UNITED NATIONS UNIVERSITY
UNU-LRT
Land Restoration Training Programme
Keldnaholt, 112 Reykjavik, Iceland

Final project 2019

THE ROLE OF MYCORRHIZAE IN THE DEVELOPMENT OF TWO GRASS SPECIES IN TWO SOIL TYPES

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ABSTRACT

A greenhouse pot experiment was carried out to evaluate the potential of arbuscular mycorrhizal fungi to enhance the growth of lyme grass and maize. The experiment was arranged using a full factorial randomized block design, a total of four treatment combinations involving two types of natural soil (Brown Andosol and Arenic Vitrisol), with or without arbuscular mycorrhizal fungi inoculum collected from mature lyme grass sand dunes. Each treatment combination was replicated five times for each plant species, resulting in a total of 40 pots. Data were collected on plant growth parameters, and at 35 days after sowing the plants were harvested and root samples taken for examining arbuscular mycorrhizal fungi root colonization. Both plant species grew better in Brown Andosol than Arenic Vitrisol, but for lyme grass this effect was only observed among un-inoculated plants. Maize plants, however, had a positive response to arbuscular mycorrhizal fungi inoculation involving leaf width. In most cases, inoculated plant roots were found to have slight arbuscular mycorrhizal fungi colonization, but un-inoculated plants were usually without any arbuscular mycorrhizal fungi. The limited arbuscular mycorrhizal fungi root colonization and weak plant responses in this experiment were possibly due to the short duration of the experiment. It likely that extended experimental time would have allowed for further mycorrhizal development and greater arbuscular mycorrhizal fungi influence on both plant species.

Keywords: Arbuscular mycorrhiza, lyme grass, maize

This paper should be cited as:

Bedaso MS (2019) The role of mycorrhizae in the development of two grass species in two soil types. United Nations University Land Restoration Training Programme [final project] https://www.grocentre.is/static/gro/publication/730/document/bedaso2019.pdf

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1. INTRODUCTION

Soils are the basis for most terrestrial biomes and are a major natural resource for human food production. This resource has been degraded for a long time through the destruction of natural vegetation, overgrazing, salinization and soil acidification, poor internal drainage and other physical and chemical land degradation (Haregeweyn et al. 2006). During land degradation, especially in the case of soil erosion, most organic matter and the clay fraction is lost, which are the most productive portion of the soil (Haregeweyn et al. 2006). In general, soil erosion is one means for reduction of soil fertility and leads to failure in the production of many crops. This impact is high in developing countries that have rapid population growth and depletion of natural resources (Feoli et al. 2002).

Ethiopia is one of the sub-Saharan African countries facing serious land degradation problems, challenging sustainable land management (Zeleke et al. 2004). Soil erosion and moisture stress are currently causing an estimated 2% annual reduction in crop yields, resulting in insufficient food availability and famine for a long period (Abebe 2018). The Ethiopian Government is addressing land degradation in its official policy of Growth and Transformation Plan (Gebreselassie et al. 2016). Different land restoration projects have been implemented, especially in the highland of Ethiopia. However, these projects have been challenged by the high mortality of plants due to low soil fertility and soil moisture stress (Gashaw et al. 2014).

The mutualistic relationship among mycorrhizal fungi and plants can reduce plant mortality and improve plant capability in stressed environments (Greipsson & El-Mayas 2000; Enkhtuya et al. 2003; Rosendahl et al. 2009). Mycorrhizal fungi improve the aggregate stability of the soil and reduce soil erosion (Jeffries et al. 2003). Besides, the employment of mycorrhizal fungi can be an effective way for improved crop production and land restoration projects and can reduce the need for expensive inputs such as chemical fertilizers (Jeffries et al. 2003; Oskarsson & Heyser 2015; Chen et al. 2018). This should encourage the utilization of mycorrhiza for improving land health and agricultural productivity. However, it is important to evaluate the potential of mycorrhizal involvement for different soil situations. This project was an exercise in assessing the potential of mycorrhizal inoculation to enhance plant growth under different soil conditions.

1.1 Goal of the study

The overall goal of the study was to evaluate the potential of mycorrhizal fungi to enhance the restoration of degraded land. Specifically, the project had the following objectives:
• To investigate the efficiency of local inoculum of arbuscular mycorrhizal fungi to enhance plant growth in a greenhouse pot experiment.
• To measure the interaction between soil types and arbuscular mycorrhizal fungi inoculum on the mycorrhizal colonization of two grass species.

2. LITERATURE REVIEW

2.1 Taxonomy and description of mycorrhizal fungi

Fungi are common eukaryotic organisms, usually found as saprophytic, parasitic or pathogenic, which reproduce by sexual and asexual spores. They are unicellular or

multicellular organisms with membrane-bound organelles (Webster & Weber 2007). Some fungi are important in decomposing undegradable organic material like lignin into usable forms (Hoorman 2011). Lignin is a natural polymer found in cell walls of woody plants and is not easily degradable by microbes. However, some types of fungi have the potential of completely degrading lignin (Jeffery et al. 2010).

Some soil fungi form a mutualistic relationship with plants called mycorrhiza. The term mycorrhiza is derived from the Latin word "mycor" = relating to fungi and "rhiza" = roots. They usually form a fine root hair-like structure called hyphae. A group of their hyphae forms a mycelium which releases oxidizing enzymes into the soil and breaks down complex molecules (Hoorman 2011). In the mutualistic relationship, the fungus takes sugar and carbon from the plant and in return, the plant receives water and nutrients absorbed by the fungi from the soil. This relationship is essential for around 80% of all world plant species (Dunn et al. 2014). Also, mycorrhizal fungi increase plant tolerance to drought and other stress conditions (Jeffery et al. 2010).

Mycorrhizae show two main characteristics in colonizing plant roots based on the location and structures of fungal hyphae. The term endomycorrhiza refers to fungal structures that are found between and inside root cells, but ectomycorrhiza refers to structures that enclose and transform plant short roots. Based on combinations of these types and the species involved, there are seven kinds of mycorrhizae: arbuscular mycorrhiza, ectomycorrhiza, ectomycorrhiza, arbutoid mycorrhiza, monotropoid mycorrhiza, ericoid mycorrhiza and orchid mycorrhiza (Smith & Read 2008). The most common type of mycorrhiza is arbuscular mycorrhizae (AM) which is endomycorrhizal. Its hyphae grow into the cell membrane of cortex root cells and form vesicles and arbuscules. Vesicles and arbuscules are involved in the exchange of resources between plants and fungi (Dunn et al. 2014).

Currently, arbuscular mycorrhizal fungi (AMF) belong to the Glomeromycota and comprise six genera known as *Glomus, Acaulospora, Entrophospora, Gigaspora,* and *Scutellospora* (Smith & Read 2008). The name 'arbuscular' refers to the typical mycorrhizal structures, the arbuscular. An arbuscular mycorrhiza consists of three important parts: a fine root hair structure which moves within and between the cells of the plant root, extraradical mycelium which moves in the soil, and the third one, the complex mycelial elements or rhizomorphs (Smith & Read 2008). AMF has been detected in nearly all terrestrial ecosystems globally (Öpik et al. 2006).

The existing diversity of mycorrhizal fungal species suggests that these fungi are highly adaptive, both environmentally and for a wide range of plant species. They play an influential role in the protection of plants against biotic and abiotic stress (Rosendahl et al. 2009). According to Cameron et al. (2013) AMF, infection stimulates biological activity in the root zone which induces plant growth-promoting bacteria that have the ability to suppress pest and diseases. AMF by itself also induced systemic resistance of plants for pathogens and pests. Furthermore, AMF colonization in the root zone enhances the production and expression of plant immunity hormone.

2.2 Interaction of arbuscular mycorrhizal fungi with grass species

Land degradation is a serious problem worldwide, including erosion, lack of soil water retention, low soil fertility, increased toxic element concentration, salinization, and reduction in soil biota coupled with a decrease in nutrient cycling and ecosystem function (Al-Karaki

2016). Land degradation is very serious in areas of low availability of soil moisture due to irregular precipitation and frequent drought. These problems are a major obstacle for successful revegetation or sustainable land restoration (Al-Karaki 2016). However, there is a consensus that microbial technology involving mycorrhizal fungi could be an important method in the restoration of degraded lands. It is because mycorrhizae are usually the principal organs for soil-plant interactions and can reduce the cost of plants for water and nutrient uptake (Smith & Read 2008).

Commonly, grass species are used to control soil erosion and restoration of the land. Greipsson and El-Mayas (2000) reported that lyme grass (*Leymus arenarius* (L.) Hochst.) is used for stabilizing drifting sand in Iceland. However, the establishment of lyme grass sowings requires high fertilizer inputs for a long period. In most findings, the inoculation of AMF was found to improve the growth of lyme grass as compared to un-inoculated plants (Greipsson & El-Mayas 1999; Enkhtuya et al. 2003; Oskarsson & Heyser 2015). Enkhtuya et al. (2003) described that lyme grass seedling establishment in the first summer was enhanced by AMF inoculation. Correspondingly, Oskarsson and Heyser (2015) found that AMF inoculation of lyme grass resulted in doubled yield in the first summer as compared to un-inoculated control plants. Greipsson and El-Mayas (2000) also found a similar mycorrhizal benefit in a pot inoculation experiment involving AMF and lyme grass. Nevertheless, studies suggest that at an early age lyme grass is not very dependent on mycorrhizae but with age the dependence increases, particularly during flowering and seed production (Oskarsson & Heyser 2015; Greipsson & El-Mayas 2000; Greipsson et al. 2002).

The benefit of AMF has also been reported for other grass species. In a field study involving turfgrass, Al-Karaki (2016) showed that AMF inoculated plants were able to establish faster and had more biomass than un-inoculated turfgrass in a water-stressed environment. He stated that AMF inoculation is beneficial for the restoration of grassland as it benefits the fastest growth and distribution of grass. It also improved the quality of grass by reducing weed development. Pezzani et al. (2006) found that the biomass of two grass species was increased by AMF inoculation. The general indication is that AMF may play significant roles in the regeneration and restoration of grass.

2.3 Response of maize to arbuscular mycorrhizal fungi

Maize (*Zea mays* L.) is by far the most significant crop, sustaining almost five billion people in low food-secure countries. Maize has a better ability in adapting to different environments as compared to other crops, increasing its global importance and multiple uses as human and livestock feed (Shiferaw et al. 2011). Moreover, this crop is a well-known host plant for many soil microorganisms. Different researchers (Ortas & Akpinar 2011; Cozzolino et al. 2013; Guo et al. 2014; Ilyas et al. 2018) found that maize rapidly forms a mutualistic association with AMF. Ortas and Akpinar (2011); and Ilyas et al. (2018) described that AMF inoculation in pot experiments significantly improves maize growth and nutrient uptake under various environmental conditions. According to Cozzolino et al. (2013), field experiments using commercially produced AMF inoculum show increased productivity of maize plants by improving nutrient uptake under low soil fertility conditions. Similarly, Guo et al. (2014) found that mycorrhizal maize crop significantly increases growth and the uptake of N, P and K as compared to non-mycorrhizal plants in a pot experiment. They found more essential plant nutrients in shoots and roots of AMF inoculated than un-inoculated plants. Ortas and Akpinar (2011) also specified that AMF inoculation enhances the P concentration in many maize genotypes.

Plants are usually more dependent on mycorrhizae under conditions of low soil fertility. The addition of P fertilizer can, for example, reduce AMF colonization of inoculated maize plants (Carrenho et al. 2007). Even though increasing fertilizer use promotes plant growth, it may be possible to increase crop yields more cost efficiently by utilizing mycorrhizal fungi because of the increasing costs of fertilizers. According to Mustafa et al. (2010), the above-ground dry weight of mycorrhizal maize plants was increased by 27.5% compared with non-mycorrhizal plants. Guo et al. (2014) also found in their greenhouse experiment that inoculation with different species of AMF increased shoots, root, and total dry weight of a maize plant by 73, 91, and 76%, respectively. Likewise, Mustafa et al. (2010) reported that inoculation with AMF increased root dry weight by 9.7-75.8% compared with non-inoculated plants. In a greenhouse experiment, Ortas and Akpinar (2011) described that non-mycorrhizal and mycorrhizal maize plants produced 14 and 31 g per pot of root dry weight, respectively. They also found that mycorrhizal plants nearly doubled their P uptake. In a field experiment, Cozzolino et al. (2013) found that mycorrhizal plants put more P in the grain than un-inoculated plants. Hence, using mycorrhizae increased the yield potential of maize even when no P was applied.

The time required for the formation of the mutualistic association affects the responses of plants to mycorrhizae. According to Mustafa et al. (2010), a significant response, i.e. the highest shoot dry weight, plant height and P content, emerged at four weeks after AMF inoculation of sweet corn. Additionally, they reported that the response of sweet corn to AMF inoculation decreased in old plants. Field AMF inoculation experiments indicate that un-inoculated and inoculated maize plants developed at a similar rate for the first 15 days after transplanting, but after 45 days more AMF colonization and a significant mycorrhizal effect was observed (Khan 1972).

Root colonization is a major parameter for signaling the effect of mycorrhizal fungi on plants. Different species of plants and mycorrhizal fungi interact differently in terms of root colonization and response to environmental factors. Many studies on maize show great differences in AMF colonization between fungal species. Ortas and Akpinar (2011) showed that there is a broad difference between maize genotypes in their mycorrhizal dependency and the influence of plant age. According to Mustafa et al. (2010), maximum AMF colonization was at 10 weeks after inoculation but root colonization at six weeks significantly affected the plant growth. This shows that time is a limiting factor to root colonization with significant effects on plant growth, in addition to other factors, such as the organisms and conditions involved, plus the inoculum potential or the amount of AMF spores and other propagules in the root environment (Smith & Read 2008).

Generally, most findings describe maize responding positively to AMF, and the practices of AMF inculcation have the potential to increase the productivity of crops and support sustainable agriculture. However, there is a need for further research related to soil types and genotype interaction with AMF.

2.4 The soils of Iceland

Iceland is a volcanic island situated on the Mid-Atlantic Ridge in the North Atlantic Ocean. The lowland climate is cold (Cfc) including cold summer temperatures, without seasonal fluctuations in precipitation, with a polar-tundra (ET) climate at higher elevations (Peel et al. 2007). The annual precipitation is around 1000 mm in the south of Iceland but less in the north (Ólafsson et al. 2007). The soils of Iceland are different from other soils in Europe due

to their fresh volcanic origin, modified by the cold climate (Arnalds 2015). The soils commonly consist of materials from volcanic eruptions called tephra, forming soil types called Andosols. Icelandic Andosols are influenced by frequent freeze-thaw cycles and the presence of eolian deposition (Arnalds 2004).

According to Arnalds (2015), Icelandic soils are classified into two main groups: soils under vegetation and desert soils. The soils under vegetation either have Andosol properties or are organic (Histosols), and desert soils have the properties of Vitrisols. Andosols are separated into Histic Andosol, Gleyic Andosol and Brown Andosol based on the dominant composition of tephra input and drainage properties (Arnalds 2015).

Based on Arnalds (2015), Brown Andosol, often in associations with other soil types, is the most common soil type under vegetation. Brown Andosol is commonly found in the older land surfaces in Iceland and is rich in amorphous clays and organic carbon (Arnalds et al. 2016). The desert soil types (Vitrisols) are generally dark grayish and contain a limited amount of organic carbon. They are infertile and exposed to erosion and dominated by basaltic tephra which makes them unique in the world (Arnalds 2015). Vitrisols of Iceland are divided into four different types: Cambic, Gravelly, Arenic, and Pumice Virtrisols (Arnalds 2015). According to Arnalds (2015), Arenic Vitrisol, which is characterized by a drifting sand surface consisting of poorly weathered basaltic glass, is relatively widespread in Iceland (Arnalds 2015). Among the few plant species thriving in this soil is lyme grass (Greipsson & El-Mayas 2000). In this study, the dominant soil types that represent vegetated land (Brown Andosol) and desert areas (Arenic Vitrisol) in Iceland were used to investigate the response of two grass species to AMF inoculation.

2.5 Role of mycorrhizal fungi in different soil types

Soil is a limited and fragile resource that needs sustainable management to conserve its health. Many unsustainable practices have resulted in the loss of soil productivity. Mycorrhizal fungi have a mostly positive impact on soil structure in the context of agricultural and land restoration (Leifheit et al. 2014). This is because of the three-dimensional matrix of fungal mycelium crosslinking soil particles, protecting the soil from erosion. Moreover, AMF improve the capacity of soil for water retention. That's why AMF is effective in improving infertile soils, vulnerable to erosion (Chen et al. 2018). AMF also reduces soil nutrient leaching by increasing nutrient sequestration by soil aggregates (Cavagnaro et al. 2015). According to Jeffries et al. (2003), mycorrhizal fungi form a widespread extraradical network that supports plants to take up nutrients and water from the soil. For this reason, the practice of adding organic matter to soil indirectly improves soil structure.

The ability of mycorrhizae to improve soil productivity is different for different soil types. This is for example due to influences of soil texture on AMF colonization (Carrenho et al. 2007). Carrenho et al. (2007) observed that AMF colonization of sorghum was significantly reduced in clay soil, probably due to its inherent fertility. Clay soils have a high capacity to absorb ions from the soil solution, and high nutrient concentration in the root zone could hinder mycorrhizal development, by reducing plant dependency on support from AMF, as shown in several studies (Carrenho et al. 2007; Joner & Jakobsen 1995). In another case, a fine soil texture with a limited pore space increased the root stress, causing breakage of the cortical root layer and a loss of colonization sites (Carrenho et al. 2007). However, porous and infertile sandy soils could be more favourable for mycorrhizae than clay soil.

The influence of soil fertility was realized by Chakravarty et al. (2018), where 48% higher mycorrhizal dependency was recorded for a wheat variety when grown in Alfisol compared to the more fertile Vertisol. The results by Guo et al. (2014) from a greenhouse experiment showed that mycorrhizal colonization was significantly affected by different soil substrates. In general, the interaction between soil types or properties and plants are the main factors influencing mycorrhizal colonization. However, more positive influences of mycorrhiza on plants are usually observed in highly stressful environments, benefitting plant establishment and early growth and facilitating improvements in soil health (Chen et al. 2018).

3. METHODS

3.1 Experimental design and treatment factors

A pot experiment was designed and carried out in a greenhouse at the Agricultural University of Iceland, Reykir campus, during the 2019 growing season. The two plant species used for the experiments were lyme grass and maize. The former species was selected for its import role in revegetating sandy deserts in Iceland, and the latter was included as a reference plant for studying mycorrhizal organelles and dependency. Two types of soil were used for the experiment: basalt sand (Arenic Vitrisol) collected from an eroded sandy desert area (GPS point 63°50'22.2"N 21°35'30.8"W) and a forest soil (Brown Andosol) collected from a forest area (GPS point 64°00'22.1"N 21°10'33.6"W) (Fig. 1). Both soil types were collected from 0-20 cm depth of soil and mixed thoroughly to form a homogeneous potting substrate before putting it into pots. For the AMF inoculation treatment, fresh lyme grass roots were collected at a depth of 10-30 cm from a coastal sand dune at Hafnarsandur in Southern Iceland (GPS point 63°52'41.3"N 21°13'20.0"W).

Figure 1. The sites where soils for the pot experiment were collected: An unvegetated site (left) at the edge of a sand dune at Hafnarsandur in Southern Iceland where the basalt sand was taken; and a Sitka spruce forest stand (right) at the Agricultural University, Reykir campus, where the forest soil was taken.

For each grass species, the experiment was arranged using a full factorial randomized block design. The analysed factors were the two soils and two AMF components, a total of four treatment combinations (Table 1). For both grass species, each treatment combination was replicated five times, resulting in a total of 40 pots. During the experiment, the greenhouse temperature was kept at 25°C/20°C (day/night). All plants were irrigated using tap water by wetting the pots to a field capacity around once every three days. Seeds of lyme grass were

provided by the Soil Conservation Service, Gunnarsholt, Iceland (seed lot: Mýrdalssandur/MEL-2016-1018-02; seed germination 80.3%). Maize seeds were purchased at a local store, a product intended for making popcorn.

Table 1. Experimental factors and treatments used for the pot experiment in the Reykir greenhouse during the 2019 growing season.

Analysed factor	Details
Arbuscular mycorrhizal inoculation	• Control (sterilized root fragments) • AMF inoculation: Mycorrhizal root fragments from lyme grass dune
Plant host	• Lyme grass • Maize
Soil types	• Forest soil (Brown Andosol) • Basalt sand (Arenic Vitrisol)

3.2 Experimental procedure

Four-litre plant pots were filled with either of the soil types. The fresh lyme grass roots containing AMF were chopped into around 1-2 cm long fragments and inserted to a depth of 4 cm, centrally in each pot (approximately 1 teaspoon per pot, see Fig. 2). Un-inoculated pots received the same amount of sterilized root fragments; the sterilization was done for a few minutes in a microwave oven. On 12th June, five seeds of either grass species were placed near the centre of each pot to the depth of 2 cm.

Nitrogen fertilizer (NH_4NO_3) was applied three times during the experiment for maize; the first application was done 2 weeks after seedlings emerged and at the later applications 3 and 4 weeks after seedling emerged, when some nitrogen deficiency symptoms had appeared. The lyme grass received N fertilizer two times, 2 weeks and 3 weeks after the seedlings emerged. The total amount of N applied to each pot was 0.306 g for maize and 0.204 g for lyme grass. No phosphorus fertilizer was applied. This was done purposely to stimulate AMF colonization.

3.3 Plant growth measurements

Plant height of lyme grass including leaf number were measured at 24 and 35 days after the seeds were sown, but for maize at 12, 24 and 35 days after sowing. The height of the tallest plant in each pot was used for plant height, and this plant also provided data for the number of leaves. For both plant species final measurements were done at harvesting time (Fig. 3). At the termination of the experiment, on 25th July, the above-ground plant biomass was harvested from each pot. The root biomass was collected by carefully washing away the soil using tap water. Randomly collected maize root samples were taken for analysis of mycorrhizae. For maize, the sizes of these samples were around 1% of the total root biomass, but for lyme grass, whole root systems were preserved for analysis of mycorrhizae. The plant biomass was dried in an oven at 80°C for 48 hours. The total dry weight of plant shoots and roots, for each pot, was weighed to the nearest milligram and shoot and root ratios were calculated. The roots of lyme grass were dried and weighed after completion of the mycorrhizal analysis.

A

B

C

Figure 2**.** A. Fresh roots collected from a lyme grass dune to be used as an AMF inoculum. B and C. Cut root fragments of applied to pots for AMF inoculation.

A

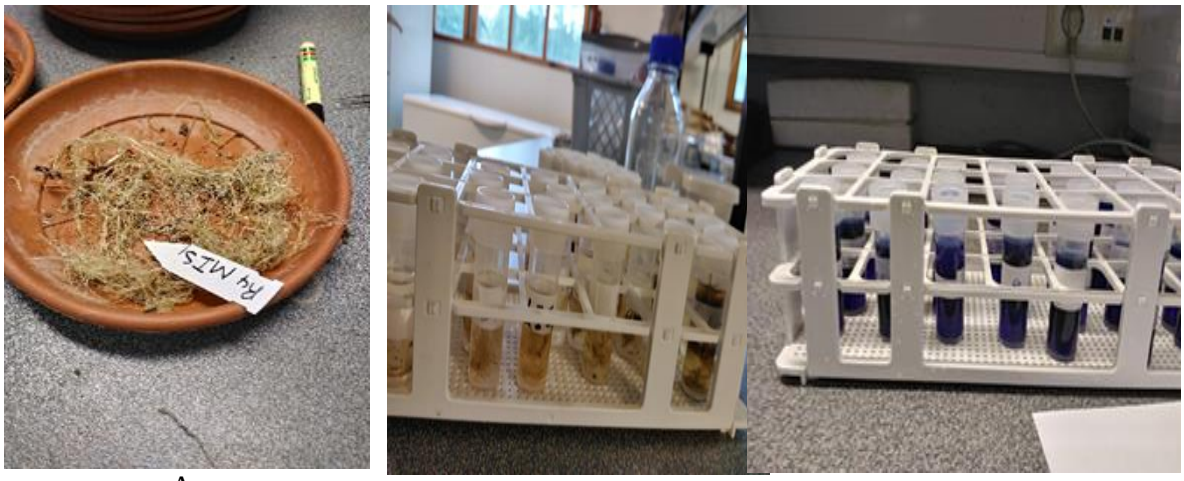
B

Figure 3. A. lyme grass and B. maize plants in the greenhouse pot experiment just before harvest.

3.4 Analysis of mycorrhizae

The 40 fresh root samples of both plant species were used for mycorrhizal analysis, each weighing around 1-2 g, were cut into 1-3 cm lengths and stored in 70% ethanol. Before the staining procedure, the roots were rinsed in tap water and cleaned in 10% KOH for 3 hours at room temperature (Fig. 4B). After this, the roots were rinsed several times with tap water and then stained in 1% Parker blue ink in lactoglycerol (1-part glycerol, 1-part lactic acid and 1-part water) for 1 hour (Fig. 4C). After staining, the root samples were examined under the microscope at 400x magnification to determine whether the root had AMF colonization or not (Fig. 5). Root samples with AMF were counted as colonized root systems and root samples without AMF were counted as non-colonized root systems. The frequency of AMF colonized, and non-colonized plants was calculated and stated as a percentage. A more detailed mycorrhizal examination was done for two replicates out of five for each experimental unit (a total of 16 root samples) under the microscope at 400x magnification, to determine the percentage length of AMF colonized root segments. The following formula was used based on Mustafa et al. (2010).

$$\text{AMF colonization (\%)} = \frac{\text{number of colonized root segments}}{\text{total number of segments examined}}$$



A B C

Figure 4. A. Fresh maize root system after harvesting. B. maize root samples in 10% KOH solution. C. maize root samples in 1% ink lactoglycerol.

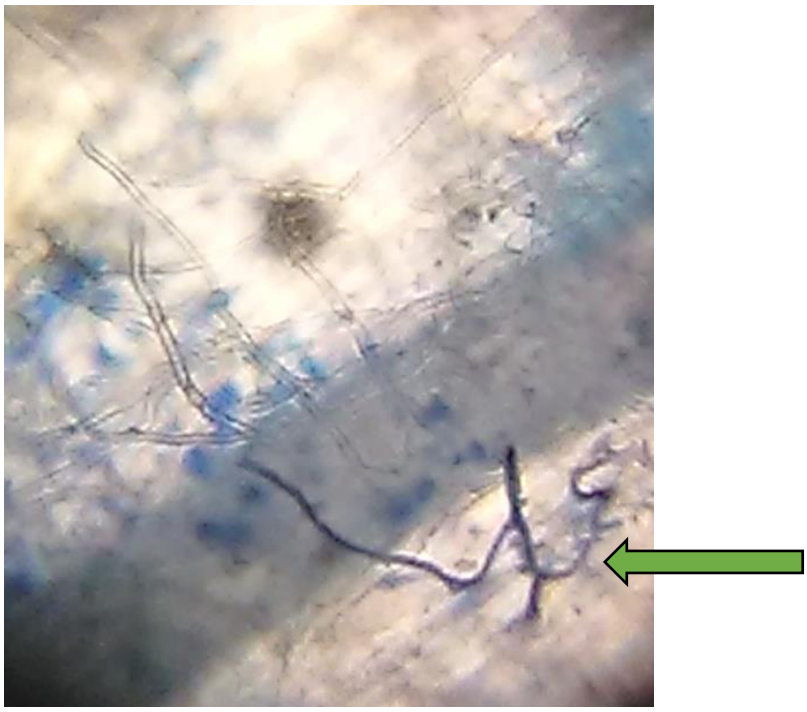


Figure 5. Root sample of lyme grass at 400x magnification under the microscope; the arrow pointing at an AMF hypha.

3.5 Data Analysis

A two-way factorial linear model was used to analyse the effects of experimental factors and their interaction on the response of variables measured in the greenhouse experiment and the laboratory. The following model was used:

$$Y_{ij} = \mu + a_i + b_j + c_k + (ab)_{ij} + \varepsilon_{ij}$$

where Y_{ij} is response variable ji, μ = mean of the measured variable, a_i = inoculum effect at i levels, b_j= soil types effect at j levels, c_k= block effect (random effect at five levels, k), $(ab)_{ij}$ = treatment interaction effect, ε_{ij} = error effect of variable response.

Mean comparison between variable responses was done using Tukey's multiple rating test for each factor and interaction between factors at the 5% significance level. The frequency of mycorrhizal colonization between experimental factors was tested using chi-square, and the summary data were presented in percentage of root with mycorrhizae.

The ANOVA and Tukey's test were done in JMP-14 .0.0 for windows (JMP 1989-2019), and the chi-square test was done in IBM SPSS 20 for windows (IBM SPSS 2011).

4. RESULTS

4.1 Growth response of lyme grass

Lyme grass seedlings emerged two weeks after sowing. The rate of lyme grass growth was not significantly influenced by the main effect of AMF inoculation (Table 2). However, at 35 days after sowing, a significant difference was observed due to the interaction between AM inoculation and soil types, and by the main effect of soil type (Table 2). The plants grew taller in Brown Andosol than in Arenic Vitrisol (Fig. 6), but leaf numbers were not significantly different between treatments (Fig. 7; Table 2). The interaction between soil types and AMF inoculation shows that this difference only appeared among the control plants (Fig. 8).

Table 2. Results (F-values) from an ANOVA model in a pot experiment of lyme grass with AMF inoculation (I) in two different soil types (S). *p <0.05; ns = not significant; df = degrees of freedom.

Parameters	**Model df=4**	**Block df= 4**	**Inoculum df=1**	**Types of soil df=1**	**Interaction (I x S) df=1**
Plant height at 24 days after sowing	0.6774ns	0.1112ns	1.1247ns	1.4710ns	0.0028ns
Plant height at 35 days after sowing	3.1067*	0.0005ns	2.7268ns	5.0508*	4.6387*
Leaf number	1.3393ns	2.1429ns	1.0714ns	1.0714ns	1.0714ns
Aboveground biomass	1.4686ns	0.0714ns	2.6792ns	2.9201ns	0.2035ns
Total dry biomass	0.9549ns	0.0004ns	2.1359ns	1.2226ns	0.2035ns
Root shoot ratio	0.9028ns	0.5075ns	0.0959ns	0.1312ns	2.8765ns

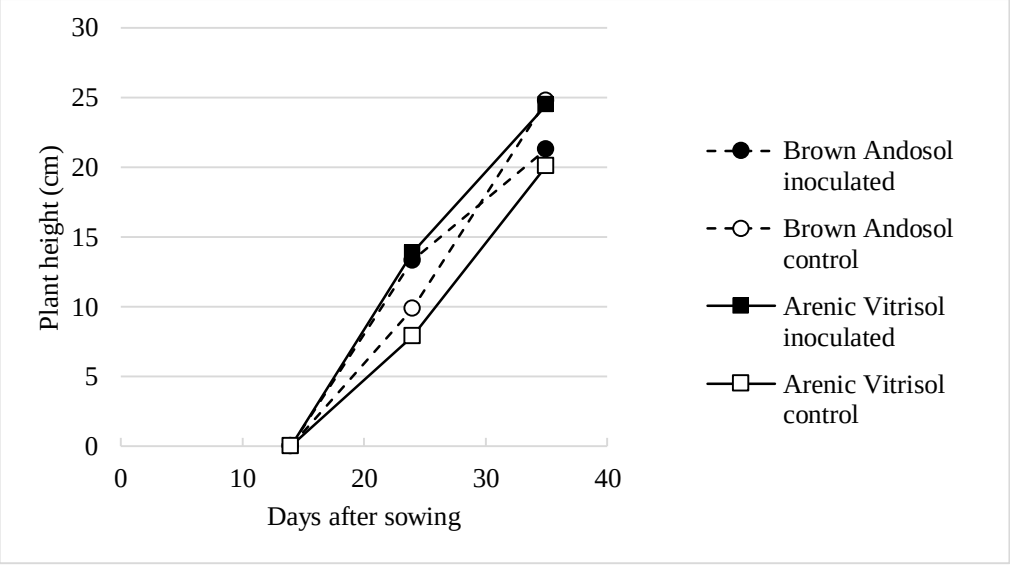


Figure 6. Lyme grass plant height at different days after sowing in a pot experiment by AMF inoculation (inoculated and control) and soil types.

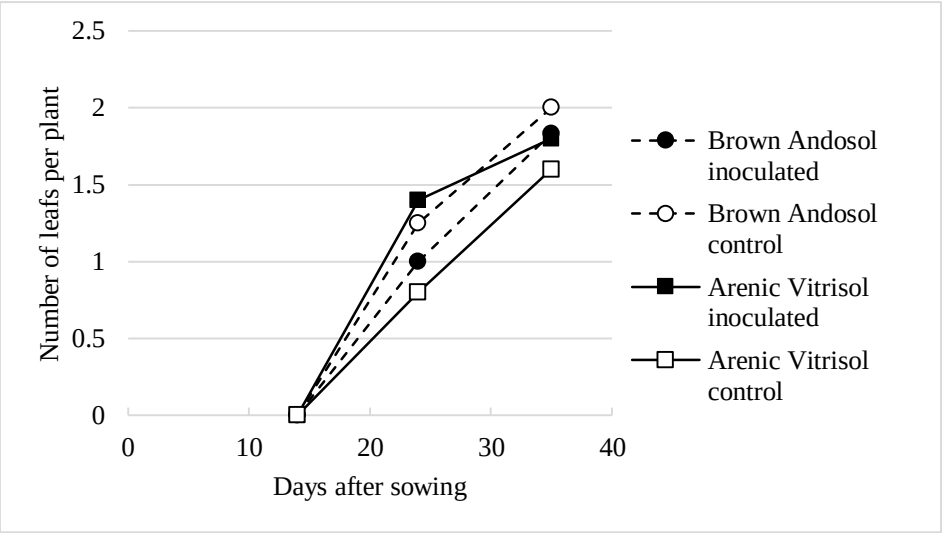


Figure 7. Lyme grass leaf number at different days after sowing in a pot experiment by AMF inoculation (inoculated and control) and soil types.

Neither total dry biomass nor dry above-ground biomass of lyme grass were significantly affected by experimental factors (Table 2). The total above-ground dry biomass per pot from Arenic Vitrisol was 0.034 g and 0.023 g for control and AMF inoculated plants, respectively; in Brown Andosol, control and AMF inoculated plant weights per pot were 0.053 g and 0.034 g, respectively.

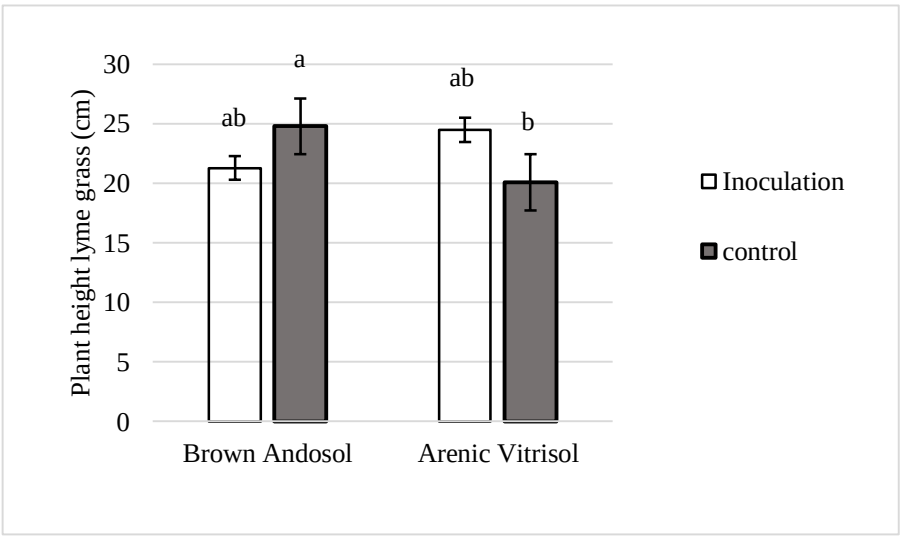


Figure 8. Lyme grass plant height at the termination of the pot experiment by AMF inoculation (inoculated and control) and soil types. Error bars indicate standard error of means, and a different letter above columns designates a significant difference of means (Tukey's test $p < 0.05$).

4.2 Lyme grass arbuscular mycorrhizal colonization

The result from root examination for AMF colonization indicated that the root samples from inoculated plants had a higher AMF occurrence than un-inoculated plants. In un-inoculated

pots for both soil types, no AMF was detected (Fig. 9). The percentage length of AMF colonized roots was 2-8% in root samples where AMF occurred.

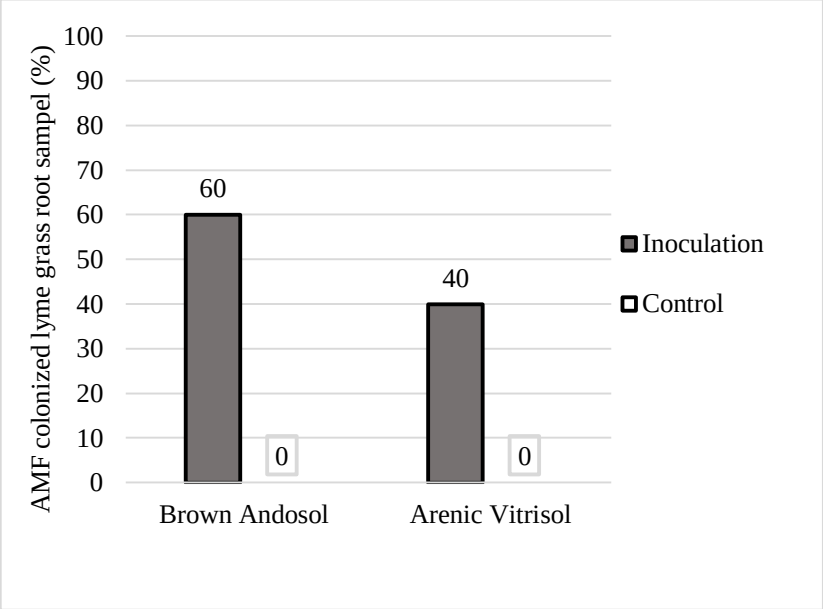


Figure 9. Percentage occurrence of AMF colonized lyme grass root systems in pot experiment involving AMF inoculation (inoculated and control) in two soil types.

4.3 Growth response of maize

Maize seedlings emerged one week after sowing. At 12 days after sowing, plant height was responding similarly to all treatments, but at 24 days and 35 days the main effect of soil types had a significant effect (Fig. 10; Table 3). Maize plants grew taller in Brown Andosol than Arenic Vitrisol (Fig. 11). The main effect of AMF inoculation did not significantly influence plant height.

Table 3. Results (F-values) from ANOVA model in a pot experiment of maize with arbuscular mycorrhizal fungi inoculation treatment (I) in two different soil type (S). ***p <0.001, **p <0.01, *p <0.05; ns = not significant; df = degrees of freedom.

Parameter	**Model df=4**	**Block df= 4**	**Inoculum df=1**	**Types of soil df=1**	**Interaction (I x S) df= 1**
Plant height at 12 days after sowing	0.7185ns	0.1288ns	0.7047ns	2.0310ns	0.0095ns
Plant height at 24 days after sowing	14.1858***	0.4226ns	1.0420ns	53.205***	2.0733ns
Plant height at 35 days after sowing	9.5548*	1.2203ns	0.8786ns	34.1054***	2.0108ns
Leaf width at 24 days after sowing	5.5220*	0.0000ns	0.0084ns	21.8694*	0.2102ns
Leaf width at 35days after sowing	13.2500***	0.0000ns	12.5000*	40.5000***	0.0000ns
Leaf number	0.5103ns	1.0000ns	0.1142ns	0.5844ns	0.5844ns
Above ground biomass	13.0620***	0.7451ns	2.3602ns	47.8496***	1.2933ns
Total dry biomass	20.9725***	0.4584ns	0.1890ns	83.0935***	0.0285ns
Shoot root ratio	22.3477***	0.1352ns	0.4963ns	87.6070***	1.1525ns

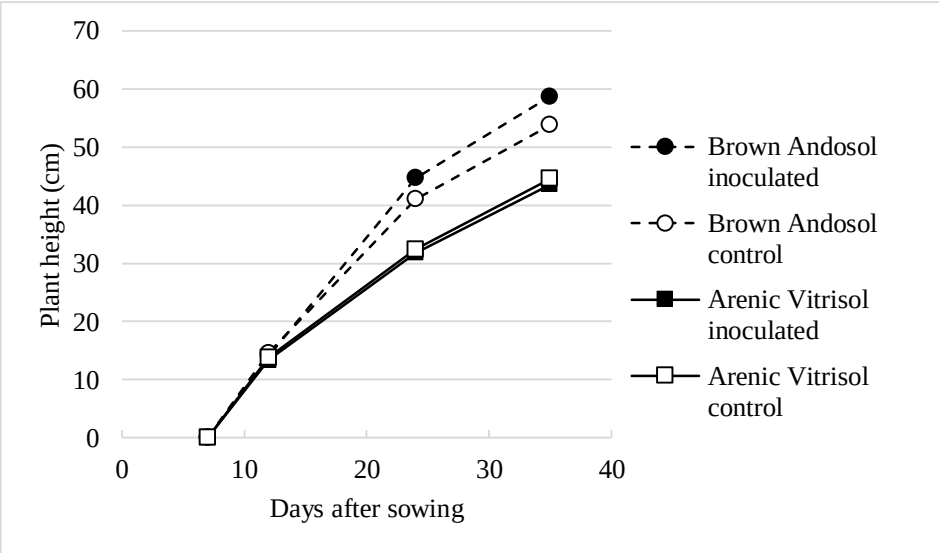


Figure 10. Maize plant height at different days after sowing from pot experiment involving AMF inoculation (inoculated and control) and soil types.

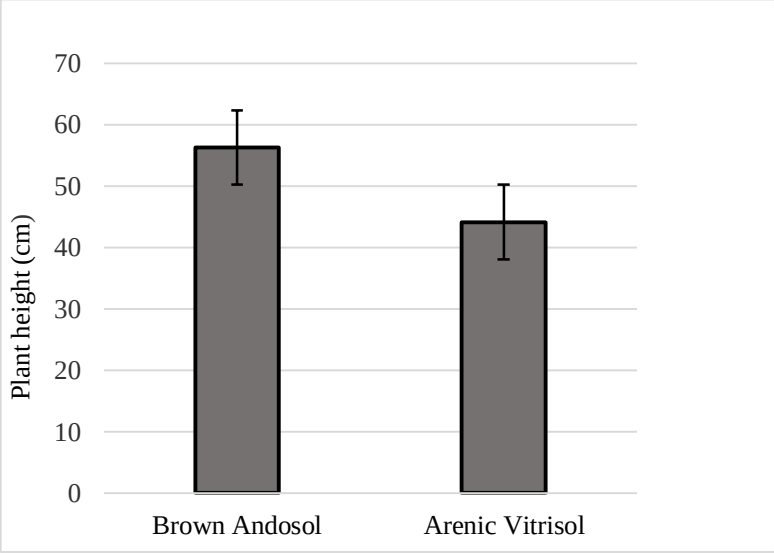


Figure 11. Maize plant height at the termination of pot experiment by soil types.

Leaf width of maize was significantly affected by the main effects of AMF inoculation and soil types (Table 3). The significant effect was larger after 35 days than 24 days (Table 3; Fig. 12).

AMF inoculated maize plants had broader leaves than un-inoculated ones (Fig. 13). Similarly, leaf width of plants in Brown Andosol was broader (2.55 mm) than in Arenic Vitrisol (1.65 mm) (not shown).

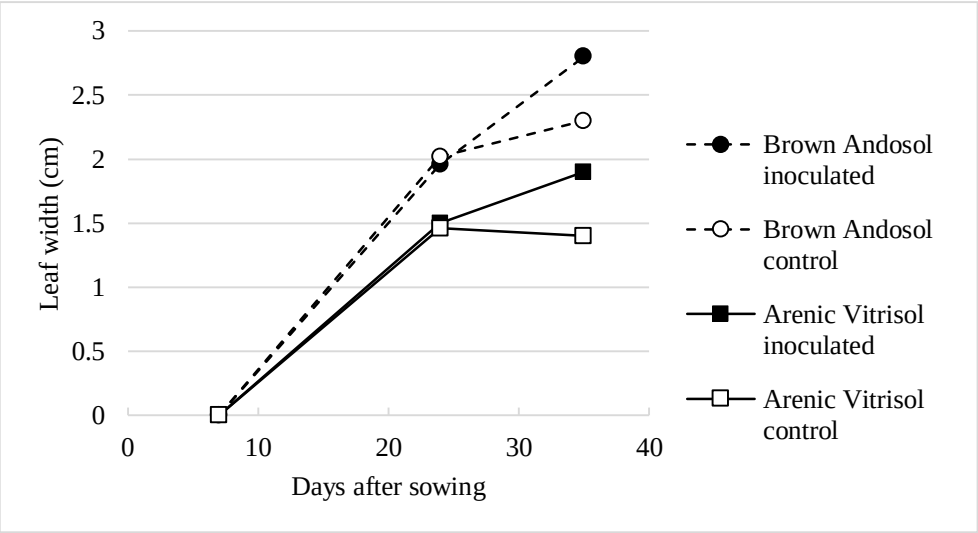


Figure 12. Maize leaf width at different days after sowing in a pot experiment involving AMF inoculation (inoculated and control) and soil types.

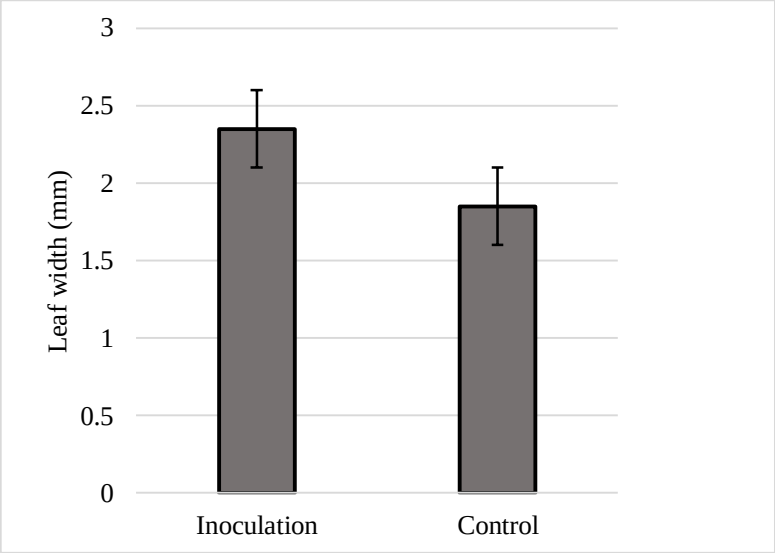


Figure 13. Maize leaf width at the termination of pot experiment by AMF inoculation

The total plant dry biomass and dry above-ground biomass of maize was neither significantly affected by AMF inoculation nor by the interaction with soil types (Table 3). However, the main effect soil type significantly influenced total dry biomass of maize (Table 3). The total dry biomass grown in Arenic Vitrisol was 2.36 g and 2.68 g for control and AMF inoculated plant, respectively. In Brown Andosol, control and AMF inoculated plants weighed 13.39 g and 14.13 g, respectively.

4.4 Maize arbuscular mycorrhizal colonization

The results from root examination for AMF colonization indicated that inoculated maize root systems had a higher AMF occurrence than un-inoculated plants. The highest occurrence (40%) of AMF colonized roots occurred in the inoculated Brown Andosol (Fig. 14), but no occurrence of AMF colonized roots was found among the un-inoculated plants in Arenic Vitrisol. The percentage of AMF colonized root lengths was 2-5% in root samples where AMF occurred.

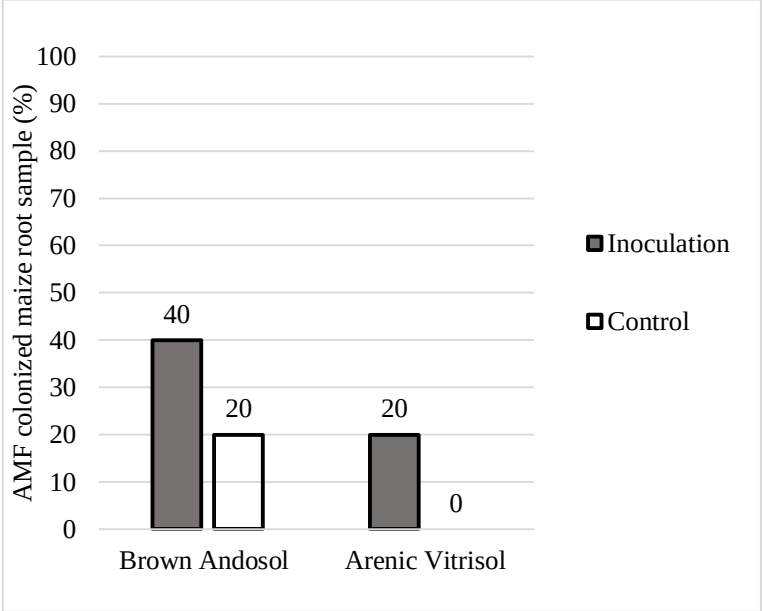


Figure 14. Percentage occurrence of maize root systems with AMF colonization, in pot experiment involving AMF inoculation (inoculated and control) in two soil types.

5. DISCUSSION

Due to time limitations, both lyme grass and maize seedlings were harvested after 35 days after sowing. Lyme grass is a perennial plant that has slow growth at the initial growth stage (Greipsson & El-Mayas 2000). At this early stage of growth, lyme grass was not affected by AMF inoculation. One of the reasons that determine potential benefits from AMF inoculation is the dependence of the plant on arbuscular fungi, which can be influenced by plant age (Mustafa et al. 2010). This agrees with previous findings (Oskarsson & Heyser 2015; Al-Karaki 2016; Khan et al. 2007; Greipsson & El-Mayas 2000; Greipsson et al. 2002) which suggest that at an early age lyme grass is not very dependent on mycorrhizae. Nevertheless, with age the dependence increases, particularly during flowering and seed production. Similarly, Mustafa et al. (2010) stated that maize plants of four weeks of age did not show significant differences between mycorrhizal and non-mycorrhizal plants; they stated that a significant response of maize to mycorrhizal treatment started at six weeks after inoculation. Graham and Syvertsen (1985) also indicated that some plant species, even the same cultivar and same species, have a different dependency on mycorrhiza.

According to Carrenho et al. (2007); Mustafa et al. (2010); and Guo et al. (2014), mycorrhizal fungi increase the leaf growth of maize plants, which is like the present results; broader leaves were observed in AMF inoculated plants.

Mustafa et al. 2010; Cozzolino et al. 2013; Guo et al. (2014) described that inoculation of mycorrhiza increased the biomass of maize plants by over 70%. But also, they indicated that the time required for the formation of the mutualistic association affects the responses of plants to mycorrhizae. Khan's (1972) AMF inoculation experiments indicate that un-inoculated and inoculated maize plants developed at a similar rate for the first 15 days after transplanting, but after 45 days more AMF colonization and a significant mycorrhizal effect were observed. This shows there is a lag period for AMF colonization and some minimum time needs to pass before the desired benefits on plant growth appear (Mustafa et al. 2010).

Root colonization is a major parameter for signalling the effect of mycorrhizal fungi on plants. The more root colonization the more effects will be observed. In the present study, the occurrence of colonized roots was higher when AMF inoculum was applied, but only a few percent of root lengths (2-8%) were colonized. According to Mustafa et al. (2010), maximum AMF root colonization (77% of root lengths colonized) was observed after 70 days from inoculation but significant effects on plant growth were observed after 42 days from inoculation when 64% of root lengths were colonized.

Plant and fungal genotypes influence root colonization and plant responses to AMF inoculation. The seed origin of maize in the present study, intended for making popcorn, may have influenced the results. In addition, other factors, such as the experimental greenhouse conditions and the inoculum potential of the natural AMF inoculum used along with the presence of other natural soil propagules and organisms in the root environment, can influence AMF colonization and plant responses (Smith & Read 2008).

The interaction between soil types and AMF inoculation significantly influenced the growth of both plants and deserves attention. Although the results are inconclusive, maize plants showed somewhat greater responses to AMF inoculation in Brown Andosol than in Arenic Vitrisol, but for lyme grass the opposite trend was observed.

Overall, the growth rate of both plant species was higher in Brown Andosol than in Arenic Vitrisol. Based on Arnalds (2015), Brown Andosol is rich in amorphous clays and organic carbon, N and other plant nutrients. However, Arenic Vitrisol contains only a very low amount of organic carbon and N, which makes it infertile and unsupportive of plant growth (Arnalds 2015).

6. CONCLUSIONS

In this research, the efficiency of local AMF inoculum to enhance the development of lyme grass and maize could not be fully realized. The AMF colonization was still at an early stage at the termination of the experiment and the host plant responses were showing a certain trend, but most were not significant. If the experiment had continued for a longer time, probably the results would have been different. Further research should take consideration of these findings and include field trials and longer greenhouse experiments.

This study has given me valuable experience, skills and knowledge on how to inoculate seedlings and how to analyse and identify mycorrhizae. I'm confident that restoration of dry land in Ethiopia could benefit from the application of mycorrhizal technology. Furthermore, this technology could potentially benefit in improving soil structure and crop productivity, and for producing better quality seedlings in forest nurseries for forestry and land restoration.

For this, it is important to include a focus on mycorrhizae in policy, counselling, and in research and educational programs.

ACKNOWLEDGEMENTS

Above all, I would like to thank God for giving me health and internal peace to complete the program and my project work, and for His care and guidance throughout my life.

I would like to express my special thanks to the managers of the UNU-LRT programme, Hafdís Hanna Ægisdóttir, Berglind Orradóttir, and Halldóra Traustadóttir and Katrín Björnsdóttir for their kind help, love, and guidance throughout the programme.

I would like to express my sincere and deepest gratitude to my supervisors Úlfur Óskarsson For his enormous guidance, professional assistance and valuable feedback starting from the project proposal up to the final project write up.

I would like to thank UNU-LRT for sponsoring me for the training and to Mekelle University for permitting me to join the programme.

My sincere thanks also go to my husband Silesh Mekonen for his love, care and support by phone and for his good care of our lovely daughter Zoe Silesh.

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