University of Zurich[UZH]

Optimization of the Production of Arbuscular Mycorrhizal Inoculum

GEO 511 Master's Thesis

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In cooperation with Agroscope

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Abstract

Land-use intensity is increasing all over the world, which leads to soil degradation and loss of biodiversity in agricultural systems. One strategy to increase the sustainability of agriculture is the inoculation of beneficial microorganisms to increase crop yield and health and to minimize the application of agrochemicals. Arbuscular mycorrhizal fungi (AMF) are a group of beneficial soil organisms that can enhance plant growth and nutrition by forming symbiotic relationships with plant roots. By introducing them into soil, their benefits could be harnessed for agricultural production. However, the production of effective inoculum with high applicability is challenging and the currently used methods are not practicable at field scale. A better production practice should be developed for a faster produced and higher concentrated AMF inoculum.

In this thesis, two greenhouse experiments were conducted to optimize AMF inoculum production. The first experiment compared the influence of the host plants maize and plantago and the depths of two container types on AMF establishment in the inoculum. In the second experiment, the effect of maize and sudan grass in different substrates on AMF root colonization and spore number was tested. The substrates where selected for beneficial traits for agricultural application, such as lightweight, homogeneity and no sharp edges. In the first experiment, maize showed significantly higher AMF root colonization and spore number compared to plantago but no significant differences between the two container depths were observed. In the second experiment, testing different substrates, it was found that lava, porlith, coarse pumice and the substrate mixture currently used at the research station Agroscope had the best AMF root colonization. Furthermore, a low P content in the substrates and a pH between 7-9 promoted AMF establishment. It was found that maize was better suited as host plant than sudan grass and had a significantly higher AMF root colonization. Even though promising AMF root establishment was observed in different substrates and maize promoted faster root colonization by AMF, further research is needed to understand the effects influencing the production of AMF inoculum and to find a sustainable material with beneficial traits for agricultural application.

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1. Introduction

1.1. Agriculture and ecological intensification

One of humanity's greatest issues is assuring food and nutrition security. The ability to utilize knowledge regarding the relationships between agricultural management, soil biodiversity, and food production is important for improving food security while avoiding further harm to the earth's ecosystem (El Mujtar et al., 2019; Hart et al., 2015; Sosa-Hernández et al., 2019; Trivedi et al., 2017). Land-use intensity is increasing all over the world, which leads to degradation (Bender et al., 2016; Stavi & Lal, 2015). Furthermore, the increased land-use caused by intensive agriculture can lead to several environmental problems, such as the eutrophication of surface water and the accumulation of pesticides (Foley et al., 2005; Tilman et al., 2002; Verbruggen et al., 2010).

However other management systems, such as conservation agriculture and organic farming result in lower yields compared to conventional systems (Trivedi et al., 2017). Intensive agriculture reduces the diversity and richness of overall soil biota and has a negative effect on soil organisms like earthworms, Arbuscular Mycorrhizal Fungi (AMF) and bacteria (De Vries et al., 2013; Kuntz et al., 2013; Stavi & Lal, 2015; Verbruggen et al., 2010).

One approach to increase the sustainability of agriculture and to minimize yield gaps is ecological intensification (Bommarco et al., 2013). The target is to manipulate soil biota, especially the microorganisms, to promote specific functions to minimize yield gaps and improve the ecosystem while reducing anthropogenic input (Bender et al., 2016; Bommarco et al., 2013; Trivedi et al., 2017). Furthermore, it aims to enhance nutrient use efficiency and sustainability of agricultural systems and to replace chemicals and fertilzers with microbial products by promoting regulatory ecosystem processes provided by microorganisms (Bender et al., 2019; Trivedi et al., 2017). The soil microbiome increases plant productivity and affects the nutrient availability and health of the plants (Frey-Klett et al., 2007). The main objectives of ecological intensification are to improve overall ecosystem service delivery and decrease the yield gaps. The manipulation aims at increasing biodiversity, which should lead to more sustainable and natural agriculture. (Bender et al., 2016; Wallenstein, 2017).

Soil ecological intensification covers all agricultural management methods supporting the soil microbiome and the ecosystem services (Bender et al., 2016). Soil ecological engineering includes inoculation of beneficial microorganisms such as AMF and N-fixing bacteria and a less intensive soil cultivation, such as crop cover and mulches (Bender et al., 2016; Pittelkow et al., 2014). Those management systems support the formation of organic substance, produce an appropriate environment for soil microbiome, and improve the soil structure (Bender et al., 2016).

1.2. Interaction of AMF and plants

Mycorrhizal fungi distinguish into two different groups depending on the interaction with the root cells. The ectomycorrhizal fungi establish an extracellular relationship whereas the arbuscular mycorrhizal fungi (AMF) establish an extra- and intracellular relationships (van der Heijden et al., 2015). AMF are biotrophic symbionts, colonizing the roots of the host plant (Schlaeppi et al., 2016). They grow inside the plant roots and spread their hyphae into the soil where they forage for nutrients which are limiting for the plants (Read & Perez-Moreno, 2003; van der Heijden et al., 2015). Thanks to the mycorrhizal network carbon and nutrients can be transferred from one plant to another through fungal hyphae (van der Heijden et al., 2015).

AMF are a group of worldwide distributed soil fungi, forming a symbiosis with most of the plant families, including many important crops (Bender et al., 2019; Fracchia et al., 2001; IJdo et al., 2011; Sosa-Hernández et al., 2019). AMF get attention because of their potential application in sustainable agriculture and ecosystem management to increase crop yield (Selvakumar et al., 2018). AMF increase the nutrient uptake especially of immobile nutrients, such as zinc and copper, and can provide up to 80 % of plants need of phosphorus (IJdo et al., 2011). Since fungal hyphae are much smaller than roots, they can fit through narrower pores to provide nutrients for the plant (Berruti et al., 2016). Furthermore, AMF increases the capacity of the plant to tolerate biotic and abiotic stress (Jung et al., 2012). AMF and the host plant must meet in the soil to establish a symbiosis. The spores germinate and form a mycelium which spreads in the soil (Besserer et al., 2006). These fungi has the potential to improve nutrient use efficiency in agricultural systems (Bender & Heijden, 2015).

1.3. Application of AMF inoculum at field scale

One strategy to increase the proportion of AMF in agriculture, is to enrich the field with AMF by inoculation, whereby plant productivity should be increased and the environmental impact of agriculture should be decreased (Bender et al., 2019; Köhl et al., 2016; Pellegrino et al., 2015; Verbruggen et al., 2010). The introduced AMF should colonize the plants fast, taking up soil nutrients and enhancing plant nutrition, whereby an effect on crop production and ecosystem functioning could be achieved (Bender et al., 2019).

In many subtropical countries the application of organisms contributing to improved plant performances is a technique already applied, due to the limited fertilizer resources (Ceballos et al., 2013). It has to be considered that the success of AMF inoculation can be soil type dependent and highly variable in temperate soils (Köhl et al., 2016). Different AMF species establish differently, depending on climatic and geomorphological conditions as well as different management systems (Oehl et al., 2010; Verbruggen et al., 2010). The inoculated fungus needs appropriate environmental conditions at the field site for a successful establishment. Different AMF species have shown that they establish under different soil

conditions, such as soil texture, pH or nutrient conditions (Oehl et al., 2010). It is assumed that the success of fungal establishment in a field relates to the amount of native AMF and the composition of their community (Köhl et al., 2016). A greater amount of native AMF lead to a smaller success of the establishment of the introduced AMF (Bender et al., 2019).

Furthermore, the distribution of AMF is dependent from the soil depth. Several studies have reported that with increasing soil depth a decrease of colonization occurs, and that the highest AMF density is found at 0-15cm (Asghari et al., 2005; Oehl et al., 2005; Säle et al., 2015). The highest spore densities in reduced tillage systems are found between 0-10 cm (Säle et al., 2015).

Furthermore, the intensification of land use causes a reduction in AMF spore abundance and AMF species diversity in the agroecosystems of Central Europe (Oehl et al., 2005; Verbruggen et al., 2010). A phosphorus-rich soil and an intensive phosphor fertilization inhibit the AMF colonization, because the plants are not dependent on the nutrient supply of the AMF (Ryan & Graham, 2002). The competitive pressure for resources like symbiotically derived carbon from host plants, soil nutrients or the available space for symbiotic interactions in the rhizosphere can be intensified (Sosa-Hernández et al., 2019).

2. AMF inoculum production

To make the application of AMF inoculum more viable in agriculture, a large-scale production of efficient and infective AMF inoculum is important, using a low-cost production and an easily accessible material (Coelho et al., 2014; Liu & Yang, 2008). The inoculum being most widely used is a sand/soil-based production system in pot cultures. It is considered as a cost-effective way to mass produce AMF inoculum for large-scale applications (IJdo et al., 2011; Liu & Yang, 2008; Selvakumar et al., 2018). The inoculum is mostly produced in greenhouses, where the conditions can be controlled in single pots (IJdo et al., 2011; Liu & Yang, 2008). One problem of pot cultures is to avoid contamination with pathogens (Liu & Yang, 2008), whereby the controlled conditions such as lighting, temperature and water availability make it possible to improve the relationship between AMF and host plant (Liu & Yang, 2008). The substrate based inoculum is often not directly usable for mechanical application in agriculture compared to other production methods (IJdo et al., 2011).

However, different systems to produce AMF inoculum were developed, such as pot culture, hydroponic culture and aeroponic culture, as well as in vitro culture systems (IJdo et al., 2011; Liu & Yang, 2008). Furthermore, few commercial viable methods to produce AMF inoculum has been developed that meets the strict symbiotic conditions and aseptic environment maintenance required for AMF cultivation, due to their obligatory biotrophic nature (Liu & Yang, 2008; Selvakumar et al., 2018). Culture systems of hydroponics and aeroponics are difficult to cultivate for producers and the quality of the AMF inoculum is challenging to monitor (Liu & Yang, 2008).

Currently the inoculum at Agroscope Reckenholz is produced in a sand/soil mixture which has a high AMF colonization but is required in large amounts for field applications and is therefore not practicable (Bender et al., 2019). However, it has rarely been addressed in the literature if substrate-based inoculum can be reliably produced using substrates other than sand/soil mixtures. Moreover, strategies to increase the concentration of AMF in the inoculum are needed to make it more suitable for agricultural applications.

2.1. Substrates

Different substrates have been tested for large-scale inoculum production, such as sandy soil (Douds & Schenck, 1990) and also less dense materials such as perlite, vermiculite and peat (Douds et al., 2006; Lee & George, 2005). These less-dense substrates, like vermiculite and perlite were also tested to dilute the sand-based substrate (Douds et al., 2005, 2006). The used substrate should have enough nutrients for successful AMF establishment, in case of absence compost or other organic substrates can be added or nutrient solution was used for plant breeding and faster plant growth (Gaur & Adholeya, 2002; Lee & George, 2005). Furthermore, the availability of phosphor and the pH of the substrate influences the

establishment of AMF (Aarle et al., 2002; Köhl et al., 2016; Liu & Yang, 2008; Oehl et al., 2010). In addition, the particle size of the used substrate is important for adequate watering of the plants, appropriate humidity and aeration of the root system, to enhance the sporulation of AMF (Gaur & Adholeya, 2002; IJdo et al., 2011). The used substrates are generally pre-treated, such as heat sterilization to avoid contamination e.g. by plant pathogens (Douds et al., 2005, 2006; Gaur & Adholeya, 2002).

2.2. Differences of host plants for AMF inoculum production

The effect of the host plants depends on the introduced fungal species (Feldmann et al., 2009; Liu & Yang, 2008). However it depends on the host and the fungus genotype as well as the environmental conditions how AMF is developing (Azcon & Ocampo, 1981; Feldmann et al., 2009; van der Heijden et al., 2015). Under ideal growing conditions, the chosen host plant should be susceptible to AMF (Feldmann et al., 2009). However, if the host plants are water stressed, it can lead to a higher colonization. Furthermore, it should be considered, that the plants have enough space in the pots for a homogenous root growth (Feldmann et al., 2009). Lastly, host-dependent sporulation is a key element that must be determined in order to produce inoculum (IJdo et al., 2011). However, in some cases the spore number can even decrease after successive propagation cycle (IJdo et al., 2011; INVAM, 2019)

Plants such as onion, leek (*Allium* spp.), maize (*Zea mais*), plantago (*Plantago lanceolata L.)* and sudan grass (*Sorghum Sudanese*) are commonly used for mass production of AMF inoculum (IJdo et al., 2011; INVAM, 2019). These plants offer a short life cycle, good colonization level by most of the AMF species, and an adequate root system (IJdo et al., 2011). Fast rooting of a plant species is important because it promotes the production of mycorrhizal roots and optimizes colonization (Liu & Yang, 2008). Currently at Agroscope, AMF inoculum is produced with plantago since this plant is strongly colonized by AMF. However, maize has a shorter life cycle as other plants such as plantago (IJdo et al., 2011).

However, it is still unclear how to predict which fungal species colonizes faster on the given host plant, without practical experience. A lot of new information on the limiting factors of host growth is necessary to make a prediction (Feldmann et al., 2009). Due to the complexity of the factors influencing AMF development, several uncertainties occur how AMF inoculum can be produced in high quality.

2.3. Applicability of AMF inoculum in agriculture

For agricultural use an inoculum should comply several requirements that the inoculation of AMF can be easily integrated into the workflow by the farmer. It is important that the substrate which is colonized by AMF could be applied with a standardized farming machine. The particles of the substrates should be colonized strongly with AMF for smaller needed quantities (Douds et al., 2005). The optimal char-

acteristics of a substrate for a seeding machine are lightweight, homogeneity and no sharp edges. Lightweight is important for smaller quantities to distribute on the fields. However, the substrate should not be too light because then problems with the seeding machine occur. In addition, homogeneity ensures an equal distribution of the inoculum in the field. It is important that the substrate has no sharp edges as otherwise the substrate could damage the seeding machine. However, it is still unclear which substrates can be colonized fast and conform to all the requirements for agricultural applicability.

3. Objectives and hypothesis

The objective of this thesis was to test, whether the quality and applicability of AMF inoculum can be improved by adapting the conditions under which AMF are propagated. This thesis aimed to answer following research questions:

- Is there a difference between shallower and deeper containers in terms of AMF root colonization and spore concentration?
- Can the inoculum concentration be increased, and can inoculum be produced faster using maize as host plant instead of plantago?
- Which substrate with potentially beneficial traits for agricultural application, such as lightweight, homogeneity, and no sharp edges results in the highest AMF concentration?
- How well is sudan grass suited as host plant for inoculum production compared to maize?
- Does the effect of the host plant on AMF differ depending on the substrate used?

Based on literature presented above, it is hypothesized that by using more shallow containers for inoculum production, the average concentration of AMF inoculum can be enhanced. Furthermore, it is assumed that the inoculum can be produced faster with maize than with plantago because of its shorter life cycle. Furthermore, it is assumed that other homogenous, low-density substrates than the currently used sand-soil mixture can be used to obtain a less dense and more homogenous material for AMF inoculum. In addition, it is hypothesized that sudan grass has the faster AMF establishment than maize.

To answer these research questions two different experiments were conducted. In the first experiment the different heights of containers and the influence of the host plants maize and plantago were compared on AMF establishment. The second experiment compared 8 different substrates and the host plant maize and sudan grass on the different concentrations of AMF.

4. Material and methods

4.1. Set-up of the AMF inoculum trials in the greenhouse

The thesis consists of two experiments to determine the effect of various factors on the establishment of AMF with the aim to optimize AMF inoculum production. All experiments of this project used the AMF *R. irregulare*.

4.1.1. Experiment 1

The first experiment focused on the vertical distribution of AMF in soil substrate and on the influence of the host plant. For this purpose, *R. irregulare* was propagated in the greenhouse using two different container types. The fist container type consisted of regular planting pots with a volume of 5 L and a height of 25 cm. The second container type consisted of boxes with a similar volume and a height of 10cm. All containers were filled with an autoclaved (129°C, 90 min) mixture (v/v) of soil (15%), sand (65%) and oil binder (20%). This mixture was called *"Agroscope mixture"* in this thesis. The soil used in the mixture was collected from a grassland site near the research station Agroscope Reckenholz (47.42741, 8.51780) and sieved with 5 mm mesh width. The autoclaved substrate was stored for 3 weeks before use. As host plants maize (*Zea mais*, Gottardo KWS) and plantago *(Plantago lanceolata L.)* were used. One half of the pots was inoculated with 5% (v/v) AMF inoculum and the other half received a similar amount of a control inoculum not containing AMF (see details below). The experiment was conducted during 3 months of plant growth. The experiment consisted of 8 treatment combinations (2 AMF x 2 container types x 2 host plants) with 7 replicates, resulting in a total of 56 experimental units.

4.1.2. Experiment 2

The second experiment compared 8 different substrates and 2 different host plants. The different substrates used were the same autoclaved Agroscope mixture used in Experiment 1 (soil (15% v/v), sand (65%) and oil binder (20%)), pure sand (< 1mm), broken lava stones (2-4 mm), porlith (2-7 mm), nutshells (0.8-1.3 mm), vermiculite (< 2mm), fine pumice stone (0.5-1 mm) and coarse pumice stone (2.5-3.5 mm). The broken lava stones are solidified magma which is crushed into 2-4 mm grain size. It is used for covering and mulching planting areas or for improving substrates and soils (Terre Suisse, Switzerland). Porlith is used for the enhancement of soils and substrates (Terre Suisse, Switzerland). It is a mineral product from oil exploitation deposited in a crater lake of volcanic origin. The material was heated during the exploitation process, resulting in a mixture of porous lava-type material, and melted red claystone residue. This production process is comparable to the ones of bricks (Flores-Ramírez et al., 2018). The substrate "nutshells" contains broken nutshells and olive and apricot kernel. They were cleaned and milled. Nutshells are commonly used as blasting media (Carlo AG, Switzerland). Pumice is a solidified lava with a low density that is formed by the rapid cooling process after a volcanic eruption. It has a low density because of the subsequent depressurization and expansion after the volcanic eruption (Flores-Ramírez et al., 2018). Vermiculite is a lightweight mineral which is often used to dilute

nutrient rich soil or substrates for inoculum production (Douds et al., 2006; IJdo et al., 2011). However, in this experiment pure vermiculite is used. In Table 1 is an overview of the desired characteristics for agricultural application and how they are met by the different used substrates.

Table 1 Overview about the used substrates regarding the 3 most important beneficial characteristics for agricultural application of AMF inoculum: lightweight, no sharp edges and homogeneity.

	lightweight	**not sharped-edged**	**homogeneous**
Agroscope mixture	-	-	-
Coarse pumice stone	+	-	+
Fine pumice stone	+	+	+
Lava	-	-	+
Nutshells	-	+	+
Porlith	-	+	+
Sand	-	+	+
Vermiculite	+	+	+

Beside the physical traits the chemical properties, potassium (K), phosphor (P) and pH are shown in Table 2. The most substrates expect the nutshells and the fine pumice stone had a low potassium value. The available phosphorous values are closer together, whereby the coarse pumice and the vermiculite showed the highest values. However, the difference of the fine and coarse pumice stone of the chemical properties is remarkable because the only difference here was the grain size.

Table 2 Overview about the potassium, phosphor, and pH values for each substrate.

	Potassium (K) [mg K/kg soil]	**Phosphor (P)** [mg P/kg soil]	**pH**
Agroscope mixture	1.86	3.7	8.41
Coarse Pumice	3.46	30.4	9.25
Fine pumice	403.15	23.9	11.21
Nutshells	210.41	20.3	4.64
Porlith	0.3	4.1	7.21
Lava	5.02	3.5	8.84
Sand	0.42	3.5	9.47
Vermiculite	1.3	39.3	9.78

All substrates were autoclaved and stored for 3 weeks before use. Substrates were filled in 3 litre planting boxes with a height of 4,5cm with the addition of 5% (v/v) AMF inoculum. On each substrate, two different host plants were planted: maize (*Zea mais*, Gottardo KWS) and sudan grass (*Sorghum Sudanese*). The experiment consisted of 16 different treatment combinations (8 substrates x 2 host plants) being replicated 6 times, resulting in a total of 96 experimental units.

4.2. Inoculum production

For both experiments *Rhizoglomus irregulare* was used, because it showed a beneficial effect in previous experiments and it is a common AMF with a worldwide distribution (Bender et al., 2019; Köhl et al., 2016). Furthermore, *R. irregulare* is abundant in a wide range of ecosystems, such as agricultural fields in Switzerland and can be introduced successfully and established under a range of different soil parameters such as pH 5.6-8.0, P availability 0.3-18.8 mg/kg, sand content 17.5-57.0% and humus content 1.0-10.5% (Köhl et al., 2016; Oehl et al., 2010).

The inoculum of *R. irregulare* was produced in a greenhouse at Agroscope Reckenholz in an autoclaved mixture (v/v) of soil (15%), sand (65%) and oil binder (20%) and with addition of AMF inoculum (5%). It was produced with plantago (*Plantago lanceolata L.)* as host plant in 7-liter pots. The pots were watered regularly. After 5 months of plant growth the pots were dried out. The shoots of plantago were removed and the roots were cut into pieces of max. 5 cm. The roots and the content of the pots were mixed. The control inoculum was produced similarly to the AMF inoculum but without AMF being added (Bender et al., 2019).

4.3. Planting and cultivation

4.3.1. Experiment 1

The first experiment was conducted in a greenhouse at Agroscope Reckenholz. The autoclaved substrate was filled in 5-liter container and inoculum (5% v/v) or control inoculum (5% v/v) was added The measured amount of inoculum was mixed in boxes with the right quantity of substrate and filled into the different containers.

In order to have conditions similar to previous inoculum production experiments, maize (*Zea mais*, Gottardo KWS) and plantago *(Plantago lanceolata L.)* was chosen for the experiments. The seeds were sterilized with sodium hypochlorite for 10 minutes, washed with distilled water and 9 seeds for each container directly planted around 1 cm below the surface. Since not all seeds had germinated, seeds were added, so that in each pot 9 maize plants grew.

The pots were watered regularly, so that the water content was 15% (m/m) of the dry substance. To reduce the effect of potential light and temperature differences between the pots in the greenhouse, they were randomized weekly. The pots were fertilized once after 45 days of cultivation with 20 ml of a modified Hoagland solution (Hoagland & Arnon, 1950) with ¼ of the normal P content. The N content was increased by adding NH_4NO_3 1 M corresponding to the addition of fertilizer of 15 kg N/ha. The aim of the fertilization was to have sufficient nutrients for plant growth but to create low P and sufficient N conditions to increase the colonization of AMF. After 3 months of growth, watering was ceased, and the pots were dried out to increase sporulation of the AMF (Selvakumar et al., 2018).

4.3.2. Experiment 2

The second experiment was conducted at Agroscope Wädenswil with an automated watering system by flood tables. The different substrates were filled into 3 litre plant boxes with a height of 4,5cm with the addition of 5% (v/v) AMF inoculum. The inoculum was added same as in the first experiment. In each substrate 12 sterilized seeds of maize (*Zea mais*, Gottardo KWS) or sudan grass (*Sorghum Sudanese*) were planted 0,5 cm below the surface. The boxes were watered once a day by flooding the tables. After three weeks of plant growth, the plants were fertilized daily with a commercial fertilizer with a 15 % stock solution. The fertilizer was a mixture of 50% Kristalon Azur containing 7,3 % NO_3, 12,7% NH_4, 5% P_2O_5, 10 % K_2O and 2% MgO and 50 % Krista-MKP containing 52% P_2O_5 and 34 % K_2O (YARA GmbH & Co. KG, Germany). The fertilizer should supply the plants with sufficient nutrients and increase the AMF colonization. Because an automated watering system was used, it was not possible to use a Hoagland solution (Hoagland & Arnon, 1950) as before. After 7 weeks of growth, watering was ceased, and the plant boxes were dried out.

4.3.3. Harvest

After drying out the pots, the plants were harvested. The plants were cut at the soil surface and shoots were stored for plant biomass analysis. The pots were emptied, and the roots were cut in pieces (< 5 cm) and mixed with the substrate (Bender et al., 2019). A representative part of the roots was taken for the analysis of AMF root colonization and stored in 50 % ethanol. In addition, 25g (Experiment 1) or 30 cm^3 (Experiment 2) of the mixed substrate of each pot was taken to quantify the spore number (Oehl et al., 2003).

4.4. Analysis

4.4.1. Spore number

The AMF spores of the pots were extracted from the substrate by wet sieving and sucrose density gradient centrifugation (Oehl et al., 2005; Säle et al., 2015). The procedure included passing 25 g or 30 cm^3, respectively of harvested, air-dried substrate through a set of sieves with 1000, 500, 125 and 32 µm mesh width. The leftovers from the 125 and 32 µm mesh were pooled into a 50ml falcon tube. The material was settled for 1 hour and then mixed with a 70% water-sucrose solution. The falcon tubes were centrifuged at 2000 rpm for 2 minutes. The supernatant solution was poured onto the 32 µm sieve, washed again with tap water and rinsed into a petri dish (Oehl et al., 2003). The spores were counted at a magnification of 16x under the binocular. The spore concentration was calculated with the respective weight or volume of the used soil sample.

4.4.2. AMF root colonization

The AMF root colonization was assessed after the end of the plant growth and after the pots were dried out. The fine roots of the plants were rinsed with distilled water and cleared with 10% KOH in an 80° water bath. The cleared roots got stained with a 5% (v/v) ink vinegar mixture and the roots were stored in a 50% glycerol-water mixture in the tubes. The fungal structures (hyphae, arbuscules and vesicles) were analysed under a microscope (Vierheilig et al., 1998). For quantification of AMF root colonization, the stained roots were aligned parallel on the microscopy slide. The colonization was analysed with a magnification of x200 and a modified line-intersection method for 100 intersections was used to obtain a representative number (McGonigle et al., 1990).

4.4.3. Plant biomass

Plants shoots were dried at 60°C for 48 hours and the dry weight of above-ground plant biomass was noted. The biomass of the inoculated plants was compared to the relative mean of the corresponding control plants to get information of the increase in biomass due to the presence of AMF (Köhl et al., 2016).

4.4.4. Nutrient analysis and pH

Nutrient analyses of the substrates were conducted by the lab of the research group environmental analytics based on the Swiss reference methods (FAL, RAC, FAW. 1996) of the federal agricultural research stations. Potassium was extracted with CO_2-saturated water, where potassium was captured which is easily soluble and immediately available to the plants. The extracted potassium from the soil was determined with flame emission (Atomic absorption spectrometer F-AAS AA 240 FS from Varian) at a wavelength of 769,9 nm.

To determine the plant-available phosphorous, the phosphate extracted with the same extraction method was transformed with ammonium molybdate, in an acid solution, into phosphor molybdenum blue. The resulting blue colour was than analysed photometric at a wavelength of 750 nm.

Substrate pH was measured after mixing the substrate with deionized water using a calibrated electrode. The grain size of the substrate for all three analyses should be smaller than 2 mm, which was not given for porlith, lava and coarse pumice.

4.5. Statistical analysis

The data was prepared in Microsoft Office Excel and analysed in R Studio 1.3.1093. First the data was checked on normal distribution with a QQ-Plot and the Shapiro-wilks test. Furthermore, the distribution of random residuals of the ANOVA was considered. In case of a large deviation of the normal distribution the data was log-transformed. A linear model (lm-function) was created for the data and analysed in a two-way ANOVA. If the ANOVA showed a significant result, a pairwise post-hoc analysis was

conducted using the TukeyHSD function. Correlations were calculated with Spearman's rank correlation if the data was not normal distributed. If the data was normally distributed the Pearson correlation coefficient was used. All statistical tests were done with a significance level of $p=0.05$.

5. Results

5.1. Experiment 1

5.1.1. Above ground biomass

The weight of the above-ground biomass was compared separately for each plant species, because the above-ground biomass of maize was much larger than of plantago. The maize plants showed a significant difference ($p<0.05$) in the weight of above-ground biomass between the two different containers. The maize plants in the box had a mean of 37,529 ± 3,431 whereas plants in the pot had a higher biomass 40,616 ± 3,312. However, it is remarkable that the non-inoculated plants of maize in the pots tended to have a greater above-ground biomass than the inoculated plants. The inoculated plants of maize in the box had the lowest above-ground biomass with a mean of 37,051 ± 2,691, this treatment had some outliers with a lower biomass.

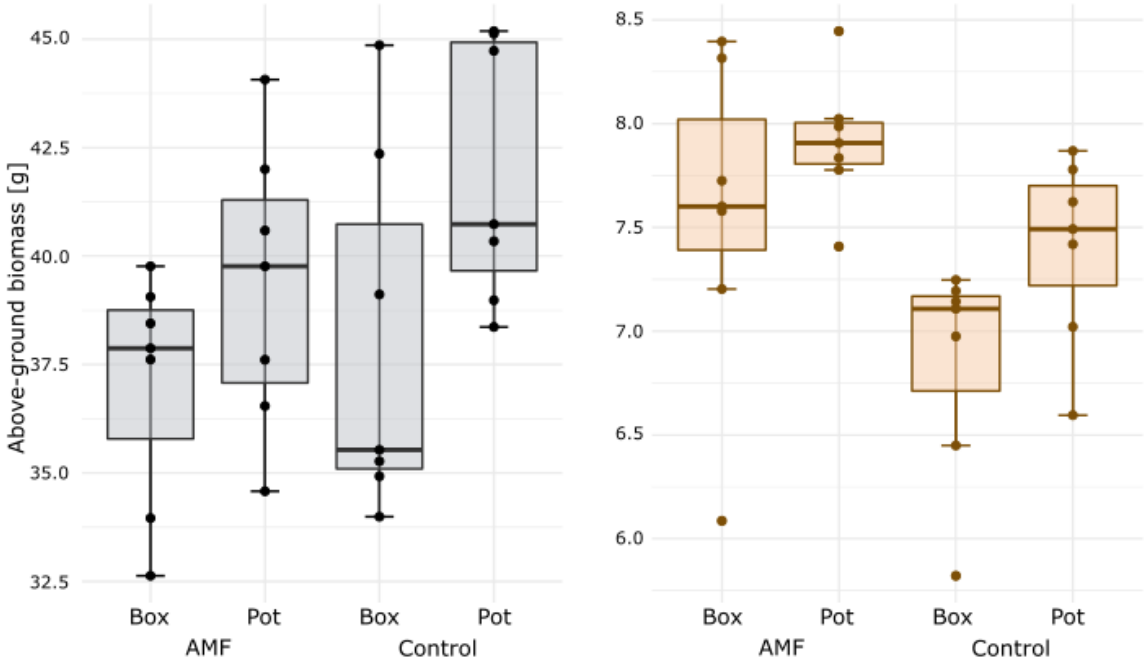

Figure 1 Above-ground biomass of the inoculated and control plants of the first experiment. The maize plants are shown in grey and the plantago in brown. There was found a significant effect for plantago and maize between the containers. The box is showing 50 % of the data with the median as line and the whiskers represent 1,5 x of the interquartile range. The points outside the whiskers are outliers.

Plantago had a significant difference in above-ground biomass ($p <0.05$) where the pots indicated a higher biomass than the boxes. The difference between the pots and the boxes for plantago was small with a mean of 7,203 ± 0,708 for the boxes and a mean of 7,655 ± 0,457 for the pots. Moreover, there was a significant difference for the treatments with plantago between the inoculated and the control plants ($p<0.01$). The control plants had a lower mean of 7,124 ± 0,551 than the inoculated plants with a mean of 7,735 ± 0,596. However, the control in the box of both, maize and plantago had the lowest biomass.

Table 3 Summary of ANOVA for the above-ground biomass as response variable separated after maize and plantago for the first experiment.

	Df	F-value	Pr(>F)	Sig
Above-ground biomass maize				
Container	1	6.102	0.021	*
Inoculum	1	2.044	0.165	
Above-ground biomass plantago				
Container	1	5.033	0.034	*
Inoculum	1	9.167	0.006	**

.Signif. codes: ='***',0.001'**, 0.01 '*',0.05'.', 0.1' '1.

5.1.2. AMF root colonization

The AMF root colonization was compared only for the inoculated plants, whereby the control plants were left out of any statistical analysis because there was no colonization on them. The AMF root colonization ranged between 87 % and 100 % for all samples.

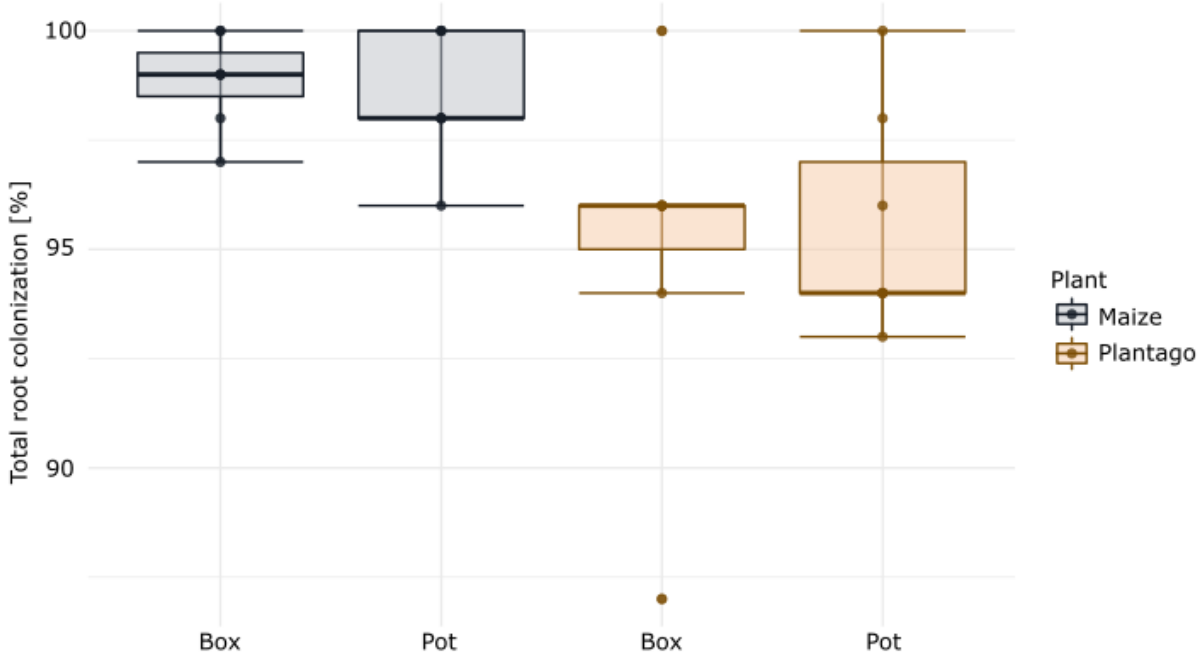

Figure 2 AMF root colonization of the inoculated plants of experiment 1. The maize plants are shown in grey and the plantago in brown. There was shown a significant difference between the plants in root colonization ($p<0.01$) but no difference between boxes or pots ($p = 0.88$). The box is showing 50 % of the data with the median as line and the whiskers represent 1,5 x of the interquartile range. The points outside the whiskers are outliers.

There was not found any significant effect between the boxes or pots in AMF root colonization. However, the difference between the plants was significant ($p<0.01$) with a mean of 98,714 ± 1,267 for maize and a mean of 95,286 ± 3,221 for plantago. Furthermore, it has to be considered that AMF colonization of the samples with plantago showed higher variance.

Table 4 Summary of ANOVA for the AMF root colonization as response variable for experiment 1.

	Df	F-value	Pr(>F)	Sig
Colonization				
Container	1	0.023	0.881	
Plant	1	13.223	0.001	**

*.Signif. codes: ='***',0.001'**, 0.01 '*',0.05'.', 0.1' '1.*

In Figure 3 the mean AMF colonization type for the different treatments is shown. The maize showed a higher mean colonization of total vesicles (70,1 %) and had more total vesicles than plantago (33 %). The treatments with maize showed also higher total arbuscules (71,4 %) than plantago (65 %). However, plantago had more single arbuscles (38,9 %) than maize (21,4 %).

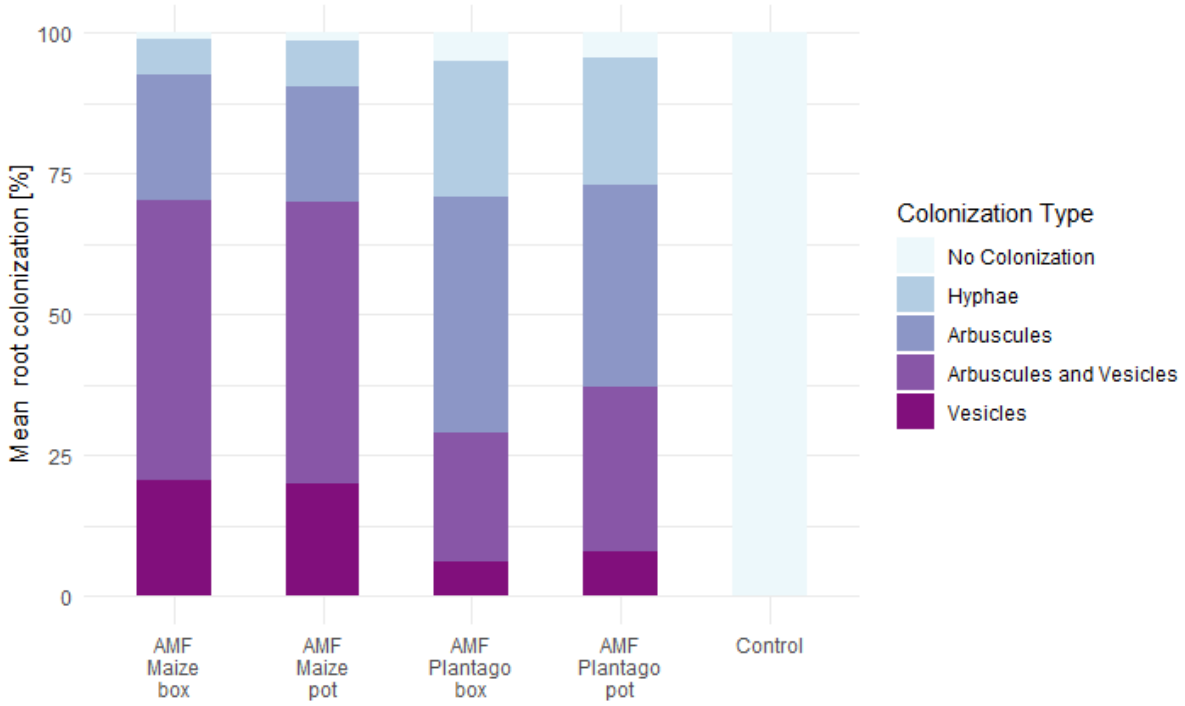

Figure 3 Mean AMF root colonization for Experiment 1 of the different experimental designs after the different colonization types: No colonization, only Hyphae, Vesicles, Arbuscules and Arbuscules and Vesicles. The control plants were summarized into one single group.

5.1.3. Spore number

Similar as for the AMF root colonization, the control plants were excluded for any statistical test to receive a better distribution of the data. The number of spores was analysed per gram of the used substrate.

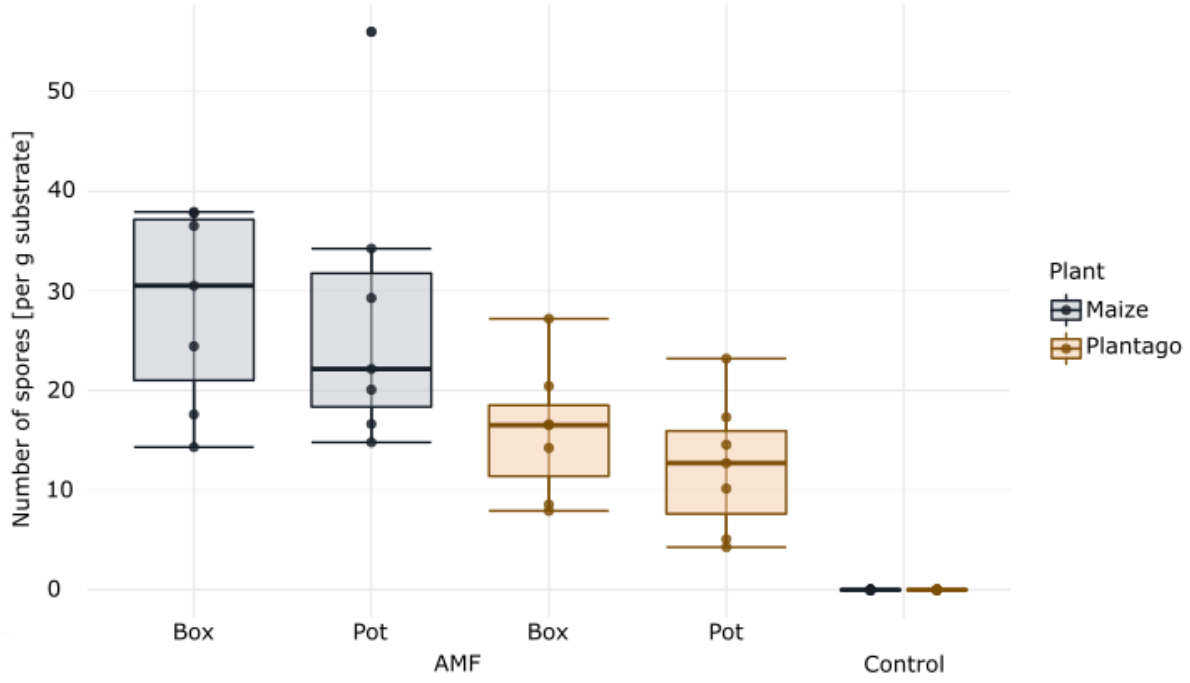

Figure 4 Number of spores per g substrate of the different experimental designs for the first experiment. The maize plants are shown in grey and the plantago in brown. The control plants were summarized into one single group because they had all no spores in the samples. The box is showing 50 % of the data with the median as line and the whiskers represent 1,5 x of the interquartile range. The points outside the whiskers are outliers.

There was no significant difference shown in spore number between the boxes or pots ($p > 0.05$). Whereas there was a significant difference ($p<0.001$) between the plants. The treatment with maize had a significantly higher spore number (28,023 ± 11,787) than the one with plantago (14,2 ± 6,687). However, it should be noted that the standard deviation for maize plants was higher than for plantago.

Table 5 Summary of ANOVA for the spore number per g substrate as response variable for experiment 1.

	Df	F-value	Pr(>F)	Sig
Spore number				
Container	1	5.033	0.563	
Plant	1	9.167	<0.001	***

*.Signif. codes: ='***',0.001'**, 0.01 '*',0.05'.', 0.1'. '1*

The total AMF root colonization and the spores had a positive correlation ($p<0.001$) after calculating the Pearson correlation coefficient (Figure 5). A higher root colonization led to a significantly higher spore number, whereas maize had the higher colonization and more spores than plantago.

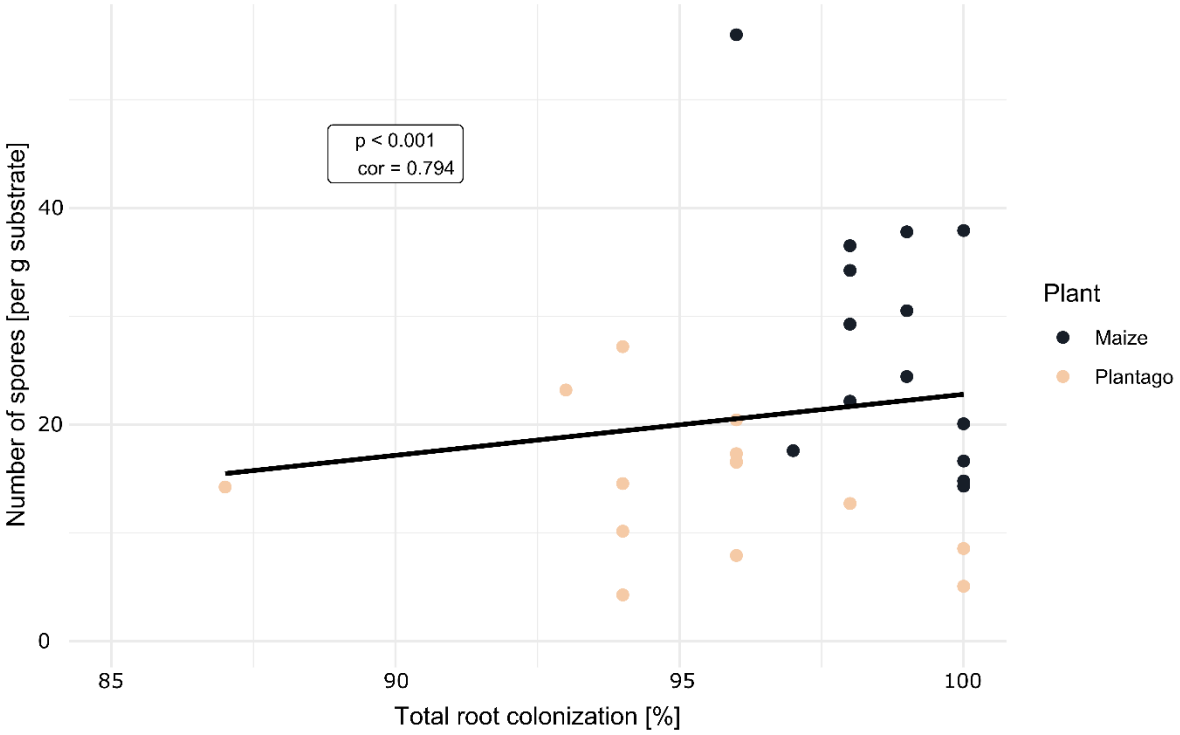

Figure 5 Relationship of the number of spores per gram substrate and the total AMF root colonization of the first experiment with a linear regression. Pearson correlation coefficient was performed. The correlation coefficient and the corresponding significance level is shown in the graph. Maize is shown in black and plantago in brown.

5.2. Experiment 2

5.2.1. Above-ground biomass

The weight of the above-ground biomass was statistically compared separately for the two plant species to obtain a better distribution of the data. The maize plants grew faster than the sudan grass and reached therefore the greater biomass. Maize and sudan grass had problems to germinate in sand. In addition, sudan grass did not grow in the fine pumice and nutshells.

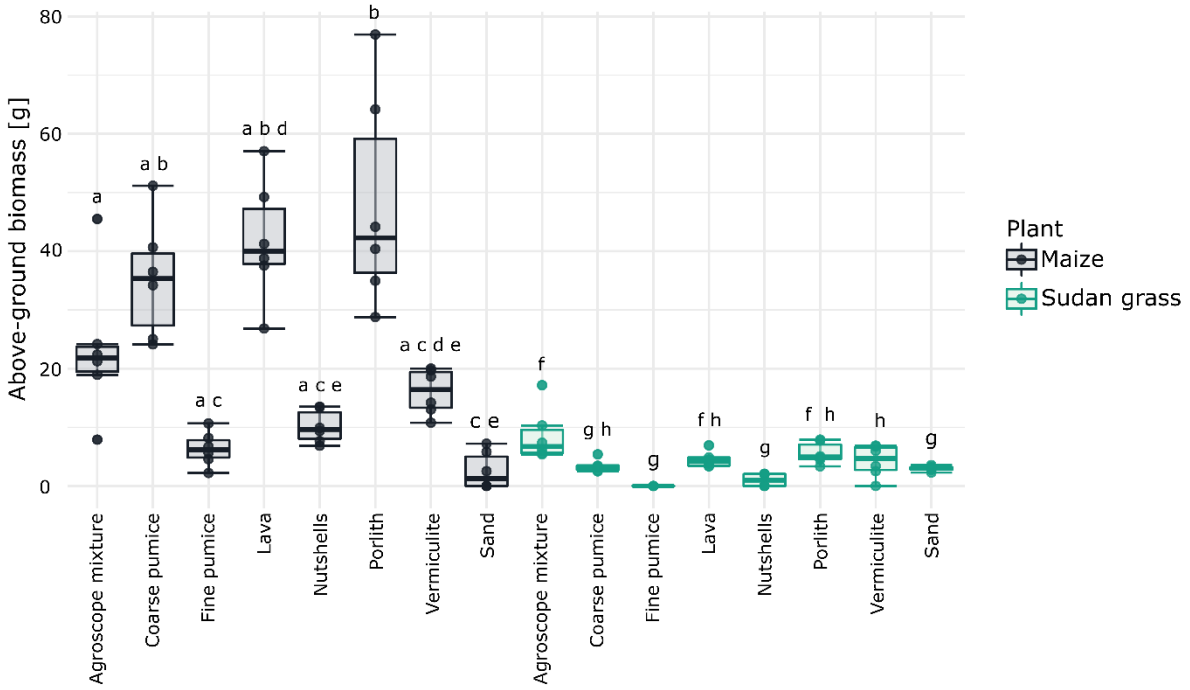

Figure 6 Above-ground biomass for maize and sudan grass for the different substrates of experiment 2. Maize is shown in grey and sudan grass in green. The letter above the box is indicating if there is a significant difference between the substrates using TukeyHSD tests. Boxplots with the same letter does not differ significantly from each other with a level of significance < 0.05. The box is showing 50 % of the data with the median as line and the whiskers represent 1,5 x of the interquartile range. The points outside the whiskers are outliers.

Maize had a significant difference ($p < 0.001$) between the different substrates for the above-ground biomass. However, the maize plants in the coarse pumice stone, lava and porlith indicated the highest biomass. The plants in the Agroscope mixture reached a high above-ground biomass. The substrates showing a high biomass, had also a higher variance within the data, such as coarse pumice stone, porlith and lava compared to the samples with a low biomass showing a smaller variance. In Figure 6, the significant differences of the above-ground biomass are shown using a post-hoc test.

Table 6 Summary of ANOVA for the above-ground biomass as response variable for experiment 2.

	Df	F-value	Pr(>F)	Sig
Above-ground biomass maize				
Substrate	7	18.844	< 0.001	***
Above-ground biomass sudan grass				
Substrate	7	9.529	< 0.001	***

*.Signif. codes: ='***',0.001'**, 0.01 '*',0.05'.', 0.1'. '1*

Similar as maize, sudan grass was showing a significant difference ($p<0.001$). The Agroscope mixture showed the highest biomass (8,637 ± 4,564) but also the standard deviation was high. The other substrates had a smaller standard deviation. Lava, porlith and vermiculite had a greater biomass for plantago compared to the other samples.

5.2.2. AMF root colonization

The AMF root colonization was tested for differences between maize and sudan grass and the different substrates. There was no colonization measured for fine pumice and the nutshells with sudan grass as host plant because there was not enough below-ground biomass. There was a significant difference ($p < 0.001$) between the two plants whereby maize showed the higher mean colonization (42,17 ± 28,09) than sudan grass (17,45 ± 10,85). In addition, the AMF root colonization was strongly depended on the substrate.

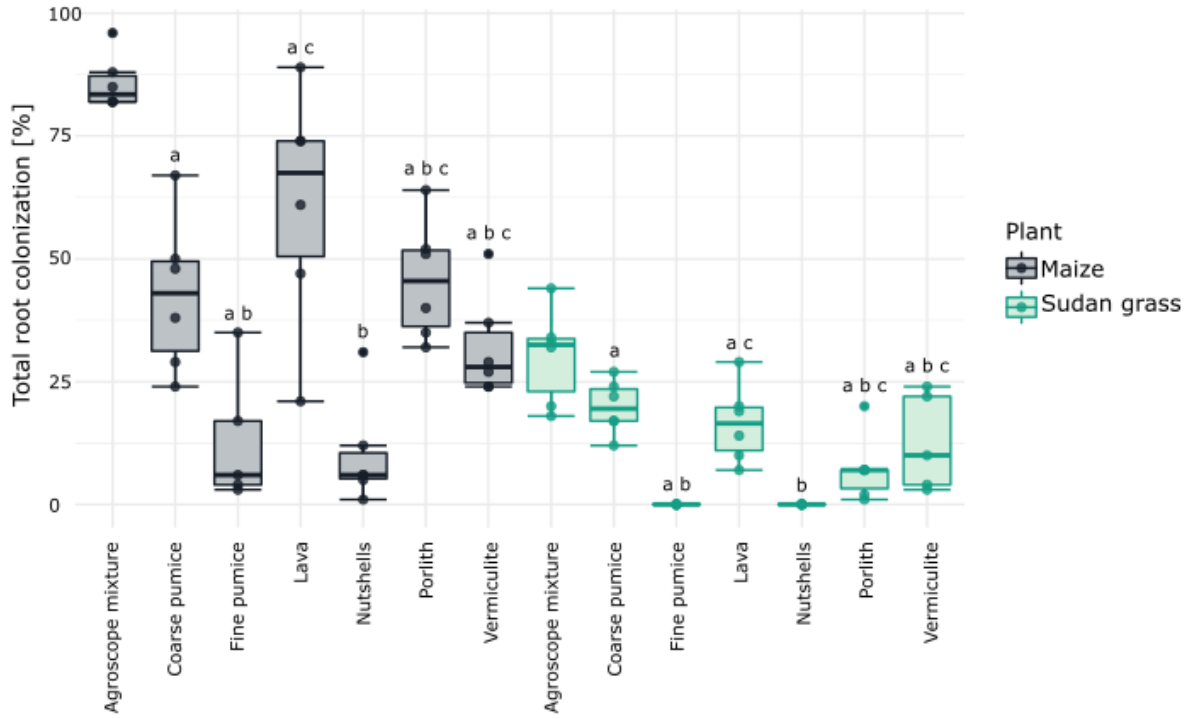

Figure 7 AMF root colonization for the different substrates and plants of experiment 2. Maize is shown in grey and sudan grass in green. The letter above the box is indicating if there is a significant difference between the substrates using TukeyHSD tests. Boxplots with the same letter does not differ significantly from each other with a level of significance < 0.05. The box is showing 50 % of the data with the median as line and the whiskers represent 1,5 x of the interquartile range. The points outside the whiskers are outliers.

The difference between the substrate was significant ($p<0.001$). The Agroscope mixture had the highest AMF root colonization, for both host plants. The colonization of the maize plants in lava and coarse pumice had a high variance. The nutshells and the fine pumice had a low colonization for the maize plants. Apart from the Agroscope mixture, the pots with sudan grass showed the best colonization for coarse pumice stone, lava, and vermiculite. Remarkable is that the porlith had a good colonization for maize but the colonization with sudan grass was low.

Table 7 Summary of ANOVA for the total root colonization as response variable of experiment 2.

	Df	F-value	Pr (>F)	Sig
Colonization				
Substrate	6	13.52	< 0.001	***
Plant	1	109.17	< 0.001	***

*.Signif. codes: ='***',0.001'**, 0.01 '*',0.05'.', 0.1'. '1*

In Figure 7, the significant differences between the substrates are shown. The Agroscope mixture with the highest colonization showed a significant difference in colonization from all other substrates used. Furthermore, there was a significant difference between the nutshells and the coarse pumice as well as between the nutshells and lava. The nutshells had the lowest colonization (Figure 7) which explains the difference.

5.2.3. Spore number

The spore number of the different substrates was widely spread. There were no significant differences between the plants nor the substrates. For the ANOVA log-transformed data was used to obtain a better distribution of the data. In addition, the number of spores was analysed for 1 ml substrate because the substrates varied strongly in density. Therefore, an indication after volume is more comparable.

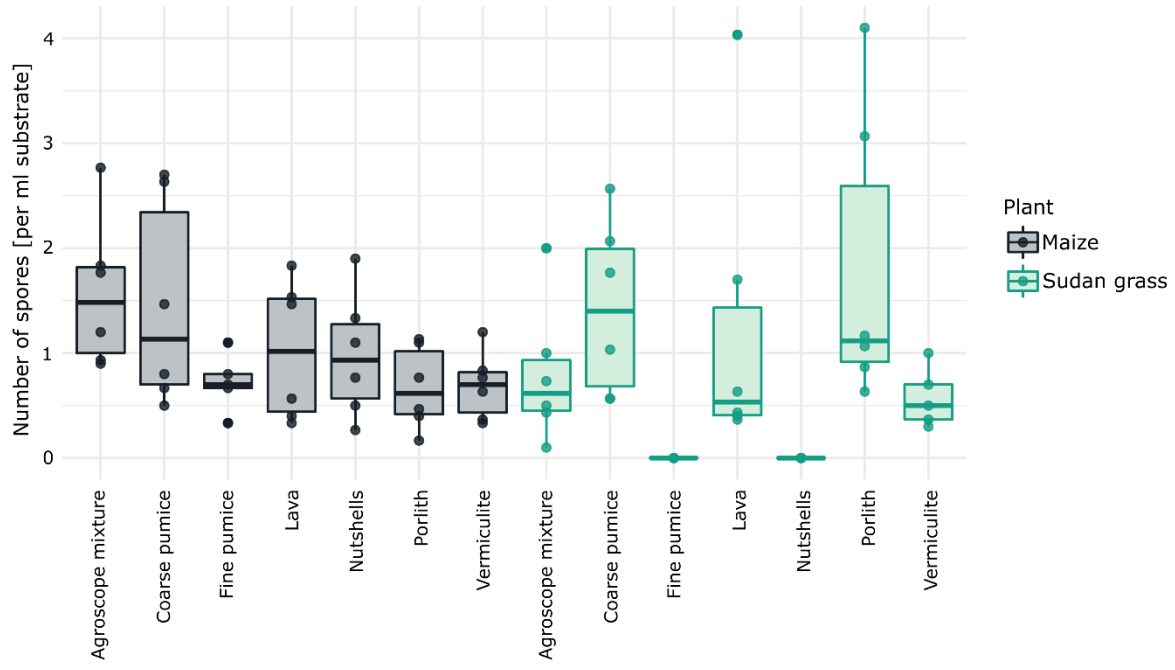

Figure 8 Spore number per ml substrate for the different substrates and plants of experiment 2. Maize is shown in grey and sudan grass in green. The box is showing 50 % of the data with the median as line and the whiskers represent 1,5 x of the interquartile range. The points outside the whiskers are outliers.

The mean spore number of sudan grass 1,20 ± 1,07 was higher than of maize 1,02 ± 0,67. It has to be considered that the standard deviation for both plants was high which is also visible in Figure 8. Sudan grass has also two outliers for the Agroscope mixture and lava as well as the number of spores of porlith had a high variance.

Table 8 Summary of ANOVA for the log-transformed spore number as response variable for experiment 2.

	Df	F-value	Pr(>F)	Sig
Spore number log				
Substrate	6	1.030	0.415	
Plant	1	0.029	0.865	

*.Signif. codes: ='***',0.001'**, 0.01 '*',0.05'.', 0.1'. '1*

5.2.4. pH and phosphor of substrates

For each substrate, only one sample was analysed for pH, phosphorous and potassium (table 2). Therefore, the values should more be seen as approximation. Furthermore, the coarse pumice, porlith and lava used in the analysis were not of the correct grain size.

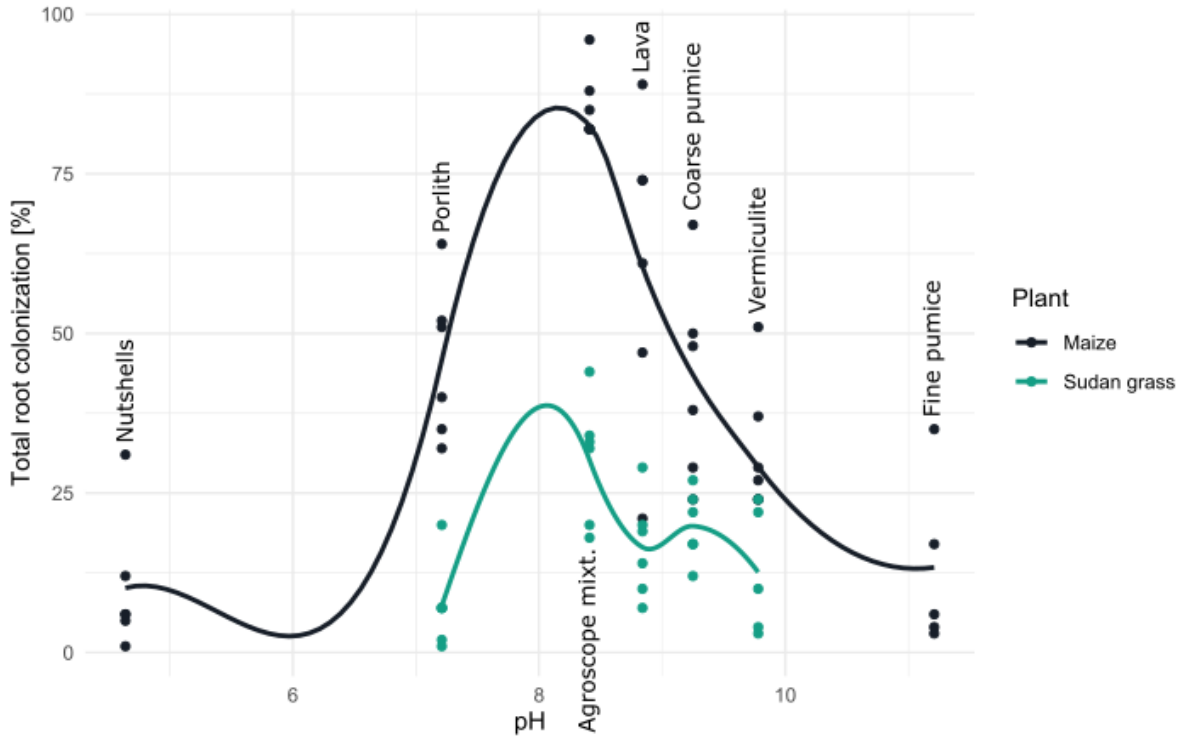

Figure 9 Relationship between total AMF root colonization and pH is represented for each substrate of experiment 2. The maize plants are shown in black and sudan grass in green.

Most of the pH of the substrates lied between 7 and 9.5. Whereas the fine pumice stone had the highest pH with 11.21. The nutshells were the only substrate with an acid pH. Regarding the relationship be-

tween pH and colonization, the colonization increased with an increasing pH until a maximal colonization for the Agroscope mixture. At a pH of 8, colonization was the highest, as shown in the Figure 9. After a pH of 8, the colonization decreased again with an increasing pH. However, there was only a colonization for maize as host plant for these two substrates with the highest pH (fine pumice) and the lowest pH (nutshells) since sudan grass did not yield enough below ground biomass for analysis.

The total root colonization and the phosphor content showed a significant correlation ($p = 0.038$) using Spearmen's rank correlation (Figure 10). With increasing phosphor content, the AMF colonization decreased. The maize plants with a low phosphor content had the higher colonization than sudan grass.

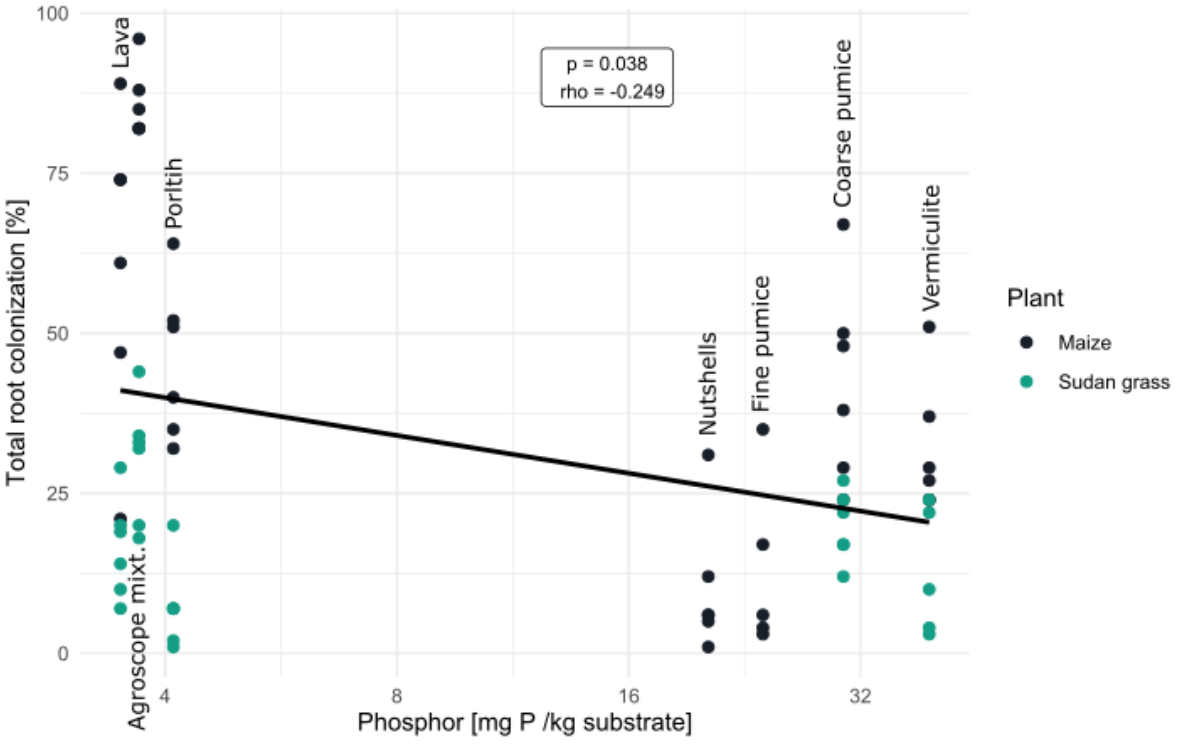

Figure 10 Relationship between AMF root colonization as percentage of root length colonized at harvest and the chemical parameter phosphor of experiment 2 is shown. Non-parametric Spearmen's Rank correlation was performed. Spearmen's rho and the corresponding significance level is shown in the graph. The maize plants are shown in black and plantago in green.

6. Discussion

This thesis investigated several factors influencing AMF inoculum production. The main aim of this thesis was to increase the AMF root colonization and spore density in a shorter cultivation cycle and to receive a more suitable material to produce AMF inoculum than the currently used Agroscope mixture. For this purpose, I assessed different container depths as well as the influence of the host plant on AMF establishment, to produce a higher concentrated AMF inoculum. Furthermore, I identified how different substrates which are more suitable for agricultural application influences the establishment success of AMF.

There was found a significantly higher root colonization and spore density for maize than for plantago, whereas there was no difference between the soil depths. As expected, the control plants had no AMF colonization or spores. In addition, maize showed significantly higher root colonization than sudan grass. Furthermore, the substrates porlith, lava, coarse pumice and the Agroscope mixture showed a significantly higher root colonization than other tested substrates.

6.1. Experiment 1

The aim of the first Experiment was to identify if boxes with 10 cm height has a higher root colonization and spore number than pots with a height of 25 cm. Furthermore, maize was tested as host plant to produce the inoculum faster and with a higher AMF concentration than plantago.

There was a significant difference for the above-ground biomass between pots and boxes for maize as well as for plantago. In addition, plantago showed a reaction to the AMF in plant growth with an increased biomass for the inoculated plants. However, the maize plants showed no significant difference between the inoculated and control plants. The results of the above-ground biomass of plantago agreed with previous experiments done, were the above-ground biomass was bigger in inoculated plants (Rafique et al., 2020). However, for both plants the difference in gram was rather small between the control and inoculated plants and the outliers may falsified the result.

The maize plants had the higher spore density and better colonization than plantago. The difference in colonization could be because of the short cultivation cycle, the faster germination and faster biomass gain of maize compared to plantago (INVAM, 2019). However, the AMF species may have an influence on this too. There are different effects of the introduced AMF species on the different plants (Feldmann et al., 2009; Liu & Yang, 2008). Therefore, maize merely had the better effect on colonization and spore number for *R. irregulare* than plantago did. Additionally, the maize or plantago may had been stressed differently due to dissimilar water requirements. The water stress could even accelerated the colonization level of the plants (Feldmann et al., 2009). Furthermore, *R. irregulare* had the better sporulation for maize than for plantago in this first experiment, which is important to consider for inoculum production

(IJdo et al., 2011). It could be that the spore number decreased after successive propagation. In a study of Gryndler et al. (2003) it was reported that the spore number and the frequency of root colonization decreased after 11 months of cultivation. However, because the cultivation of the pots was only three months, no decrease of spore number is assumed (INVAM, 2019).

There was no difference between the containers in AMF colonization or spore density. This could be because the height difference between the boxes and the pots was only 15cm. The homogenous distribution of the starting inoculum, on the other hand, may have resulted in a more uniform distribution of AMF in the containers. However, the spore abundance was only examined in field soils, whereas in pot culture spore number may not differ (Oehl et al., 2005).

6.2. Experiment 2

The second experiment aimed to get a substrate for inoculum production which has the beneficial traits for agricultural application, such as lightweight, homogeneity and no sharp edges and is promoting the AMF establishment. Furthermore, the influence of the substrate and the differences in AMF root colonization and spore density depending on the host plant maize and sudan grass was tested.

The above-ground biomass of maize and sudan grass differed significantly depending on the substrate. For the treatments with sand, the plants grew only a little or did not germinate at all. Maybe the seeds where too far below the surface or the aeration was limited (Chen & Maun, 1999). In addition, sudan grass grew only a little or did not germinate in the fine pumice and the nutshells. However, the maize plants had no problem with growing in these two materials. The nutshells and the fine pumice had the most extreme pH values of 4.64 and 11.21 (Table 2). This could be an explanation that sudan grass cannot handle with these pH values and did not germinate. The maize seeds germinated faster than the sudan grass, which are better properties for the application for the inoculum production (IJdo et al., 2011). Generally over all different used substrates, maize grew faster and showed the higher above-ground biomass than sudan grass and is therefore more suitable for a fast inoculum production, regarding a fast rooting system and a fast plant growth (IJdo et al., 2011; Liu & Yang, 2008). The plants in the Agroscope mixture, lava, porlith and coarse pumice showed the greatest above-ground biomass and also a greater rooting system, which promotes fast colonization by AMF and the production of mycorrhizal roots (Liu & Yang, 2008). The shallow planting boxes may prevent a fast-rooting system of the plants because the roots had limited spaces. Nevertheless, the roots had the ability to grow out of the planting boxes at the bottom. It should always be considered to have space for unlimited root growth for the best AMF establishment (Feldmann et al., 2009). In addition, led the limited space in the planting boxes to a higher number of roots in less substrate than in experiment 1. The maize plants had a higher dependency of the above-ground biomass depending on the substrate than sudan grass. However, the higher dependency on the substrate could also arise from the greater biomass and higher variance maize had.

The AMF root colonization was lower than in the first experiment due to the shorter cultivation period. Maize had the higher colonization than sudan grass. Therefore, it can be assumed that *R. irregulare*, used for these two experiments, colonizes maize faster than sudan grass and plantago. As discussed in literature, the effect of AMF differs between the introduced fungi species and is depending on the host plant (Feldmann et al., 2009; IJdo et al., 2011; Liu & Yang, 2008). However, the Agroscope mixture showed the highest colonization for both plants. Sudan grass had a lower biomass than maize and a lower root colonization. As reported in literature, a greater biomass can have an influence on the AMF root colonization (Gaur & Adholeya, 2000). However, there was an exception, such as the Agroscope mixture with maize. These treatments showed the highest colonization but the treatment with lava, coarse pumice and porlith had the higher biomass. This indicates that other factors influenced the AMF root colonization stronger.

The spore number was not significantly different between plants or substrate. The data had a high variance and the samples with sudan grass had several outliers. Compared to the first experiment where a high colonization led to a higher spore number (Figure 5), there was no such relationship observable in the second experiment. However, for coarse pumice stone was an even higher spore number observable for sudan grass than for maize. Sudan grass produced the spores faster or equal to maize compared to the plant growth or AMF root colonization. Consequently, the host dependent sporulation should always be determined in the beginning and it is important to asses it for an effective inoculum production (IJdo et al., 2011). Furthermore, the pots were only dried out for 10 days compared to the first experiment, where the pots were dried out over 21 days. However, it may make sense to dry out the pots for a longer time, so that the AMF have more time to build spores. Another possibility would be to observe the spore number during the drying process and dry the pots as long as the spore number does not vary (Feldmann et al., 2009).

The difference of AMF root colonization distinguished between the used substrates. The particle size of the different substrate and the available nutrients for the plant influence the AMF root colonization (Gaur & Adholeya, 2002; IJdo et al., 2011). The particle size of the substrates had an influence on the plant growth and colonization of the different pots. The substrates with a larger grain size (coarse pumice, lava and porlith) had a positive influence on the colonization and plant growth. However, the sporulation was not significantly higher for those substrates. Maybe other grain sizes of the substrates would lead to other results in colonization, as it was shown for coarse and fine pumice (Gaur & Adholeya, 2000; IJdo et al., 2011). Therefore, if the same substrates were tested in several grain sizes different results of AMF root colonization and spore number may occur (IJdo et al., 2011). Furthermore, it has to be considered that the density of the material should be rather low, to produce a lightweight inoculum for agricultural application. Greater grain sizes lead to lower densities because there is more air between the grains (Coelho et al., 2014).

6.3. Watering system

The used drainage system in the second experiment watered every substrate the same way as it was automated, even if the substrates had a different water holding capacity due to grain size or other properties of the material. Smaller grain sizes led to a better water holding capacity (Flores-Ramírez et al., 2018). However, if the different substrates were watered differently, it may have led to a more appropriate humidity. Resulting in better plant growth and AMF colonization of the roots for the fine grained materials (Chen & Maun, 1999; Feldmann et al., 2009; Gaur & Adholeya, 2002; Liu & Yang, 2008). Furthermore, the aeration could be limited due the automated watering system in the fine grained materials (Chen & Maun, 1999; Gaur & Adholeya, 2002; IJdo et al., 2011). The contamination of the watering system may have been higher because the same water is used for all treatments which should be prevented (Liu & Yang, 2008). Furthermore, the plants were less water-stressed than in the first experiment which could also cause a lower colonization in this second experiment (Feldmann et al., 2009).

6.4. Nutrient supply

In the second experiment another fertilizer as the normally used Hoagland solution (Hoagland & Arnon, 1950) was applied, due to the automated watering system. Plant bowls with only 4 cm height and 3 litres of substrate for 12 plants were used. Therefore, a nutrient supply with commercial fertilizer was necessary to guarantee that the used substrate had enough nutrients for plant growth and improved AMF root colonization (Gaur & Adholeya, 2000; Lee & George, 2005). However, it could be that the fertilizer mixture led to an inadequate nutrient supply of the substrates or to other phosphor conditions which changed the AMF root colonization. It is reported that the application of phosphor fertilizer directly influences AMF root colonization (Rafique et al., 2020). To evaluate the effect of the fertilizer, experiments with various fertilizers should be made to evaluate the effect of the fertilizer on AMF establishment.

6.5. pH and phosphor of substrates

The pH of the substrates influenced the establishment of AMF. The substrates porlith, lava and the Agroscope mixture had a pH between 7 and 9 and had the highest AMF root colonization for the maize plants in the second experiment (Figure 9). Whereas in literature, a pH between 5,6- 8,0 is reported as optimal (Aarle et al., 2002; Köhl et al., 2016; Oehl et al., 2010). However, a pH which is too acidic (pH = 4,64) prevents a successful establishment of AMF, which is the case for the treatments with the nutshells. This corresponds with Abbott & Robson (1985) that the root colonization was hindered or failed when the soil pH was lower than 5.3. Whereas the treatments in an alkaline (pH < 9,5) substrate also showed a low AMF root colonization compared to the other substrates. Consequently, the AMF root

colonization is depended on the pH value of the substrate (Verbruggen et al., 2010). It has to be considered that the pH measurements were not very precise because no measurement repetitions were done. Furthermore, lava and the coarse pumice were not the correct grain size (>2mm) for pH measurements.

The nutrient availability, especially the phosphorous content of the substrates influences the colonization level of the roots (Gaur & Adholeya, 2002; Lee & George, 2005; Ma et al., 2018). Porlith, lava, sand, and the Agroscope mixture had a low phosphor availability between 3,5 - 4,1 mg P/kg. Confirming to literature, the optimal phosphor availability for a successful establishment of AMF lies between 0,3 - 18,8 mg P/ kg (Köhl et al., 2016; Oehl et al., 2010). The substrates that have a higher phosphor availability resulted in lower AMF root colonization. Several studies reported that AMF root colonization increases when phosphor availability decreases (Collins & Foster, 2009; Ma et al., 2018; Ryan & Graham, 2002; Verbruggen et al., 2010). Nevertheless, the coarse pumice has the second highest phosphor content with 30,4 mg P/kg but had a good AMF root colonization. However, porlith, lava and the Agroscope mixture shows a higher AMF root colonization. There is an influence of phosphor availability of the substrates depending on the AMF establishment in the substrates and roots. However, the phosphor content of the substrates may play a minor role in this experiment because other factors such as fast plant-growth, adequate aeration of the root system and appropriate humidity of the substrate were more important (Gaur & Adholeya, 2002; IJdo et al., 2011; Liu & Yang, 2008). This analysis was only conducted once for each substrate and the different substrates were used in the original grain size and not in the optimal grain size (<2mm). Therefore, measurement inaccuracies could falsify the results.

6.6. Different Substrates

The different tested substrates showed a promising colonization. However, the substrates had the highest AMF root colonization did not posses the beneficial traits for agricultural application, which are lightweight, homogeneity and no sharp edges. Vermiculite and fine pumice which had all these 3 properties showed a too low AMF establishment. Whereas lava and the Agroscope mixture had the highest AMF root colonization have none of the beneficial traits Figure 1. Porlith and coarse pumice possess two of the beneficial traits and showed a promising AMF root colonization with maize.

The used substrates were differently produced and available. The aim of a substrate beside the beneficial traits for agricultural application is that it should be easily available and low-cost for an economical viable inoculum production (Ceballos et al., 2013; Douds et al., 2005; Feldmann et al., 2009). A low-cost material is important to keep the production as cheap as possible. However, lava, pumice, vermiculite and porlith are not as easily accessible for inoculum production as previously used sand or soil. Furthermore, it is important to obtain a sustainable product for agriculture where the production of the used substrate is ecological. However, the production of porlith needs a lot of energy because of the heating of the material (Flores-Ramírez et al., 2018). Pumice and lava are both from volcanic origin and

therefore in some regions of the world not easily accessible (Flores-Ramírez et al., 2018). Vermiculite and the nutshells are also produced industrially. Substrates such as soil are therefore better accessible and often more sustainable and cheaper than industrially produced products.

6.7. Limitations

There are several limitations in these two experiments. Firstly, a longer cultivation time in the second experiment would lead to better results. Furthermore, it would be interesting to compare the AMF root colonization and spore density after different growing times to see how the AMF colonization and the spore number is developing. In addition, a comparison of the first and second experiment would be interesting to evaluate the differences between the two watering systems and the different containers used and to find out which method is more practicable. However, this was not possible due to different growing periods.

Furthermore, it would be interesting to get data of the below-ground biomass to see if there is any correlation between AMF root colonization and root biomass. A higher below-ground biomass can lead to an increased AMF root colonization and the infectivity of the inoculum is directly proportional to the mass of the colonized roots produced by the host plants (Gaur & Adholeya, 2000).

Measurement repetitions of the data would lead to higher statistical power with less outliers, due to possible outlier analysis. For example, the results of the spore number in the second experiment would become more precise if measurements had been repeated to evaluate the outliers. Furthermore, the spore number has to be considered critically. Some bigger spores could not fit through the finer sieve and are therefore neglected in the analysis (Oehl et al., 2005). In addition, in the second experiment some problems in extracting with the sucrose-density gradient occurred because of the less-dense materials such as vermiculite and the pumice. Those materials did not settle as the others did and therefore it was more difficult to count the spores for these samples.

The colonization and the spore number are a method to evaluate how the establishment of the AMF inoculum is in the given substrates. However, to evaluate how efficient a substrate-based inoculum is, it should always be tested to evaluate the effectivity (Coelho et al., 2014; Feldmann et al., 2009).

7. Conclusion and outlook

It was found that the AMF root colonization and spore number did not vary between the two different heights of containers. However, maize showed faster root colonization and a higher spore density than plantago within the same growing period. Therefore, it can be assumed that the AMF inoculum can be produced faster with a higher AMF concentration using maize instead of plantago.

In terms of different substrates, it was found that lava, the Agroscope mixture, porlith and coarse pumice showed promising AMF root colonization, whereas the spore density of the substrates did not show any significance difference. However, lava and the Agroscope mixture possess only one or none of the beneficial traits for agricultural application, such as lightweight, homogeneity and no sharp edges, whereas porlith and coarse pumice had two of these characteristics. Nevertheless, it has to be considered that the production of porlith needs a lot of energy. Therefore, porlith is not applicable at a larger scale if the inoculum product should be sustainable. Coarse pumice is compared to porlith more sustainable due to the natural occurrence. In addition, the substrates used for inoculum production with *R. irregulare* should have beneficial traits for agricultural application as well as neutral to alkaline pH-levels and a low phosphor content. However, these recommendations may have to be adapted depending on the fungal species used.

It was found that maize was better suited as host plant than sudan grass. Maize was growing faster and had a higher AMF root colonization. Nevertheless, sudan grass did not show a significantly different spore density compared to maize. The effect of the host plants on AMF establishment differed depending on the used substrate. However, for both host plants, the Agroscope mixture had the highest AMF root colonization. It can be concluded that maize showed the most promising AMF establishment of all three tested host plants.

In further studies, it might be interesting to test the effect of AMF establishment in different substrates under different cultivation factors, such as the watering systems and the fertilizers influencing the production of a highly concentrated AMF inoculum. For a successful analysis of the factors and a more efficient and economical viable AMF inoculum production, the different factors should be evaluated separately from each other to obtain precise information about which factor has which influence on AMF establishment in the inoculum. Furthermore, the performance of the produced AMF inoculum once applied to the field should be tested.

In summary, further research is needed to understand the interaction between AMF establishment, the different substrates and the host plants, which are influenced by the cultivation methods to make the production of AMF inoculum more efficient and the inoculum easily applicable in agriculture.

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Appendix

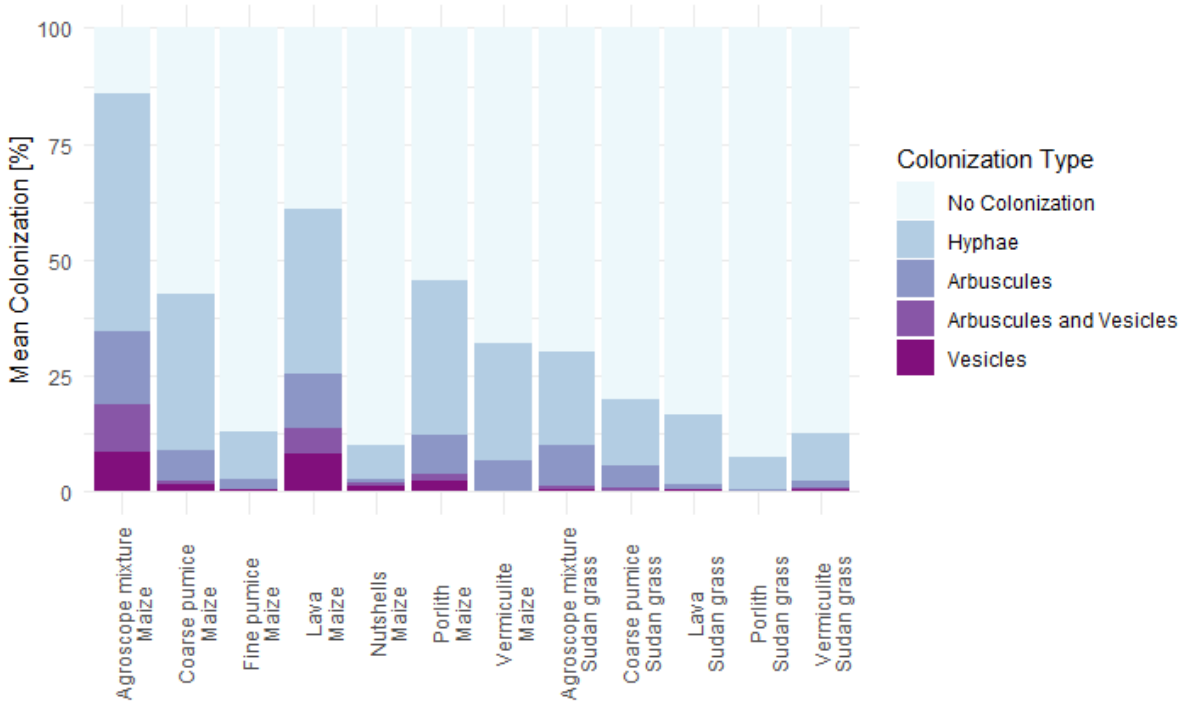


Figure 11 Mean AMF root colonization of the different experimental designs of the second experiment after the different colonization types: No colonization, only Hyphae, Arbuscules and Arbuscules and Vesicles, Vesicles.

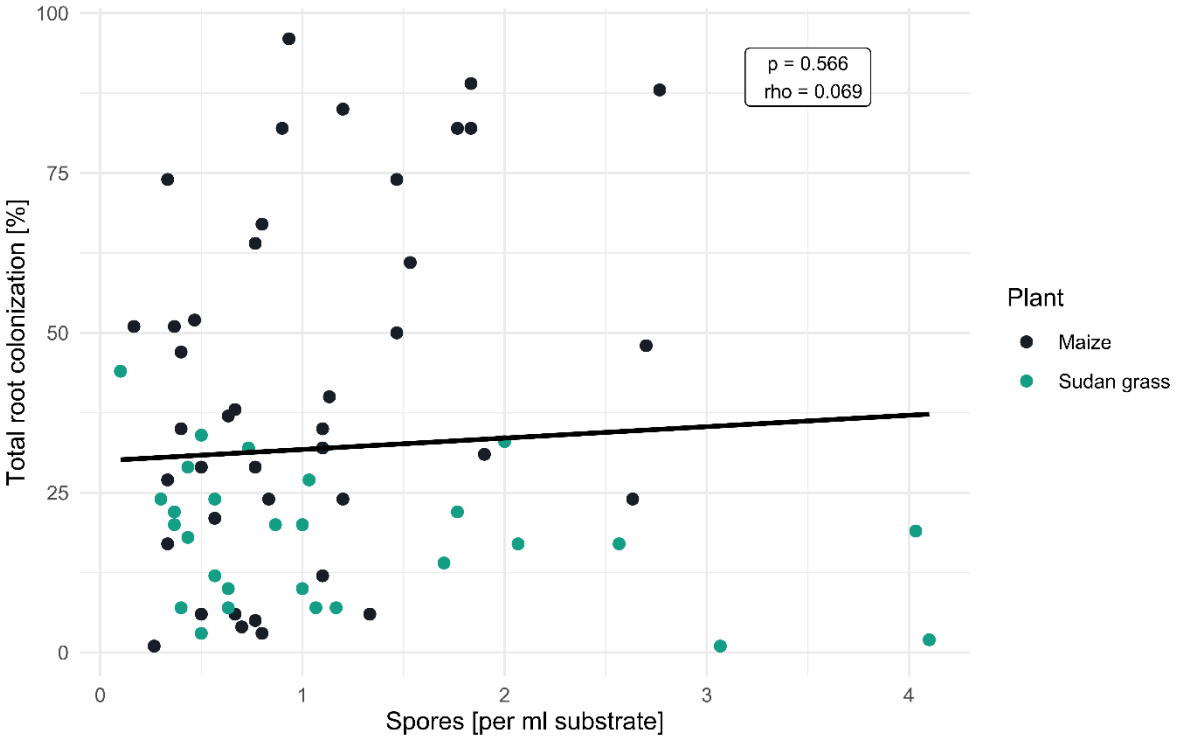
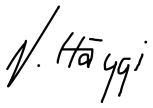


Figure 12 Relationship between AMF root colonization as percentage of root length colonized at harvest and spore number per ml substrate is shown. Non-parametric Spearmen's Rank correlation was performed. Spearmen's rho and the corresponding significance level is shown in the graph. The Maize plants are shown in black and plantago in green.

Personal declaration

I hereby declare that the material contained in this thesis is my own original work. Any quotation or paraphrase in this thesis from the published or unpublished work of another individual or institution has been duly acknowledged. I have not submitted this thesis, or any part of it, previously to any institution for assessment purposes.



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Zürich, 26. August 2021