf N mass fractions were higher in seedlings of *A. etbaica* than in seedlings of *A. senegal*. *Boswellia* seedlings were without nodules. The hypothesis that the interactive effect of AM and poor soil will improve the performance of *Acacia* and *Boswellia* seedlings was supported for *Boswellia* seedling only.

We hypothesized that higher growth in AM plants is achieved by the maintenance of stomatal conductance, transpiration, and assimilation at higher water levels, because the plants remained better hydrated. Higher growth in AM is also achieved by more effective nutrient acquisition. This hypothesis was fully supported by this experiment. Inoculation with AM positively modified the biomass growth of *Boswellia* under moisture stress, and with better moisture availability for *A. senegal* and *A. etbaica* seedlings. *Boswellia* seedlings with AM under low water had higher biomass growth compared to the other treatments. The increase in photosynthesis when inoculated with AM under moisture stress conditions for *Boswellia* seedlings was due to an increase in stomatal conductance and transpiration rate (Figure 4-3). The higher water use efficiency (Table 4-3), mid-day water potential (Table 4-4), and NPK levels (Table 4-5) under AM might contribute to the higher photosynthesis recorded for moisture-stressed *Boswellia* seedlings. AM plants often show higher photosynthetic rates than non-mycorrhizal ones, which is consistent with AM effects on stomatal conductance (Garcia et al., 2011, Augé, 2001, Ruiz-Lozano et al., 1995). Under drought stress, mycorrhizal *Olea europaea* seedlings showed higher photosynthetic and transpiration rates, stomatal conductance and foliar P concentration than similar-sized non-mycorrhizal plants (Caravaca et al., 2003). The protection of mycorrhizal plants against water stress is also related to the increased uptake of K (in chapter 3, seedlings lost most K in the dry season and didn't show an increased response to water stress). Potassium plays a key role in plant water stress as the cationic solute which is responsible for stomatal movement in response to changes in bulk leaf water status (Ruiz-Lozano et al., 1995). Stomatal conductance and leaf water potential are functionally linked as a change in one usually drives a change in the other (Augé, 2001). The higher mid-day water potential with AM seedlings helped in moisture conservation, and increased K levels helps in the regulation. The effect of AM on stomatal conductance could be partly nutritional (increased P and K) and partly non nutritional (increased leaf water potential).

AM inoculated *Acacia* and *Boswellia* seedlings had higher shoot and root nutrient levels than non-mycorrhizal ones (Figure 4-3). Moreover, AM increased the shoot NPK levels in *A. etbaica* and *A. senegal* seedlings, while in *Boswellia* seedlings roots get more than shoots. Also other studies showed that AM inoculation increased plant tissue P, K and

N mass fraction (Giri et al., 2005, Huat et al., 2002, Tarafdar and Kumar, 1996, Ruiz-Lozano et al., 1995, Reena and Bagyaraj, 1990). AM inoculated *Araucaria angustifolia* leaf concentrations of P, K, Na, and Cu (Zandavalli et al., 2004), and shoot N and P concentration of *Dyera polyphylla* and *Aquilaria filarial* seedlings (Turjman et al., 2006) were higher than in non-mycorrhizal plants. This increase in nutrient concentration in plant tissue is attributed to a higher nutrient absorption rate by mycorrhizal plants (Smith and Read, 2008, Haselwandter and Bowen, 1996, Jasper et al., 1989). Most of the nutrients in dry infertile soils are immobile and the rate of absorption by roots from the soil solution through diffusion is limited. AM enhances nutrient uptake by shortening the distance that nutrients have to diffuse from the soil to the roots (Kungu, 2006, Bolan, 1991). In addition to enlarging the depletion zone, mycorrhizal hyphae are able to penetrate soil pores inaccessible to roots (Smith and Read, 2008). In the dry deciduous woodlands the dry season lasts for more than nine months which makes resource acquisition difficult for young plants. Nutrient and moisture acquisition of seedlings using their root system only may not be a good strategy for survival and development. The symbiosis with AM will enable the seedlings to scavenge more nutrients to survive during drought and to continue growth. At low P availability both P acquisition and biomass increase with AM colonization, supporting earlier results that demonstrated that AM improve the growth of indigenous tropical tree seedlings through an increase of P uptake (Moyersoan et al., 1998, Habte and Fox, 1989, Michelsen, 1993, Fagbola et al., 2001). The simultaneous increase in gas exchange, water relations and nutrient levels for inoculated *Boswellia* and *A. senegal* seedlings indicate that these species benefit from AM colonization, while *A. etbaica* does not.

We hypothesized that the colonization of roots by AM increases with increasing soil and moisture stress. Root colonization increased with soil moisture for both *Acacia* species but decreased with moisture in *Boswellia* seedlings. This might be related to the root structure and its acquisition strategy. *Boswellia* stores most resources, including water, below-ground in the coarse root. This water storage may allow mycorrhizal activity at low water availability (Birhane et al., Chapter 3). In contrast, the *Acacia* species allocate most carbon to shoots and do not store resources and water below-ground. Under limited water application both plant and mycorrhizal fungus could then be limited by water, resulting in reduced colonization with increasing dryness. Dryness then imposes an annual cycle on the mycorrhizal fungus, resulting in increased sporulation. At the start of the rainy season

these spores germinate and establish new mycorrhizal colonization and the rainy season results in increased resource availability and the association can start again.

Conclusion

Our results give experimental evidence that the mycorrhizal symbiosis improves the nutrient and water status of *Acacia* and *Boswellia* seedlings. AM helps in moisture and nutrient acquisition. AM symbiosis helps *Acacia* and *Boswellia* seedlings to have higher biomass increment through enhancing gas exchange, water relations and nutrient levels. AM helps both *Acacia* and *Boswellia* species to better adapt to degraded soils. The species, however, responded differently to water availability. The rapidly growing *Acacias* benefited from AM at higher water availability, possibly because they accounted for additional water and nutrient acquisition at high root density and resource availability. The slowly growing *Boswellia*, however, benefited from AM at low levels of water availability. This species, with its large coarse root and few fine roots, benefits at low root density and resource availability from AM, enlarging the water and nutrient uptake capacity. Our studies thus show that acquisitive and conservative species both benefit from AM, but that the acquisitive *Acacias* mainly benefit at the highest water availability level, whereas the conservative *Boswellia* benefits at water or nutrient-stressed conditions. Further research is required on how these conservative and acquisitive strategies are modified by AM when they occur together in competition.

Chapter 5

*Mycorrhiza alters competitive interactions of Acacia and Boswellia seedlings in
drought pulsing*

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Abstract

Arbuscular mycorrhizal (AM) fungi can have a substantial effect on moisture and nutrient uptake and therefore influence plant competition in stressful environments where resource availability is controlled by rainfall pulses. In this study we examined the influence of drought pulsing and AM colonization on the competitive relationships between seedlings of the acquisitive species *Acacia etbaica* and the conservative species *Boswellia papyrifera*. Both seedlings benefited from AM when grown alone, though the effect was stronger for *Boswellia* seedlings. AM affected the competitive performance of the two species. The conservative species was not affected by intraspecific competition, whereas the acquisitive species was strongly negatively affected by intraspecific competition. This effect was even stronger in the presence of AM. In interspecific competition, the acquisitive AM species outcompeted the conservative species. Water pulsing and the presence of AM fungi did not affect the outcome of interspecific competition, and the aggressivity index of *Acacia* remained unchanged. Water pulsing strongly negatively affected *Acacia* seedlings. Colonization decreased with water pulsing in *Acacia* seedlings but increased in *Boswellia* seedlings. The extent to which AM influenced plant competition in water pulsed environment depends on the nature of the root system of the species. This study shows that both water pulsing and AM can modify the balance between intraspecific and interspecific competition. These results show that AM and water pulsing can adjust plant interactions and determine the establishment and survival of seedlings.

Key-words: arbuscular mycorrhizal fungi; competition; relative yield; conservative; acquisitive; colonization; *Acacia etbaica*; *Boswellia papyrifera*.

Introduction

Competition is one of the biotic factors shaping plant communities (Pietikainen and Kytoviita, 2007, Aerts, 1999). Many studies have examined the impact of competition on plant growth. These studies were often executed without explicit attention for soil biota, and this could have resulted in outcomes that may be unrepresentative of the species' behavior under natural conditions. For instance, the effect of the arbuscular mycorrhizal (AM) symbiosis on competition has not often been addressed. While most plants (around 80% of all terrestrial plant species, (Brundrett, 2009, Smith and Read, 2008, Muthukumar et al., 2003) form AM, different plant species differ in the degree to which they depend on and response to AM fungi. This differential response will likely affect the competitive relationship between plant species (Danieli-Silva et al., 2010, Scheublin et al., 2007).

The effect of the AM symbiosis on individual plants is fairly well established (Kytoviita et al., 2003, Allsopp and Stock, 1992) and could be both positive and negative (Jones and Smith, 2004). AM colonization usually increases nutrient and moisture acquisition which then results in increased growth (Moyersoen et al., 1998), improved drought tolerance (Augé, 2001), and enhanced resistance against diseases and insect herbivores (Newsham et al., 1995). AM facilitates moisture stress alleviation through increased uptake of nutrients, but even at the same size and nutrient status mycorrhizal plants have often higher assimilation and transpiration rates and higher stomatal conductance than non-mycorrhizal plants due to hormonal effects, sink stimulation of photosynthesis and improved access to soil micropores (Augé, 2001, Ruiz-Lozano & Aroca, 2010).

In arid ecosystems, water and soil nutrients go through periods (pulses) of high and low abundance. Such pulses occur on several temporal scales. On an annual basis a very long dry season (more than 9 months) alternates with short rainy season (less than 3 months). Also in the rainy season, pulses occur due to irregularity of rainfall events, as a consequence of which there may be no rainfall in a period of one or two weeks (Schwinning and Sala, 2004). Plants vary in their strategies to acquire and conserve these pulsed resources. Following proposals by Díaz et al. (2004) we have suggested two major strategies to cope with water pulses in such environments (Birhane et al., Chapter 4). Fast-growing species with an acquisitive strategy acquire and deplete resources rapidly, while slow-growing species with a conservative strategy acquire these resources more slowly, and effectively store them for harsh periods (Roumet et al., 2006). Strategy theory is based on above-ground properties; however work by Liu et al. (2010) has shown substantial

coordination between above-ground and below-ground organs that are involved in resource acquisition. Acquisitive species can therefore be hypothesized to produce more roots, in particular fine and branched roots with abundant long root hairs that allow them to rapidly acquire soil resources (Poorter, 1994, Reich et al., 1998, Tjoelker et al., 2005, Brundrett, 1996). In contrast, conservative species are likely to have large unbranched coarse roots with few short root hairs. As a consequence of different root morphologies, plants that exhibit a conservative strategy are likely to depend more on and to respond more to the mycorrhizal symbiosis than plants with an acquisitive strategy (Perez and Urcelay, 2009). Consequently, the competitive superiority of the acquisitive strategy under non-mycorrhizal conditions could be reversed under more stressful conditions and/or when plants are mycorrhizal (Lavorel et al., 2006, Roumet et al., 2006, Craine et al., 2001, Zangaro et al., 2005, Huat et al., 2002, Haselwandter and Bowen, 1996). Due to their ability to store resources in the harsh season, mycorrhizal plant species with a conservative strategy are supposed to better survive these harsh periods with low resource availability and consequently are able to outcompete the acquisitive species (Roumet et al., 2006). A study by Scheublin et al. (2007) is in support of these hypotheses: in the absence of mycorrhiza, the grass *Festuca ovina* was competitively superior to the legume *Lotus corniculatus*, but in the presence of mycorrhiza the latter species was competitively superior. In other cases the presence of AM fungi had smaller impacts on plant competition. A competition experiment between the mycorrhiza-responsive species *Lafoensia capari* (Lythraceae) and the non-responsive (or even negative responsive) species *Cabralea canjerana* (Meliaceae) showed that the AM symbiosis did not influence the competitive outcome between these species. However, intraspecific competition (the degree of mycorrhizal responsiveness as a function of plant density) was significantly different between both species (Danieli-Silva et al., 2010). Reduction in plant growth (and reduction in mycorrhizal responsiveness) at increasing plant densities has been repeatedly observed (Van der Heijden, 2002, Allsopp and Stock, 1992, Koide, 1991).

In this study we focus on interspecific competition between *Acacia etbaica* (*Acacia*) and *Boswellia papyrifera* (*Boswellia*) that have distinctive nutrient and water acquisition and conservation strategies (Birhane et al., Chapter 4). Both plants grow in the same ecosystem where moisture and nutrient availability are pulsed. We compare the strength of interspecific competition with that of intraspecific competition. Both the effects of arbuscular mycorrhiza and of water pulses (water limitation) are assessed. We hypothesized that:

1. *Acacia* seedlings will deplete soil resources more rapidly and will therefore be less responsive to mycorrhiza than *Boswellia* seedlings.
2. Because of their acquisitive strategy, intraspecific competition in *Acacia* will be stronger than that in *Boswellia*. Increased plant density will reduce mycorrhizal responsiveness in *Acacia* but not in *Boswellia*.
3. Because of its acquisitive strategy *Acacia* will outcompete *Boswellia* in the absence of mycorrhiza at ample water supply. At more limiting water and / or when mycorrhizal, the competitive superiority of *Acacia* will become less, and will allow for species coexistence.

Methodology

A competition experiment with *Acacia* and *Boswellia* seedlings was conducted in Mekelle, northern Ethiopia. The experiment was conducted in a greenhouse located at 13°3'N 39°E at an altitude of 2000 m a.s.l. The mean daily temperature of the greenhouse was 25 °C during the day and 22 °C during the night with mean daily average relative humidity of 51% for the study period. The study was conducted for 16 months from 01 June 2008 to 01 October 2009. This period included a rainy season (first four months, June to September) followed by a prolonged dry season (eight months, October to May) and another rainy season (four months, June to September). A factorial experimental set up was employed with two factors: AM (present or absent) and water (regular watering or water pulse). There were five planting combinations where *Acacia* and *Boswellia* seedlings were planted singly, where two individuals of the same species were planted in a single pot (intraspecific competition), and where both species were planted in a single pot (interspecific competition). Every treatment was replicated 8 times. Seedlings were harvested after 16 months and measured for biomass and root colonization. Gas exchange measurements were done at the end of the experiment before harvest.

Seedling preparation

Seeds of *Acacia* and *Boswellia* were collected in March 2007 from adult *Boswellia* and *Acacia* 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 were pretreated to accelerate germination. The seeds of *Boswellia* were soaked in cold water for 12 hours (Rijkers et al., 2006, Taye,

2002). *Acacia* seeds were immersed in boiled water and allowed to cool and soaked for 24 hours (Bein et al., 1996). Germination took place in plastic trays filled with autoclaved pure river sand under greenhouse conditions. All seeds germinated within 5-15 days. 350 germinants were individually transplanted to plastic pots, 8cm diameter and 15cm high. Potted seedlings were placed on metal mesh benches and were watered regularly using micro-sprinkler irrigation every other day to field capacity until the plants were ready (30 days) for the experiment that exposed them to the water pulse experiment. Dimethoate was sprayed to ward off ants and aphids which were observed on leaves. In June 2008, 256 seedlings of uniform size were transplanted to larger perforated 20 litre plastic containers, either one or two seedling per container, filled with 15 kg autoclaved soil.

Experimental design and treatments

We simulated seasonality by supplying water for 4 months followed by 8 month without water supply and also simulated the second rainy season with water supply for another 4 months. To simulate short-term water pulses (infrequent rains during the rainy season) we supplied experimental seedlings with watering for 14 days, followed by a dry period of 14 days (as in Chapter 3). All pots therefore received the same amount of water. A factorial experimental design was used. The factors were Arbuscular Mycorrhiza (colonized= AM+ and not colonized= AM-), two water levels (water control=WC and short water pulse= SP), and five species combinations (single *Acacia* = A, two *Acacia* = AA, *Acacia* and *Boswellia* = AB, two *Boswellia* = BB, and single *Boswellia* = B per pot). The treatment units were arranged on greenhouse benches in a completely randomized design. There were 8 replications giving a total of 160 treatment units (256 experimental seedlings).

AM inoculum and potting soil

We collected soil samples from the dry deciduous woodlands. The soil and root fragments that were collected during the dry season from the rhizosphere of dry deciduous woodland trees, (mainly from the rhizosphere of *Boswellia*) were trapped and reared with sorghum. Based on visual observations on spore morphology and wall structure, members of the genus *Glomus* 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-colonized *Sorghum bicolor* plants. About 50 g fungal inoculum was added per pot 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, similar habitat where *Acacia* and *Boswellia* trees naturally grow. Before the inoculation procedure, the soils were sieved and sterilized by an autoclave at 121°C for two-hours. Control seedlings were planted in sterilized soils.

Seedling biomass measurements

Seedlings were harvested after 16 months. Harvested seedlings were divided into root and shoot and their dry mass (g) was determined after oven-drying the samples at 80 °C until constant weight was achieved. We calculated root to shoot ratio of each seedling. Relative yield total and the aggressivity index, a parameter that shows which tree species is competitively superior, were calculated according to Scheublin et al. (2007).

Assessment of mycorrhizal colonization

Mycorrhizal colonization was determined using the grid line intersection method (Giovannetti and Mosse, 1980). Subsamples of non-suberized roots were collected, cleared with 10% KOH, and stained with 0.01 trypan blue in lactoglycerol (Brundrett et al., 1996). Roots were then divided into 1 cm pieces and mounted lengthwise on a microscope slide. Six slides per treatment per species containing nine root pieces per slide were examined by making 3 microscope observations (top, middle and bottom) per 1 cm root piece at 400x magnification. Colonization was expressed as percentage of the root colonized. Total mycorrhizal colonization, arbuscular, vesicular and hyphal colonization in the root cortex were separately recorded.

Plant nutrient analysis

Mineral status of the plants was determined by conducting elemental analysis for all seedlings. After sun-drying, shoot and root samples were oven-dried at 80 °C for 48 hours. Samples were then wet-digested and analyzed for N, P, and K. Total N was determined using the standard Kjeldahl method, P colorimetrically by spectrophotometer and K by Flame Photometry (Anderson and Ingram 1993).

Gas exchange measurements

Gas exchange was measured at the end of the experiment. Measurements were made between 0900 and 1200 h from five mature fully expanded leaves two times per leaf under full sunlight using a LCP-002 portable photosynthesis system (LC Pro ADC Bioscientific Ltd.). Measurements included net photosynthesis rate ($\mu\text{mol C m}^{-2} \text{ s}^{-1}$), stomatal

conductance ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$), and transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$). Photosynthetic water use efficiency ($\mu\text{mol C mmol H}_2\text{O}^{-1}$) was calculated as the ratio between the photosynthetic rate and transpiration rate.

Data analysis

Total biomass, shoot and root biomass, root to shoot ratio, gas exchange and plant nutrient levels were analyzed using ANOVA with mycorrhiza and water pulse for each planting combination as main effects and AM \times water pulse as the interaction effect. Fractional colonization was analyzed with one-way ANOVA with water pulse as treatment. Means were compared using Tukey test when F-test from ANOVA was significant. Gabriel post-hoc test for unequal sample was performed. In order to meet the assumptions of normal distribution and homogeneity of variances, data on stomatal conductance and water use efficiency were log transformed before statistical analysis. All statistical analyses were performed with SPSS (PASW statistics 18) software.

Results

Growth of single seedlings

The growth of *Acacia* seedlings was enhanced by mycorrhiza (mycorrhizal plants had 24% more biomass than non-mycorrhizal plants) and reduced by water pulsing (Figure 5-1). More carbon was allocated to shoots than to roots (root: shoot ratio < 1), especially in the mycorrhizal condition (Table 5-1). Assimilation and transpiration rates, stomatal conductance and water use efficiency were not affected by mycorrhiza or water pulsing (Figure 5-2). Shoot mass fractions of N, P (though not significantly so) and K were higher in mycorrhizal plants than in non-mycorrhizal plants.

The growth of *Boswellia* seedlings was also enhanced by mycorrhiza (mycorrhizal plants had 74% more biomass than non-mycorrhizal plants) and, contrary to *Acacia*, also enhanced by water pulsing (Figure 5-1). Almost all carbon (80-90%) was allocated below-ground and stored in the coarse root (Table 5-2). Assimilation and transpiration rates were enhanced in the presence of mycorrhiza and by water pulsing, whereas stomatal conductance and water use efficiency were not affected by the treatments (Figure 5-2). Shoot mass fractions of N, P and K (marginally so) were higher in mycorrhizal plants than in non-mycorrhizal plants and were higher in water-pulsed plants than in plants that received continuous water (Table 5-2).

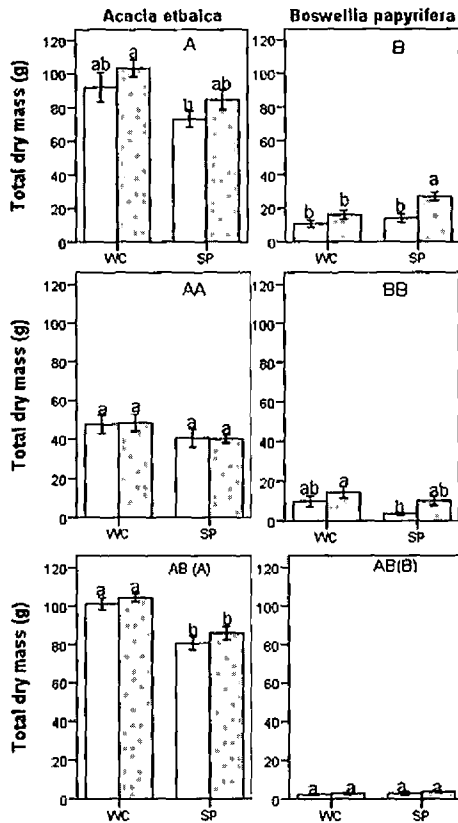


Figure 5-1 Dry mass (\pm SE) of seedlings of *Acacia* and *Boswellia*. Seedlings inoculated with AM are shown by solid bars and seedling without AM are with open bars. Seedlings were provided water to field capacity during the growing season as water control (WC) or pulsed for two weeks as a short pulse (SP). Seedlings were planted singly per pot (A = *Acacia*, B = *Boswellia*), two seedlings of the same species per pot for intraspecific competition (AA = two *Acacia*, BB = two *Boswellia*), and both *Acacia* and *Boswellia* species per pot for interspecific competition (AB (A) = *Acacia* seedling grown with *Boswellia*, AB(B) = *Boswellia* seedling grown with *Acacia*). Bars with different letters indicates significant differences between treatments at $P < 0.05$.

Intraspecific competition

For seedlings of *Acacia* intraspecific competition was very strong. Total biomass of two plants per pot was only fractionally higher than when plants were grown singly. In the absence of mycorrhiza weight of two plants per pot was 3-11% higher than in the single-seedling treatment, whereas in the presence of mycorrhiza intraspecific competition even

reduced total biomass production by 6% (Figure 5-1). Assimilation and transpiration rates were reduced when two seedlings were growing in one pot. When two plants were growing in a pot, the effect of mycorrhiza on shoot mass fractions of N, P and K was not any more significant (Table 5-1).

For seedlings of *Boswellia* intraspecific competition was strongly dependent on water availability. Under continuous watering total biomass of two plants per pot was almost double (increase +80%) than when plants were growing singly (Figure 5-1). However, under water pulsing intraspecific competition was very strong. In fact, biomass reduction was reduced by 25-50%. Assimilation rates and stomatal conductance were much lower with two plants per pot than when plants grew singly when plants were non-mycorrhizal; while the reduction was much less in the mycorrhizal condition (Figure 5-2). Shoot mass fractions of N, P and K were (marginally) significantly higher in mycorrhizal than in the non-mycorrhizal plants (Table 5-2). There were no effects of intraspecific competition on shoot nutrient mass fractions, compared to when plants grew singly.

Interspecific competition

In interspecific competition *Acacia* was clearly superior (Figure 5-1). Dry mass of one *Acacia* seedling in competition with a *Boswellia* seedling was slightly more than double that of a seedling in intraspecific competition. Mycorrhizal responsiveness was very low (< 5%) and comparable to the condition of intraspecific competition. However, assimilation and transpiration rates and stomatal conductance were significantly higher for mycorrhizal seedlings compared to non-mycorrhizal seedlings (Figure 5-2). There were no effects of mycorrhiza or water pulsing on shoot mass fractions of N, P and K.

Boswellia performed very poorly in interspecific competition. Total dry mass of *Boswellia* was 20-40% of biomass of a seedling when growing in intraspecific competition; only non-mycorrhizal seedlings with a water pulse showed a smaller reduction in performance, possibly due to the very poor performance in intraspecific competition (Figure 5-1). There was neither an effect of mycorrhiza nor of water pulsing on *Boswellia* weight. Assimilation and transpiration rates and stomatal conductance were all very strongly reduced in interspecific competition (Figure 5-2). However, mycorrhizal plants maintained (marginally) significant higher values for these parameters. There were no effects of mycorrhiza or water pulsing on shoot mass fractions of N and K, but mycorrhizal plants had higher P mass fractions than non-mycorrhizal plants (Figure 5-3).

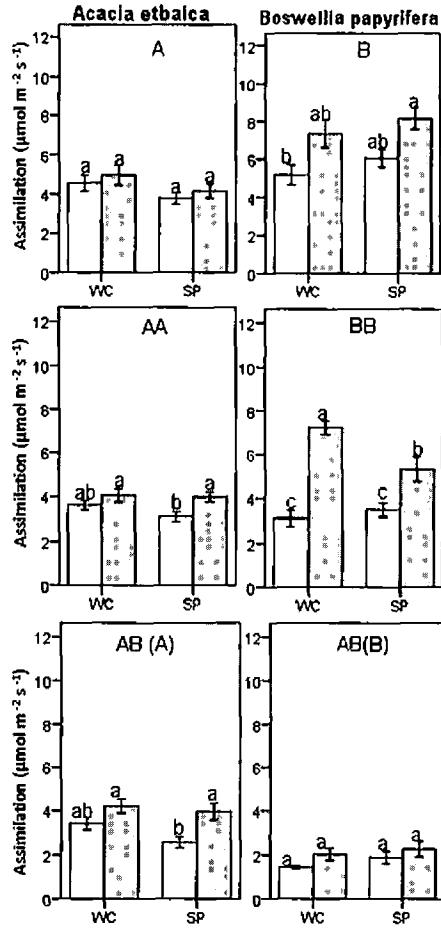


Figure 5-2 Assimilation rate (\pm SE) of seedlings of *Acacia* and *Boswellia*. Seedlings inoculated with AM are shown by solid bars and seedling without AM are with open bars. Seedlings were provided water to field capacity during the growing season as water control (WC) or pulsed for two weeks as a short pulse (SP). Seedlings were planted singly per pot (A = *Acacia*, B = *Boswellia*), two seedlings of the same species per pot for intraspecific competition (AA = two *Acacia*, BB = two *Boswellia*), and both *Acacia* and *Boswellia* species per pot for interspecific competition (AB (A) = *Acacia* seedling grown with *Boswellia*, AB(B) = *Boswellia* seedling grown with *Acacia*). Bars with different letters indicates significant differences between treatments at P < 0.05.

Relative yield total was larger than one in all treatments and ranged from 1.18 to 1.39. RYT was somewhat larger in cases of water pulses than with continuous water supply (1.32 versus 1.18), whereas there was no effect of mycorrhiza on RYT. The

aggressivity index showed the competitive superiority of *Acacia*. The aggressivity index between *Acacia* and *Boswellia* was close to 1, except in the non-mycorrhizal treatment with water pulsing where it was 0.6.

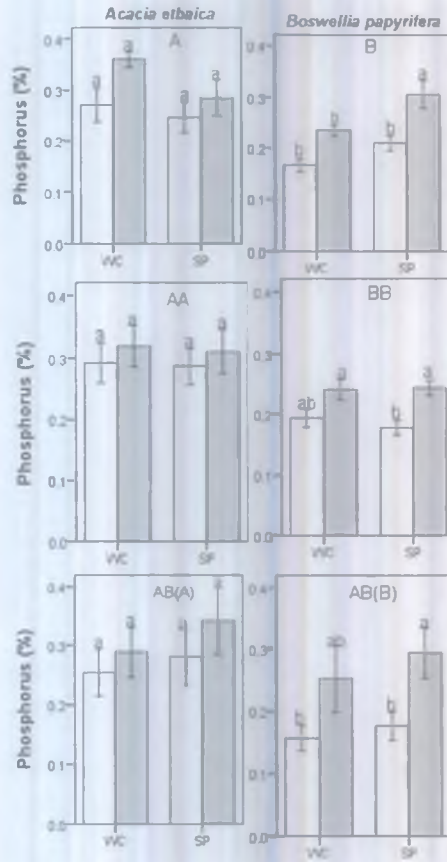


Figure 5-3 Phosphorus level (\pm SE) of seedlings of *Acacia* and *Boswellia*. Seedlings inoculated with AM are shown by solid bars and seedling without AM are with open bars. Seedlings were provided water to field capacity during the growing season as water control (WC) or pulsed for two weeks as a short pulse (SP). Seedlings were planted singly per pot (A = *Acacia*, B = *Boswellia*), two seedlings of the same species per pot for intraspecific competition (AA = two *Acacia*, BB = two *Boswellia*), and both *Acacia* and *Boswellia* species per pot for interspecific competition (AB (A) = *Acacia* seedling grown with *Boswellia*, AB(B) = *Boswellia* seedling grown with *Acacia*). Bars with different letters indicates significant differences between treatments at $P < 0.05$.

AM colonization

None of the seedlings grown in the sterile soil were colonized by AM fungi except in one pot of *Boswellia* planted without competition in short water-pulse treatment. All seedlings received AM treatments were colonized by AM fungi and contained intraradical hyphae, arbuscules and/or vesicles. In *Acacia*, average root colonization was 70-92% when planted without competition, 77-89% in intraspecific competition and 64-87% in interspecific competition. Colonization levels in *Boswellia* ranged from 45-81% when planted without competition, 59-79% in intraspecific competition and 69-90% in interspecific competition. Pulsing significantly decreased colonization in *Acacia* seedlings while it increased colonization in *Boswellia* seedlings in all treatments (Figure 5-4).

Discussion

In this study, we showed how seedlings of *Acacia* and *Boswellia* responded differently to AM, pulse water supply and competition. These differences are related to differences in resource acquisition strategies. *Acacia* is an acquisitive species. It grows quickly, and allocates more carbon above-ground than below-ground. In the dry season it is deciduous. It depletes below-ground resources effectively through its fine roots: it shows a small response to mycorrhiza. Due to its deciduous habit, it probably enforces an annual life cycle upon the associated AM fungi, where in the rainy season mycorrhizal colonization is initiated from spores. With decreasing water availability (through pulsed water application) mycorrhizal colonization decreased. *Boswellia* is a conservative species. It grows slowly and allocates most (often 90%) of its carbon below-ground, where it is stored in its coarse root. In the dry season seedlings die-back above-ground. However, while seedlings are dormant above-ground they maintain below-ground activity. As a consequence of its coarse root system it is more responsive to mycorrhiza than *Acacia*. Storage of carbon and possibly water in the coarse root system allows mycorrhizal activity in the dry season, and therefore the mycorrhizal network is more of a permanent nature. It responds to decreasing water availability (through water pulses) with increased biomass accumulation and increased mycorrhizal colonization. Such a strategy has been reported for plants adapted to soil resource limited environments (Hodge, 2009, Hermans et al., 2006), since it allows seedlings to persist in the root zone area, gradually get access to more soil resources and they survive (Roumet et al., 2006). These species-specific differences were also noted in our earlier studies (Birhane et al., Chapters 3 and 4) and confirmed hypothesis 1.

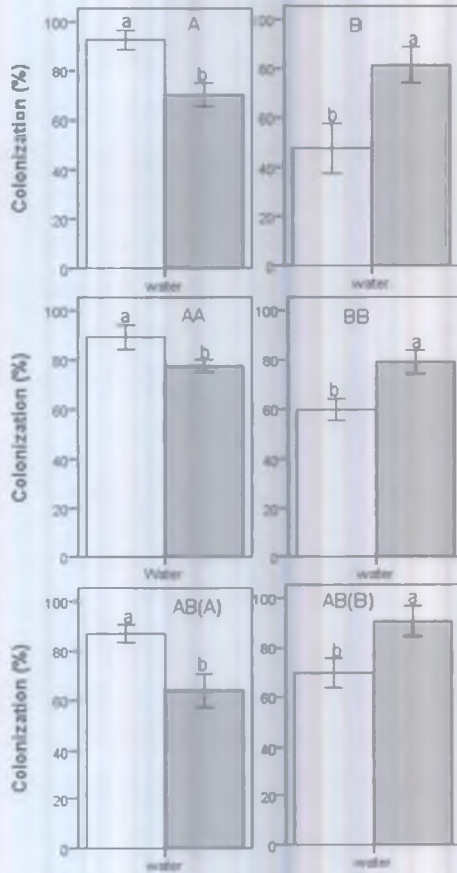


Figure 5-4 Fractional root colonization (\pm SE) of seedlings of *Acacia* and *Boswellia*. Seedlings under water control are shown by open bars and seedling under water pulsing are with solid bars. Seedlings were provided water to field capacity during the growing season as water control (WC) or pulsed for two weeks as a short pulse (SP). Seedlings were planted singly per pot (A = *Acacia*, B = *Boswellia*), two seedlings of the same species per pot for intraspecific competition (AA = two *Acacia*, BB = two *Boswellia*), and both *Acacia* and *Boswellia* species per pot for interspecific competition (AB (A) = *Acacia* seedling grown with *Boswellia*, AB(B) = *Boswellia* seedling grown with *Acacia*). Bars with different letters indicates significant differences between treatments at $P < 0.05$.

The question we addressed in this paper is how the mycorrhizal symbiosis and water availability affects competition between these species with their different resource acquisition strategies. In order to understand interspecific competition, we also included

intraspecific competition. Most studies on the role of the mycorrhizal symbiosis during interspecific competition were executed with grasses and forbs in temperate ecosystems. Similar studies on tropical forest trees have been rarely done (Danieli-Silva et al., 2010). Knowledge on the competitive interactions between *Acacia* and *Boswellia* as affected by mycorrhiza and water availability is also important for successful rehabilitation and maintenance of *Boswellia*, a tree of major ecological and economic (frankincense) importance.

Boswellia showed a higher mycorrhizal responsiveness than *Acacia*, especially under more water-stressed conditions. A higher responsiveness to mycorrhiza in the coarse-rooted species with its conservative strategy supports the earlier proposal by Bayliss (1975) and St. John (1980) that plants with magnolioid roots benefit more from mycorrhiza than plants with graminoid roots. In a comparison of 78 tree species in southern Brazil, Zangaro et al. (2005), however, obtained opposite results. They showed that pioneer and early successional species with thin and branched roots with many root hairs were more responsive to mycorrhiza than late-successional and climax species with coarse unbranched roots with few root hairs. However, in that study mycorrhizal responsiveness was also inversely correlated with seed size (carbon reserves in the seed and cotyledons), which may be a determinant of mycorrhizal responsiveness at a times when plants have only little biomass. Zangaro et al. (2005) grew their plants for four to six months, much shorter than our experimental period of 16 months, which included two rainy seasons of four months and a dry period of eight months that could still have allowed mycorrhizal activity in *Boswellia*. Zangaro et al. (2005) also noted that mycorrhizal responsiveness correlated with mycorrhizal colonization of the root system. That observation was largely confirmed in our study after water pulsing. Mycorrhizal colonization of *Boswellia* increased after water pulsing and this coincided with an increase in mycorrhizal responsiveness. Mycorrhizal colonization of *Acacia* decreased after water pulsing and mycorrhizal responsiveness did not change.

Because of its ability to deplete resources much more quickly, both in the absence and in the presence of mycorrhiza, we predicted that intraspecific competition in *Acacia* seedlings would be much stronger than in *Boswellia* seedlings. This was confirmed by our experiment. Total biomass of both *Acacia* seedlings was only fractionally higher than that of one seedling when grown singly in the non-mycorrhizal condition, and even somewhat smaller in the mycorrhizal condition. Decreased mycorrhizal responsiveness with increasing plant density has been reported before (Koide and Dickie, 2002) and negative

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Table 5-1: Means (\pm SE) and statistical analysis of biomass (g), assimilation ($\mu\text{mol C m}^{-2} \text{ s}^{-1}$), transpiration ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), stomatal conductance ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), water use efficiency ($\mu\text{mol C mmol H}_2\text{O}^{-1}$) and nutrient levels (%) of seedlings of *Acacia* grown singly (A), grown together (AA), and mixed with *Boswellia* (AB(A)) either with mycorrhiza (AM+) or without mycorrhiza (AM-) under water control (WC) and short water pulse (SP) conditions. Statistical values tabulated are F ratios from one-way ANOVA testing treatment combinations. Means followed by different letters are significantly different at $p < 0.05$.

Species	Parameters	AM-		AM+		F	P
		WC	SP	WC	SP		
A	Shoot dry mass	53.09(5.7)ab	39.17(3.8)b	65.10(5.3)a	49.5(4.6)ab	4.678	.009
	Root dry mass	38.81(3.8)	33.82(2.6)	38.13(1.7)	35.05(2.4)	.745	.534
	Total dry mass	91.91(8.7)ab	73.00(4.8)b	103.24(5.1)a	84.61(5.9)ab	4.004	.017
	Root: shoot ratio	0.78(0.09)	0.90(0.08)	0.61(0.05)	0.74(0.07)	2.422	.087
	Assimilation	4.56(0.4)	3.77(0.3)	4.98(0.5)	4.63(0.4)	1.582	.194
	Transpiration rate	3.87(0.21)	3.31(0.25)	4.13(0.23)	3.83(0.23)	2.463	.063
	Stomatal conductance	0.11(0.00)	0.11(0.01)	0.12(0.00)	0.12(0.01)	.569	.636
	Water use efficiency	1.34(0.10)	1.55(0.18)	1.37(0.11)	1.28(1.11)	.705	.550
	Nitrogen	2.04(0.3)ab	1.54(0.23)b	2.75(0.29)a	1.67(0.20)ab	3.933	.023
	Phosphorus	0.27(0.03)	0.24(0.03)	0.35(0.01)	0.28(0.03)	2.691	.074
	Potassium	2.08(0.20)b	1.81(0.21)b	3.08(0.24)a	2.03(0.18)b	6.945	.002
AA	Shoot dry mass	29.02(3.7)	25.27(3.8)	29.91(3.1)	23.41(1.3)	.929	.432
	Root dry mass	18.52(1.4)	15.29(1.4)	18.48(1.4)	16.56(1.0)	1.330	.273
	Total dry mass	47.55(4.6)	40.56(4.5)	48.39(4.3)	39.97(1.9)	1.226	.308
	Root: shoot ratio	0.75(0.08)	0.81(0.13)	0.67(0.04)	0.73(0.05)	.471	.704
	Assimilation	3.62(0.20)ab	3.10(0.20)b	4.05(0.30)a	3.99(0.21)a	3.349	.019
	Transpiration	3.12(0.11)bc	2.74(0.14)c	3.95(0.18)a	3.49(0.14)ab	11.576	.000
	Stomatal conductance	0.10(0.00)b	0.08(0.00)b	0.10(0.00)ab	0.12(0.00)a	6.261	.000
	Water use efficiency	1.39(0.08)	1.79(0.27)	1.27(0.11)	1.47(0.12)	1.746	.156
	Nitrogen	1.88(0.28)	1.75(0.20)	2.01(0.27)	1.92(0.24)	.182	.908
	Phosphorus	0.29(0.03)	0.28(0.02)	0.31(0.03)	0.31(0.01)	.218	.883
	Potassium	2.18(0.23)	2.07(0.22)	2.27(0.23)	2.27(0.24)	.167	.918
AB (A)	Shoot dry mass	62.20(3.2)a	44.84(3.2)b	63.34(1.5)a	50.25(2.8)b	10.465	.000
	Root dry mass	38.92(2.7)	35.83(2.06)	41.07(1.1)	35.67(2.4)	1.429	.255
	Total dry mass	101.12(3.2)a	80.67(3.4)b	104.41(2.1)a	85.93(3.4)b	13.337	.000
	Root: shoot ratio	0.64(0.06)	0.84(0.09)	0.65(0.02)	0.72(0.06)	1.801	.170
	Assimilation	3.42(0.28)ab	2.57(0.25)b	4.21(0.31)a	3.97(0.38)a	5.254	.002
	Transpiration	2.81(0.17)bc	2.20(0.18)c	4.89(0.24)a	3.55(0.23)b	29.062	.000
	Stomatal conductance	0.07(0.00)b	0.06(0.00)b	0.14(0.01)a	0.12(0.01)a	19.961	.000
	Water use efficiency	1.17(0.08)ab	1.43(0.12)a	0.94(0.07)b	1.38(0.15)a	3.594	.014
	Nitrogen	1.27(0.14)	1.73(0.29)	1.82(0.31)	2.18(0.42)	1.455	.257
	Phosphorus	0.25(0.03)	0.28(0.04)	0.28(0.04)	0.34(0.05)	.598	.624
	Potassium	1.62(0.11)	2.06(0.32)	2.21(0.33)	2.62(0.44)	1.613	.218

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Table 5-2: Means (\pm SE) and statistical analysis of biomass (g), assimilation ($\mu\text{mol C m}^{-2} \text{ s}^{-1}$), transpiration ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), stomatal conductance ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), water use efficiency ($\mu\text{mol C mmol H}_2\text{O}^{-1}$) and nutrient levels (%) of seedlings of *Boswellia* grown singly (B), grown together (BB), and mixed with *Acacia* (AB(B)) either with mycorrhiza (AM+) or without mycorrhiza (AM-) under water control (WC) and short water pulse (SP) conditions. Statistical values tabulated are F ratios from one-way ANOVA testing treatment combinations. Means followed by different letters are significantly different at $p < 0.05$.

Species	Parameters	AM-		AM+		F	P
		WC	SP	WC	SP		
B	Shoot dry mass	0.76(0.2)	2.83(0.9)	1.87(0.5)	3.68(2.0)	1.153	.345
	Root dry mass	9.95(2.2)b	11.32(1.8)b	14.45(2.2)b	23.36(1.2)a	9.418	.000
	Total dry mass	10.72(2.2)b	14.15(2.4)b	16.33(2.5)b	27.05(2.4)a	8.445	.000
	Root: shoot ratio	21.06(6.5)	10.47(3.2)	9.14(1.3)	29.73(13.9)	1.499	.236
	Assimilation	5.19(0.52)b	6.06(0.45)ab	7.35(0.73)ab	8.14(0.55)a	5.433	.002
	Transpiration	2.90(0.16)c	4.11(0.18)b	4.75(0.43)b	6.19(0.21)a	37.963	.000
	Stomatal conductance	0.16(0.01)	0.14(0.00)	0.16(0.00)	0.16(0.00)	.891	.448
	Water use efficiency	1.81(0.18)	1.48(0.08)	1.86(0.44)	1.36(0.09)	2.424	.069
	Nitrogen	0.97(0.08)b	1.36(0.16)ab	1.64(0.19)a	1.77(0.15)a	5.031	.009
	Phosphorus	0.16(0.01)b	0.20(0.01)b	0.23(0.01)b	0.30(0.02)a	11.029	.000
	Potassium	1.35(0.13)	1.51(0.16)	1.89(0.24)	2.06(0.17)	3.082	.051
BB	Shoot dry mass	1.72(0.4)b	0.90(0.2)b	4.07(0.6)a	1.81(0.4)b	7.958	.000
	Root dry mass	8.16(2.2)	2.61(0.5)	10.48(2.8)	8.49(2.0)	2.601	.060
	Total dry mass	9.89(2.6)ab	3.51(0.6)b	14.55(2.9)a	10.31(2.4)ab	3.716	.016
	Root: shoot ratio	5.98(1.3)	7.44(2.4)	3.49(1.0)	6.11(1.6)	.963	.416
	Assimilation	3.11(0.35)c	3.50(0.29)c	7.23(0.31)a	5.32(0.55)b	14.666	.000
	Transpiration	3.39(0.28)b	2.61(0.12)b	6.13(0.06)a	5.84(0.28)a	49.972	.000
	Stomatal conductance	0.08(0.00)c	0.08(0.00)c	0.20(0.00)a	0.15(0.01)b	33.647	.000
	Water use efficiency	0.86(0.06)b	1.66(0.16)a	1.18(0.05)ab	1.03(0.13)ab	6.559	.000
	Nitrogen	1.29(0.12)	1.23(0.12)	1.73(0.19)	1.69(0.21)	2.461	.077
	Phosphorus	0.19(0.01)ab	0.17(0.01)b	0.24(0.01)a	0.24(0.01)a	5.568	.003
	Potassium	1.51(0.12)	1.52(0.10)	1.81(0.13)	1.95(0.16)	2.766	.054
AB(B)	Shoot dry mass	0.23(0.02)b	0.60(0.12)ab	0.80(0.09)a	0.50(0.17)ab	4.544	.010
	Root dry mass	2.05(0.4)	2.18(0.9)	2.10(0.4)	3.18(0.1)	.878	.464
	Total dry mass	2.28(0.4)	2.79(1.0)	2.91(0.4)	3.69(0.3)	.930	.439
	Root: shoot ratio	8.96(1.9)ab	4.54(1.4)ab	2.70(0.5)b	9.91(2.16)a	4.454	.011
	Assimilation	1.00(0.08)	1.89(0.27)	2.03(0.26)	2.27(0.35)	2.529	.062
	Transpiration	0.97(0.11)	1.03(0.14)	1.74(0.44)	1.12(0.05)	2.599	.056
	Stomatal conductance	0.01(0.00)b	0.02(0.00)b	0.025(0.00)a	0.03(0.00)a	4.004	.010
	Water use efficiency	1.17(0.12)b	2.98(0.42)a	2.47(0.34)ab	1.95(0.31)ab	4.049	.009
	Nitrogen	1.12(0.27)	1.04(0.12)	1.56(0.36)	1.75(0.26)	1.616	.219
	Phosphorus	0.15(0.02)b	0.17(0.02)b	0.25(0.05)ab	0.31(0.04)a	7.10	.002
	Potassium	1.54(0.22)	1.27(0.09)	2.05(0.43)	2.11(0.29)	1.916	.161

responsiveness of mycorrhizal plants has often been related to small pot volumes in relation to plant size (Bååth and Hayman, 1984). Negative responsiveness has also been related to carbon costs, but the possibility of nutrient immobilization in the mycorrhizal mycelium is a more plausible alternative, considering that mycorrhizal plants increase their photosynthesis through sink stimulation (Kaschuk et al., 2009). The biomass of *Boswellia* seedlings on the other hand almost doubled (increase 80%) when two seedlings were grown in a pot rather than one. While this almost doubling suggests that a part of the soil volume was still not exploited by the mycorrhizal plant, the negative effect of water pulsing during intraspecific interactions (compared to when plants were grown singly) suggest intense competition for water at higher plant density and at lower water availabilities. Our results are in agreement with those by Danieli-Silva et al. (2010) who noted intensive intraspecific competition in the tree *Cabralea canjerana* (that showed negative mycorrhizal responsiveness) and relatively little intraspecific competition in the tree *Lafoensia pacari* that showed positive responsiveness to mycorrhiza. A consequence of the differential behavior of *Acacia* and *Boswellia* could be that seedling emergence during the rainy season will result in much more intense competition and much more density-dependent mortality (self-thinning) in *Acacia* than in *Boswellia*. However, in periods with little rain (water pulses) during the rainy season, seedlings of *Boswellia* may also suffer from intense intraspecific competition and enhanced density-dependent mortality.

In interspecific competition *Acacia* clearly outcompeted *Boswellia*. Neither the presence of mycorrhiza nor the water pulse influenced that outcome, as the aggressivity index of *Acacia* was close to 1 under all conditions. Only in the non-mycorrhizal condition with water pulsing was *Acacia* less aggressive (aggressivity index 0.6), but this lower value was not due to lower performance or aggressivity of *Acacia* but to the poor performance of non-mycorrhizal *Boswellia* under water pulsing. Lack of a mycorrhizal effect on interspecific competition was unexpected. The study by Scheublin et al. (2007) showed a reversal in aggressivity index between the grass *Festuca ovina* (that was dominant in the non-mycorrhizal condition) and the legume *Lotus corniculatus* (that was dominant in the mycorrhizal condition). The authors suggested that competition in the presence of AM fungi would favor the species with the greatest responsiveness. A similar result has been noted in competition experiments between the forbs *Fragaria vesca* and *Centaurea jacea* (Zobel & Moora, 1995). The latter species was competitively superior in the non-mycorrhizal condition and benefitted also more from the mycorrhizal symbiosis.

Consequently, the AM symbiosis amplified interspecific competition. Altered competitive relationships between a legume (*Medicago sativa*) and two grasses (*Bromus inermis*, *Phleum pratense*) where the more responsive legume increased its competitive ability were noted by Hamel et al. (1992). However, this beneficial effect only occurred during one of the five harvests in their experiment. Danieli-Silva et al. (2010) noted that the AM symbiosis did not affect competitive relationships between two tropical tree species, even though both species differed widely in mycorrhizal responsiveness. As noted by these authors, a major reason for the different results is that the relative importance of intraspecific and interspecific competition has been different in the various experiments. In our experiment there were huge size differences between the seedlings of both species. These size differences created competitive asymmetries that were very large, and which mycorrhizal fungi could not revert. In fact, intraspecific competition was so large for *Acacia* seedlings that the species was released from competition when planted together with the much smaller *Boswellia*. For *Boswellia* the opposite was likely valid. It suffered very little from intraspecific competition, but in combination with the large-sized *Acacia* seedlings, it could not take up nutrients. The differences in size probably resulted in substantial shading in *Boswellia*. In interspecific competition assimilation rates and stomatal conductance were very strongly reduced. The shading effect of *Acacia* seedlings might suppress the amount of light reaching the leaves of *Boswellia* seedlings. *Acacia* seedlings were tall and branchy with small but dense leaves while *Boswellia* seedlings were short and stunted. Similar results have been reported for plants in interspecific interaction (Danieli-Silva et al., 2010, Van der Heijden, 2002).

However, we also observed that the relative yield total for the two species was greater than one, indicating that, while competing for resources, there was complementarity in resource use. Possibly *Boswellia* could still derive some benefit from its mycorrhizal symbiosis in the long dry season when *Acacia* was leafless and inactive, while *Boswellia* was dormant above-ground but still maintaining mycorrhizal activity through carbohydrates and water stored in the coarse roots.

Implications

The results of our study invite an extrapolation towards field conditions. In northern Ethiopia both species can co-occur. Both tree species were found in the same sites in Abergelle and Humera (Birhane et al., 2010). Our result show that seedlings of *Boswellia*

are competitively inferior to seedlings of *Acacia*; and neither the presence of AM fungi nor a stronger water limitation (through pulsing) affect the outcome of the interspecific competition. What would then be the chances for sustained regeneration and growth of *Boswellia* in sites where *Acacia* also occurs? While this experiment suggests that *Boswellia* will always be outcompeted by *Acacia* we suggest that this conclusion may be preliminary. The ability of *Boswellia* to be active during the long dry season combined with its lower sensitivity to water pulsing could suggest that with more severe dry seasons or with longer intermittent dry spells during the rainy season the competitive balance could shift. Considering the differential vulnerability to fire and grazing of both species with their different above-ground and below-ground strategies, more extreme conditions may well lead to different result, especially over the longer term. Field experiments on competition between both species while manipulating the mycorrhizal symbiosis and water availability are recommended. However, our study does suggest that regeneration and maintenance of *Boswellia* stands may be more difficult in the future, if the climate in northern Ethiopia gets somewhat more humid, as predicted by some global change models (Hulmel et al., 2001). Also soil erosion may lower the chances for *Boswellia* as a lower mycorrhizal inoculum potential and a lower nutrient level negatively impact on the seedlings growth (Birhane et al., Chapter 4).

General Discussion and Synthesis



Dry woodlands cover 4% of the forest area globally of which 60% is found in Africa. In Ethiopia, dry woodlands cover more than 50% (Mayaux et al., 2004). These woodlands provide both economic and ecological benefits to the people residing in these drylands. Trees and shrubs such as *Boswellia papyrifera* and various species of *Acacia* provide incense and gum respectively, used both locally and as export products. Dry woodlands, which often have low soil fertility, are prone to degeneration due to poor management, which results in lack of regeneration of these important tree species.

Arid environments are characterized by limited and variable rainfall. Often rainfall events are pulsed, resulting in pulsed resource supply (Chesson et al., 2004). Pulsed supply of water and nutrients determines the dynamics of plant growth (Ogle and Reynolds, 2004, Reynolds et al., 2004, Schwinning and Sala, 2004, Ludwig et al., 2005, Loik et al., 2004). Periodic rainfall events trigger pulses of growth alternated with prolonged periods where the plants are physiologically dormant, at least above-ground (Muldavin et al., 2008). Plant establishment and survival in these ecosystems depend ultimately on the ability of plants to utilize these pulsed resources during short rain fall periods. Vulnerability of such plants is particularly high in the seedling stage. One adaptive strategy consists of allocating almost all carbon acquired during the wet season below-ground (Campbell, 1996, Ogbazghi, 2001) and showing not only leaf fall (deciduousness) but also complete above-ground stem die-back. Such morphological adaptation also entails physiological adaptation. Through these mechanisms seedlings minimize their vulnerability to grazing and fire in dry periods. This strategy may continue for several years, until sufficient carbohydrates have been stored that allow the plant to grow within one season to a height or stem diameter that protects it against grazing damage and fires.

This strategy ('*waiting in the underground*') can only lead to rapid vegetative growth at the onset of the rainy season if sufficient amounts of nutrients (nitrogen, phosphorus, potassium) are simultaneously stored. As the rainy season usually lasts only for a short time, uptake of nutrients at the onset of the wet period could result in a substantial delay in above-ground performance; it may also result in strong competition between above-ground and below-ground sinks. I therefore hypothesized that such plants temporarily uncouple carbon and nutrient investment and expenditure in nutrient-absorbing organs (roots, mycorrhizal fungi) and photosynthesising organs (leaves). Because of the need to efficiently acquire nutrients and water, it is likely that the mycorrhizal association

of these trees plays a major role in their adaptation. The importance of mycorrhiza is also likely considering that some of these trees possess a root system consisting of relatively coarse roots with few short root hairs.

In this dissertation I applied field and greenhouse experiments to study mechanisms of seedling performance in the dry deciduous woodlands of Ethiopia, with special emphasis on the role of the arbuscular mycorrhizal (AM) symbiosis. The dissertation provides new insights on the role of the AM symbiosis on the performance of dry woodland species. The major focus is on *Boswellia papyrifera* (henceforth *Boswellia*), the frankincense tree. I compared the strategy of this tree with a different strategy, exhibited by several species of *Acacia*. Next to understanding the mycorrhizal role while *Boswellia* seedlings wait in the underground, my study addresses the question how rehabilitation efforts could be improved with understanding of these below-ground processes. The following four questions were specifically addressed:

1. What is the mycorrhizal status (mycorrhizal type, levels of colonisation, abundance of propagules) of the dominant trees and shrubs in various sites in the *Boswellia*-dominated dry deciduous woodlands both in the dry and rainy season?
2. How do non-mycorrhizal and mycorrhizal *Boswellia* seedlings respond to water pulsing treatments?
3. How do Arbuscular Mycorrhiza, water deficit, and soil fertility interact in influencing carbon acquisition and allocation, and gas exchange of *Boswellia* and *Acacia* seedlings?
4. How does AM influence the competition between *A. etbaica* and *Boswellia* seedlings at different levels of water availability?

In the following section I will briefly summarize the main results from chapters 2 to 5. After that I will come back to each of the four research questions, answer them in a synthesis and provide suggestions for further research.

The AM symbiosis in *Boswellia*-dominated dry deciduous woodlands

Few studies have been conducted on the AM status of different tree species of the dry forests in Ethiopia. When I started my research the mycorrhizal status of *Boswellia* was still not known. In **chapter 2** I studied the AM status of 43 woodland species. I also investigated the effect of management (exclosures), soil depth (topsoil, 0-15; and subsoil 15-30 cm) and season (dry season, rainy season) on AM fungal spore density and colonization.

Boswellia and all other species studied formed arbuscular mycorrhiza (Table 2-3). Root colonization was low on average (17%) and ranged from 0-95%. Colonization was higher in sites in Abergelle and Humera that are more water-stressed and have shallower soils compared to the less harsh sites of Metema (Figure 2-3b). The different sites also showed a contrasting seasonal pattern, with colonization levels being higher in the dry season than in the rainy season in Abergelle and Humera, but being lower in the dry season compared to the rainy season in Metema. Spore density of AM fungi ranged between 8-69 spores per 100 g of soil, with species of *Glomus* being dominant (Table 2-3). Spore densities were higher in the dry season in all three sites (Figure 2-3a, Table 2-4). Spore density was lower than that reported in other studies in tropical forests, although comparable to spore densities reported in other studies in Ethiopia. Possibly soil degradation resulting in erosion resulted in the loss of spores together with the topsoil. Exclosures in Humera supported higher spore density than the grazed sites (Figure 2-4). However, in Abergelle no effect of exclosures was found, probably due to low effectiveness of fencing in Abergelle. Prevention of grazing in exclosures not only reduced erosion, but the herbaceous cover (mainly grasses) could also have resulted in increased sporulation by AM fungi. Exclosures can therefore be practical tools to increase both AM spore density and root colonization (Table 6-1). Spore densities were not significantly different between both soil depths.

Increased levels of mycorrhizal colonization in the harshest sites in Abergelle and Humera (but not in Metema) suggest that the mycorrhizal symbiosis in these sites shows unique adaptations to the pulsed environment. As this pattern was observed in various plant species, I propose that this strategy is fungal-species specific. Increased colonization levels in the dry season also suggest mycorrhizal activity, allowing plants to acquire essential resources during the season in which their above-ground parts are seemingly inactive. Such adapted fungi could therefore be important when seedlings are waiting in the underground.

Seedlings of *Boswellia* benefit more from the mycorrhizal symbiosis under conditions of water stress

Studies that quantify the interaction between AM fungi and different levels of water availability (water pulses) are rare. In **chapter 3** I investigated the interaction between moisture pulses and AM Fungi on *Boswellia* seedling growth and physiological responses.

Mycorrhizal *Boswellia* seedlings had higher growth rates, particularly for the root, and thus achieved higher biomass and size than non-mycorrhizal seedlings (Figure 3-2, Table 3-1). More than 90% of biomass was allocated to the coarse storage root. Moreover, stomatal conductance and phosphorus mass fraction in shoots and roots were significantly higher for mycorrhizal than for non-mycorrhizal seedlings (Table 3-1). Mycorrhizal colonization was higher in plants that were subjected to water pulses than in plants that received continuous water supply (Table 3-2), suggesting that drought stress increases mycorrhizal colonization as already reported in Chapter 2. I observed a significant interaction between mycorrhiza and water pulsing. Non-mycorrhizal seedlings showed poorer growth at more irregular water supply, but mycorrhizal seedlings performed better under water pulsing than under conditions of continuous watering (Figure 3-2). Both a larger leaf area (Figure 3-3a) and higher assimilation rates (Figure 3-3c) contributed to higher plant dry mass. Transpiration rate was also significantly higher for seedlings under short water pulse with AM (Figure 3-3b). Remarkably, however, the stomatal conductance was not highest for this treatment (Figure 3-3f). *Boswellia* seedlings store a large part of the carbon and minerals harvested during the short rainy season in the coarse root system. Mycorrhizal plants stored even more carbon and nutrients below-ground. The ability to store large carbon reserves (and large amounts of water) in the root system allows AM Fungi to continuously use carbon during the dry periods. Therefore these mycorrhizal fungi are perennial under climatic conditions (drought) that would enforce an annual life cycle on them. Higher root colonization observed during the dry season supports this observation of continuous mycorrhizal activity (Figure 2-3). This mechanism shown by seedlings (waiting in the underground during the dry period) is part of its strategy of adaptation (Tables 6-1 and 6-2). However, not all plants display this strategy and a comparison of seedling performance of *Boswellia* with those of *Acacia* species could provide more insight into the various adaptive strategies employed by tree seedlings in the harsh and pulsed climate.

The arbuscular mycorrhizal symbiosis supports both conservative and acquisitive seedling strategies

I compared the growth of seedlings of *Acacia* (*A. etbaica*, *A. senegal*) and *Boswellia* species in order to understand how the mycorrhizal symbiosis supports different plant strategies to acquire nutrients in this pulsed environment. I grew seedlings of these species for six months in a full factorial randomized design under four levels of water deficit, on

richer topsoil versus poorer subsoil, and inoculated with AM fungi or under non-mycorrhizal conditions. In chapter 4 I evaluated the effects of AM, water deficit and soil fertility, and their interactions on biomass acquisition, gas exchange, water relations, nutrient mass fractions (N, P, and K) and root colonization of seedlings of these three species.

The AM symbiosis increased the growth of *Acacia* and *Boswellia* seedlings (Table 4-2). *Boswellia* was more responsive to inoculum addition than both *Acacia* species. Mycorrhizal plants showed higher assimilation rates, stomatal conductance and transpiration rates than non-mycorrhizal plants (Table 4-3). The higher midday water potential of mycorrhizal plants reflected an increased water uptake by mycorrhizal seedlings (Table 4-4). Mycorrhizal plants showed higher leaf mass fractions of N, P, and K. Higher nutrient levels likely contributed to the higher assimilation rates of mycorrhizal plants. Furthermore, higher levels of K could have contributed to maintaining osmotic pressure in the leaves (Table 4-5).

Seedlings performed significantly better on the topsoil compared to subsoil. This effect of soil was most pronounced for *Boswellia* seedlings. Water availability had contrasting effects on the different species. *Acacia* seedlings performed better with increasing moisture while *Boswellia* seedlings performed better when they were provided with less water (Tables 4-2 to 4-5). Similarly mycorrhizal colonization increased with increasing moisture for *Acacia* seedlings, and increased with decreasing soil moisture for *Boswellia* seedlings (Figure 4-4, Table 4-5). The data on *Boswellia* confirmed results described in Chapters 2 and 3 that showed increased mycorrhizal activity during dry conditions. The slow-growing *Boswellia* seedlings follow a conservative strategy: they grow slowly and carbon allocation is mostly below-ground. Leaf mass fraction is low, fine root mass fraction is equally low, and most of the carbon (up to 90%) ends up in the coarse storage root (Table 6-2). This conservative strategy is also water-saving, which might explain why mycorrhizal plants performed better at lower levels of water availability. The seedlings of both *Acacia* species on the other hand show an acquisitive strategy. They are fast growing, and allocate more carbon above-ground (up to 52% for *A. etbaica* and 60% for *A. senegal*) and have more fine roots. Consequently transpiration is higher, which explains their positive response to increasing moisture.

Mycorrhizal plants had higher stomatal conductance and photosynthesis values compared to non-mycorrhizal plants, especially in *Boswellia* seedlings under low moisture and in *Acacia* seedlings at higher soil moisture (Figure 4-3).

The different strategies of *Acacia* and *Boswellia* raise the question how both plants would compete for resources, especially water, and the extent to which competitive interactions between both plants are modified in the presence of mycorrhiza.

The impact of Arbuscular Mycorrhiza on competition between *Acacia* and *Boswellia* seedlings under drought stress

In chapter 5 I studied biomass, gas exchange, nutrient levels and colonization of seedlings of *Acacia etbaica* and *Boswellia papyrifera* planted singly, and in intraspecific and interspecific interaction both when mycorrhizal and non-mycorrhizal and with and without water pulse in a factorial experiment in the greenhouse.

Intraspecific competition decreased overall performance of *A. etbaica* seedlings. The mycorrhizal symbiosis did not modify the competitive impact (Figure 5-1). The negative impact of intraspecific interaction was due to root overlap in these seedlings. *Boswellia* seedlings were not impacted by intraspecific competition. Mycorrhizal *Boswellia* seedlings, when grown singly, showed better growth under pulsed conditions, but when competing with a conspecific neighbour seedlings provided with more moisture grew bigger (Figure 5-1 and 5-2). Apparently, competition for water became more important at higher plant density. The presence of mycorrhiza had a beneficial effect on intraspecific competition. Interspecific competition benefitted *A. etbaica* seedlings as those seedlings achieved the same biomass as when grown singly, and achieved much more biomass when competing with a conspecific neighbour. *Boswellia* seedlings had lower biomass in competition with *A. etbaica* than when growing singly (Figure 5-1 and 5-2). Neither the presence of AM Fungi nor water pulsing did affect the outcome of the competition. Reducing moisture availability reduced mycorrhizal colonization of *A. etbaica* but increased colonization in *Boswellia* in all treatments (Figure 5-4). This result may suggest that in the presence of enough moisture *A. etbaica* always outcompetes *Boswellia* seedlings, but that under conditions when water is more limiting (longer dry season, longer dry pulses during the rainy season) competitive performance of *Boswellia* seedlings could possibly be enhanced through the presence of AM Fungi (Table 6-1).

Table 6-1. Variables considered in this study and their implication on seedling performance ((+), positive influence; (-) negative influence; (+/-) either positive or negative influence; (n.i.) not investigated).

Chapters	Factors	Species			Implication	
		<i>Boswellia papyrifera</i>	<i>Acacia etbaica</i>	<i>Acacia senegal</i>		
Woodland scale	2	Exclosures	+	+	+	Reduce disturbance and protect seedlings from grazing and fire. increase spore density
	2	Erosion	-	-	-	Removal of top soil and spore which affects survival of seedlings by limiting root colonization and resource availability
	2	Soil depth	+	+	+	Increase both colonization through storing more spore propagule as seed bank and support more root growth.
	2	Dry season	+/-	-	-	Increase sporulation
Plant scale	2,4,5	drought	+/-	-	-	Limit growth of seedlings. AM increases growth of seedlings, particularly for <i>Boswellia</i> seedlings.
	2,3,4,5	AM	+	+/-	+	Increase growth through increased resource acquisition, but not always confirmed for <i>A. etbaica</i> seedlings.
	3,5	Water pulsing	+	-	n.i.	Increase growth of <i>Boswellia</i> when AM. AM bridged the inter pulse dry period during the short rainy season and facilitate uptake.
	4	Moisture deficit	+	-	-	Increase colonization and growth of <i>Boswellia</i> with AM
	4	Soil fertility	+	+	+	Increase growth and survival through increased resource availability
5	Competition	+/-	+/-	n.i.	Intraspecific decrease growth of <i>Acacias</i> due to root overlap, but increase growth of <i>Boswellia</i> . Interspecific benefits <i>Acacia</i> seedlings when planted with <i>Boswellia</i> . AM positively modify intraspecific for <i>Boswellia</i> but negatively affect <i>Acacia</i> seedlings.	

SYNTHESIS – THE WAY FORWARD

What are the major environmental factors determining mycorrhizal functioning in *Boswellia*-dominated dry deciduous woodlands?

Seasonal effects in the levels of mycorrhizal colonization were very prominent between the different research sites. Higher colonization levels during the dry season, as observed in Abergelle and Humera, have not often been reported before. A high colonization level at the time when the trees had shed their leaves and were therefore not or only slightly (in the case of photosynthetic stems) gaining carbon was surprising. This observation confirmed our initial hypothesis that under these severe conditions carbon gain by the tree and carbon expenditure by the fungus was temporally uncoupled. High mycorrhizal activity in the dry season suggests that mycorrhizal networks could be more important than spore inoculum of mycorrhizal fungi, and this implication could be important for restoration efforts. However, it is unknown what drives this peculiar behaviour in the mycorrhizal symbiosis. While data in Chapter 2 suggests that this behaviour is not primarily determined by plant traits (higher colonization in the dry season was found in the three plant species that occurred at all three sites), the experiments executed subsequently suggested that increased mycorrhizal activity under conditions of water stress was observed for *Boswellia* but not for *Acacia*. Those experiments therefore suggest that plant strategies (conservative versus acquisitive resource capture strategies) are important as well. However, lower colonization in the dry season in Metema (with its somewhat better water availability and deeper soils) suggests that the species composition of the AM fungal community could be important. As I only morphotyped spores (which showed a prominence of members of the genus *Glomus* in all sites and with all plant species), I cannot address this question. Further investigations towards species composition of the AM fungal community are therefore essential. Such investigations should look at site (climate, soil) effects, but also at the question whether different plant species are colonised by different AM fungi. Discovery (and subsequent isolation) of AM fungi that are extremely drought resistant and that have the ability to remain active and to maintain a mycorrhizal network during periods of extended drought would be very important for rehabilitation purposes.

The presence of exclosures (if well managed) resulted in increased spore numbers. Exclosures reduce risks of soil erosion and loss of mycorrhizal fungal spores that are present in the topsoil. The establishments of plants (mainly grasses) could also result in increased sporulation in the rhizosphere. Both factors might be beneficial for rehabilitation efforts; however, increased plant cover could also increase competition for water. When

Boswellia with its conservative strategy competes with plants with an acquisitive strategy, it is likely to be outcompeted (Chapter 5). It would be important also to study the competition between *Boswellia* seedlings and herbaceous plants under levels of variable mycorrhizal inoculum density and at various levels of water availability.

In arid ecosystems water pulses determine plant activity. Rainfall in the dry deciduous woodland is seasonal. There are intermittent dry spells, lasting several weeks to one or two months during the rainy season; and a long dry period of up to 8-10 months (Murphy and Lugo, 1986). Most plants are deciduous and acquire carbon during the short rainy season. Only trees with photosynthetic bark could also gain carbon in the dry period, provided water availability is sufficient. Dry spells during the rainy season may further limit carbon gain. AM fungi might play two major roles. During dry spells in the rainy season they could maintain water and nutrient uptake through better soil-hyphal contact. AM Fungi have the capacity to penetrate into micropores that increase access to water. During the long dry season, the active mycelial network could continue to take up nutrients. That latter role might be especially prominent in *Abergelle* and *Humera*, in plants that have a conservative strategy of resource acquisition (such as *Boswellia*), and where a large root with sufficient carbohydrate and water storage allows mycorrhizal activity in dry periods. This hypothesis is supported by the pulsing experiment in chapter 3, the water limitation experiment in chapter 4, and competition experiment in chapter 5. Some species, such as *Boswellia papyrifera* and *Sterculia setigera*, can photosynthesise with their stem during the dry season (Atkilt, unpublished data). This may provide an additional mechanism for carbon gain during the long dry period and might contribute to maintenance of the mycorrhizal network in that period. Quantifying stem photosynthesis in dry and wet periods and determining its importance as an additional carbon source for AM fungi is a major question for future research.

How do *Boswellia papyrifera* seedlings respond when they are exposed to AM and water pulsing treatments?

AM fungi increased the growth of *Boswellia* seedlings by enhancing photosynthesis and water use efficiency in a prolonged drought pulse. Various mechanisms have been proposed that explain improved drought tolerance in mycorrhizal plants. Increased uptake of nutrients through the mycorrhizal symbiosis will result in larger plants. This effect could be explained by the increased contribution of the AM uptake when the soil dries (Smith et al., 2010). As the soil dries out uptake of moisture and nutrients through diffusion

decreases and access will be limited. Higher mass fractions of essential nutrients (N, P, and K) increase photosynthesis on a leaf area basis, creating a multiplier effect. Increased photosynthesis could, however, result in increased transpiration and therefore in higher chances that water limitation will negatively feedback on photosynthetic rates. Increased stomatal conductance and water use efficiency by mycorrhizal plants may be an additional benefit from the mycorrhizal symbiosis (Allen, 2007, Augé et al., 2004, Querejeta et al., 2003, Augé, 2001).

In dry areas plants may provide part of the carbohydrate stored during the rainy season to AM fungi in response of increased resource acquisition during the dry season. The root system of seedlings of *Boswellia* (with most carbon allocated below-ground in a coarse structure that stores carbohydrates and water that allow a year-round mycorrhizal network to be active) is the prerequisite for the plant's successful strategy of *waiting in the underground*. While waiting in the underground, the plant apparently remains active, as mass fractions of N and P of 12-month old seedlings were much higher than those of 4-months old seedlings, whereas those of K were significantly lower. Due to higher nutrient mass fractions that are stored below-ground, especially in mycorrhizal seedlings, these seedlings re-sprouted more quickly compared to non-mycorrhizal seedlings before the rainfall started in the next rainy season. Differences in plant quality then become amplified during this 8-month period of above-ground dormancy, resulting in higher fitness of mycorrhizal plants and in a shorter time period before these plants can show height growth that allows them to escape from grazers and fires. It would be important in future investigations to quantify the carbon and nutrient changes while the seedling is dormant above-ground and waits in the underground.

Though the AM plants were apparently performing well in this long dry season, the mechanism how the plants persistently sustain the AM during the dry season is not clear. Allen (2007) suggested that there is a hydraulic re-distribution from plant to fungus that could play a critical role in sustaining hyphae through drought. Nocturnal water translocation from oak trees to ectomycorrhizal fungi occurred in association with hydraulic lift during severe soil drying (Querejeta et al., 2003, 2007). These observations require a critical experimental study on how dry woodland species, specifically *Boswellia* can sustain AM during the long dry season and benefit from the persistent colonization. The question if AM fungi survive the dry season because of the ability to acquire water from residual soil moisture, or as a result of some water moving out of the coarse root and from there flowing into the hyphae as suggested by Querejeta et al. (2003), needs to be

answered. The waiting strategy of *Boswellia* seedlings changes with increasing age (Figure 3-2). Root mass fraction dropped from 90% to 82% with increasing age (Chapters 3 and 5).

Re-sprouting seedlings of *Boswellia* follow changes in atmospheric humidity. 79% of the seedlings re-sprouted before they were given water at the end of May (Figure 6-1). Mycorrhizal seedlings re-sprouted more often than non-mycorrhizal seedlings before watering was resumed, even though the difference was not significant.

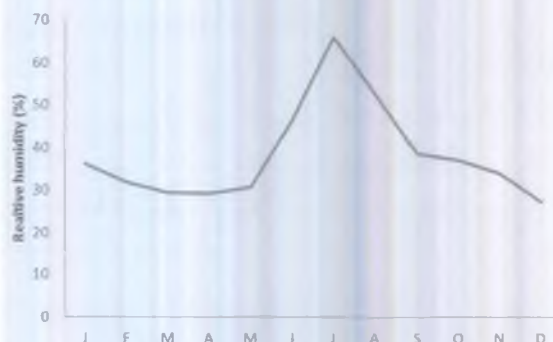


Figure 6-1: Relative humidity in the greenhouse during the study period. Note that part of the plants had emerged above-ground (re-sprouted) before watering was resumed.

How does AM, water deficit and soil fertility influence the growth, allocation and gas exchange of *Boswellia* and *Acacia* seedlings?

In chapter 4 it was noted that AM-mediated carbohydrate allocation within the plant is species dependent. The effect of AM was more pronounced in aerial biomass for the *Acacia* species but in root biomass for *Boswellia* seedlings. AM colonization caused a proportional greater allocation of carbohydrate to the shoot system in acquisitive *Acacia* seedlings whereas to the root system in conservative *Boswellia* seedlings. Mycorrhizal plants normally have a smaller root/shoot ratio than non-mycorrhizal plants (the fungus takes over), but drought results in a higher root/shoot ratio in both mycorrhizal and non-mycorrhizal plants, as predicted by the theory of the functional equilibrium (Brouwer 1963). This observation requires a further study on many species of similar behaviour in their natural environment. In the literature data are contradictory, with both increases and decreases in root: shoot ratio due to the mycorrhizal symbiosis (Davies et al., 2002, Zandavalli et al., 2004, Boulet, 2005). One would expect lower root to shoot ratio, because the fungus takes over the role of the root system. However, when mycorrhizal and non-

mycorrhizal plants have different sizes, root: shoot ratio is also affected by allometric changes during growth, and this effect of plant size may override the mycorrhizal effect. In order to fully understand the role of mycorrhiza in differential allocation patterns it will be vital to model a larger number of species with distinct growth forms that lead to different resource acquisition strategies.

The mid-day water potential of AM seedlings was higher than of non-mycorrhizal ones. This confirms that under drought conditions the leaf water potential is often higher in mycorrhizal plants because of the higher water uptake. In this study I find it difficult to back up the claim on osmotic adjustment. When it comes to the role of K in osmotic adjustment, we have to conclude that shoot K levels were high at 4 and 16 months, and very low at 12 months, suggesting that plants have lost almost all their K while waiting in the underground (Chapter 3). Remarkably, stomatal conductance and water use efficiency were not correlated. No significant differences were observed in relative water content and leaf water potential among treatments within the same age class (Table 3-1). Does it suggest that AM maintains, but not improves, internal water potential, and that AM thereby contributes to drought avoidance rather than drought tolerance? The data from chapter 3 and chapter 4 on the effect of mycorrhiza on relative water content and water potential were inconsistent. Studying the effect of mycorrhizal dry woodland species in relation to soil water potential, stem water potential and leaf water potential provides an evidence on how mycorrhiza enables plants to resist drought.

Mycorrhizal *Acacia* and *Boswellia* seedlings had a contrasting response to moisture. AM increase the growth of *Acacia* seedlings with increasing moisture whereas it increased growth of *Boswellia* seedlings with decreasing moisture. The differential moisture use between the two plant species enables them to use moisture in different times. When there is high availability of moisture AM enables *Acacia* plants to maximize their uptake as they require higher threshold levels (higher moisture availability) and when the moisture reduces to lower threshold *Boswellia* seedlings use the residual moisture which *Acacia* seedlings have no longer access to. This species-dependent AM-mediated moisture use strategy is intriguing. Examining the relationship between drought-adapted AM fungi and plant species with different resource use strategies (as related to the variation in the root system and its effect on plant water relations) is a topic for further detailed study. A detailed modelling work on the relationship between plant moisture threshold levels and response of AM fungi could indicate how AM is critically supporting plants that are adapted to dry conditions. The drought-adapted AM fungi used for the experimental study

was mainly collected from the rhizosphere of *Boswellia* trees. The differential effect could be due to differences in mycorrhizal fungal species specificity. Investigating the native fungal community, its specificity and specific drought adaptation will be crucial to study how this helps explaining differences between species and tree species coexistence.

There is a relatively small difference in soil fertility between the upper and lower soil layers. This study found a huge effect of soil depth in a greenhouse study, whereas the field study showed similar levels of mycorrhizal inoculum in both layers (Birhane et al., 2010). Considering the relatively small differences in soil fertility, one could propose that the difference in the soil effect may not be related to nutrient levels only but also to other (physical) properties of the soil. The effect of mycorrhiza on plants is often studied. The effect of mycorrhiza on the soil itself requires further study.

Does AM influence competition between *Acacia etbaica* and *Boswellia papyrifera* seedlings at different water availability?

The results of chapter 5 showed that the presence of arbuscular mycorrhiza had very little impact on the competition between *Boswellia* and *Acacia* seedlings. In both the mycorrhizal and non-mycorrhizal conditions, and at both water availabilities, *Acacia* was a consistent winner in competition due to its faster growth and ability to acquire more resources. However, it could be dangerous to extrapolate these results of one-year-old seedlings to larger time spans. The increased benefit of moisture stress on mycorrhizal *Boswellia* seedlings in combination with reduced benefit for *Acacia* seedlings under those conditions, suggest that persistent dry periods may allow *Boswellia* to escape from competition. AM colonization did not release *Boswellia* from the negative effect of *Acacia* in interspecific interactions. The presence of AM did not benefit the more responsive species (*Boswellia*) compared to the less responsive species (*Acacia*). Danieli-Silva et al. (2010) noted that the AM symbiosis did not affect competitive relationships between *Cabralea canjerana* and *Lafoensia pacari*, even though both species differed widely in mycorrhizal responsiveness. A major reason for the different results is the relative importance of intraspecific and interspecific competition. In our experiment there were huge size differences between the seedlings of both species. These size differences created competitive asymmetries that were very large, and which mycorrhizal fungi could not revert. Moreover, as the symbiosis between plants and AM fungi shows varying degrees of specificity, competing plant species can be interconnected by mycorrhizal hyphal networks and benefit from the network in the dry season. This requires further study.

Establishment and survival of seedlings in the dry deciduous woodlands

The field study and the greenhouse experiments indicated the importance of AM for seedling growth and survival under stress conditions. Seedlings require AM to better grow and survive under conditions of pulsed resource availability. The mycorrhizal symbiosis both enhances survival and growth during the long dry season (when seedlings have died back above-ground but are still active below-ground) and during intermittent dry spells during the rainy season (where mycorrhizal plants actually benefitted from irregular water supply). The outcome of each experiment and its implication is summarized (Table 6-1).

Contrasting functional traits and ecological strategies of dry woodland species

Species in the dry woodland ecosystem of northern Ethiopia showed two different adaptive strategies (Table 6-2). *Boswellia* is an example of a species that follows a conservative strategy whereas both *Acacia* species follow an acquisitive strategy. Here I summarize the ecological strategies of these dry deciduous woodlands species and forward relevant research questions from the classification (Table 6-2).

The conservative strategy of *Boswellia* can be called '*waiting in the underground*'. In the seedling stage *Boswellia* allocates most carbon to and stores most nutrients and water in the coarse root system. Water storage in the root system likely allows *Boswellia* to be physiologically active during the dry season and to strongly benefit from the AM symbiosis. Querejeta et al. (2003) described how in the dry savannah an ectomycorrhizal tree (*Quercus*) in the dry season exudates water (that has been taken up from deeper soil layers) that allows continued hyphal activity of the ectomycorrhizal fungi. Likely *Boswellia* uses stored water to allow hyphal activity of AM fungi during the long dry season. Continuous activity of mycorrhizal fungi results in a perennial fungal life cycle (De Carvalho et al., 2010). Mycorrhizal plants therefore can store more resources, which shortens the time that seedlings annually die-back above-ground, before growing to a small tree of a size where it can escape grazing and fire. AM fungi also facilitate uptake of resources during intermittent dry period in the short rainy season. This mycorrhizal benefit also leads to shortening of the time that seedlings stay below-ground.

The acquisitive strategy exhibited by *Acacia* species is a radically different solution to such pulsed environment. Seedlings of these species avoid drought stress through shedding of leaves during the dry season. Most carbon is allocated above-ground. This strategy allows the plant to rapidly grow to a size where it can escape from adverse effects of fire and grazing, but makes it vulnerable to prolonged periods of drought.

Table 6-2: Summary of an acquisitive and conservative strategy of dry woodland species

Traits	<i>Boswellia</i> (Conservative species)	<i>Acacia</i> (Acquisitive species)
Shoot	Die back in seedling stage, deciduous when adult; small number of leaves with large size	Deciduous in seedling and adult stage; high number of leaves with small size
Stem	Soft, succulent	Strong and thorny
Root	High root mass fraction, few fine roots, large coarse root, shallow rooted	Low root mass fraction, many fine roots, small coarse root, deep rooted
AM	More responsive to AM, especially with irregular water supply	Less responsive to AM, even lower mycorrhizal benefit at irregular water supply
N-fixation	Do not fix	Fix nitrogen
Allocation	More to the root	More to the shoot
Phenology	Shoot die back	Leaf shedding
Light	Light demanding	Light demanding
Growth rate	Slow	Fast
Regeneration	Seedling bank, vegetative	Seed/seedling bank

While both *Acacia* species did show some positive response to the presence of AM fungi, their mycorrhizal benefit declined at lower water availability and at higher plant density (intraspecific competition). It is likely that the root system does not contain sufficient stored resources (carbon, water) to allow mycorrhizal activity in the dry period. Consequently, *Acacia* species impose an annual life cycle upon the AM fungi (De Carvalho et al., 2010). At the start of the rainy season, spores of AM fungi germinate and establish a new association. However, an annual life cycle (compared to the perennial cycle as shown by *Boswellia*) shortens the window of opportunity during the short wet season, as at the start of the rainy season there is increased competition between above-ground and below-ground sinks. By temporally separating sink competition (due to mycorrhizal activity in the dry season), mycorrhizal *Boswellia* enhances its chances of gaining sufficient resources during the short rainy season. *Acacia* seedlings benefit from

the additional association with nitrogen-fixing bacteria. It provides high root nitrogen concentration that is associated with high respiration of fine roots (indicating metabolic activity) that facilitates uptake of nutrients and assimilation in fast-growing species (Tjoelker et al., 2005). Competition experiments between seedlings of these different strategies would suggest that the acquisitive strategy is superior over the conservative strategy, irrespective of water pulsing and the level of mycorrhizal inoculum (Chapter 5). However, this conclusion may be premature and requires further study. Under increasing drought stress (both the irregularity of rains during the wet season and the length of the wet season) the acquisitive strategy may become increasingly vulnerable.

Management Recommendations

The future of *Boswellia* as a tree species of major economic and ecological importance is not certain. Overgrazing and topsoil erosion has locally resulted in loss of soil organic matter and soil fertility, and may also have reduced mycorrhizal inoculum potential. Considering the strong differences in mycorrhizal responsiveness at relatively small differences of soil organic matter levels (topsoil versus subsoil, Chapter 4), such soil quality decline may jeopardise *Boswellia*. Possibly exclosures, which prevent topsoil erosion and loss of nutrients and mycorrhizal inoculum, could enhance seedling survival and growth. Exclosures could also further augment mycorrhizal inoculum and activity. Increased mycorrhizal activity possibly increases levels of glomalin, a glycoprotein produced by the hyphae of AM fungi (Rillig and Mummey, 2006), that contributes to soil aggregate stability and improves water retention of soils. Work by Augé et al. (2004) has shown that improved drought tolerance of mycorrhizal plants is both due to root colonization by AM fungi and by soil colonization; and that both factors can be equally important.

Hence exclosures are practical tools that facilitate restoration of dry deciduous woodlands through protection of interference and increasing resource availability to young seedlings. This study showed that under the current climatic condition seedlings in the dry deciduous woodlands, particularly *Boswellia* can successfully establish if they become colonized by AM fungi. In order to establish nurseries for *Boswellia*, inoculation of seedlings will increase the chance of establishment and survival. However, AM fungal species choice could be critical, as AM fungi show a large range of drought adaptation and only some species seem adapted to these specific conditions (Marandula et al., 2006). Also specificity of AM fungi towards different tree species may be a relevant property that

needs to be studied. Specificity of AM fungi could also affect the competitive balance between seedlings of *Boswellia* and those of trees with acquisitive strategies such as *Acacia*.

This study is especially relevant and timely as water conservation is an issue in the rehabilitation of dryland areas and fulfilling the demand of rapidly growing populations. It is critical to understand how something that seems tiny as a fungus could possibly be important in the management of soil water and facilitate rehabilitation efforts.



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Summary

Arid areas are characterized by a seasonal climate with a long dry period and relatively short rainy periods. In such ecosystems resources become available in pulses. Plants have to cope with these periodic changes and particularly in the seedling stage they are vulnerable. Some plants are adapted by allocating the carbon acquired during the wet season below ground and dying back aboveground during the dry season. Complete disappearance above-ground may protect such plants against fires, grazing and drought, until sufficient carbohydrates have been stored that allow the plant to grow rapidly aboveground and escape such calamities. Drought pulses can be overcome through increased access to moisture and nutrients. Arbuscular mycorrhizal (AM) fungi enhance access to moisture and nutrients and thereby influence plant performance in stressful environments where resource availability is controlled by rainfall pulses.

In this dissertation I applied field observations and greenhouse experiments to study the mechanisms that characterized how seedlings survive in these dry deciduous woodlands. The following questions were addressed; 1) What are the major environmental factors influencing AM incidence in the *Boswellia*-dominated dry deciduous woodlands? 2) How do *Boswellia* seedlings respond when they are exposed to AM fungi and water pulsing and their interaction? 3) How do AM fungi, water deficit and soil fertility influence the growth, allocation and gas exchange of *Boswellia* and *Acacia* seedlings? 4) Does the AM symbiosis influence competition between *Acacia* and *Boswellia* seedlings at different water pulse levels?

The present study showed that (almost) all woodland plants in northern Ethiopia are colonized by AM fungi. Root colonization levels in dry and wet seasons demonstrated that in the sites with the harshest conditions, AM plants and fungi apparently adapt to pulsed resource availability by temporally disconnecting carbon gain by the plant and carbon expenditure by the fungus. Consequently, we studied below-ground processes in conferring adaptation to highly pulsed resources in *Boswellia* seedlings.

The strong interaction between the presence of AM fungi and water pulse showed that mycorrhizal *Boswellia* benefits from drought pulses during the short rainy season. Water pulsing increased leaf area and phosphorus mass fractions in root and shoots, resulting in increased assimilation rates. Water use efficiency was highest in mycorrhizal seedlings under conditions of water pulses. *Boswellia* showed a conservative resource acquisition strategy. This strategy of acquiring carbon and water after rain events and

storing them in coarse roots is beneficial in this harsh climate. By this strategy seedlings disconnect carbon gain from nutrient gain, and store reserves below-ground till they are able to produce shoots that grow sufficiently high to escape the impacts of grazing and fire. From this observation we carried out an experiment to test whether other *Acacia* trees which remained aboveground, show a more acquisitive strategy which is also successful in these condition.

AM symbiosis helps *Acacia* and *Boswellia* seedlings to have higher biomass increase through enhancing gas exchange, water relations and nutrient levels and to better adapt to shallow and poor soils. The rapidly growing *Acacias* benefited from AM fungi at higher water availability, possibly because they achieved additional water and nutrient acquisition at high root density and resource availability. The slowly growing *Boswellia* with its large coarse root and few fine roots, benefited at low water availability from AM fungi, by enhancing water and nutrient uptake. Our studies thus show that acquisitive and conservative species both benefit from the AM symbiosis, but that the acquisitive *Acacias* mainly benefit at higher water availability, whereas the conservative *Boswellia* benefits at water or nutrient-stressed conditions. This output led us to investigate how mycorrhiza and water availability affect competition between plants with different resource acquisition strategies in these drylands.

Seedlings of *Boswellia* are competitively inferior to seedlings of *Acacia*. Neither the presence of AM fungi nor a stronger water limitation (through pulsing) affected the outcome of the interspecific competition. The ability of *Boswellia* to be active during the long dry season combined with its lower sensitivity to water pulsing could suggest that with more severe dry seasons or with longer intermittent dry spells during the rainy season the competitive balance shifts. Considering the differential vulnerability to fire and grazing of both species with their different above-ground and below-ground strategies, more extreme conditions may well lead to different competitive outcome over the longer term. However, our study imply that regeneration and maintenance of *Boswellia* stands may be more difficult in the future, if the climate in northern Ethiopia gets somewhat more humid, as predicted by some global change models. Moreover, soil erosion may lower the chances for *Boswellia* as a lower mycorrhizal inoculum potential and a lower nutrient level negatively impact on seedling growth.

Samenvatting

Droge tropische vegetaties worden gekenmerkt door een seizoensgebonden klimaat met een lang droog seizoen van rond de 8 maanden, en een relatief kort nat seizoen. In deze ecosystemen worden water en nutriënten beschikbaar voor planten in de perioden tijdens en na neerslag in het natte seizoen, en is dus afhankelijk van het optreden van zogenaamde water pulsen. De planten in deze gebieden zijn aangepast aan dergelijke veranderingen, maar blijven vooral als zaailing kwetsbaar. Planten passen zich aan door opgenomen bouwstoffen tijdens water pulsen op te slaan in de wortels onder de grond. Bovengrondse scheuten van deze planten kunnen volledig afsterven boven de grond tijdens het droge seizoen, maar groeien weer uit met behulp van de opgeslagen reservestoffen tijdens het natte seizoen. Een dergelijke groei strategie beschermt deze planten tegen droogte, begrazing en vuur gedurende het droge seizoen. Tijdens droogte pulsen gedurende het natte seizoen (tussen sporadische buien), kunnen associaties met "arbusculaire mycorrhiza" (AM) deze planten voordelen bieden, omdat AM beter toegang geven tot de opname van het resterende vocht, en de nutriënten, in de bodem.

In de proefschrift presenteer ik experimenteel onderzoek en veldonderzoek om de mechanismen te begrijpen waarmee deze zaailingen overleven in deze droge gebieden. Ik richt me op de wierookboom (*Boswellia papyrifera*) in de droge gebieden van Ethiopië. Deze soort levert als grote boom belangrijke bosbijproducten, onder andere wierook. In de laatste 2 hoofdstukken wordt de wierookboom vergeleken met 1 of 2 Acaciasoorten, die samen met de *Boswellia* in de droge gebieden voorkomen. De volgende vragen zijn besproken: 1) Wat zijn de belangrijkste ecologische factoren die van invloed zijn op zaailing associaties met AM in deze droge gebieden? 2) Hoe reageren zaailingen op AM en water pulsen tijdens het natte seizoen? 3) Hoe beïnvloeden AM associaties, waterbeschikbaarheid en bodemvruchtbaarheid de fotosynthese en groei van zaailingen van *Boswellia* en *Acacia*? 4) Hoe bepaalt AM de concurrentie tussen *Acacia* en *Boswellia* zaailingen?

Mijn studie laat zien dat bijna alle struiken en bomen van de onderzochte droge gebieden gekoloniseerd zijn door AM. Terwijl de associaties tussen plant en AM sterk zijn gedurende het regenseizoen, worden deze associaties waarschijnlijk losgekoppeld gedurende het lange droge seizoen. Verder is in detail naar bodemprocessen gekeken om te kijken hoe de zaailingen zich aanpassen aan gepulste omstandigheden.

Boswellia zaailingen profiteren van de aanwezigheid van AM gedurende de korte perioden van droogte (in de orde van dagen, soms weken), dus na de regenbuien. Planten met AM maken onder dergelijke gepulste omstandigheden meer bladoppervlak, en slaan meer fosfor op in de wortel en scheut, en ze bereiken hogere fotosynthese per blad oppervlak. Uiteindelijk namen deze zaailingen meer water op uit hun omgeving dan zaailingen onder constante water condities, waarschijnlijk omdat de associatie met AM de wateropname efficiënt deed verlopen. Na de water pulsen, die sporadische regenbuien imiteren, slaan de planten de extra verworven koolstof en water op in grove wortels, waarschijnlijk om perioden van droogte of schaarste te kunnen overleven. De verwachting is dat ze in een veel later stadium deze reserve stoffen gebruiken om snel bovengronds door te groeien, en zo het risico op schade door vuur en begrazing zo kort mogelijk te houden. Deze conservatieve groeistrategie staat in groot contrast met de snelle, meer verkwistende, groeistrategie van *Acacia* soorten.

AM symbiose helpt zowel *Boswellia* als *Acacia* om tot snellere biomassaproductie te komen, doordat AM de gasuitwisseling en opname van water en voedingsstoffen verbetert. *Acacia* soorten profiteren echter voornamelijk bij constante natte condities, zonder droogte perioden tussendoor, waarschijnlijk omdat ze dan ondanks de aanwezigheid van een dicht fijn wortel netwerk, nog efficiënter de voedingsstoffen en het water kunnen opnemen. De veel tragere groeier *Boswellia*, gekenmerkt met grove wortels en weinig fijn wortels, profiteert van AM bij lagere waterbeschikbaarheid.

In een concurrentie situatie leggen zaailingen van *Boswellia* het af tegen zaailingen van *Acacia*, en de aan-of afwezigheid van AM, en een hoge of lage water beschikbaarheid verandert daar weinig aan. De observatie dat *Boswellia* het relatief beter dan *Acacia* doet bij droogte, in zowel nat als droog seizoen, suggereert dat deze soort een concurrentie voordeel zou kunnen halen bij langdurige droogte. De klimaatmodellen voorspellen echter nattere omstandigheden voor de toekomst, waardoor *Boswellia* het mogelijk steeds sneller gaat afleggen tegen een snelle groeier als *Acacia*. De voortschrijdende bodem erosie, waarbij er verliest aan AM kan optreden, maakt de situatie er waarschijnlijk niet beter op.

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Short biography

Emiru Birhane was born on 01 July 1976 in Tigray, Ethiopia. He got a scholarship at the age of 5 from his uncle Ato Abay Hizikias and was taken to Addis Ababa to start primary school. He attended Bole senior secondary school. He joined Alemaya (Haromaya) University in September 1995 and graduated in forestry sciences in July 1998. He worked at the Tigray Bureau of Agriculture and Natural Resources as a junior forestry and agroforestry expert for 18 months. In October 2001 he started the joint masters programme between Swedish University of Agriculture and Wondogenet College of Forestry and graduated in farm forestry in July 2002. He studied the "Actual and potential contribution of area enclosures to enhance bio-diversity in the drylands of Eastern Tigray with particular emphasis to woody species". In 2003, he joined Mekelle University, Department of Land Resources Management and Environmental Protection (LaRMEP) as a lecturer until August 2006. He taught different forestry and natural resource conservation and management courses and researched the rehabilitation of degraded dryland ecosystems. In September 2006 he joined the Forest Ecology and Forest management Group and the Soil Quality Group of Wageningen University as a PhD student in the sandwich scheme. In this period he studied the Mycorrhizal symbiosis and seedling performance of the frankincense tree (*Boswellia papyrifera*). During this time he was also teaching at Mekelle University as assistant professor of forest ecology. Emiru Birhane is married with Gidey Gebreyohannes in September 2000 and they have two boys.



List of publications

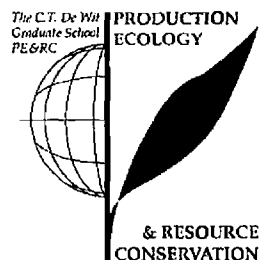
1. **Emiru Birhane**, Thom Kuyper, Frank Sterck, Frans Bongers. 2010. Mycorrhizal associations in frankincense-tree dominated dry deciduous woodlands of Ethiopia. *Forest Ecology and Management* 260: 2160–2169
2. **Emiru Birhane**, Ermias Aynekulu, Wolde Mekuria, Degitu Endale. 2011. Management, Use and Ecology of Medicinal plants in Tigray, Northern Ethiopia. *Journal of Medicinal Plants Research* 5: 309-318.
3. Wolde Mekuria, Mastewal Yami, **Emiru Birhane**, Mitiku Haile, Kindeya Gebrehiwot. 2011 (in press). Impact of Exclosures on woody biomass production and fuel wood supply: The case of Douga Tembien, Tigray, Ethiopia. *Journal of Natural Resources*
4. **Emiru Birhane**, Mulubrhan Balehegn, Daniel Kiros and Diress Tsegaye 2011 (in press). Distribution, animal preference and nutritive value of browse species in Abala woreda. Northern Afar. *Journal of Agriculture and Technology*
5. Molla Mekonnen, Kindeya Gebrehiwot, **Emiru Birhane**, Sarah Tewoldeberhan. 2011 (in press). Regeneration, Density and diversity of woody vegetation in Awash National park, Ethiopia. *Journal of the drylands*.
6. Molla Mekonnen, Kindeya Gebrehiwot, **Emiru Birhane**, Sarah Tewoldeberhan 2011 (in press). Impact of interference on species diversity of large wild mammals in Awash National Park Ethiopia. *Journal of the drylands*.
7. Ermias Aynekullu, **Emiru Birhane**, Nigussu Begashaw, Wolday Wubneh. 2006. Monitoring and evaluating land use/cover change using participatory geographic information system (PGIS): A case study in Begasheka watershed, Tigray, Ethiopia. *EJISDC* 25 (3), 1-10.
8. **Emiru Birhane**, Demel Teketay & Pia Barklund. 2007. Enclosures to Enhance Woody Species Diversity in the Drylands of Eastern Tigray. *East African Journal of sciences* 1(2) 136-147.
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Education Certificate

PE&RC PhD Education Certificate

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)



Review of literature (5.6 ECTS)

- Arbuscular Mycorrhizal symbiosis in the dry tropics, with special attention for the role of Mycorrhiza in water and nutrient relations

Writing of project proposal (4.5 ECTS)

- Waiting in the underground: survival strategy of *Boswellia papyrifera* in a highly pulsed environment

Post-graduate courses (8.6 ECTS)

- Soil ecology; PE&RC (2007)
- Survival analysis; PE&RC (2007)
- What is up in tropical community ecology?"; PE&RC (2009)
- Plant physiological ecology; Groningen University (2010)
- The legume-rhizobium symbiosis: from molecules to farmers; PE&RC/PPS (2010)
- Introduction to R; PE&RC (2010)
- Summer school "Rhizosphere signalling"; EPS (2010)

Laboratory training and working visits (2.5 ECTS)

- Photosynthesis and leaf water potential measurement techniques; Addis Ababa (2007)
- Techniques for the study of arbuscular Mycorrhizal symbiosis (characterization, identification, spore counting and colonization measurement, inoculation practices, maintaining cultures); WUR, Soil Quality Group (2007)
- Root assessment; Addis Ababa University (2007)

Deficiency, refresh, brush-up courses (9 ECTS)

- Basic statistics (2006/07)
- Ecological methods (2007)

Competence strengthening / skills courses (1.5 ECTS)

- Scientific publishing; Language services (2007)
- PhD Competence assessment; PE&RC (2007)
- Information literacy PhD + Endnote introduction; WGS (2010)
- How to write a world class paper; WUR (2010)

PE&RC Annual meetings, seminars and the PE&RC weekend (2.4 ECTS)

- PE&RC Weekend (2007)
- PE&RC Days: Multiple views in scales and scaling and Selling science (2007 and 2010)
- Scenario building for *B. papyrifera* management; Ethiopia (2008)
- Current PhD researches on tropical ecology (2010)

Discussion groups / local seminars / other scientific meetings (5.1 ECTS)

- Forest and conservation ecology (2006/07 and 2009/10)
- Forest rehabilitation and conservation in northern Ethiopia; Mekelle University (2007/08)
- Popularization of *B. papyrifera* tapping techniques in Ethiopian (2008)
- Wood biology and dendro-chronology seminar ; with presentation; Belgium (2009)
- Functional ecology and sustainable management of mountain forests in Ethiopia (2009)
- A world in Transition; WUR (2010)

International symposia, workshops and conferences (6.3 ECTS)

- International congress: Mycorrhizal symbiosis: ecosystem and environment of Mediterranean area (2010)
- International Workshop "Mycorrhizae: a biological tool for sustainable development in Africa (2011)
- Association for Tropical Biology and Conservation & Africa Section of the Society for Conservation Biology: Adaptability to Climate Change and Attaining the Millennium Development Goals for Tropical Ecosystems(2011)

FRAME: Frankincense, Myrrh and gum arabic: sustainable use of dry woodland resources in Ethiopia

More than half of the total land area in Ethiopia is covered by arid to semiarid woodlands with marginal agricultural potential. These woodlands are commonly overexploited for their natural resources, which reduces the local livelihood options for a rapidly expanding population. Climate change (e.g. drought) may intensify this negative trend. Consequently, there is an urgent need for improved land-use strategies that will make the vast arid and semiarid woodland resources optimally contribute to the livelihoods of local people and national development goals.

The dry woodlands in Ethiopia are not resource poor as they host several woody species that hold economically well recognized aromatic products such as gum arabic, frankincense and myrrh, which are widely used locally and in several of today's commercial industries such as cosmetic, pharmacological and food industries. Frankincense and myrrh are among the oldest internationally traded commercial tree products. Ethiopia is worldwide the main producer of frankincense and myrrh, and exports much gum arabic. Gum/resin production could significantly contribute towards sustainable development of these dry woodland areas. However, the overexploitation of natural resources by intensive grazing and intensive resin/gum harvesting and the lack of land management threatens the sustainability of the woody vegetation, and as a result of that also the long-term gum/resin production. Local communities may also enhance the productive capacity of the natural vegetation by establishing protected enclosures and by cultivation of trees. Such production systems may have a lower status regarding biodiversity and natural ecosystem functioning, but maintain ecological buffering capacity and improve production for human benefit.

The FRAME program addresses the following main research question: in what way dry land forests in Ethiopia can be made productive while maintaining ecosystem integrity in terms of sustainability of production and vegetation cover, with special attention to resin and gum resources?

FRAME uses a multidisciplinary approach involving scientific disciplines ranging from landscape-level geo-information studies to village-level socio-economic studies, plot level ecological and harvesting technology studies to tree-level ecophysiological studies with a strong contribution of local knowledge in answering the central research question. FRAME thus establishes a scientific basis for the sustainable management, including cultivation, of gum and resin yielding tree species and their habitat, the dry woodlands in the Horn of Africa. FRAME is actually involved in development of long-term scenarios for proper use and selection of suitable areas of dry woodland resources in Ethiopia.

The current PhD thesis is part of this FRAME program. A large part of this integrated FRAME research program was financially supported by NWO-WOTRO (Netherlands Organization for Scientific Research- Science for Global Development), grant W01.65.220.00.



