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The effect of maize cultivars and microbial inoculation on soil aggregation and soil parameters

–Bachelor's thesis–

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Table of Contents

Abstract

Humans started using soil for agriculture around 23,000 years ago and today soils provide us with 95% of our food. However, because of soil degradation and urbanization, the amount of soil available to meet human demands is decreasing. Soil quality can be improved through management and is determined by soil properties like structure, organic matter (OM) content, water stability of aggregates, pH, and hydraulic properties.

After the green revolution in the 1960s new crop cultivars were bred and sown aiming higher crop yields. Modern and older bred *Zea mays* (maize) genotypes may also differ in microorganism recruitment, root growth, and plant biomass, which are factors that can be related to soil quality and the sustainability of the agricultural systems.

The objective of this study was to determine whether different maize breeding origins and inoculation with *Rhizophagus irregularis* and *Azospirillum brasilense* in an artificial soil influenced shoot biomass and soil parameters, including soil aggregates (size and water stability), water repellency (WR), water holding capacity (WHC), and pH.

The experiment followed a factorial design with three maize breeding origins: modern Brazilian hybrids (MBH), modern German hybrids (MGH), and old maize cultivars (OMC) before the green revolution, and two treatments: one inoculated with microorganisms and one non-inoculated treatment. The aggregate size distribution (ASD) was determined by the size fractions 0–250 µm, 250–500 µm, 500 µm–1 mm, 1–2 mm, and 2–4 mm by dry sieving. The percentage of water-stable aggregates (WSA) was determined for aggregates with a diameter of 250 µm–1 mm and 1–4 mm by wet sieving. WR of the soils was examined by the water infiltration method. Shoot biomass, WHC, and pH of the soils were also evaluated.

Older cultivars had fewer aggregates with a diameter of 0–250 µm, more WSA with a diameter of 250 µm–1 mm, less WSA with a diameter of 1–4 mm, and less shoot biomass compared to modern cultivars. Inoculated modern cultivars showed a decrease in WSA (1–4 mm) and an increase in shoot biomass, whereas older cultivars were not affected by inoculation. The hydraulic properties and pH showed no differences between modern and older cultivars.

In conclusion, it was demonstrated that the percentage of aggregates with a diameter of 0–250 µm, the percentage of WSA (250 µm–1 mm and 1–4 mm), and shoot biomass differed between modern and older cultivars. Also, they responded differently to inoculation with *A. brasilense* and *R. irregularis* regarding the amount of WSA with a diameter of 1–4 mm and shoot biomass. It could be suggested that these differences are due to different exudates of modern and older cultivars and their subsequent interactions with the microorganisms. The exudates and their interactions with the microorganisms should be investigated in future studies. So, this knowledge could be a guide for plant breeding, with the aim to improve the soil structure in agricultural fields.

List of abbreviations

AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
ASD	Aggregate size distribution
CODA	Compositional data analysis
MBH	Modern Brazilian hybrids
MGH	Modern German hybrids
OM	Organic matter
OMC	Old maize cultivars
VWF	Van der Waals attractive forces
WHC	Water holding capacity
WIT	Water infiltration time
WR	Water repellency

1. Introduction

Soil was formed about 850 million years ago (Knauth & Kennedy, 2009), enabling the transition and evolution of complex life from aquatic to terrestrial systems (Schrenk, 2008). Humans started using soil for agriculture around 23,000 years ago, allowing us to settle in cities and to build complex societies (American Friends of Tel Aviv University, 2015).

Today 95% of our food is derived from agriculture (Busscher, 2012). But, although 25% of the coverage of the earth's surface is soil, only about 7.5% of it is suitable for agriculture (Moore et al., 2020). Other than for food, humans use soil as it supports the growth of plants for lumber, biofuel, fiber, paper, clothing, and animal production. As the human population is growing rapidly, so too the demand for agricultural and forestry products. However, the amount of soil capable to meet these demands is decreasing, due to soil degradation, urbanization, and the need to conserve areas and parks for the preservation of natural biodiversity (Weil & Brady, 2017).

Apart from supporting plant growth by giving plant roots the space and the nutrients to grow, soil provides many other environmental services such as acting as habitat for numerous organisms, regulating water supply, interacting with the atmosphere, cycling nutrients, and organic wastes, and serving as a medium for human engineering e.g., for the construction of streets, roads, and buildings (Weil & Brady, 2017). All these ecosystem services and functions are linked to soil quality (Barrios, 2007; Wall & Nielsen, 2012). Soil quality describes the ability of the soil to perform ecological functions. Characteristics like soil structure and organic matter (OM) content determine soil quality and can be influenced by management (Weil & Brady, 2017). Today, mankind faces the challenge of restoring degraded soils and maintaining the habitat of diverse organisms.

An important aspect of soil quality is soil structure. It describes how the mineral particles arrange with organic material to form aggregates and porosity (Finch et al., 2014). Soil structure is influenced by the quality and quantity of OM, which includes depositions by root exudates and by biological activities. OM in turn is dependent on the planted crops.

One of the most important world crops is *Zea mays L.* (maize). Maize is not only an important food source for humans but also important as feed for livestock and producing biofuels. Maize interacts with soil microorganisms, including the arbuscular mycorrhizal fungus *Rhizophagus irregularis* (Błaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler 2010 and the plant promoting bacteria *Azospirillum brasilense Sp7* Tarrand et al. 1979 (Approved Lists 1980) emend. Hördt et al. 2020 (e.g., Battini et al., 2017; Marks et al., 2015; Quiroga et al., 2019;

Zeffa et al., 2019). It is assumed that plant breeding in the last century, and especially after the green revolution, may have changed how maize interacts with soil microorganisms, and how maize exudates may influence soil structure (Brisson et al., 2019; Chen et al., 2019; Favela et al., 2021; Schmidt et al., 2020).

This thesis focuses on the effect of different maize cultivars (old maize cultivars (OMC), modern Brazilian hybrids (MBH), and modern German hybrids (MGH)) and inoculation with the arbuscular mycorrhizal fungi (AMF) *R. irregularis* and the bacteria *A. brasilense* on soil parameters, including aggregation and water holding capacity (WHC).

1.1 Brief review of soil aggregation

As per definition, a soil aggregate consists of particles that adhere to one another stronger than to the surrounding particles. It is composed of OM, minerals, and primary soil particles (Kemper & Rosenau, 2018), it is the basis of soil structure and strongly influences soil porosity and WHC of soils.

Aggregates can be organized hierarchically. Macroaggregates (> 250 µm) are composed of smaller microaggregates (< 250 µm) and OM. Whereas microaggregates are formed out of primary soil particles (< 53 µm) by physico-chemical forces and binding agents, macroaggregates are bound by more temporary agents, like roots, fungal hyphae, and microorganisms (Oades, 1984; Six et al., 2004; Tisdall & Oades, 1982).

The aggregation process can be divided into physical-chemical and biological processes.

1.1.1 Physical-chemical processes

1.1.1.1 Flocculation

The attraction between clay particles and organic molecules is called flocculation. Colloids carry negative and positive electrostatic charges on their inner and outer surfaces, although in most cases negative charges predominate (Weil & Brady, 2017). Clay particles store mineral nutrients, like Ca^{2+} and K^{+}. When two clay platelets are close enough to each other, the cations in the interlayer attract the negative charges on the surface of each clay particle, acting as a binding bridge between the particles. This leads the clay particles to flocculate into clay domains. Therefore, the formation of clay domains is promoted by polyvalent cations and humus provides long-term stability for smaller microaggregates (< 250 µm). While some cations encourage flocculation, others do not. Generally, di- and trivalent cations, like Ca^{2+}, Fe^{2+}, Al^{3+} are better flocculating agents than monovalent cations, especially Na^{+}. If monovalent cations dominate, the attractive forces will be too weak, to overcome the repulsive behavior of the clay particles toward

each other and the clay particles will not be able to approach each other sufficiently to flocculate and will instead stay dispersed (Weil & Brady, 2017). In such cases, a gel-like soil, impermeable for water and air and undesirable for plant growth, is what remains.

1.1.1.2 Swelling and shrinking

Swelling and shrinking occur in wet-dry and freeze-thaw cycles. These cycles create cracks and pressures that alternately break large soil masses and compact soil particles into defined structural peds (Weil & Brady, 2017). Furthermore, plant roots remove moisture from the soil, which also leads to soil cracking and shrinkage, hence influencing soil aggregation (Nichols & Halvorson, 2013).

1.1.2 Biological processes

1.1.2.1 Physical stabilization

Due to the movement of earthworms and termites and from the growth of roots and hyphae, soil particles come closer together encouraging aggregate formation (Weil & Brady, 2017).

1.1.2.2 Exudates

Plant roots, fungal hyphae, and some bacteria exude extracellular polymorphic substances (e.g., Allison, 1968). Shamoot et al. (1968) found, that regardless of the species, plants release 0–49 g organic material per 100 g harvested root. According to Naveed et al. (2017), maize root exudates consist of 27.8% organic acids, 24% phosphoric acid, 17.8% sugars, sugar acids and alcohols, 13% fatty acids, 9.6% urea, and 5.7% amino acids. These polysaccharides act as organic glues (Allison, 1968; Allison & Jastrow, 2006; Six et al., 2004) and are involved in stabilizing aggregates with a diameter of less than 50 µm (Tisdall & Oades, 1982). They form a sticky network, holding clay particles together and binding microaggregates into macroaggregates, even after the root or hyphae has died (Tisdall & Oades, 1980b; Weil & Brady, 2017).

When the outer surface of an aggregate is occupied with active organic compounds, the force causing coherence between different clay particles and adjacent aggregates becomes too weak to hold these aggregates together. Furthermore, the particles within the aggregate are orientated to each other and are in close contact, leading to a strong cohesive force between them. This causes the aggregate to be stable rather than to disperse (Martin et al., 1955).

Thus, fungi, bacteria, and roots contribute to macroaggregate stabilization (Harris et al. 1966; Tisdall & Oades, 1980a).

1.1.3 Water infiltration time, WHC, and pH

Soil quality is not only determined by soil aggregate formation and stability but also by soil hydrological properties and pH.

1.1.3.1 Water infiltration time and WHC

Water infiltration time (WIT) can be a proxy for water repellency (WR). The higher the WIT the higher the WR. Naveed et al. (2018) demonstrated that maize root exudates at a 4.6 mg g^{-1} concentration increased WR for sandy loam soils. Exudates can form hydrophobic films that cover the surface of aggregates, making them be more stable and more water repellent (Wessels, 1996; Young, 1998). In 2019 Naveed et al. found out that maize root exudates increased soil water retention.

1.1.3.2 pH

The pH of root exudates influences the pH of the soil and thus determines soil quality, stability, and aggregation. Maize root exudates in aqueous solutions at 2.6 mg g^{-1} concentration had a pH of 9.35 (Naveed et al., 2017). Root-mediated changes in pH can have a big impact. The bioavailability of nutrients and toxic elements as well as the physiology of the roots and microorganisms are influenced by pH. The release or uptake of ions by roots can change the pH of the surrounding soil (Riley & Barber, 1969, 1971). 75% of maize root exudates are transformed by microbial respiration into CO_2 (Helal & Sauerbeck, 1989). An increased CO_2 concentration can lead to a pH decrease because carbonic acid will be formed in the rhizosphere, which can dissociate in neutral to alkaline soils and thus lead to a decrease in pH (Hinsinger et al., 2003).

1.2 Background information on the experimental design

1.2.1 The different maize breeding origins

During the green revolution in the 1960s and 1970s new crop cultivars were bred and sown with the purpose of higher yields to fight global famine (Mann, 1997). Since modern and older cultivars have different genotypes, they do not only differ in crop growth, but also in rhizosphere microbiome recruitment (Favela et al., 2021), enzyme activities, root growth, shoot morphology, biomass, and nitrogen uptake and redistribution (Feil, 1992). Based on that, this study examines the effect of maize breeding origins on soil properties like aggregation and WR. Many studies revealed different responses to inoculation with AMF or *Azospirillum* among different cultivars (e.g., Chu et al., 2013; Walker et al., 2011), thus differences in the response to inoculation between modern and older cultivars are expected in this experiment.

1.2.2 The inoculants: AMF and *Azospirillum*

1.2.2.1 Role of mycorrhizal fungi in soil aggregation

Mycorrhizal fungi live in symbiosis with the roots of many plants (Smith & Smith, 2011; Wang & Qiu, 2006). The plants provide the fungi with sugars and in return, the fungi help the plants with nutrient uptake. Fungal hyphae can reach places where the roots cannot grow and can help the plants acquire nutrients outside of their depletion zones (Parniske, 2008).

In this experiment, AMF were used. AMF belong to the phylum *Glomeromycota* and it is estimated that they evolved 460-600 Ma ago (Redecker et al., 2000; Redecker & Raab, 2006; Schüβler et al., 2001). Their hyphae penetrate the cortical root cell walls and form small, branched structures called arbuscules inside the plant cell, which transfer sugars and nutrients between plant and fungus (Weil & Brady, 2017). Hyphal growth is not only influenced by the host plant and fungi but also impacted by soil pH, nutrient levels, and water (Helgason & Fitter, 2009; Johnson et al., 2003; Parniske, 2008). AMF hyphae have been found to correlate with soil stability (Wilson et al., 2009). Mycorrhiza produce glomalin, which is a protein that is believed to aggregate soil (Caesar-TonThat et al., 2010; Nichols & Halvorson, 2013; Weil & Brady, 2017). In an in vitro study, Rillig et al. (2010) found out that in the presence of AMF mycelium soil WR increased and WSA were maintained. An overall positive effect of AMF on soil aggregation was also revealed in a meta-analysis (Leifheit et al., 2014).

1.2.2.2 Role of *Azospirillum* in soil aggregation

Bacteria used in this experiment belong to the genus *Azospirillum*. They are gram-negative and vibrion-shaped rods, have a diameter of 1 µm, are very motile, have a long, polar flagellum and sometimes peritrichous flagella (Okon, 1985). *Azospirilla* can fix N_2 from the air as a nitrogen source for growth and they colonize primarily forage and grain grasses such as maize (Okon, 1985). Okon (1985) showed that in the presence of *Azospirillum sp.* yields of forage and cereal grasses increased. The bacteria contribute to improved root development, hence increasing water and nutrient uptake and soil aggregation, and they support plant growth by biological nitrogen fixation (Okon, 1985; Okon & Kapulnik, 1986; Sarig et al., 1984). Furthermore, bacteria have charges on their cell walls, which promote flocculation of clay particles and some bacteria produce organic binding agents like polysaccharides, which promote aggregation (Weil & Brady, 2017).

1.2.3 Conceptual model about the study of soil aggregation

During lab processing, the agents and forces that contribute to aggregate formation and/or stabilization change (Figure 1). In the wet soil core, the Van der Waals attractive forces (VWF) and ionic interactions between clay particles, more precisely chains of water dipoles and interactions of exchangeable cations between clay particles (via hydrogen bonding or direct bridging) (Mazurak, 1950) and organic material, contribute to the stabilization and formation of aggregates (Martin et al., 1955). The OM includes polysaccharides that bind via hydrogen bonding of alcoholic hydrogen to soil particles, polar long-chain molecules that tie or bridge soil particles together (Nichols & Halvorson, 2013), and roots and hyphae that form aggregates via physical forces. When dried, new aggregates form due to decreasing water content which leads to shrinking and cracking of the soil (Nichols & Halvorson, 2013). As soil moisture decreases, the cohesion between clay particles through water dipoles is lost. When the roots are removed, the hyphae die and subsequently the stabilization of soil aggregates through roots and hyphae is lost.

If the soil is sieved, mainly the ionic interactions between clay particles and the organic material are responsible for aggregate stabilization. The VWF are lost, due to the applied physical forces during sieving (Martin et al., 1955). It may be possible though, that new VWF appear directly after the sieving. Regarding WR, the hydrophobicity of the material (e.g., proteins, fatty acids) and the OM associated with the clay particles act as the binding forces and repelling agents. WSA are stabilized by ionic interactions between clay particles, especially exchangeable cations (Mazurak, 1950), and the organic material associated with the clay particles (Robinson & Page, 1951). VWF are possibly involved in stabilization as well.

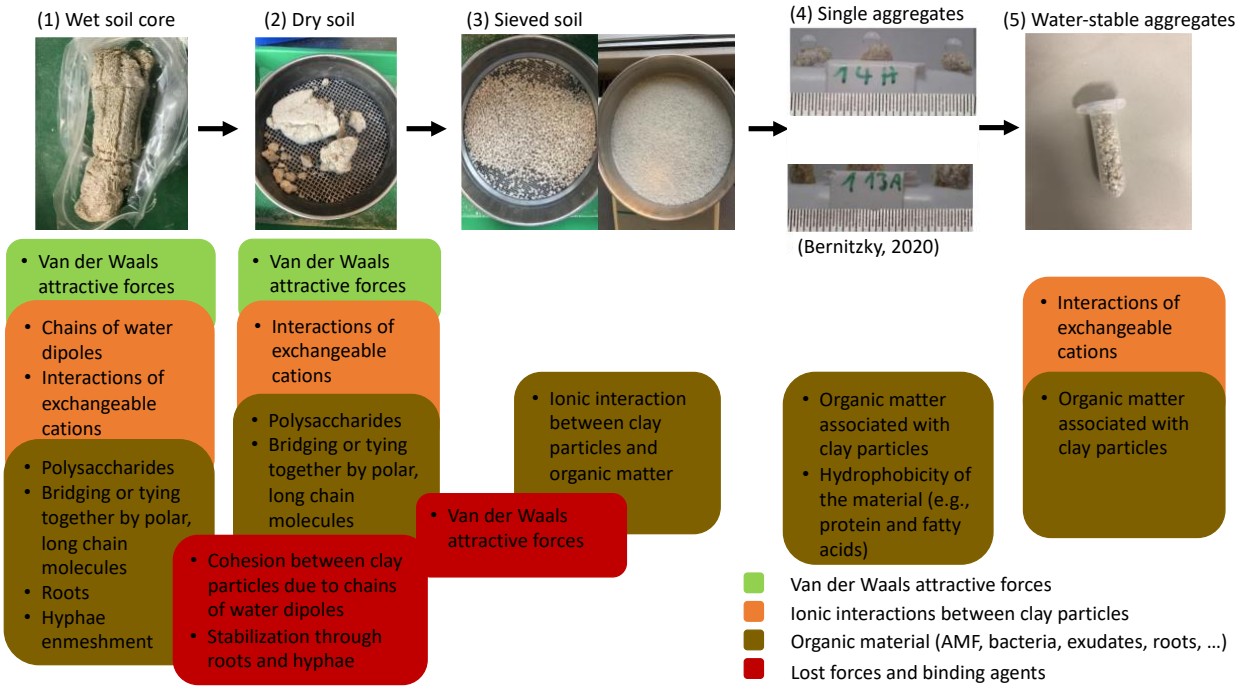


Figure 1. *Conceptual figure depicting the forces and agents that contribute to aggregate formation and/or stabilization during each step of the experiment: (1) wet soil core (2) dry soil (3) sieved soil (4) single aggregates (5) water-stable aggregates. A more detailed explanation can be found in section "1.2.3 Conceptual model about the study of soil aggregation".*

1.3 Objective

The objective of this study was to determine if different maize breeding origins (OMC, MBH, and MGH) and inoculation with *R. irregularis* and *A. brasilense* influence aggregate size, water stability, WR, WHC, and pH of artificial soil as well as shoot biomass.

1.4 Hypotheses

1) Aggregate size distribution (ASD), WSA, WR, WHC, pH, and shoot biomass will vary between modern and older cultivars.
   Because of the green revolution, it is expected that they differ in root/shoot and exudate allocation (Feil, 1992), causing different behavior on ASD in the soil, the formation of WSA, WR and WHC of the soil, pH, and shoot growth.

2) Modern and older maize cultivars will respond differently to inoculation with *A. brasilense* and *R. irregularis*, in line with previous studies which have found that cultivars with different genotypes respond differently to *A. brasilense*- and *R. irregularis*-inoculation (Boleta et al., 2020; Chamam et al., 2013; Chu et al., 2013; Walker et al., 2011).

2. Material and Methods

2.1 Material

2.1.1 Maize Cultivars

In this study the following maize cultivars of three different breeding origins were examined: A) OMC (before the green revolution): 1. Golden Bantam, 2. Bear Paw and 3. Negro Cine, B) MBH: 1. L940, 2. J2M88 and 3. RK3115 and C) MGH: 1. Saludo, 2. Fred and 3. Robertino.

2.1.2 Treatments

Both inoculated and non-inoculated maize cultivars were included in the study. Inoculated cultivars were infected with *R. irregularis* and *A. brasilense Sp7*. Artificial soil pots without plants were kept as negative controls, and here there were also inoculated and non-inoculated treatments.

2.2 Experimental design

The experiment followed a factorial design with three maize breeding origins (with three cultivars each) and two treatments (+/-): inoculated with microorganisms (treat +) or non-inoculated (treat –). Pots without plants, both inoculated and non-inoculated, served as a control. The experiment had six replicates. The plants were sown in 400 g of artificial soil (70% sand, 28% clay-kaolinite, and 2% of cellulose, watered with Hoagland solution with reduced N and P) in cone-tainers, and according to the treatments. The plants were grown in the greenhouse of the Institute of Biology at the Freie Universität Berlin. Artificial soil was used because it is the best way to reduce interference of soil OM in mass spectrometry analysis (future analysis).

After six weeks the samples were harvested. The shoots were cut at the base, and the seeds and roots were removed with a tweezer. Both shoots and roots were kept for further analysis. The soil samples were air-dried in the greenhouse for three days. Later, the samples were analyzed as described below.

2.2.1 Dry sieving into two fractions: 0–2 mm and 2–4 mm

A 2 mm and a 4 mm sieve were stacked in this order on top of a solid bottom container. First, the soil sample was pushed carefully through the 4 mm sieve, slightly breaking the aggregates. When the whole sample was pushed through, the stacked sieves were shaken five times in circular movements. Aggregates bigger than 2 mm were weighed. The bottom container, containing aggregates smaller than 2 mm, was weighed and the aggregates were placed back in the bag with the aggregates bigger than 2 mm. Afterward, the content of the bag was mixed by shaking.

If roots were found during the procedure, they were removed with a tweezer and put into Eppendorf tubes. Because of time pressure, the removal of the roots was minimized for most samples.

Roughly 40 g of each sample were put into small plastic bags for further analysis.

The weights of the bottom containers were subtracted from the measured values of the aggregates with a diameter smaller than 2 mm. All values were transformed into percentages for better understanding [1] [2].

$$a_{2\_4mm} = \frac{m_{a2\_4mm}}{m_a} \quad [1]$$

a_{2_4mm}: *percentage of the aggregates with a diameter of 2–4 mm*
m_{a2_4mm}: *mass of the aggregates with a diameter of 2–4 mm*
m_a: *mass of the full sample*

$$a_{0\_2mm} = \frac{m_{a+c} - m_c}{m_a} \quad [2]$$

a_{0_2mm}: *percentage of the aggregates with a diameter of 0–2 mm*
m_{a+c}: *mass of the aggregates with a diameter of 0–2 mm plus the mass of the bottom container*
m_c: *mass of the bottom container*

2.2.2 Dry sieving into five fractions: 0–250 µm, 250–500 µm, 500 µm–1 mm, 1–2 mm and 2—4 mm

To have more precise data, the samples were sieved again into five soil size fractions.

About 10 g of each sample were taken from the prepared 40 g and filled into 15 ml tubes. A 250 µm, a 500 µm, a 1 mm and a 2 mm sieve were stacked in this order on top of a weighing boat. The samples were placed on the 2 mm sieve and sieved by shaking the sieves and the weighing boat ten times in circular movements. The aggregates bigger than 2 mm were placed in a prepared weighing boat and the remaining sieves, including the weighing boat, were shaken another ten times. All fractions were placed in prepared weighing boats and weighed.

If the samples were still wet, they were placed in the oven at 35°C until they were dry and then sieved.

The weights of the weighing boats were subtracted from the measured values. All values were transformed into percentages for better understanding [3]. For further analysis, the 2–4 mm fraction from this sieving was neglected, so that the other fractions give a more precise insight of the 0–2 mm fraction from the first sieving (2.2.1). Here we consider the sample minus the aggregates with a diameter of 2–4 mm as the whole sample.

$$a_x = \frac{m_{a+wb} - m_{wb}}{m_{all}} \quad [3]$$

a_x: percentage of the aggregate fraction
m_{a+wb}: mass of the aggregates plus the mass of the weighing boat
m_{wb}: mass of the weighing boat
m_{all}: mass of the whole sample (without the 2–4 mm fraction)

2.2.3 Wet sieving of two aggregate size ranges: 250 µm–1 mm and 1–4 mm

As a preparation for the wet sieving, the weight of the aggregates bigger than 2 mm was adjusted according to the percentage measured in the first dry sieving (2.2.1). Then, the aggregates bigger than 2 mm were added to the weighing boat containing the aggregates with a diameter of 1–2 mm. The aggregates with a diameter of 500 µm–1 mm were added to the weighing boat, containing the aggregates with a diameter of 250–500 µm. Aggregates smaller than 250 µm were discarded.

A wet sieving apparatus was used, sieving eight samples at once. The aggregates with a diameter of 250 µm–1 mm were put into 250 µm sieves and the aggregates with a diameter of 1–4 mm were put into 1 mm sieves of the machine and placed in 100 ml of deionized water for 30 s. Then the samples were taken out of the water to drain off for 1 min. The machine sieved the samples

for 3 min. The WSA were washed out of the sieve with deionized water into the prepared weighing boats. All samples were dried in the incubator at 60°C. The WSA with a diameter of 250 µm–1 mm were weighed with the weighing boats. Then, to remove the sand, the aggregates were moistened with deionized water and carefully broken by hand. They were then sieved with a 250 µm sieve by stirring the aggregates manually so that the water could run off and shaking the sieve two times in circular movements. Soil particles bigger than 250 µm (sand) were washed out of the sieve with deionized water into weighing boats, dried in the incubator for two days at 60°C, and then weighed. The WSA with a diameter of 1–4 mm were sieved again with a 1 mm sieve, placed in an Eppendorf tube, and then weighed.

If roots were found in the samples, they were removed with a tweezer.

Since the sand was only removed in the smaller WSA, the values cannot be compared to the values of the bigger WSA. The sand was removed because some sand particles are bigger than 250 µm and thus could be mistaken as WSA. But it should be noted that this technique for removing sand is not very precise, as it also leads to the removal of the sand inside of the broken aggregates.

The weights of the weighing boats and Eppendorf tubes were subtracted from the measured values. All values were transformed into percentages for better understanding [4].

$$WSA = \frac{m_{wsa+p} - m_p}{m_a} \quad [4]$$

WSA: percentage of WSA

m_{wsa+p}: mass of WSA plus the mass of the weighing boat or Eppendorf tube

m_p: mass of the weighing boat or Eppendorf tube

m_a: mass of the aggregates before wet sieving

For the calculation of the 250 µm–1 mm WSA, the mass of the sand was subtracted [5].

$$m_S = m_{s+ps} - m_{ps}$$

$$WSA = \frac{(m_{wsa+p} - m_p) - m_s}{m_a - m_s} \quad [5]$$

m_s: mass of the sand

m_{s+ps}: mass of the sand plus the mass of the weighing boat that contained the sand

m_{ps}: mass of the weighing boat that contained the sand

2.2.4 Measuring WIT and WHC

A funnel with a filter paper was placed on the opening of a 50 ml tube, that was weighed before. About 10 g of the soil sample were placed in the funnel and 10 ml of water were added. Infiltration time was measured with a chronometer from the moment the first drop of water reached the soil surface until the last visible drop of water was absorbed by the soil. The tube containing the water that had run through the sample was weighed. Three samples were observed at a time. Three glass funnels were used. Two of them had a diameter of 5 cm and one had a diameter of 5.2 cm. The filters used were Rotilabo® round funnels, type 111A made of cellulose with a membrane diameter of 90 mm. To determine the water absorption of the filter paper, this procedure was run in advance without the soil samples.

WHC was calculated by dividing the mass of water that stayed in the sample through the mass of soil [6].

$$m_{ws} = m_w - (m_{wt} - m_t) - m_{wf}$$

$WHC = \frac{m_{ws}}{m_s}$ [6]

m_{ws}: mass of water that stayed in the sample
m_w: mass of water that was initially poured on the sample
m_{wt}: mass of water that went through the sample with the weight of the tube
m_t: mass of the 50 ml tube
m_{wf}: mass of the water that is absorbed by the filter
WHC: WHC in percent
m_s: mass of the soil sample

2.2.5 Measuring the pH of the soil samples

After WIT and WHC were measured, the filter paper and the sample were put into the 50 ml tube. The tube was filled up with deionized water to 25 ml and stored in the fridge overnight. The samples were shaken in the water sieving machine for 15 min and then centrifuged for 1 min at 4,000 rpm so that the samples and the filter papers precipitated. The pH was measured with a pH meter. Before the samples were analyzed, the pH of the water and the pH of water with a filter paper were measured as a control.

2.2.6 Weighing of the maize shoot biomass

The maize shoots were weighed in a beaker. The weight of the beaker was then subtracted from the measured values [7].

$$m_{shoot} = m_{s+b} - m_b \quad [7]$$

m_{shoot}: mass of the shoot
m_{s+b}: mass of the shoot plus mass of the beaker
m_b: mass of the beaker

2.3 Data presentation and statistical analysis

Data analysis and plotting were done with RStudio.
ASD data are presented as stacked bar charts, displaying the mean percentage of the respective aggregate sizes. The non-compositional data are presented as boxplots, whereby the means and single data points are plotted.
While analyzing the different breeding origins, all data points outside of the whiskers of the boxplots were identified as single construct outliers and treated as NAs following the single construct technique (Aguinis, 2013). Exceptions were made, when otherwise there would be less than four repetitions. In this case, the outlier that was closer to the mean was left in the analysis. These samples were J2M88 +B in the first sieving (0–2 mm) and L940 −A (250–500 µm), J2M88 +D (500 µm–1 mm), and J2M88 +C (1–2 mm) in the second sieving. Sample J2M88 +F was removed completely from the analysis because it had almost no plant growth. Also, some other replicates were lost and treated as NAs.
For statistical analyses, the two-way analysis of variances (ANOVA) from the package 'car' was used. Before performing the ANOVA, the data were tested to ensure that they would fulfill all the assumptions. Homogeneity of variance was tested using the Breusch-Pagan test (Breusch & Pagan, 1979). The other assumptions were examined using the 'DHARMa' diagnostic functions from the 'DHARMa' package in R. If the data did not meet these assumptions they were transformed with the Box-Cox transformation (Box & Cox, 1964).
The Scott-Knott test was applied to compare differences between the breeding origins and treatments (Scott & Knott, 1974). This is a hierarchical algorithm that partitions treatments into distinct groups. The advantage of this test compared to other tests (e.g., the Tukey test) is that the groups do not overlap. The significance level was set at $p = .05$, except for shoot biomass, where the ANOVA and the Scott-Knott test results contradicted and thus for this variable the significance level was set at $p = .069$. The results are presented as letters, where different letters refer to statistically significant differences between the groups (Jelihovschi et al., 2014; McHugh, 2011).

3. Results

3.1 ASD considering two fractions: 0–2 mm and 2–4 mm

The influence of different maize breeding origins and inoculation with *R. irregularis* and *A. brasilense* on ASD of aggregates with a diameter of 0–2 mm and 2–4 mm was observed (Figure 2). Breeding origin and treatment influenced both fractions (0–2 mm: breeding origin: $p \approx .006$, treatment: $p \approx .010$; 2–4 mm: breeding origin: $p \approx .011$, treatment: $p \approx .018$, Figure 2). Further, the influence of the breeding origin on aggregates with a diameter of 2–4 mm was affected by the treat ($p \approx .036$, Figure 2).

The Scott-Knott test shows that all breeding origins exhibited a higher percentage of aggregates with a diameter of 0–2 mm compared to the control (MBH –: $\overline{x} \approx (74.1 \pm 1.3)\%$, MGH –: $\overline{x} \approx (75.5 \pm 0.7)\%$, OMC –: $\overline{x} \approx (74.4 \pm 0.9)\%$, control –: $\overline{x} \approx (68.6 \pm 2.9)\%$) and accordingly a lower percentage of aggregates with a diameter of 2–4 mm (MBH –: $\overline{x} \approx (25.9 \pm 1.3)\%$, MGH –: $\overline{x} \approx (24.5 \pm 0.7)\%$, OMC –: $\overline{x} \approx (26.1 \pm 1.0)\%$, control –: $\overline{x} \approx (31.4 \pm 2.9)\%$) (Figure 2). Furthermore, only MGH showed a difference between treatments having a decreased percentage of aggregates with a diameter of 0–2 mm when inoculated (MGH +: $\overline{x} \approx (70.4 \pm 1.3)\%$, MBH +: $\overline{x} \approx (73.1 \pm 1.1)\%$, OMC +: $\overline{x} \approx (73.9 \pm 0.6)\%$, control +: $\overline{x} \approx (69.6 \pm 1.1)\%$), and an increased percentage of aggregates with a diameter of 2–4 mm (MGH +: $\overline{x} \approx (29.6 \pm 1.3)\%$, MBH +: $\overline{x} \approx (26.9 \pm 1.1)\%$, OMC +: $\overline{x} \approx (26.1 \pm 0.6)\%$, control +: $\overline{x} \approx (30.4 \pm 1.1)\%$) (Figure 2).

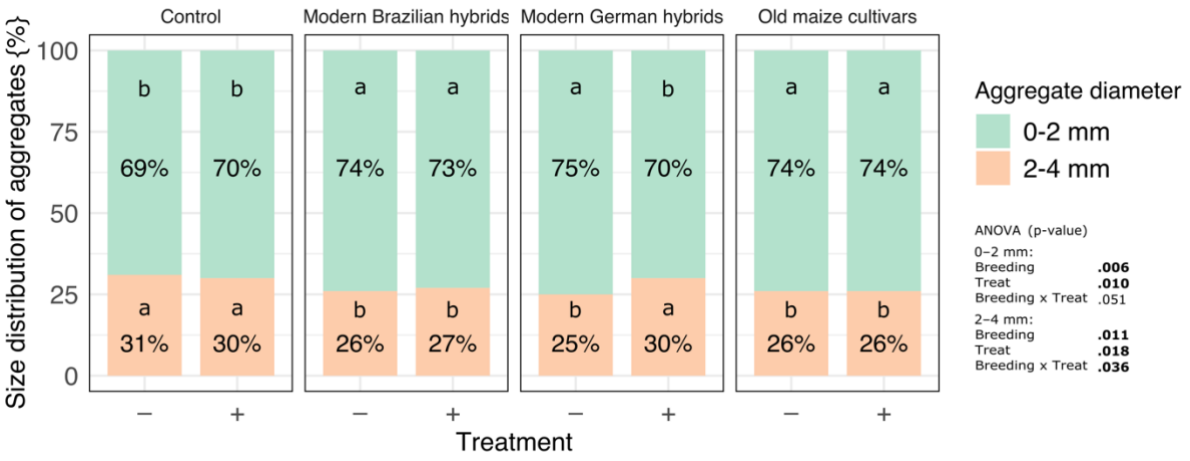


Figure 2. *Aggregate size distribution according to different maize breeding origins and treatments in percent, after sieving the samples into two fractions: 0–2 mm (green) and 2–4 mm (orange). The percentages were rounded. Same letters for each size range indicate no statistically significant difference by the Scott-Knott test ($p < .05$). The ANOVA results (p-values) are presented on the right.*

3.2 ASD considering four fractions: 0–250 µm, 250–500 µm, 500 µm–1 mm and 1–2 mm

The influence of different maize breeding origins and inoculation with *R. irregularis* and *A. brasilense* on ASD of aggregates with a diameter of 0–250 µm, 250–500 µm, 500 µm–1 mm and 1–2 mm was examined (Figure 3). Breeding origin showed an influence on the percentage of aggregates with a diameter of 0–250 µm ($p < .001$, Figure 3) and aggregates with a diameter of 1–2 mm ($p \approx .010$, Figure 3). For these fractions, the treatment influenced the effect of the breeding origin (0–250 µm: $p \approx .019$, 1–2 mm: $p \approx .007$, Figure 3). Treatment had no effect on any fraction (0–250 µm: $p \approx .129$, 250–500 µm: $p \approx .095$, 500 µm–1 mm: $p \approx .449$, 1–2 mm: $p \approx .957$, Figure 3). The percentage of aggregates with a diameter of 250–500 µm and 500 µm–1 mm was not influenced by the breeding origin (250–500 µm: $p \approx .360$, 500 µm–1 mm: $p \approx .246$, Figure 3).

The Scott-Knott test results show that the OMC examined a lower percentage of aggregates with a diameter of 0–250 µm compared to the control and the modern maize cultivars, whereby the modern cultivars did not differ from the control (OMC –: $\overline{x} \approx (1.9 \pm 0.2)\%$, MBH –: $\overline{x} \approx (3.0 \pm 0.3)\%$, MGH –: $\overline{x} \approx (3.1 \pm 0.4)\%$, control –: $\overline{x} \approx (2.6 \pm 0.5)\%$) (Figure 3). For all breeding origins, a lower percentage of aggregates with a diameter of 1–2 mm compared to the control was observed (MBH –: $\overline{x} \approx (15.6 \pm 0.8)\%$, MGH –: $\overline{x} \approx (18.2 \pm 0.9)\%$, OMC –: $\overline{x} \approx (18.1 \pm 1.0)\%$, control –: $\overline{x} \approx (23.7 \pm 2.3)\%$) (Figure 3). Only the control differed between inoculated and non-inoculated samples, having an increased percentage of aggregates with a diameter of 0–250 µm when inoculated (control +: $\overline{x} \approx (6.1 \pm 1.3)\%$) and a decreased percentage of aggregates with a diameter of 1–2 mm (control +: $\overline{x} \approx (17.5 \pm 1.2)\%$) (Figure 3).

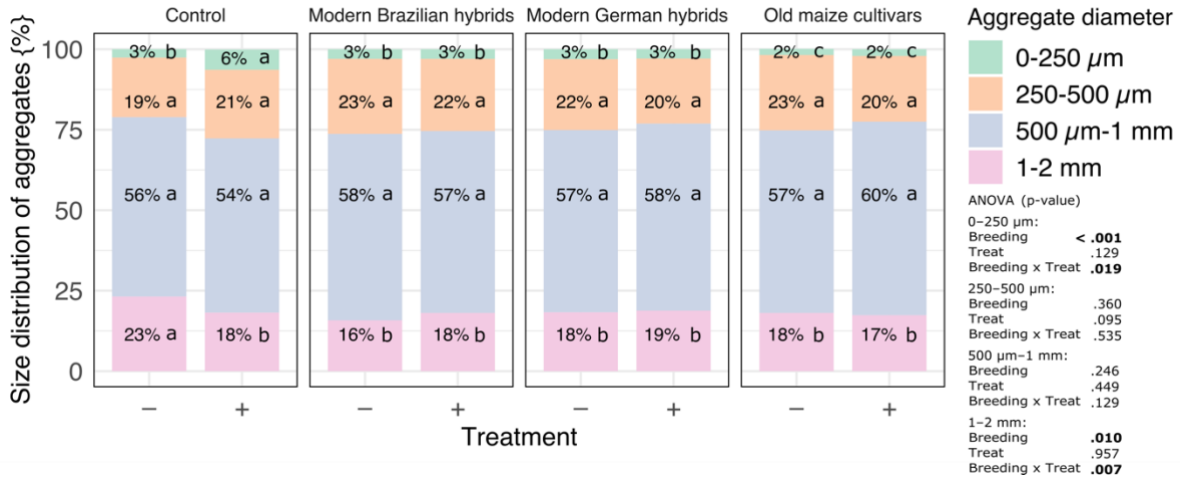


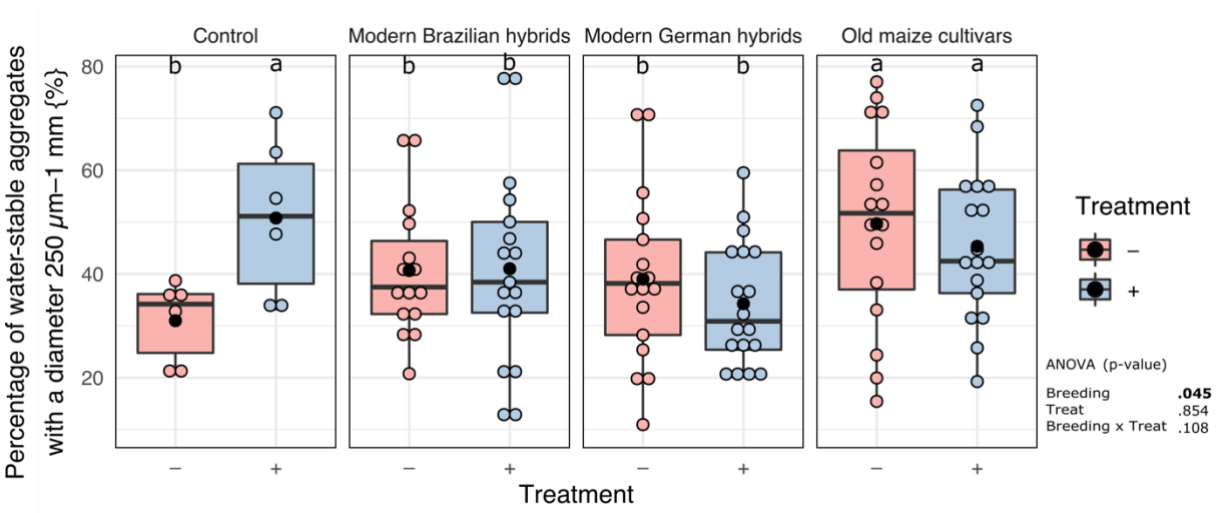
Figure 3. *Aggregate size distribution according to different maize breeding origins and treatments in percent, after sieving the samples into five fractions: 0–250 µm (green), 250–500 µm (orange), 500 µm–1 mm (blue), and 1–2 mm (pink). The percentages were rounded. Same letters for each size range indicate no statistically significant difference by the Scott-Knott test (p < .05). The ANOVA results (p-values) are presented on the right.*

3.3 WSA of two aggregate size ranges: 250 µm–1 mm and 1–4 mm

The influence of different maize breeding origins and inoculation with *R. irregularis* and *A. brasilense* on the percentage of WSA of two different aggregate sizes was examined (Figure 4). The percentage of WSA of both size fractions (250 µm–1 mm and 1–4 mm) was influenced by the breeding origin (250 µm–1 mm: $p \approx .045$, 1–4 mm: $p < .001$, Figure 4). Treatment had no effect on the percentage of WSA (250 µm–1 mm: $p \approx .854$, 1–4 mm: $p \approx .073$, Figure 4).

The results from the Scott-Knott test show that only the OMC showed an increase in WSA with a diameter of 250 µm–1 mm compared to the control (OMC –: $\overline{x} \approx (49.7 \pm 4.9)\%$, MBH –: $\overline{x} \approx (40.7 \pm 3.4)\%$, MGH –: $\overline{x} \approx (39.0 \pm 4.0)\%$, control –: $\overline{x} \approx (31.0 \pm 3.2)\%$) (Figure 4). Also, only the OMC showed a decrease in WSA with a diameter of 1–4 mm compared to the control (OMC –: $\overline{x} \approx (9.7 \pm 2.1)\%$, MBH –: $\overline{x} \approx (16.8 \pm 1.5)\%$, MGH –: $\overline{x} \approx (19.1 \pm 2.0)\%$, control –: $\overline{x} \approx (23.0 \pm 1.1)\%$) (Figure 4). Regarding the WSA with a diameter of 250 µm–1 mm, only the control showed a difference between inoculated and non-inoculated samples having a higher percentage of WSA when inoculated (control +: $\overline{x} \approx (50.8 \pm 6.3)\%$, OMC +: $\overline{x} \approx (45.3 \pm 3.6)\%$, MBH +: $\overline{x} \approx (41.0 \pm 4.7)\%$, MGH +: $\overline{x} \approx (34.3 \pm 2.9)\%$) (Figure 4). A lower percentage of WSA with a diameter of 1–4 mm was found in the inoculated modern cultivars compared to the non-inoculated samples, while OMC and the control were not affected by inoculation (MBH +: $\overline{x} \approx (13.8 \pm 1.5)\%$, MGH +: $\overline{x} \approx (13.8 \pm 1.5)\%$, OMC +: $\overline{x} \approx (11.0 \pm 1.7)\%$, control +: $(18.8 \pm 5.1)\%$) (Figure 4).

1)



2)

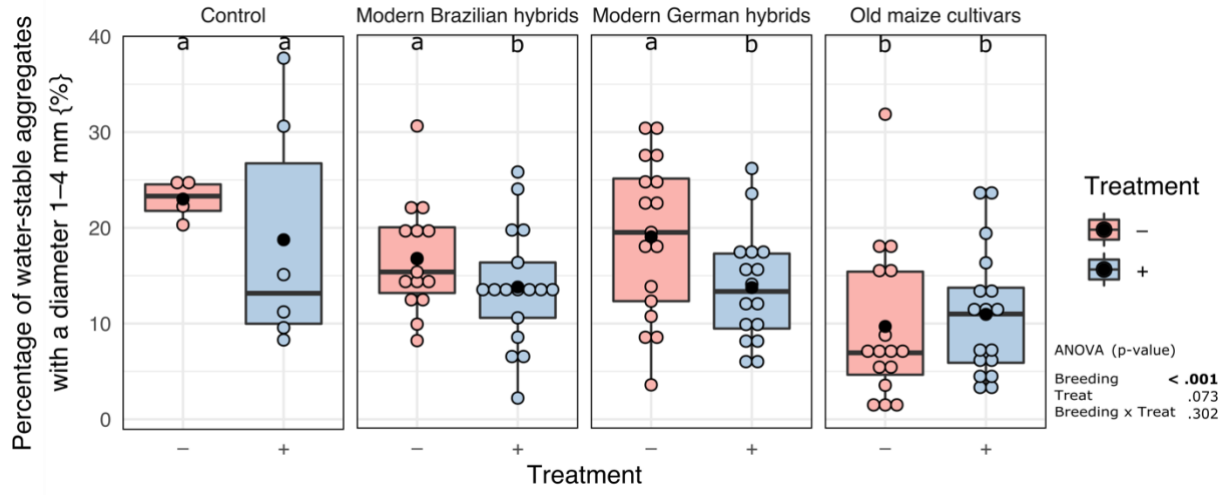


Figure 4. *The percentage of water-stable aggregates with 1) a diameter of 250 µm–1 mm and 2) a diameter of 1–4 mm according to different maize breeding origins and treatments. Same letters for each size range indicate no statistically significant difference by the Scott-Knott test ($p < .05$). The ANOVA results (p-values) are presented on the right.*

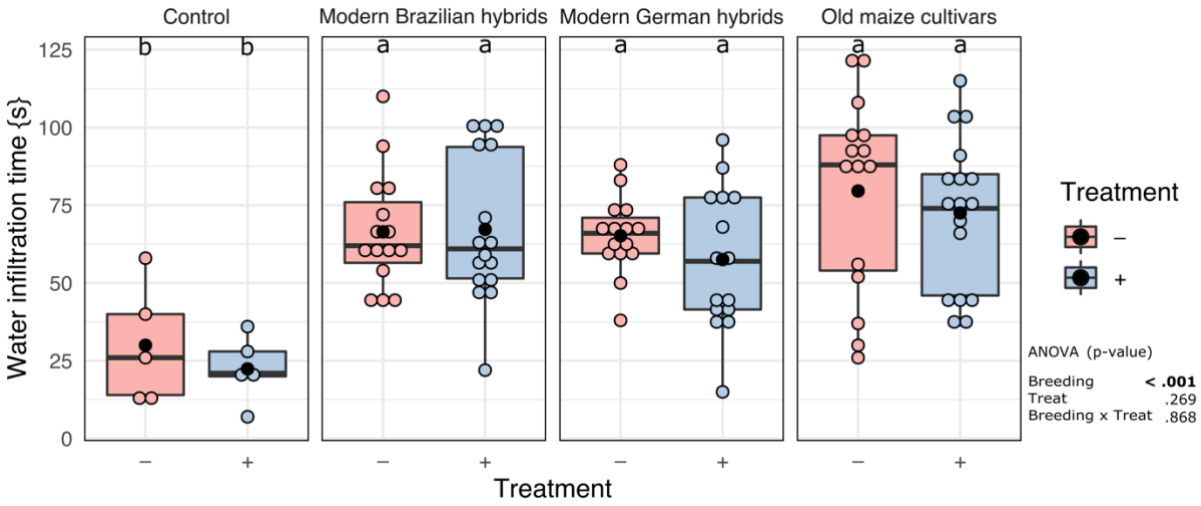
3.4 WR and WHC

The influence of different maize breeding origins and inoculation with *R. irregularis* and *A. brasilense* on WR and WHC of the soil was observed (Figure 5). Breeding origin influenced WR ($p < .001$, Figure 5) and WHC ($p \approx .018$, Figure 5) of the soil. The treatment influenced WHC ($p \approx .019$, Figure 5) but had no effect on WR ($p \approx .269$, Figure 5).

For all breeding origins an increase in WR of the soil compared to the control was observed (MBH –: $\overline{x} \approx (66 \pm 5)\ s$, MGH –: $\overline{x} \approx (65 \pm 4)\ s$, OMC –: $\overline{x} \approx (80 \pm 9)\ s$, control –: $\overline{x} \approx (30 \pm 9)\ s$) (Figure 5). Neither the breeding origins nor the control showed a difference in WR among treatments (MBH +: $\overline{x} \approx (67 \pm 6)\ s$, MGH +: $\overline{x} \approx (58 \pm 6)\ s$, OMC +: $\overline{x} \approx (73 \pm 6)\ s$, control +: $\overline{x} \approx (22 \pm 5)\ s$) (Figure 5).

All breeding origins showed an increased WHC of the soil compared to the control (MBH –: $\overline{x} \approx (31.9 \pm 1.7)\%$, MGH –: $\overline{x} \approx (35.0 \pm 1.4)\%$, OMC –: $\overline{x} \approx (32.0 \pm 1.2)\%$, control –: $\overline{x} \approx (24.0 \pm 5.5)\%$) (Figure 5), whereas only the control showed a difference based on inoculation, having a higher WHC when inoculated (control +: $\overline{x} \approx (33.2 \pm 2.9)\%$, MBH +: $\overline{x} \approx (35.0 \pm 1.1)\%$, MGH +: $\overline{x} \approx (34.8 \pm 1.2)\%$, OMC +: $\overline{x} \approx (34.8 \pm 1.1)\%$) (Figure 5).

1)



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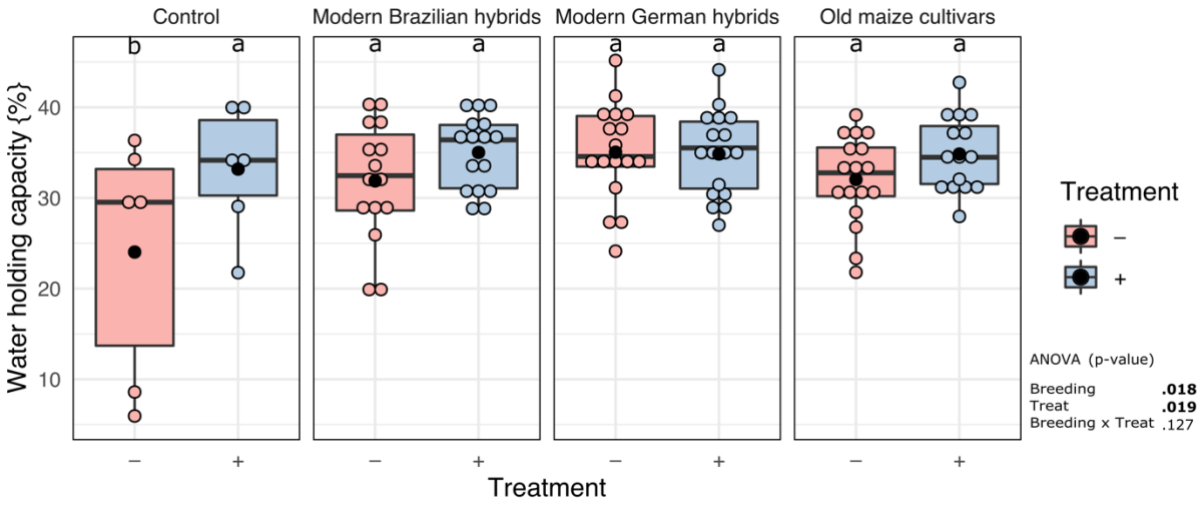

Figure 5. *1) Water repellency measured as water infiltration time in seconds and 2) water holding capacity in percent according to different maize breeding origins and treatments. Same letters for each size range indicate no statistically significant difference by the Scott-Knott test ($p < .05$). The ANOVA results (p-values) are presented on the right.*

3.5 pH of the soil

The influence of different maize breeding origins and inoculation with *R. irregularis* and *A. brasilense* on soil pH was examined (Figure 6). Breeding origin exhibited an effect on soil pH ($p < .001$, Figure 6), whereas treatment had no effect ($p \approx .524$, Figure 6).

The Scott-Knott test results show that soil pH was increased for all breeding origins compared to the control (MBH –: $\overline{x} \approx 6.4 \pm 0.1$, MGH –: $\overline{x} \approx 6.6 \pm 0.1$, OMC –: $\overline{x} \approx 6.6 \pm 0.1$, control –: $\overline{x} \approx 6.0 \pm 0.4$), but there was no difference between modern and older cultivars (Figure 6). No difference in soil pH between treatments was observed (MBH +: $\overline{x} \approx 6.6 \pm 0.1$, MGH +: $\overline{x} \approx 6.5 \pm 0.2$, OMC +: $\overline{x} \approx 6.7 \pm 0.1$, control +: $\overline{x} \approx 5.6 \pm 0.3$) (Figure 6).

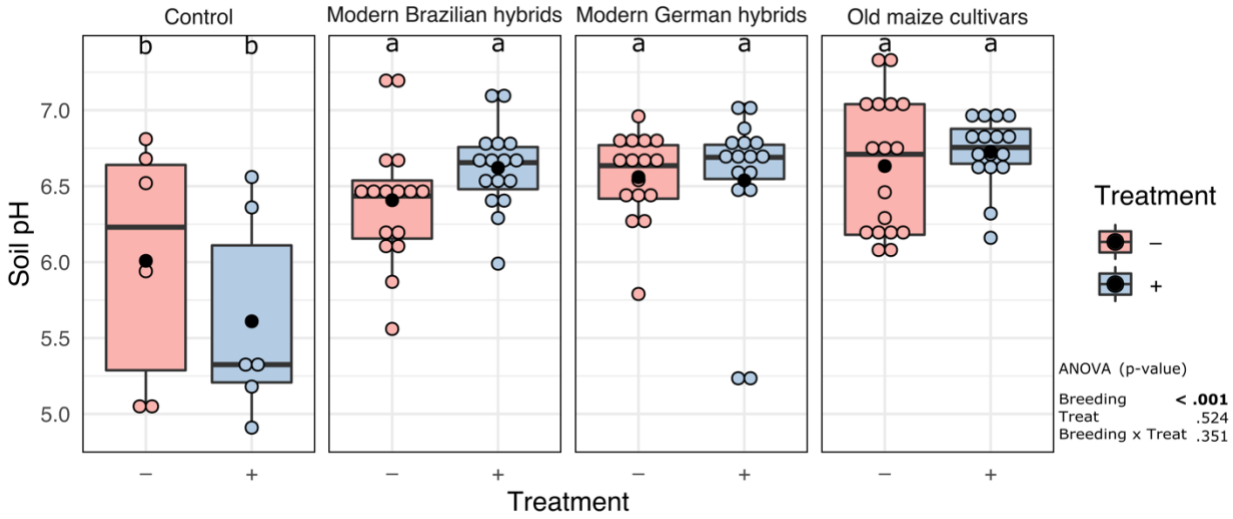

Figure 6. *Soil pH according to different maize breeding origins and treatments. Same letters for each size range indicate no statistically significant difference by the Scott-Knott test (p < .05). The ANOVA results (p-values) are presented on the right.*

3.6 Shoot biomass

The influence of different maize breeding origins and inoculation with *R. irregularis* and *A. brasilense* on shoot biomass was examined (Figure 7). Breeding origin and treatment had an influence on shoot biomass (breeding origin: $p < .001$, treatment: $p \approx .027$, Figure 7).

The Scott-Knott test shows that the OMC had a decrease in shoot biomass compared to the modern cultivars (MBH –: $\overline{x} \approx (233 \pm 9)\, mg$, MGH –: $\overline{x} \approx (278 \pm 25)\, mg$, OMC –: $\overline{x} \approx (136 \pm 14)\, mg$) (Figure 7). Especially Negro Cine had the lowest shoot biomass among all cultivars (Negro Cine –: $\overline{x} \approx (85 \pm 12)\, mg$, e.g., Golden Bantam –: $\overline{x} \approx (172 \pm 23)\, mg$, Table 1). Inoculation led to higher shoot biomass of modern cultivars, whereas OMC did not respond to inoculation (MBH +: $\overline{x} \approx (305 \pm 28)\, mg$, MGH +: $\overline{x} \approx (332 \pm 27)\, mg$, OMC +: $\overline{x} \approx (153 \pm 24)\, mg$) (Figure 7).

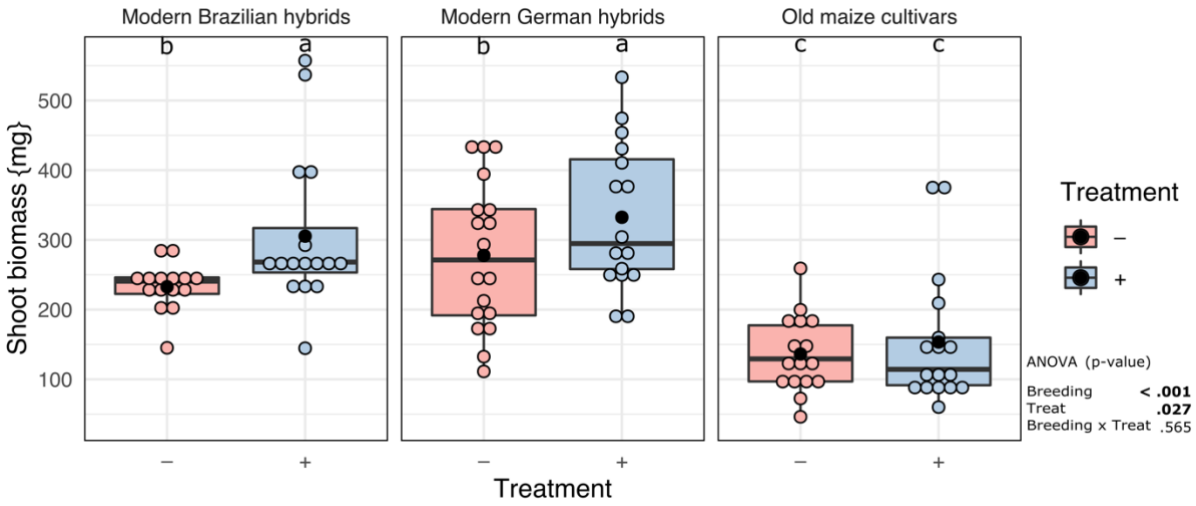

Figure 7. *Shoot biomass in milligram according to different maize breeding origins and treatments. Same letters for each size range indicate no statistically significant difference by the Scott-Knott test (p < .069). The ANOVA results (p-values) are presented on the right.*

3.7 The effect of maize cultivars and inoculation

The influence of the different maize cultivars and inoculation with *R. irregularis* and *A. brasilense* on all tested variables was examined and the results are summarized in Table 1. They will not be further analyzed, as this goes beyond the scope of this thesis.

Table 1. *Soil parameters and shoot biomass for each maize cultivar with and without inoculation with A. brasilense and R. irregularis.*

Breeding origin		Modern Brazilian hybrid						Modern German hybrid						Old maize cultivar							
Maize Cultivar		L940		J2M88		RK3115		Saludo		Fred		Robertino		Golden Bantam		Bear Paw		Negro Cine		Control	
Treat		–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+
Aggregate size distribution (%)	0–2 mm	68.3 ± 2.4 b	72.0 ± 1.6 b	77.3 ± 0.8 a	78.2 ± 1.6 a	75.9 ± 0.6 a	70.8 ± 1.0 b	74.9 ± 1.0 a	64.9 ± 1.8 c	75.5 ± 0.9 a	75.7 ± 1.3 a	76.0 ± 1.4 a	70.5 ± 0.8 b	75.6 ± 2.0 a	75.0 ± 1.0 a	74.5 ± 1.0 a	72.5 ± 1.5 a	72.8 ± 0.9 a	74.1 ± 0.6 a	68.6 ± 2.9 b	69.6 ± 1.1 b
	2–4 mm	31.7 ± 2.4 b	28.0 ± 1.6 b	22.7 ± 0.8 c	21.8 ± 1.6 c	24.1 ± 0.6 c	29.2 ± 1.0 b	25.1 ± 1.0 c	35.1 ± 1.8 a	24.5 ± 0.9 c	24.4 ± 1.3 c	24.0 ± 1.4 c	29.5 ± 0.8 b	24.4 ± 2.0 c	25.0 ± 1.0 c	26.7 ± 1.5 c	27.5 ± 1.5 c	27.2 ± 0.9 c	25.9 ± 0.6 c	31.4 ± 2.9 b	30.4 ± 1.1 b
	0–250 µm	2.5 ± 0.2 c	3.5 ± 0.4 b	3.7 ± 0.6 b	3.0 ± 0.3 b	2.8 ± 0.5 b	2.2 ± 0.2 c	3.8 ± 0.5 b	2.9 ± 0.6 b	3.6 ± 0.9 b	2.2 ± 0.2 c	1.9 ± 0.4 c	3.5 ± 0.6 b	1.7 ± 0.2 c	1.7 ± 0.2 c	2.3 ± 0.4 c	2.2 ± 0.2 c	1.7 ± 0.3 c	2.5 ± 0.4 c	2.6 ± 0.5 c	6.1 ± 1.3 a
	250–500 µm	17.9 ± 1.5 b	21.2 ± 0.2 b	27.8 ± 1.6 a	27.7 ± 1.5 a	22.2 ± 1.7 b	19. 3 ± 2.9 b	22.5 ± 1.4 b	19.7 ± 1.0 b	23.5 ± 1.2 a	17.5 ± 1.3 b	20.0 ± 3.3 b	23.3 ± 1.7 a	19.3 ± 1.0 b	17.3 ± 1.3 b	24.9 ± 2.6 a	21.8 ± 0.7 b	25.3 ± 1.7 a	22.1 ± 1.4 b	19.0 ± 1.2 b	20.4 ± 2.6 b
	500 µm–1 mm	56.9 ± 0.7 b	56.4 ± 1.4 b	54.1 ± 3.3 b	53.5 ± 1.7 b	59.6 ± 2.2 a	58.2 ± 1.8 a	53.3 ± 1.4 b	60.7 ± 0.9 a	52.1 ± 0.9 b	60.9 ± 1.2 a	62.2 ± 3.3 a	52.9 ± 2.4 b	58.2 ± 2.7 a	63.6 ± 1.3 a	56.7 ± 2.6 b	62.1 ± 1.5 a	55.7 ± 1.8 b	54.2 ± 1.3 b	57.0 ± 1.9 b	51.9 ± 1.8 b
	1–2 mm	17.5 ± 1.8 c	18.7 ± 1.4 b	14.4 ± 1.6 c	17.5 ± 1.4 c	15.4 ± 0.9 c	17.5 ± 0.6 c	20.4 ± 1.1 b	16.7± 1.1 c	18.5 ± 1.7 b	19.4 ± 1.9 b	15.9 ± 1.0 c	20.3 ± 1.0 b	22.7 ± 1.6 a	17.6 ± 1.5 c	15.3 ± 0.9 c	14.9 ± 1.0 c	16.5 ± 1.1 c	19.1 ± 1.0 b	23.7 ± 2.3 a	17.5 ± 1.2 c
Water-stable aggregates (%)	250 µm–1 mm	34.4 ± 2.6 b	32.6 ± 5.5 b	41.9 ± 7.7 b	32.2 ± 7.1 b	43.8 ± 5.5 a	56.7 ± 7.5 a	25.6 ± 4.7 b	36.7 ± 4.6 b	35.1 ± 2.0 b	36.3 ± 6.4 b	55.6 ± 5.3 a	29.8 ± 3.8 b	50.9 ± 10.8 a	46.3 ± 6.6 a	52.9 ± 2.6 a	54.8 ± 5.4 a	45.0 ± 9.6 a	36.5 ± 4.5 b	31.0 ± 3.2 b	50.8 ± 6.3 a
	1–4 mm	11.1 ± 1.3 b	13.0 ± 3.0 b	20.3 ± 3.0 a	10.8 ± 1.8 b	17.7 ± 1.5 a	17.2 ± 2.4 a	23.4 ± 1.4 a	12.1 ± 3.8 b	21.5 ± 3.5 a	16.6 ± 2.1 a	13.0 ± 3.5 b	12.8 ± 1.9 b	6.3 ± 0.8 b	6.8 ± 1.8 b	11.3 ± 4.8 b	12.9 ± 3.4 b	11.1 ± 3.4 b	13.5 ± 2.9 b	23.0 ± 1.1 a	18.8 ± 5.1 a
Water infiltration time (s)		59 ± 9 b	57 ± 5 b	73 ± 13 a	83 ± 10 a	66 ± 4 a	63 ± 13 a	55 ± 6 b	76 ± 5 a	68 ± 3 a	47 ± 5 b	72 ± 5 a	50 ± 12 b	76 ± 15 a	91 ± 7 a	93 ± 3 a	75 ± 10 a	74 ± 19 a	52 ± 8 b	30 ± 9 c	22 ± 5 c
Water holding capacity (%)		24.3 ± 2.6 b	34.6 ± 2.0 a	33.8 ± 1.5 a	36.0 ± 1.7 a	35.4 ± 2.4 a	34.5 ± 2.0 a	34.9 ± 1.9 a	32.5 ± 2.0 a	34.4 ± 3.4 a	35.2 ± 1.4 a	36.0 ± 1.1 a	36.9 ± 2.3 a	34.2 ± 1.3 a	34.0 ± 1.7 a	33.3 ± 2.1 a	39.6 ± 1.2 a	27.9 ± 2.3 b	32.0 ± 0.7 a	24.0 ± 5.5 b	33.2 ± 2.9 a
pH		6.7 ± 0.3 a	6.6 ± 0.2 a	6.2 ± 0.2 a	6.6 ± 0.1 b	6.4 ± 0.2 a	6.6 ± 0.1 a	6.5 ± 0.2 a	6.3 ± 0.4 a	6.7 ± 0.1 a	6.8 ± 0.1 a	6.4 ± 0.1 a	6.6 ± 0.1 a	6.5 ± 0.2 a	6.8 ± 0.1 a	6.7 ± 0.2 a	6.9 ± 0.1 a	6.8 ± 0.3 a	6.6 ± 0.2 a	6.0 ± 0.4 b	5.6 ± 0.3 b
Shoot Biomass (mg)		236 ± 5 a	330 ± 80 a	233 ± 7 a	320 ± 40 a	230 ± 23 a	264 ± 3 a	280 ± 60 a	300 ± 60 a	270 ± 40 a	266 ± 10 a	290 ± 40 a	409 ± 25 a	172 ± 23 b	240 ± 50 a	145 ± 18 b	129 ± 14 b	85 ± 12 c	84 ± 6 c	- -	- -

The means of the replicates and the standard errors are presented. Same letters in a line indicate no statistically significant difference in the values by the Scott-Knott test ($p < .05$).

4. Discussion

This thesis evaluated the effect of maize breeding origins, before and after the green revolution (MBH, MGH, and OMC), and inoculation with *R. irregularis* and *A. brasilense* on soil aggregation and soil parameters (ASD, WSA, WR, WHC, pH), and shoot biomass. We expected that modern and older maize cultivars would differ in the observed variables and their response to inoculation. However, overall, we detected that this was only true for some parameters and will discuss details below.

4.1 Hypothesis 1 - ASD, WSA, WR, WHC, pH, and shoot biomass will vary between modern and older cultivars

The percentage of aggregates with a diameter of 0–250 µm (Figure 3), the amount of WSA of both size fractions (Figure 4), and shoot biomass (Figure 7) were the only variables that differed between older and modern cultivars. These differences could be explained by the variable root/shoot and exudate allocation between modern and older cultivars (Feil, 1992). Also, the different maize cultivars have different exudates with specific properties, influencing soil aggregation and aggregate stability (Preece & Peñuelas, 2020), which could have led to the measured differences.

Since there were no differences among the breeding origins in either WR or WHC (Figure 5), it could be implied that their exudates provide the aggregates with similar hydraulic properties. Both high WR and high WHC are desired soil traits for agriculture since WR prevents soils from slacking and erosion and correlates with more stable aggregates (Goebel et al., 2005; Sullivan, 1990). Additionally, a high WHC increases the water supply for plant growth (Bhadha et al., 2017). Hence, none of the breeding origins has preferable traits regarding WR and WHC compared to the others.

A higher soil pH was observed for all breeding origins compared to the control (Figure 6), which contradicts hypothesis 1. However, these findings agree with the fact that roots mainly exude acids (Naveed et al., 2017). Indicating, that the exudates of modern and older cultivars have a similar pH.

Modern cultivars had higher shoot biomass than older cultivars (Figure 7). The grain yield of wheat correlates positively with shoot dry matter (Tolley and Mohammadi, 2020). Since the green revolution aimed for higher yields (Weil & Brady, 2017), this is in consent with our findings.

4.2 Hypothesis 2 - Differences in the response to inoculation with *A. brasilense*- and *R. irregularis* between modern and older cultivars will be observed

Regarding the amount of WSA with a diameter of 1–4 mm (Figure 4) and shoot biomass (Figure 7) modern and older cultivars responded differently to inoculation, which was predicted in hypothesis 2. These findings agree with other studies, stating that different maize cultivars differ in their response to inoculation (e.g., Chamam et al., 2013). In both cases, modern cultivars did respond to inoculation, while older cultivars did not (Figure 4, Figure 7). This could be explained by different root exudates among the cultivars, which differ in their interaction with the inoculants (e.g., Chamam et al., 2013).

Azospirillum was proven to promote plant growth by hormone regulation (Bashan & de-Bashan, 2010; Thuler et al., 2003) and to improve plant nutrient uptake, by increasing root growth (Okon, 1985; Okon & Kapulnik, 1986; Sarig et al., 1984). This leads to changes in plant root/shoot allocation (Veresoglou & Menexes, 2010), which could explain the increased shoot biomass of inoculated modern cultivars (Figure 7).

The decrease in WSA with a diameter of 1–4 mm that was observed for inoculated modern cultivars (Figure 4) could be explained by the mutualism-parasitism paradigm (Mandyam and Jumpponen, 2015). It states that host and fungal genotypes are very important for their symbioses. The hosts' responses depend on their genotype and therefore are very unpredictable. Thus, the symbioses range from mutualism to parasitism. In other words, although it is known that inoculation with *R. irregularis* commonly increases aggregate stability (e.g., Harris et al. 1966), the host genotype could change the interaction into parasitism, which could lead to a decrease in aggregate stability, which was observed here. It is also interesting, that inoculation did not affect the control, but it did affect the modern cultivars. It could be hypothesized that the symbiosis between modern plants and inoculants and/or the interaction between root exudates and the inoculants are crucial for the effect inoculation has on WSA formation.

As shown in previous studies, inoculation leads to an increase in larger aggregates and a decrease in smaller aggregates (e.g., Leifheit et al., 2014; Okon, 1985), which was only found in the ASD (0–2 mm and 2–4 mm) of MGH (Figure 2). It could be implied that inoculation compensated the effect that MGH had without inoculation. Without inoculation, all breeding origins had a higher percentage of smaller aggregates and a lower percentage of bigger aggregates compared to the control, whereas inoculated MGH were not different from the control anymore (Figure 2). According to Tisdall & Oades (1982), the presence of aggregates with a diameter of 1-10 mm improves the quality of soil structure, needed for crop growth. This would indicate that when only considering aggregate size, inoculated MGH would be the best-suited cultivars for crop

growth. The cultivar Saludo responded especially well to inoculation (Table 1). However, no clear difference between modern and older cultivars was found here. ASD (0–2 mm and 2–4 mm) was the only measured variable with a difference among both modern hybrids. It can be assumed that the exudates of MGH and/or their roots interact differently with the inoculants and their exudates, influencing aggregate size (Boleta et al., 2020; Chamam et al., 2013; Chu et al., 2013; Walker et al., 2011).

Regarding the ASD of the five fractions (0–250 µm, 250–500 µm, 500 µm–1 mm and 1–2 mm) (Figure 3), WSA with a diameter of 250 µm–1 mm (Figure 4), WR, WHC (Figure 5), and soil pH (Figure 6), no difference in the response to treatments was found between older and modern cultivars. This implies that their exudates interact similarly with the inoculants when it comes to the properties that influence these variables. These findings contradict many studies that observed an influence of inoculation with *R. irregularis* and *A. brasilense* on the measured soil properties (e.g., Hudson, 1994; Rillig et al., 2010; Thuler et al., 2003). For instance, while treatment had an effect in the first sieving, it had none in the second (Figure 2, Figure 3). One explanation for this could be that the root and hyphae exudates and the compounds produced by the bacteria are not involved in the stabilization of aggregates with the observed diameters. For example, polysaccharides are only involved in stabilizing aggregates with a diameter of less than 50 µm (Tisdall & Oades, 1982). Alternatively, the exudates could have only been temporary binding agents. Macroaggregates are bound by more transient binding agents, like fine roots and fungal hyphae (Six et al., 2004; Tisdall and Oades, 1982), from which the roots were removed after the first sieving. Also, since the second sieving was performed three weeks after the first sieving it could be assumed that the binding agents were already degraded (Jastrow et al., 2007; Oades, 1984; Six et al., 2004). Either way, the ASD of the five fractions (Figure 3), the WSA with a diameter of 250 µm–1 mm (Figure 4) and the WHC (Figure 5) of the control did respond to inoculation, implying that the effect of inoculation was compensated by the presence of the plant. This could have been the result of an interaction between plant exudates and inoculants. Apart from that, our results contradict what has been found in previous studies, e.g., that WR is increased by inoculation with AMF (Rillig et al., 2010) and that pH is decreased by bacteria (Helal & Sauerbeck, 1989; Hinsinger et al., 2003). Implying that the interaction between *R. irregularis* and *A. brasilense* could have different effects on soil properties than when inoculated alone (Mar Vázquez et al., 2000; Rashid et al., 2016). However, the increased WR and WHC (Figure 5) in the non-inoculated planted samples compared to the control could be explained by the presence of OM, like root exudates, which are known to increase WHC and WR, through e.g., hydrophobic coating on soil particles (e.g., Rillig et al. 2010; Wessels, 1996).

4.3 Discussion of the method and possible error sources

Since lab processing (i.e., sieving) alters the forces acting between soil particles (Figure 1), it is questionable, if the used methods yield adequate results for conclusions compared to undisturbed soils. New methods are promising for the delineation of aggregates in intact soils using X-ray imaging (e.g., Koestel et al., 2021), however, these methods are still expensive and time-demanding for analyzing several samples, as is the case of this thesis.

Some samples were contaminated with fungal growth during the greenhouse experiment. Since no clear pattern was seen regarding which samples were affected, the fungus probably came from outside the experimental setting. This fungus could have influenced the samples and thus likely affected the variability among replicates and the standard deviation of the measured parameters. This could be avoided in future experiments by conducting the experiment in sterile conditions.

Further, aggregate size data is compositional, since the parts are constrained to the total, and so compositional data analysis (CODA) of the ASD data should be further performed and could improve the statistical analysis of the data.

5. Conclusion and outlook

In conclusion, it was shown that some of the examined soil parameters and the effect of inoculation with *R. irregularis* and *A. brasilense* differed between modern and older maize cultivars. But also, the response to inoculation often differed from what we predicted. For example, WR and pH were not affected by inoculation, although it is known that AMF-inoculation increases WR (Rillig et al., 2010) and bacteria decrease pH (Helal & Sauerbeck, 1989; Hinsinger et al., 2003). All these findings indicate that there are important differences in modern and older maize cultivars, which are likely related to root exudates. We assume that different interactions between the exudates and the inoculants and/or the soil particles influenced the soil parameters.

The exudates inside the soil samples should be further identified so that the interactions between root exudates and inoculants can be used systematically to improve soil structure for agriculture. Also, the influence of the inoculants and the different breeding origins on root growth should be examined in further studies, to learn more about root/shoot and exudate allocation. Additionally, methods to study soil properties in undisturbed soils should be further developed to get results that describe the conditions in situ better (e.g., Koestel et al., 2021).

When the effect of different maize cultivars on soil structure is better understood, these results could influence future plant breeding aiming for improved soil structure and thus enabling sustainable and profitable agriculture, which is needed now more than ever.

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8. Appendix

Table 2. *ANOVA Table (Type II tests) related to the soil properties and shoot biomass according to maize breeding origin and treatment.*

		Explanatory variable	Sum Sq	Df	F	p
Aggregate size	0–2 mm	Breeding	208.75	3	4.3448	**.006**
		Treat	109.73	1	6.8517	**.010**
		Breeding x Treat	128.92	3	2.6832	.051
		Residuals	1553.48	97		
	2–4 mm	Breeding	192.64	3	3.9406	**.011**
		Treat	94.52	1	5.8004	**.018**
		Breeding x Treat	144.26	3	2.9509	**.036**
		Residuals	1596.94	98		
	0–250 µm	Breeding	9.287	3	8.3094	**< .001**
		Treat	0.871	1	2.3386	.129
		Breeding x Treat	3.887	3	3.4778	**.019**
		Residuals	37.998	102		
	250–500 µm	Breeding	75.12	3	1.0830	.360
		Treat	65.53	1	2.8344	.095
		Breeding x Treat	50.74	3	0.7316	.535
		Residuals	2335.22	101		
	500 µm–1 mm	Breeding	122.22	3	1.4053	.246
		Treat	16.73	1	0.5770	.449
		Breeding x Treat	168.24	3	1.9345	.129
		Residuals	2956.94	102		
	1–2 mm	Breeding	146.84	3	4.0006	**.010**
		Treat	0.04	1	0.0030	.957
		Breeding x Treat	157.47	3	4.2901	**.007**
		Residuals	1223.51	100		
Water-stable aggregate size	250 µm–1 mm	Breeding	2031.5	3	2.7705	**.045**
		Treat	8.3	1	0.0341	.854
		Breeding x Treat	1520.0	3	2.0729	.108
		Residuals	25420.6	104		
	1–4 mm	Breeding	1086.9	3	0.0002533	**< .001**
		Treat	169.5	1	0.0733063	.073
		Breeding x Treat	191.3	3	0.3021416	.302
		Residuals	5174.5	100		
Water infiltration time		Breeding	19363	3	12.7889	**< .001**
		Treat	625	1	1.2387	.269
		Breeding x Treat	363	3	0.2399	.868
		Residuals	47944	95		
Water holding capacity		Breeding	356.5	3	3.5054	**.018**
		Treat	192.9	1	5.6878	**.019**
		Breeding x Treat	197.8	3	1.9438	.127
		Residuals	3425.2	101		
Soil pH		Breeding	47751145	3	8.6329	**< .001**
		Treat	752869	1	0.4083	.524
		Breeding x Treat	6101377	3	1.1031	.351
		Residuals	186221117	101		
Shoot biomass		Breeding	145.661	2	39.1088	**< .001**
		Treat	9.383	1	5.0383	**.027**
		Breeding x Treat	2.138	2	0.5741	.565
		Residuals	171.327	92		

The response variables are ‘Size distribution of aggregates in percent’, ‘percentage of water-stable aggregates in percent’, ‘water infiltration time in seconds’, ‘water holding capacity in percent’, ‘soil pH’, and ‘shoot biomass in milligram’. ‘Breeding’ contains the factors ‘Old maize cultivars‘, ‘Modern Brazilian hybrids‘, and ‘Modern German hybrids‘. ‘Treat’ contains the factors ‘treat +’ and ‘treat –‘. (p-values smaller than .05 represent a statistically significant effect of the explanatory variable on the response variable).