The effect of selective breeding and genetic manipulation on the microbiome surrounding maize roots

by

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Abstract

Maize (*Zea mays* L.) is a major staple crop whose wild ancestor was domesticated about 9,000 years ago (Beadle, 1939). Long-term breeding for more desirable traits ultimately resulted in the maize we see today. This long-term breeding likely impacted the processes within the rhizosphere of maize, however, to what extent is not well understood. This study examined the microbial communities between an inbred maize line (B73), a hybrid of two isogenic lines (B73xMo17), and two genetically modified maize hybrids (DKC63-55RIB and DKC64-69RIB) to determine if the plant's ability to attract beneficial microbes changed with breeding. The hypothesis was that the isogenic cultivar forms better relationships with bacteria and fungi compared to the newer cultivars, especially in low P soil. It was also expected that the greater the difference between the cultivars the more distinct their soil microbiome. To test these hypotheses, experiments were conducted under greenhouse and field conditions. Analyses consisted of root staining to test symbiotic relationships, phospholipid fatty acid analysis (PLFA) for microbial communities, total plant and root biomass, and nutrient content to understand plant responses. Based on the field results, there was no impact on root and shoot biomass and nutrient content by differences in cultivar. Differences in cultivar did impact arbuscular mycorrhizal fungi (AMF) colonization, which decreased over time and depth for all. Soil AMF also saw a significant effect by cultivar. Other microbial groups were not impacted by cultivar, were greatest in the control, and decreased over time. Greenhouse results showed a cultivar by time interaction for root and shoot biomass. Soil P also impacted shoot biomass, but not root biomass. Shoot nutrient content was greater in high P soil, while roots only saw an impact for root P. No cultivar effect was found for soil microbial groups except for fungi, while all microbial groups were reduced in the control soil. Most soil microbial groups were also impacted by soil P as

indicated by reduced concentrations in low P soil. AMF was the only microbial group that was not negatively impacted by limited soil P. In addition, all soil microbial groups increased over time, although fungi saw a decrease at R1. No significances were observed for percent AMF colonization.

Table of Contents

List of Figures

List of Tables

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Dedication

I dedicate this work to my daughter, Athena Crabtree, who has spent a lot of her childhood on campus while I pursued my education. No matter what the situation, you always made the best out of it. You are a wonderful person and I will never be able to thank you enough for everything.

Chapter 1 - Introduction and Literature Review

With an anticipated 9.5 billion people by 2050 and the increasing concern about climate change, there is a need for enhanced crop production that has less of an impact on the environment (e.g. greenhouse gas emissions, soil erosion, nutrient losses, etc.). This will be a challenging task as climate change brings with it a series of interacting issues such as changes in precipitation patterns, increased drought periods, increased temperatures, shorter and milder winters, increased insect herbivory, and increased plant disease. Because of this, crop yields will reduce to levels below current annual production. These reductions in crop yields will be detrimental as production must increase to meet future food demands. Increasing production will require more novel approaches to current agricultural practices. One much-neglected aspect of agriculture can provide some solace, which is the belowground interaction between plants and the soil environment. Previous research indicated that microbial interactions can benefit plant health and that selective pressures in agriculture can result in changes in root morphology and physiology, which therefore impacts the microbiome that surrounds them (Graaff et al., 2013). Knowing this, there is potential in manipulating these aspects to achieve improved plant-microbial interactions.

Maize is ideal for such research as it is produced all over the world under diverse climates in many soil types, and is impacted by the pressures of agriculture.

History of Maize

Archaeological evidence from the highlands of Mexico dates the domestication of the wild ancestor of maize back to 9,000 years ago (Beadle, 1980). There have been several names for the wild ancestor of maize, such as cincocopi, atzitzintle, and acecintle (Blake, 2015). Today, however, we refer to the wild ancestor as teosinte (*Zea mays* ssp. parviglumis), a name of

Nahuátl Indian origin (Doebley, 2004). Teosinte plants do not look like the maize (*Zea mays* ssp. mays) we see today. This is likely the result of several genetic mutations, which were more desirable and thus selected for (Blake, 2015). Eventually, maize was successfully cultivated and entered western and northern Mexico before expanding into the southwestern United States (Boyd et al., 2006). By 1493, maize was introduced into southern Spain by Columbus not long after he entered the New World (Rebourg et al., 2003). Historians and linguists considered this to be the source of all maize in Europe, however, that particular maize was adapted to warmer climates (Dubreuil et al., 2006). The fact that maize was successfully cultivated in Germany in 1539 (Finan, 1948), not even a decade later, means that there were other introductions of maize into Europe that came from different parts of the New World (Rebourg et al., 2003).

Modern Maize Production

The 20th Century saw great advancements in crop production. In 1953, hybrid maize seeds were first sown and research towards the development of domestic maize hybrids started (Stojaković et al., 2010) as they showed an increase in yield. Soon after, starting in the 1960s, the green revolution occurred and was powered by increased investments in research and market development as well as better policy support (Pingali, 2012). This ultimately led to the hybridization of the two isogenic inbred lines B73 [released 1972 (Russell, 1972)] and Mo17 [released in 1973 (Zuber, 1973)], producing the popular hybrid B73xMo17 in 1973 (Troyer, 1999). This hybrid has been the parental line for many modern maize cultivars we see today. Despite the continuous efforts to improve grain yield, maize production remains vulnerable to factors such as disease, nutrient deficiency, water stress, herbivory, and others. This meant there was a need for further research into improving crop production.

Introduction of Biotechnology

The use of biotechnology has assisted crop production in many ways, and the development of biotechnology has consisted of several overlapping discoveries. Starting in the 1970s, the use of a model plant system was initiated with *Arabidopsis thaliana* because of its several advantageous characteristics (Bahadur et al., 2015). The improved understanding of plant systems ultimately led to the use of biotechnology in cropping systems. In the early 1980s, work was being done by two scientists on the genetic manipulation of *Nicotenia tabacum*. Lloyd W. Ream and Milton P. Gorden (1982) found that the *Agrobacterium tumefaciens* can infect this plant via wounds in the plant tissue. Their idea was that genetic modification of the bacteria could thus introduce new genes into the plant DNA as well, resulting in a genetically modified plant. The introduction of manipulated DNA via *Agrobacterium* was not successful for all plants and thus other methods to introduce new DNA were developed, one of those being particle bombardment (Lundquist and Walters, 2007). This provided several new opportunities to the field of agriculture and has since become a very important aspect of modern crop production, especially for maize.

In 1995, due to the high annual costs associated with crop losses caused by the European corn borer, two biotechnological companies (Mycogen Corporation and Ciba Seeds) were approved by the U.S. Environmental Protection Agency (EPA) to genetically engineer maize seeds using a gene from the soil bacterium *Bacillus thuringiencis* (Bt) (Ward, 1995). The success in improving crop yields using biotechnology ultimately led to the Panzea project. This project was funded by NSF in 1999. Through this project, researchers are able to gather and study vast amounts of genetic information on the *Zea* genus (Blake, 2015).

The continuous efforts towards improving maize have helped maize production greatly. Compared to the 91.39 million metric tons produced in the U.S. in 1961 (FAOSTAT, 2016), data for 2014-2015 shows that there was a global maize production of 1,012.84 million metric tons of which the United States (U.S.) produced the largest share with 361.09 million metric tons (USDA ERS, 2016; FAS, 2016). This is an increase of 269,702 million metric tons in the U.S. alone.

Future of Maize Production

The future of crop production is unsure. Agriculture today is very demanding of soil and crops to provide food for an exponentially growing population. Crop production means some level of soil disturbance, depending on the management practices. This disturbance-dependent agriculture has resulted in soil erosion, nutrient losses, soil biodiversity losses, weed challenges, and reduced water intake and retention (Crews et al., 2016). With the rapidly growing population, it is estimated that there will be 9.5 billion people by 2050, an estimate established by the Agrimonde foresight study (Paillard et al., 2014). This is 2.2 billion more people than the global population of 7.3 billion in 2015 (He et al., 2016). To feed this population, crop production will have to increase. This will be challenging because the degradation of already cultivated land and the decline of available land means that the increase of crop production will have to be achieved through improving crop yield. Furthermore, there are challenges such as climate change, depleting aquifers, and other constraints on crop productions that are pushing the need for a sustainable intensification of agriculture (Baulcombe, 2010). In other words, another green revolution is needed.

Genetic modification of crops has been and still is one of the main ways of enhancing not only yield but also nutritional value. Despite its negative image, this technology will help to

provide food for a future under less than ideal environmental conditions. These conditions are predicted to include, but are not limited to, greater soil erosion, water stress, heat stress, increased insect herbivory, nutrient deficiency, and increased plant disease. The use of genetically modified crops alone will not be sufficient and should be used with measures that will improve the environmental conditions, such as better management practices. These practices also improve yields. This is clear when using a no-till system as the improved soil structure allows for better water use efficiency, fewer greenhouse gas emissions (Hobbs et al., 2008), and greater microbial biodiversity.

The Importance of Soil Microorganisms in Crop Production

For many years, crops have been bred for specific desirable traits. However, this breeding for aboveground traits may have impacted belowground interactions between the plant and the soil environment. The increasing need to optimize crop yields while reducing the environmental impact associated with enhanced agriculture has led scientists to examine the belowground aspect in search of improving plant-microbial interactions (van der Heijden and Wagg, 2013). This is especially true for maize plants, as maize is a major crop that is produced globally and is often associated with monoculture, an agricultural practice that focuses on one crop in an area. In addition, because of the crop's great phenotypic and genetic variations, maize can be used to better understand the impact of plant genetic variation on soil microbial communities (Peiffer et al., 2013).

Soil Microbial Communities

Soils hold a wide variety of biodiversity with complex interactions. Microbes are a large part of the soil and are an important part of agriculture. Their associations with the plant vary and can be beneficial, neutral, or detrimental to the plant. Understanding how the plant and soil

microbiome interact is important in assessing overall plant health and productivity (Turner et al., 2013). Plants have a great influence on the microbial communities within the soil by modifying the soil environment via their roots. Through the exudation of chemical compounds, plant roots can promote or interfere with the growth of microbial species, thus changing the soil microbiome surrounding plant roots from that of the bulk soil. A better understanding of how plant roots manipulate the soil to form beneficial microbial communities could provide a great resource for improving plant growth and plant health (Bouffaud et al., 2011). As the plant grows, the roots exchange inorganic and organic materials with the surrounding soil, which is heavily populated by soil-borne microorganisms (Ryan et al., 2017). The soil directly surrounding roots or the soil that is affected by plant roots is also commonly referred to as the rhizosphere (Pinton et al., 2007). Associated microorganisms aid in the exchanging of materials by catalyzing the biogeochemical processes between the soil and the plant and are essential for plant growth (Gomes et al., 2001). Furthermore, the microorganisms that are associated with the plant can act as a protective barrier against certain phytopathogens and improve the growth of the plant by producing phytohormones. In addition, the microorganisms can boost the plant's ability to withstand environmental stressors such as heat, water, and salinity. To fully exploit these benefits, plants can create a more desirable rhizosphere by altering the pH and producing exudates that attract a particular group of microorganisms. Abiotic stress such as salinity, temperature, and drought can impact soil microorganisms and their function as well. Under these and some biotic stress conditions, the plants overproduce the growth regulating plant hormone, ethylene. Bacteria that produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase can lower the ethylene production in plants by degrading its precursor ACC (Yang et al., 2015). The ACC deaminase-producing bacteria lower the concentrations of ACC outside of the root. This

stimulates the plant to release more ACC into the rhizosphere and thereby reducing the amount available to be converted into ethylene. By doing this, the ACC deaminase containing bacteria help regulate and lower the levels of ethylene in the plant, thereby boosting plant growth. Not all soils, however, contain bacteria that produce ACC deaminase.

Soil Microbial Diversity

Microbial diversity often refers to the taxonomic and functional diversity of different microbial groups (Banu et al., 2004; Bouffaud et al., 2011) and their relative abundance within a microbial community (Dey et al., 2012). In terrestrial ecosystems, this diversity aids in the multifunctionality of the soil, or the ability to perform multiple ecosystem functions and services at the same time (Delgado-Baquerizo et al., 2016). The importance of diversity is evident when it is reduced as this impacts ecosystem function, soil erosion, crop quality/quantity, and more. A diverse microbial group will have dominant taxa and rare taxa, where dominant microbial groups are responsible for most of the microbial activity. Rare microbial groups, although less in numbers, are a source of genetic and functional diversity. This allows soil to adapt to changing environments (Bent and Forney, 2008), which is of importance in a changing climate. To assess microbial diversity within a soil, it is important to know that culture-based assessments are very limited because less than 1% of all soil microorganisms can be cultured (Ward et al., 1990). Because of this, researchers utilize molecular techniques such as sequencing of 16S rRNA, DNA fingerprinting, and PCR (Banu et al., 2004). Nonetheless, non-culturing techniques have their limitations as well and to obtain a true representation of soil microbial diversity, it is best to combine different methods (Zhang and Xu, 2008).

In the rhizosphere, microbial diversity is different from that of the bulk soil. Research by Li et al. (2014) compared microbial diversity in both rhizosphere and bulk soil of modern maize.

Their results indicate that the rhizosphere is selective and reduces microbial diversity while increasing functional diversity compared to that of the bulk soil. These findings were supported by research on the maize rhizosphere by García et al. (2001). To what extent genetic manipulation and long-term breeding for more desirable traits have altered the rhizosphere processes is not well known. However, microbial diversity is influenced by the physical and chemical properties of the soil which are influenced by the genetics of the plant (Peiffer et al., 2013). Planting sequence and plant age can also impact the microbial diversity within the rhizosphere. A study on wheat showed less microbial diversity surrounding young wheat roots which increased over time with significant differences between the vegetative and reproductive stage (Donn et al., 2014).

Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal fungi (AMF) are microorganisms that are known to form associations with plants that are mutually beneficial to both plant and fungi (Blackwell, 2000). This symbiosis likely aided plants in their invasion of nutrient-poor terrestrial environments long ago (Pirozynski and Malloch, 1975) while they co-evolved over the span of more than 400 million years (Hamel and Plenchette, 2007). Of all terrestrial plants that are currently in existence, more than 80% can form these beneficial associations (Smith and Read, 2010). Nutrient acquisition is greatly improved by the presence of AMF (Dighton, 2016), especially for phosphorus (P) (Fitter, 1990). Other nutrients that experience enhanced uptake are nutrients such as copper (Cu), zinc (Zn), potassium (P), and nitrogen (N) (Gildon and Tinker, 1983; Marschner and Dell, 1994). The root colonization of AMF also impacts water uptake of plants because more soil can be explored with the presence of fungal hyphae.

The Mechanism behind AMF Colonization

The process of root exudation allows plants to send out signals to the surrounding soil microbiome to protect themselves against harmful organisms or to attract beneficial microorganisms (Bouwmeester et al., 2007). Several factors affect root exudation: plant species, plant age, temperature, light, plant nutrition, microorganisms, medium supporting the roots, soil moisture, and root damage (Rovira, 1969). This is evident in the biosynthesis and responsiveness of phytohormones such as strigolactones, as these are involved in the ability of plant roots to sense and respond to a low P soil environment (Umehara et al., 2010; Mayzlish-Gati et al., 2012).

Arbuscular mycorrhizal fungi are obligate plant symbionts from the phylum Glomeromycota (Lekberg et al., 2013). The establishment of a symbiotic relationship between a plant and AMF is a mutual effort that starts before there is even physical contact. During the presymbiotic stage, the plant exudates signaling compounds into the rhizosphere (Buee et al., 2000) that induce spore germination and hyphal branching of AMF (Giovannetti et al., 1993). Strigolactones are one example of such exudates. These plant hormones are branching factors (BF) responsible for the triggering of hyphal growth and extensive hyphal branching (García et al., 2001; Koltai, 2011). The increased branching of the AMF hyphae ensures close contact with the plant roots after which the hyphae develop hyphopodia (Bonfante et al., 2000), allowing the AMF to penetrate the outer root tissues (Genre et al., 2005). While the plant produces exudates to attract AMF, the AMF release chitooligosaccharides. These compounds induce calcium spiking in the nucleus of receptive plant cells, triggering the relocation of the nucleus, and allows for the plant cells to form an intracellular accommodation structure (Gutjahr and Parniske, 2013) also known as the prepenetration apparatus (PPA) (Genre et al., 2005). After PPA formation, the

fungus enters the cell, forms an arbuscule trunk, and differentiates into a mature arbuscule (Gutjahr and Parniske, 2013).

The Impact of AMF on Soil Health

When thinking of soil health, it is important to compare the soil to a complex living system that respires, requires moisture, and can support life to other organisms. Like a living organism, each soil system is unique and complex as they are formed under a different climate, topography, or parent material. These varying environments can impact the chemical and physical properties of a soil, such as soil structure, nutrient exchange capacity, water holding capacity, etc. To assess soil health, it is therefore important to look at the physical, and chemical components. However, biological components of the soil are important as well. These three can be examined by looking at the four key indicators of soil health: soil organic carbon, soil structure, water infiltration and retention, and soil biodiversity (Singh et al., 2011).

Soil Organic Carbon

Soil organic carbon (SOC) is an indirect product of organic material decomposition. Fungi and bacteria are the primary factors in the decomposition of organic material. The rate at which they can decompose depends on several factors such as climate, soil conditions, and the quality of the plant material (Kibblewhite et al., 2008). The plant material that gets decomposed will become part of what is known as soil organic matter, which can get decomposed further by other microorganisms. Through the immobilization and mineralization of the nutrients that are in the organic matter, soil microorganisms contribute to nutrient cycling within soil. Not all of the organic matter will be broken down and taken up by plants; some will contribute to the formation of soil aggregates and thereby protect SOC through encapsulation. Other organic matter may form intimate bonds with soil minerals, making the extraction and study of soil organic matter

difficult (Lehmann and Kleber, 2015). Soil organic carbon is a great indicator of soil health as high SOC often correlates with higher crop yields, however, there are disadvantages. In some instances, high SOC can interfere with nitrogen (N) availability to plants as well as require greater soil pesticide applications (Letey et al., 2003; Sojka et al., 2003; Bennet et al., 2010).

Soil Structure

Structure is one of the main physical components of soil and by soil pedology standards, refers to the size, shape, and orientation of soil peds or aggregates (Lal, 2006). Soil aggregates consist of different sized soil particles such as sand, silt, and clay and are held together by a variety of biological byproducts. Aggregation of soil is an important aspect of soil structure because it impacts multiple soil functions such as water infiltration, soil aeration, carbon (C) storage, soil moisture retention, etc. The formation of aggregates and the stabilization of these can be improved by the presence of AMF. In 1996, researchers found that glomalin, a glycoprotein that is secreted by AMF, has an important role in soil aggregation (Wright and Upadhyaya, 1996; Wright et al., 1999) because it glues the soil particles together (Rillig et al., 2002). The protein can last between 6 to 42 years in soil and adds up to 15% of stable organic carbon to grasslands (Miller et al., 2000) and has a greater impact on soil aggregation than the presence of fungal hyphae alone (Rillig et al., 2002). Aside from glomalin production, under constant abiotic factors, the hyphae of AMF are one of the most important factors that affect soil aggregation followed by the effect of roots, soil bacteria, and soil fauna (Oades, 1984). This could be because AMF hyphae tend to have a longer life span in soils since grazers prefer to feed on saprobic fungal hyphae (Klironomos and Kendrick, 1996). In addition, AMF can grow using the C provided by plants and can, therefore, extend into C poor bulk soil, unlike saprobic fungi (Smith et al., 2011).

Water Infiltration and Retention

Improved soil structure increases water infiltration and enhances drainage. With changing precipitation patterns, precipitation distribution and intensity will change, and the survival of plants will therefore largely depend on the plants' ability to tolerate drought. Since plants gather water *via* the roots, an important soil volume to focus on is that of the rhizosphere, or the volume of soil directly surrounding the roots (Haichar et al., 2014). Soil moisture is largely impacted by soil stability. Management practices therefore have a large impact on how crops will endure drought. Under healthy soil conditions, there will be greater biodiversity and a larger variety of soil aggregates. The main drivers of soil aggregation are the wetting and drying cycles of the soil in combination with the deposition of organic compounds, burrowing by small creatures, roots, and mineral surface bonding (Dexter, 1988).

Soil that is in the direct vicinity of roots has greater aggregate stability and is more stable. This is evident when pulling roots out of the soil and finding soil adhering to the roots. Roots produce mucilage, a gel-like polysaccharide that is secreted by the root cap cells to help roots penetrate the soil. The amount of mucilage that gets produced depends on how much resistance the root tips experience. In a study using glass beads, they found that root exudation of young corn plants increased when roots faced obstructions (Boeuf-Tremblay et al., 1995). Root mucilage is also an important factor in hydraulic conductivity. In the presence of water, the mucilage will expand and take up water, thus improving the hydraulic conductivity between the roots and the bulk soil (McCully and Boyer, 2006). Hydraulic conductivity is important, as water is needed to generate turgor pressure within the root cells, which ultimately drives further root growth. This is shown by a study performed by Taylor and Gardner (1963), indicating that cotton taproots were less likely to penetrate the soil when the soil water pressure potential was at -

0.2MPa. The optimum water pressure potential differs among plants as shown by a study performed by Clark et al. (2003). Corn and grain sorghum (*Sorghum bicolor* L.), for example, have a reduced total root weight under dry conditions compared to soybean (Mayaki et al., 1976). Other crops, such as chickpea (*Cicer arietnum* L.) and field pea, tend to have a greater total root biomass deeper within the soil profile when experiencing water stress (Benjamin and Nielsen, 2006).

Soil Biodiversity

The biodiversity of a soil covers all living organisms living within (Zhang and Xu, 2008). This includes organisms such as earthworms, mites, ants, fungi, algae, bacteria, and many more. A healthy soil will have a complex system with a broad variety of organisms of different sizes. This diversity allows for optimal utilization of resources and is essential in nutrient cycling. Arbuscular mycorrhizal fungi take up a large portion of the soil biome and their benefits are many. In a healthy soil, the AMF present can form symbiotic relationships with a large portion of plant species and enhance their nutrient and water uptake as well as increase their resistance to soil pathogens and to abiotic stresses. In addition, AMF can also assist in soil structural improvement, providing more stable aggregates (Magurno et al., 2014).

Unhealthy soils are often found when management practices include tillage. Under conventionally tilled soils, AM fungal hyphae are broken up, aggregates are destroyed, captured carbon gets released, soil structure degrades, and less water can infiltrate and thus be retained. These soils tend to be more prone to soil erosion.

The Impact of AMF on Abiotic Stresses

Some studies suggest that AMF can alleviate some of the stressors in soil that impact plant growth (Doubková et al., 2013; Yang et al., 2015) such as heavy metal contamination,

drought and saline soil. To protect plants against heavy metal toxicity, the AMF either binds the metals in their cell wall, actively restricts influx, actively enhances efflux, chelates the heavy metals from the cytoplasm, and/or stores them into vacuoles so that there is minimal uptake of the metals by the plant (Leyval et al., 1997). In saline soils, AMF have been shown to reduce the impact of salt stress upon plants (Poss et al., 1985; Al-Karaki, 2000; Yamato et al., 2009) by decreasing the uptake of sodium (Na) (Al-Karaki, 2000) while also increasing the uptake of nutrients (Ruiz-Lozano et al., 1996). The AMF also helps the plant to accumulate proline solutes to allow for osmotic adjustment in the plant so that the plant does not lose water via the roots (Paleg et al., 1984). Another important abiotic stress, drought, is becoming more prominent due to climate change. Since AMF improve soil structure, they allow for water penetration deep into the soil profile. The aggregates that are tightly bound together and are full of organic matter and other soil particles are also able to hold onto moisture. The soil's ability to store moisture because of the better structure aids plants when drier periods come along. Besides the above, the AMF tend to enhance root growth, which benefits water uptake as well (Wu et al., 2013).

The Impact of AMF on Nutrient Uptake

The fine AMF hyphae that serve as a skeletal structure in the soil make the direct physical link between the plant root and the soil nutrient resources (Jastrow J. D. and Miller R. M., 1993). The main factor in the enhanced uptake of nutrients when AMF are present is the increased surface area that the hyphae of AMF can explore (Harley and Smith, 1983). In addition, the fungi can deliver the nutrients directly to the cortical cells within the root (Smith et al., 2011). This delivery has been demonstrated for nutrients such as phosphorus (P), ammonium (NH_4^+), nitrate (NO_3^-), calcium (Ca), copper (Cu), zinc (Zn), and iron (Fe). Moreover, the delivery of nutrients *via* hyphae can far exceed that of the roots, which is especially true for P

and Cu (Marschner and Dell, 1994). Phosphorus fertilization generally tends to decrease AMF colonization. Hence, in a P rich soil, AMF colonization tends to be lower as well. This is because the plants are investing most of the carbon (C) into their aboveground biomass (Marschner et al., 1996) and not into the roots. Nitrogen fertilization, on the other hand, can have a positive impact on AMF colonization if the P content of the soil remains limited (Karanika et al., 2007).

Arbuscular mycorrhizal fungi require plant hosts for nutrition and reproduction (Cheeke et al., 2013), however, this relationship is not always symbiotic and can at times fluctuate between symbiotic and parasitic form (Johnson et al., 1997; Johnston-Monje et al., 2014). A parasitic relationship between AMF and the plant host is considered when the cost to maintain the symbiotic relationship exceeds the benefit that the plant gets from the AMF colonization (Smith and Smith, 2013).

The Colonization of Maize Roots by AMF

Maize (*Zea mays* L.), a C4 plant that is more apt at growing under hot and dry conditions due to its unique photosynthetic pathway in which photorespiration is minimized, is an important globally produced crop that readily forms associations with AMF (Hamel and Smith, 1991). Assessing root colonization is essential in AMF studies. The extent to which roots are colonized is usually expressed in a percentage or as the proportion of a known root length that is colonized by AMF (Smith, 1997). To be able to assess roots correctly, it is important to realize that AMF have more than 200 species under the phylum Glomeromycota (Redecker et al., 2013) and the morphology of the main AMF structures (vesicles, arbuscules, hyphae, etc.) can differ between these species (Walker and Schüßler, 2004) as well as their functional diversity (Tian et al., 2013). Aside from species, the extent of colonization can also be impacted by other things such as soil management practices and soil properties (Pereira et al., 2014). In addition, the level to

which land use, the environment, and soil conditions impact AMF species can be seen in the AMF's overall prevalence (Stürmer and Siqueira, 2011).

It is not clear if maize is species-specific when it comes to AMF colonization, however, research by Tian et al. (2013) indicates that maize can form symbiotic relationships with more than one species. The AM fungal hyphae present in the soil are attracted to root exudates and will grow more rapidly near exuding roots. Upon contact, AMF are able to penetrate the root tissue without triggering defensive reactions from the plant (Gadkar et al., 2001).

The Impact of Plants on Rhizosphere Microorganisms

Plant roots secrete vast amounts of small molecular weight compounds known as exudates. There are several factors that affect root exudation which are: plant species, plant age, temperature, light, plant nutrition, microorganisms, medium supporting the roots, soil moisture, and root damage (Rovira, 1969). Secretion of exudates allows the plant roots to influence the rhizosphere, with most secretion occurring around the root tips. These exudates can be grouped into low molecular compounds and high molecular compounds. The low molecular compounds are believed to make up the largest portion of the root exudates and consist of amino acids, sugars, phenolics, organic acids, and other secondary metabolites. The high molecular compounds are mostly made up out of polysaccharides and proteins (Walker et al., 2003).

The carbon secreted by roots as exudates is about 0.4% of the total carbon that is photosynthesized (Rovira, 1969). Despite still losing valuable photosynthetically fixed carbon through rhizodeposition, there are many benefits to the plant. Exudates can stimulate mutually beneficial interactions between plants and microorganisms and between plants, which can help make nutrients more available. In addition, root exudates are a vital defense mechanism. This is because plants face a variety of natural enemies and are exposed to the perils of nature

constantly. The negative factors, or stresses, can be biotic or abiotic. When under biotic stress, there is a large chemical diversity of exudates that can aid in protecting the plant. These can be classified into phenolics and terpenoids, which are general chemical classes with strong antibacterial and antifungal abilities. The phenolic metabolites act as an antibacterial agent but can also attract certain microbial communities (Baetz and Martinoia, 2014). An *in vitro* study done by Hudak et al. (2000) indicated that plant roots can also secrete proteins that defend against potential soil-borne pathogens. The PAP-H protein, for example, is a ribosome-inactivating protein that can recognize and break down the ribosomes of pathogenic fungi (Hudak et al., 2000). It is important to note that the exudate that suppresses one microbial community may stimulate the growth of another (Pereira et al., 2014).

Most root exudation is found in actively growing roots (García et al., 2001). Baetz and Martinoia (2014) discovered that this process is more active than initially thought, meaning that plants regulate exudation. This active process involves primary and secondary transport systems to allow for the transport of exudates across the plasma membrane. There are two main protein families involved in this transport, which are known as multidrug and toxic compound extrusion (MATE) and ATP-binding cassette (ABC) (Baetz and Martinoia, 2014). Transport functions of these protein families can help in aspects such as signaling for disease resistance, protection of the root from toxic compounds, and aluminum tolerance (Yazaki et al., 2008). The ATP-binding cassette mediates many transport functions and most of the proteins are primary pumps. Multidrug and toxic compound extrusion, on the other hand, are mainly secondary transport pumps. The phytochemicals that are assigned to these transport pumps are diverse and include compounds such as heavy metal chelates, alkaloids, lipids, inorganic acids, peptides, and polysaccharides (Theodoulou, 2000).

Root exudates can also act as chemoattractants; by producing chemicals that a variety of bacterial species are interested in, they enhance the movement of these bacteria towards the roots. This behavior is known as positive chemotaxis. Some of the molecules secreted by the roots that are of bacterial interest include sugars, amino acids, and aromatic compounds. Maize, for example, produces aromatic metabolites that attract *Pseudomonas putida* bacteria. These are found in the maize rhizosphere and are known to have beneficial traits to the plant (Neal et al., 2012). Changing the microbial community structure within the rhizosphere will affect nutrient cycling. As plants senesce, the plant residues become soil organic matter, which then gets decomposed by microbes. This cycles N, P, and S so that it is readily available to plants again. In addition, soil microbes can derive nutrients from elsewhere such as the atmosphere, available nutrients, and primary and secondary minerals. To do this, microbes transform the elements through mineralization and immobilization, reduction and oxidation, solubilization, volatilization, and detoxification (Paul, 2006). To gather nutrients such as N from the atmosphere, a symbiotic relationship can form between certain plants, such as legumes, and N-fixing bacteria. For this to happen, the plant roots will first secrete exudates such as flavonoids. The flavonoids are secreted continuously into the rhizosphere by the legume roots and increase the presence of the rhizobia. The bacteria then enter the root and nodules are formed (Haichar et al., 2014).

As soil microorganisms decompose root mucilage over time, separate areas of the roots will have different amounts of mucilage and thus soil-water relations. This is where the microbial secondary metabolites such as exopolysaccharides play an important role because of their similar water-holding capacity to mucilage (Czarnes et al., 1999). The secondary

metabolites from soil microbes also aid in soil aggregation, which, as stated before, improves soil aeration and water infiltration.

The Impact of Breeding on Soil Microorganisms

Crops today do not resemble their ancestors. This is a direct result of domestication and selection for desirable traits over a long-term breeding process (Bouffaud et al., 2011) and this process has been sped up by the advancements in biotechnology. The aboveground changes have unintentionally affected belowground aspects such as shoot morphology, root morphology, root anatomy, plant physiological processes (Schmidt et al., 2016), and changes in the reproductive organs. Changes in root morphology and physiology also impacted the microbial interactions (Germida and Siciliano, 2001).

Selective Breeding

After the domestication of crops, farmers continued selecting landraces that were suitable for the environment and the soil type (Johnston-Monje et al., 2014). Crop breeding for desirable traits is still a major aspect of agriculture. Varying breeding programs even go as far as gathering seeds from different countries if the plant in question has traits that are more desirable. Crops in tropical regions, for example, will have a greater ability to withstand heat stress and with a changing climate, this may prove to be vital in ongoing crop production in other regions of the world.

Each plant species has unique soil microbiomes as they manipulate the soil based on their individual needs and abilities. More recent research is showing that plant genotype within a plant species can alter the soil microbiome, especially as it relates to microbial function and microbial community structure (Mazzola et al., 2004). Based on this information, it is evident that breeding for more desirable aboveground traits has impacted the rhizosphere of several crops. These

changes are likely due to different root morphologies and thus root exudations, which affect the soil environment and the microbial communities (Bais et al., 2006; Van Overbeek and Van Elsas, 2008). In modern wheat and canola, endophytic microorganisms (found within plant tissue) and those within the rhizosphere, have different relationships compared to their ancestral lines (Siciliano et al., 1998). For wheat, the ancestral lines had greater amounts of certain bacteria, such as *Pseudomonads*, in the rhizosphere compared to the modern lines. Although less rich in the rhizosphere compared to the ancestral lines, those bacteria constituted the most dominant endophytes in the modern lines (Germida and Siciliano, 2001). Differences were also found among modern and ancestral rice cultivars of which wild cultivars had lower microbial diversity in the rhizosphere (Elbeltagy et al., 2000). This is different from maize because a comparison between teosinte, the ancestor of maize, and sweet corn showed greater microbial diversity in teosinte. Moreover, despite bulk soil normally having greater microbial diversity compared to that of the rhizosphere, the rhizosphere of teosinte did not see a decrease in microbial diversity (Szoboszlay et al., 2015). The ability of teosinte to utilize beneficial microbial groups while maintaining rhizosphere microbial diversity may be of interest for future crop production.

Hybridization

Hybridization is a natural process between two closely related species. The process often results in a hybrid that is morphologically different from the parental lines. When trying to breed for more desirable traits, a combination of two parental lines with specific beneficial traits could result in a hybrid that has both desired traits. An example of this would be the study by Picard et al. (2006), in which they crossed two inbred maize lines (Lo964 and Lo1016). Each inbred was chosen for their different root morphology; inbred line Lo964 had deep roots with fewer lateral

roots, while Lo1016 had shallow roots with extensive lateral branching. The resulting hybrid, Lo964xLo1016, had deep roots and extensive lateral branching, a beneficial trait to find water and nutrient resources. Other maize hybridization advantages between a variety of inbred lines (e.g. B73 and Mo17) include yield increase, shorter growing season, lower rate of root and stalk lodging, and lower grain moisture (Stojaković et al., 2010). Since hybridization would impact the plant's genotype, it is not surprising that they found hybridization of maize to also impact the root microbiome. The roots of hybrids tend to establish beneficial microbial relationships sooner than those of the parental lines. This is the case for rhizobacteria that fix nitrogen and produce antibiotic compounds, as well as for AMF. An earlier study by Picard and Bosco (2006) also provided evidence that maize hybrids can select specific lines of *Pseudomonas* capable of producing antibiotic compounds such as 2,4-diacetylphloroglucinol (DAPG), whereas the parental lines could not.

Other cereal crops, such as rice (*Oryza sativa)*, have also seen benefits from hybridization. One report by Saeed et al. (2013) stated a shorter growing period with a yield increase of more than 60% from a field study in Punjab, Pakistan. It is important to note that yield increases from rice hybridization vary over time and space (e.g. 15% increases in China and 30% increases in India). Despite these benefits, there were significant drawbacks. There was greater cost related to growing hybrid rice due to the greater cost of hybrid seeds, increased use of pesticides, fertilizer, etc. In addition, hybrid rice was found to be of lesser quality with an inferior taste.

Yield increases were also found in sorghum (*Sorghum bicolor*) (Rao et al., 2013), however, not much information was found on hybridization of other cereal crops.

Genetic Modification for Bt

Genetic modification has been an important factor in improving modern crop production, including the modification that causes crops such as maize to express the Crystal (Cry) or Cytolitic (Cyt) protein from the gram-positive bacterium, *Bacillus thuringiensis* (Bt) (Bravo et al., 2007; Tan et al., 2011). This naturally occurring insecticidal toxin has been an asset in battling insect damage to the plant, with no known harmful effects for humans and other vertebrates. Most Bt corn hybrids express the Cry1Ab protein, a protein that was initially developed to battle the European corn borer (*Ostrinia nubilalis*) in 1995, however, it is also effective against other insect pests (Icoz and Stotzky, 2008). Once the insect ingests the Bt plant material, the Cry protein breaks down midgut epithelial cells, resulting in the death of the insect (Bravo et al., 2007). Because of its toxicity to insects, questions remain on how toxic it is to the soil microbiome as it enters the soil via root exudates, pollen, and decomposition of residues (Icoz et al., 2008).

The Impact of the Bt Protein on Soil Microbial Communities

Microorganisms contribute to the majority of the soil biological factor. They take part in multiple processes such as organic matter decomposition, nutrient mineralization, plant pathogen control, soil structure improvement (Bruinsma et al., 2003), as well as forming mutualistic relationships with plants. They are known to be essential in the functioning and stability of agricultural ecosystems; however, their response to genetically modified crops is not well understood. Based on a study by Velasco et al. (2013), genetically modified crops tend to increase overall microbial activity in the rhizosphere as well as nitrogen mineralization.

The Impact of the Bt Protein on AMF

The impact of the Cry protein on AMF has, per some studies, a negative impact on AMF in that the non-Bt corn shows greater overall root colonization compared to the Bt corn (Turrini et al., 2005; Castaldini et al., 2005). In contrast, newer studies show that the impact of the protein on AMF is not significant (de Vaufleury et al., 2007; Knox et al., 2008; Verbruggen et al., 2012; Cheeke et al., 2013). It is important to note that the effect of Bt is highly varied between different plant varieties and the type of Bt protein (Tan et al., 2011) and is also greatly impacted by environmental conditions (Cheeke et al., 2013).

Despite the many research findings concerning AMF colonization, there is a lack of information about how breeding has impacted maize root colonization. In addition, with contradicting findings concerning the impact of Bt corn on AMF, a comparison between landraces, hybrids, and genetically modified corn that expresses the Cry protein, may provide a better understanding of any potential differences in root colonization. To look at differences due to breeding, it is important to select inbred lines and their hybrid. The genetic modification of this hybrid to express the Cry protein may then further clarify differences due to genetic manipulation. As plants acquire different amounts of specific nutrients over different growth stages, several sampling times would be beneficial. During these sampling times, roots should be collected as well as the aboveground biomass. Root staining for microscope analysis will allow for an evaluation of the percent colonization. Root and plant biomass can be ground and analyzed to look at nutrient content, especially for P, to see if the amount of root colonization correlates.

Genetic Modification for Drought Tolerance

Drought is one of the many natural phenomena that plagues agriculture globally with about 38% of the terrestrial environment undergoing some form of water stress (Dilley et al.,

2005). Climatic factors such as precipitation and temperature, influence drought and these factors will become more variable as the climate changes. Climate change models predict drought frequencies to increase the amount of drought-affected areas (Li et al., 2009). The potential for increased crop losses due to drought incited a need for more drought-tolerant crops, especially in already stressed areas.

The ability of plants to survive depends on their ability to deal with stresses. Drought resistance covers four main mechanisms: the ability to avoid drought stress, the ability to deal with drought stress, the ability to stop drought stress, and the ability to recover from drought stress (Fuganti-Pagliarini et al., 2017). Through physiological and biochemical ways as well as cellular and molecular ways, the plant can deal with the induced stress (Shinozaki and Yamaguchi - Shinozaki, 2007). Multiple genes that differ in their initial response and prolonged tolerance to water stress influence these methods. Therefore, using conventional breeding techniques would be difficult (Fuganti-Pagliarini et al., 2017). Genetic modification, however, has provided a way to create drought-tolerant plants by using transcription factors that can find, activate, and regulate specific genes in DNA sequences that regulate cellular demise under water stress (Shinozaki and Yamaguchi - Shinozaki, 2007). Several plants that were subject to the introduction of the transcription factor that regulates drought-responsive genes have been shown to perform better compared to non-modified counterparts when under drought (Fuganti-Pagliarini et al., 2017).

The impact of genetic modification for drought on the soil microbial communities likely depends on morphological and physiological changes of the plant.

Summary

Crop production is a complex interactive system in which many factors must be considered. These factors include, but are not limited to, soil moisture, soil structure, soil fertility, soil texture, type of crop, insects, pathogens, and microbial interactions within the soil. The microbial interactions within the soil have for many years been a much-neglected part of the crop for several reasons. First, the idea that microbes can benefit crop health and productivity is new in the general timeline of crop production. Second, techniques to study microbes were for a long time restricted to culture-based research, which limited the study of the microbial diversity. The introduction of molecular techniques has made a new way for researchers to study different microorganisms, however, they also have limitations. To obtain a good understanding of which organisms are present in a soil, it is best to overlap several microbial research techniques. Third, to obtain microorganisms that have an impact on the plant, the focus should be on rhizosphere soil. This soil is directly impacted by the root and adheres to the root when roots are removed. Rhizosphere soil is often defined by sampling methodology and is commonly obtained by first extracting roots, then vigorously shaking the roots to dislodge attached soil. Soil that is easily removed is considered the soil matrix, while that which remains attached is considered the "rhizosphere". This method is labor intensive, time consuming, error prone, and often influenced by soil texture and soil moisture content at the time of sampling (Böhm, 1979).

Despite the challenges, microbial interactions with plants have been and are currently being studied. From these studies came an understanding about the importance of microorganisms in relation to plant nutrient acquisition, soil moisture retention, pathogen resistance, and stress resistance. This understanding has provided a window of opportunity to come up with agricultural practices that require less input by improving current plant-microbial

interactions thus assisting in the mitigation of the effects of climate change. From the research findings stated in this literature review, each plant is unique, and their soil microbiome depends not only on their environment but also on their genotype. The breeding of crops, be it through selective breeding, hybridization, or genetic modification, impacts the genotype and therefore the microbial communities that the plant interacts with. To what extent crop breeding has impacted these microbial interactions is, however, not yet well established. What is known, is that the domestication of crops and their long-term breeding for desirable aboveground traits has changed their morphology and physiology and thereby their influence on the rhizosphere and the microorganisms within.

References

Al-Karaki, G.N. 2000. Growth of mycorrhizal tomato and mineral acquisition under salt stress. Mycorrhiza 10(2): 51–54. doi: 10.1007/s005720000055.

Baetz, U., and E. Martinoia. 2014. Root exudates: the hidden part of plant defense. Trends Plant Sci. 19(2): 90–98. doi: 10.1016/j.tplants.2013.11.006.

Bahadur, B., M.V. Rajam, L. Sahijram, and K.V. Krishnamurthy. 2015. Plant Biology and Biotechnology: Volume II: Plant Genomics and Biotechnology. Springer, New Delhi.

Bais, H.P., T.L. Weir, L.G. Perry, S. Gilroy, and J.M. Vivanco. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annu. Rev. Plant Biol. 57: 233–266.

Banu, N.A., B. Singh, and L. Copeland. 2004. Microbial biomass and microbial biodiversity in some soils from New South Wales, Australia. Soil Res. 42(7): 777–782. doi: 10.1071/SR03132.

Baulcombe, D. 2010. Reaping benefits of crop research. Science 327(5967): 761–761. doi: 10.1126/science.1186705.

Beadle, G.W. 1980. The ancestry of corn. Sci. Am. 242(1): 112–119.

Benjamin, J.G., and D.C. Nielsen. 2006. Water deficit effects on root distribution of soybean, field pea and chickpea. Field Crops Res. 97(2–3): 248–253. doi: 10.1016/j.fcr.2005.10.005.

Bennet, L.T., P.M. Mele, S. Annett, and S. Kasel. 2010. Examining links between soil management, soil health, and public benefits in agricultural landscapes: An Australian perspective. Agric. Ecosyst. Environ. 139(1–2): 1–12. doi: 10.1016/j.agee.2010.06.017.

Bent, S.J., and L.J. Forney. 2008. The tragedy of the uncommon: understanding limitations in the analysis of microbial diversity. ISME J. 2(7): 689–695. doi: 10.1038/ismej.2008.44.

Blackwell, M. 2000. Terrestrial life--fungal from the start? Science 289(5486): 1884–1885. doi: 10.1126/science.289.5486.1884.

Blake, M. 2015. Maize for the Gods: Unearthing the 9,000-Year History of Corn. Univ. of California Press.

Boeuf-Tremblay, V., S. Plantureux, and A. Guckert. 1995. Influence of mechanical impedance on root exudation of maize seedlings at two development stages. Plant Soil 172(2): 279–287. doi: 10.1007/BF00011330.

Böhm, W. 1979. Methods of Studying Root Systems. Springer, Berlin, Heidelberg.

Bonfante, P., A. Genre, A. Faccio, I. Martini, L. Schauser, J. Stougaard, J. Webb, and M. Parniske. 2000. The Lotus japonicus LjSym4 Gene Is Required for the Successful Symbiotic Infection of Root Epidermal Cells. Mol. Plant. Microbe Interact. 13(10): 1109–1120. doi: 10.1094/MPMI.2000.13.10.1109.

Bouffaud, M.L., M. Kyselková, B. Gouesnard, G. Grundmann, D. Muller, and Y. Moënne-Loccoz. 2011. Is diversification history of maize influencing selection of soil bacteria by roots? Mol. Ecol. 21(1): 195–206. doi: 10.1111/j.1365-294X.2011.05359.x.

Bouwmeester, H.J., C. Roux, J.A. Lopez-Raez, and G. Bécard. 2007. Rhizosphere communication of plants, parasitic plants and AM fungi. Trends Plant Sci. 12(5): 224–230. doi: 10.1016/j.tplants.2007.03.009.

Boyd, M., C. Surette, and B.A. Nicholson. 2006. Archaeobotanical evidence of prehistoric maize (Zea mays) consumption at the northern edge of the Great Plains. J. Archaeol. Sci. 33(8): 1129–1140. doi: 10.1016/j.jas.2005.12.003.

Bravo, A., S.S. Gill, and M. Soberón. 2007. Mode of action of Bacillus thuringiensis Cry and Cyt toxins and their potential for insect control. Toxicon 49(4): 423–435. doi: 10.1016/j.toxicon.2006.11.022.

Bruinsma, M., G.A. Kowalchuk, and J.A. van Veen. 2003. Effects of genetically modified plants on microbial communities and processes in soil. Biol. Fertil. Soils 37(6): 329–337. doi: 10.1007/s00374-003-0613-6.

Buee, M., M. Rossignol, A. Jauneau, R. Ranjeva, and G. Bécard. 2000. The pre-symbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant root exudates. Mol. Plant-Microbe Interact. MPMI 13(6): 693–698. doi: 10.1094/MPMI.2000.13.6.693.

Castaldini, M., A. Turrini, C. Sbrana, A. Benedetti, M. Marchionni, S. Mocali, A. Fabiani, S. Landi, F. Santomassimo, B. Pietrangeli, M.P. Nuti, N. Miclaus, and M. Giovannetti. 2005. Impact of Bt Corn on Rhizospheric and Soil Eubacterial Communities and on Beneficial Mycorrhizal Symbiosis in Experimental Microcosms. Appl. Environ. Microbiol. 71(11): 6719–6729. doi: 10.1128/AEM.71.11.6719-6729.2005.

Cheeke, T.E., M.B. Cruzan, and T.N. Rosenstiel. 2013. Field evaluation of arbuscular mycorrhizal fungal colonization in Bacillus thuringiensis toxin-expressing (Bt) and non-Bt maize. Appl. Environ. Microbiol. 79(13): 4078–86. doi: 10.1128/AEM.00702-13.

Clark, L.J., W.R. Whalley, and P.B. Barraclough. 2003. How do roots penetrate strong soil? p. 93–104. *In* Roots: The Dynamic Interface between Plants and the Earth. Developments in Plant and Soil Sciences. Springer, Dordrecht.

Crews, T.E., J. Blesh, S.W. Culman, R.C. Hayes, E.S. Jensen, M.C. Mack, M.B. Peoples, and M.E. Schipanski. 2016. Going where no grains have gone before: From early to mid-succession. Agric. Ecosyst. Environ. 223: 223–238. doi: 10.1016/j.agee.2016.03.012.

Czarnes, S., S. Hiller, A.R. Dexter, P.D. Hallett, and F. Bartoli. 1999. Root:soil adhesion in the maize rhizosphere: the rheological approach. Plant Soil 211(1): 69–86. doi: 10.1023/A:1004656510344.

Delgado-Baquerizo, M., F.T. Maestre, P.B. Reich, T.C. Jeffries, J.J. Gaitan, D. Encinar, M. Berdugo, C.D. Campbell, and B.K. Singh. 2016. Microbial diversity drives multifunctionality in terrestrial ecosystems. Nat. Commun. 7: 10541. doi: 10.1038/ncomms10541.

Dexter, A.R. 1988. Advances in characterization of soil structure. Soil Tillage Res. 11(3–4): 199–238. doi: 10.1016/0167-1987(88)90002-5.

Dey, R., K.K. Pal, and K.V.B.R. Tilak. 2012. Influence of soil and plant types on diversity of rhizobacteria. Proc. Natl. Acad. Sci. India Sect. B Biol. Sci. 82(3): 341–352. doi: 10.1007/s40011-012-0030-4.

Dighton, J. 2016. Fungi in Ecosystem Processes, Second Edition. CRC Press, New York.

Dilley, M., R.S. Chen, U. Deichmann, A.L. Lerner-Lam, M. Arnold, J. Agwe, P. Buys, O. Kjekstad, B. Lyon, and G. Yetman. 2005. Natural Disaster Hotspots: A Global Risk Analysis. World Bank Publications, Washington, D.C.

Doebley, J. 2004. The genetics of maize evolution. Annu. Rev. Genet. 38(1): 37–59. doi: 10.1146/annurev.genet.38.072902.092425.

Donn, S., J.A. Kirkegaard, G. Perera, A.E. Richardson, and M. Watt. 2014. Evolution of bacterial communities in the wheat crop rhizosphere. Environ. Microbiol. 17(3): 610–621. doi: 10.1111/1462-2920.12452.

Doubková, P., E. Vlasáková, and R. Sudová. 2013. Arbuscular mycorrhizal symbiosis alleviates drought stress imposed on plants in serpentine soil. Plant Soil 370(1–2): 149–161. doi: 10.1007/s11104-013-1610-7.

Dubreuil, P., M. Warburton, M. Chastanet, D. Hoisington, and A. Charcosset. 2006. More on the introduction of temperate maize into Europe: Large-scale bulk SSR genotyping and new historical elements. Maydica 51(2): 281–291.

Elbeltagy, A., K. Nishioka, H. Suzuki, T. Sato, Y.-I. Sato, H. Morisaki, H. Mitsui, and K. Minamisawa. 2000. Isolation and characterization of endophytic bacteria from wild and traditionally cultivated rice varieties. Soil Sci. Plant Nutr. 46(3): 617–629. doi: 10.1080/00380768.2000.10409127.

FAOSTAT. 2016. Food and Agriculture Organization of the United Nations Statistics Division.

FAS. 2016. Grain, world markets, and trade. Foreign Agricultural Services, Washington, D.C.

Finan, J.J. 1948. Maize in the great herbals. Ann. Mo. Bot. Gard. 35(2): 149–191. doi: 10.2307/2394391.

Fitter, A.H. 1990. The role of ecological significance of vesicular-arbuscular mycorrhizas in temperate ecosystems. Agric. Ecosyst. Environ. 29(1–4): 137–151. doi: 10.1016/0167-8809(90)90268-I.

Fuganti-Pagliarini, R., L.C. Ferreira, F.A. Rodrigues, H.B.C. Molinari, S.R.R. Marin, M.D.C. Molinari, J. Marcolino-Gomes, L.M. Mertz-Henning, J.R.B. Farias, D. Oliveira, M.C. N, N. Neumaier, N. Kanamori, Y. Fujita, J. Mizoi, K. Nakashima, K. Yamaguchi-Shinozaki, and A.L. Nepomuceno. 2017. Characterization of soybean genetically modified for drought tolerance in field conditions. Front. Plant Sci. 8. doi: 10.3389/fpls.2017.00448.

Gadkar, V., R. David-Schwartz, T. Kunik, and Y. Kapulnik. 2001. Arbuscular mycorrhizal fungal colonization. factors involved in host recognition. Plant Physiol. 127(4): 1493–1499. doi: 10.1104/pp.010783.

García, J.A.L., C. Barbas, A. Probanza, M.L. Barrientos, and F.J.G. Mañero. 2001. Low molecular weight organic acids and fatty acids in root exudates of two Lupinus cultivars at flowering and fruiting stages. Phytochem. Anal. 12(5): 305–311. doi: 10.1002/pca.596.

Genre, A., M. Chabaud, T. Timmers, P. Bonfante, and D.G. Barker. 2005. Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in Medicago truncatula root epidermal cells before infection. Plant Cell 17(12): 3489–3499. doi: 10.1105/tpc.105.035410.

Germida, J., and S. Siciliano. 2001. Taxonomic diversity of bacteria associated with the roots of modern, recent and ancient wheat cultivars. Biol. Fertil. Soils 33(5): 410–415. doi: 10.1007/s003740100343.

Gildon, A., and P.B. Tinker. 1983. Interactions of vesicular-arbuscular mycorrhizal infection and heavy metals in plants. I. The effects of heavy metals on the development of vesicular-arbuscular mycorrhizas. New Phytol. 95(2): 247–261.

Giovannetti, M., C. Sbrana, L. Avio, A.S. Citernesi, and C. Logi. 1993. Differential hyphal morphogenesis in arbuscular mycorrhizal fungi during pre-Infection stages. New Phytol. 125(3): 587–593.

Gomes, N.C.M., H. Heuer, J. Schönfeld, R. Costa, L. Mendonça-Hagler, and K. Smalla. 2001. Bacterial diversity of the rhizosphere of maize (Zea mays) grown in tropical soil studied by temperature gradient gel electrophoresis. Plant Soil 232(1–2): 167–180. doi: 10.1023/A:1010350406708.

Graaff, M.-A. de, J. Six, J.D. Jastrow, C.W. Schadt, and S.D. Wullschleger. 2013. Variation in root architecture among switchgrass cultivars impacts root decomposition rates. Soil Biol. Biochem. 58: 198–206. doi: 10.1016/j.soilbio.2012.11.015.

Gutjahr, C., and M. Parniske. 2013. Cell and developmental biology of arbuscular mycorrhiza symbiosis. Annu. Rev. Cell Dev. Biol. 29(1): 593–617. doi: 10.1146/annurev-cellbio-101512-122413.

Haichar, F. el Z., C. Santaella, T. Heulin, and W. Achouak. 2014. Root exudates mediated interactions belowground. Soil Biol. Biochem. 77: 69–80. doi: 10.1016/j.soilbio.2014.06.017.

Hamel, C., and C. Plenchette. 2007. Mycorrhizae in Crop Production. HFAPP/Haworth Food & Agricultural Products Press, Binghamton, NY.

Hamel, C., and D.L. Smith. 1991. Plant development in a mycorrhizal field-grown mixture. Soil Biol. Biochem. 23(7): 661–665. doi: 10.1016/0038-0717(91)90080-4.

Harley, J.L., and S.E. Smith. 1983. Mycorrhizal Symbiosis. San Diego.

He, W., D. Goodkind, and P. Kowal. 2016. International Population Reports. United States Census Bureau.

van der Heijden, M.G.A., and C. Wagg. 2013. Soil microbial diversity and agro-ecosystem functioning. Plant Soil 363(1–2): 1–5. doi: info:doi/10.1007/s11104-012-1545-4.

Hobbs, P.R., K. Sayre, and R. Gupta. 2008. The role of conservation agriculture in sustainable agriculture. Philos. Trans. R. Soc. B Biol. Sci. 363(1491): 543–555. doi: 10.1098/rstb.2007.2169.

Hudak, K.A., P. Wang, and N.E. Tumer. 2000. A novel mechanism for inhibition of translation by pokeweed antiviral protein: Depurination of the capped RNA template. RNA 6(3): 369–380.

Icoz, I., D. Saxena, D.A. Andow, C. Zwahlen, and G. Stotzky. 2008. Microbial populations and enzyme activities in soil in situ under transgenic corn expressing Cry proteins from Bacillus thuringiensis. J. Environ. Qual. 37(2): 647–662. doi: http://dx.doi.org.er.lib.k-state.edu/10.2134/jeq2007.0352.

Icoz, I., and G. Stotzky. 2008. Fate and effects of insect-resistant Bt crops in soil ecosystems. Soil Biol. Biochem. 40(3): 559–586. doi: 10.1016/j.soilbio.2007.11.002.

Jastrow J. D., and Miller R. M. 1993. Neighbor influences on root morphology and mycorrhizal fungus colonization in tallgrass prairie plants. Ecology 74(2): 561–569. doi: 10.2307/1939316.

Johnson, N.C., J.H. Graham, and F.A. Smith. 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytol. 135(4): 575–586.

Johnston-Monje, D., W.K. Mousa, G. Lazarovits, and M.N. Raizada. 2014. Impact of swapping soils on the endophytic bacterial communities of pre-domesticated, ancient and modern maize. BMC Plant Biol. 14: 233.

Karanika, E.D., D.A. Alifragis, A.P. Mamolos, and D.S. Veresoglou. 2007. Differentiation between responses of primary productivity and phosphorus exploitation to species richness. Plant Soil 297(1–2): 69–81.

Kibblewhite, M.G., K. Ritz, and M.J. Swift. 2008. Soil health in agricultural systems. Philos. Trans. Biol. Sci. 363(1492): 685–701.

Klironomos, J.N., and W.B. Kendrick. 1996. Palatability of microfungi to soil arthropods in relation to the functioning of arbuscular mycorrhizae. Biol. Fertil. Soils 21(1–2): 43–52. doi: 10.1007/BF00335992.

Knox, O.G.G., D.B. Nehl, T. Mor, G.N. Roberts, and V.V.S.R. Gupta. 2008. Genetically modified cotton has no effect on arbuscular mycorrhizal colonisation of roots. Field Crops Res. 109(1–3): 57–60. doi: 10.1016/j.fcr.2008.06.005.

Koltai, H. 2011. Strigolactones' ability to regulate root development may be executed by induction of the ethylene pathway. Plant Signal. Behav. 6(7): 1004–1005. doi: 10.4161/psb.6.7.15501.

Lal, R. 2006. Encyclopedia of soil science. 2nd edition.. Taylor & Francis, New York.

Lehmann, J., and M. Kleber. 2015. The contentious nature of soil organic matter. Nature 528(7580): 60–68. doi: 10.1038/nature16069.

Lekberg, Y., S.M. Gibbons, S. Rosendahl, and P.W. Ramsey. 2013. Severe plant invasions can increase mycorrhizal fungal abundance and diversity. ISME J. 7(7): 1424–1433. doi: 10.1038/ismej.2013.41.

Letey, J., R.E. Sojka, D.R. Upchurch, D.K. Cassel, K.R. Olson, W.A. Payne, S.E. Petrie, G.H. Price, R.J. Reginato, H.D. Scott, P.J. Smethurst, and G.B. Triplett. 2003. Deficiencies in the soil quality concept and its application. J. Soil Water Conserv. 58: 180–187.

Leyval, C., K. Turnau, and K. Haselwandter. 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7(3): 139–153. doi: 10.1007/s005720050174.

Li, X., J. Rui, J. Xiong, J. Li, Z. He, J. Zhou, A.C. Yannarell, and R.I. Mackie. 2014. Functional potential of soil microbial communities in the maize rhizosphere. PloS One 9(11): e112609.

Li, Y., W. Ye, M. Wang, and X. Yan. 2009. Climate change and drought:: a risk assessment of crop-yield impacts. Clim. Res. 39(1): 31–46.

Lundquist, R., and D. Walters. 2007. Fertile transgenic corn plants. https://patents.google.com/patent/US20070300315/en (accessed 12 April 2018).

Magurno, F., Z. Sasvári, and K. Posta. 2014. From monoculture to the Norfolk system: assessment of arbuscular mycorrhizal fungi communities associated with different crop rotation systems. Symbiosis 64(3): 115–125. doi: 10.1007/s13199-014-0309-8.

Marschner, H., and B. Dell. 1994. Nutrient uptake in mycorrhizal symbiosis. Plant Soil 159(1): 89–102. doi: 10.1007/BF00000098.

Marschner, H., E.A. Kirkby, and I. Cakmak. 1996. Effect of mineral nutritional status on shoot-root partitioning of photoassimilates and cycling of mineral nutrients. J. Exp. Bot. 47(Special_Issue): 1255–1263. doi: 10.1093/jxb/47.Special_Issue.1255.

Mayaki, W.C., L.R. Stone, and I.D. Teare. 1976. Irrigated and nonirrigated soybean, corn, and grain sorghum root systems. Agron. J. 68(3): 532–534. doi: 10.2134/agronj1976.00021962006800030028x.

Mayzlish-Gati, E., C. De-Cuyper, S. Goormachtig, T. Beeckman, M. Vuylsteke, P.B. Brewer, C.A. Beveridge, U. Yermiyahu, Y. Kaplan, Y. Enzer, S. Wininger, N. Resnick, M. Cohen, Y. Kapulnik, and H. Koltai. 2012. Strigolactones are involved in root response to low phosphate conditions in Arabidopsis. Plant Physiol. 160(3): 1329–1341. doi: 10.1104/pp.112.202358.

Mazzola, M., D.L. Funnell, and J.M. Raaijmakers. 2004. Wheat cultivar-specific selection of 2,4-Diacetylphloroglucinol-producing fluorescent Pseudomonas species from resident soil populations. Microb. Ecol. 48(3): 338–348. doi: 10.1007/s00248-003-1067-y.

McCully M. E., and Boyer J. S. 2006. The expansion of maize root-cap mucilage during hydration. 3. Changes in water potential and water content. Physiol. Plant. 99(1): 169–177. doi: 10.1111/j.1399-3054.1997.tb03445.x.

Miller, R.M., M. Kling, Environmental Research, and Swedish Univ. of Agricultural Sciences. 2000. The importance of integration and scale in the arbuscular mycorrhizal symbiosis. Plant Soil 226(2): 295–309. doi: 10.1023/A:1026554608366.

Neal, A.L., S. Ahmad, R. Gordon-Weeks, and J. Ton. 2012. Benzoxazinoids in root exudates of maize attract Pseudomonas putida to the rhizosphere (recruitment of beneficial bacteria by maize roots). PLoS ONE 7(4): e35498. doi: 10.1371/journal.pone.0035498.

Oades, J. 1984. Soil organic matter and structural stability: mechanisms and implications for management. Plant Soil 76(1): 319–337. doi: 10.1007/BF02205590.

Paillard, S., S. Treyer, and B. Dorin (Eds). 2014. Agrimonde – Scenarios and Challenges for Feeding the World in 2050. Springer Netherlands, Dordrecht.

Paleg, L.G., G.R. Stewart, and J.W. Bradbeer. 1984. Proline and glycine betaine influence protein solvation. Plant Physiol. 75(4): 974–8. doi: 10.1104/pp.75.4.974.

Paul, E.A. 2006. Soil Microbiology, Ecology and Biochemistry. 3rd ed. Elsevier Science.

Peiffer, J.A., A. Spor, O. Koren, Z. Jin, S.G. Tringe, J.L. Dangl, E.S. Buckler, and R.E. Ley. 2013. Diversity and heritability of the maize rhizosphere microbiome under field conditions. Proc. Natl. Acad. Sci. U. S. 110(16): 6548–6553. doi: 10.1073/pnas.1302837110.

Pereira, C.M.R., D.K.A. da Silva, A.C. de A. Ferreira, B.T. Goto, and L.C. Maia. 2014. Diversity of arbuscular mycorrhizal fungi in Atlantic forest areas under different land uses. Agric. Ecosyst. Environ. 185: 245–252. doi: 10.1016/j.agee.2014.01.005.

Picard, C., and M. Bosco. 2006. Heterozygosis drives maize hybrids to select elite 2,4-diacethylphloroglucinol-producing Pseudomonas strains among resident soil populations. FEMS Microbiol. Ecol. 58(2): 193–204. doi: 10.1111/j.1574-6941.2006.00151.x.

Pingali, P.L. 2012. Green Revolution: Impacts, limits, and the path ahead. Proc. Natl. Acad. Sci. 109(31): 12302–12308.

Pinton, R., Z. Varanini, and P. Nannipieri. 2007. The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface, Second Edition. CRC Press.

Pirozynski, K.A., and D.W. Malloch. 1975. The origin of land plants: A matter of mycotrophism. BioSystems 6(3): 153–164. doi: 10.1016/0303-2647(75)90023-4.

Poss, J., E. Pond, J. Menge, and W. Jarrell. 1985. Effect of salinity on mycorrhizal onion and tomato in soil with and without additional phosphate. Plant Soil 88(3): 307–319. doi: 10.1007/BF02197488.

Rao, S., J. Patil, A. Umakanth, J. Mishra, C. Ratnavathi, G. Prasad, and B. Rao. 2013. Comparative performance of sweet sorghum hybrids and open pollinated varieties for millable stalk yield, biomass, sugar quality traits, grain yield and bioethanol production in tropical indian condition. Sugar Tech 15(3): 250–257. doi: 10.1007/s12355-013-0224-y.

Ream, L.W., and M.P. Gordon. 1982. Crown gall disease and prospects for genetic manipulation of plants. Science 218(4575): 854–859. doi: 10.1126/science.218.4575.854.

Rebourg, C., M. Chastanet, B. Gouesnard, C. Welcker, P. Dubreuil, and A. Charcosset. 2003. Maize introduction into Europe: the history reviewed in the light of molecular data. Theor. Appl. Genet. 106(5): 895–903. doi: 10.1007/s00122-002-1140-9.

Redecker, D., A. Schüßler, H. Stockinger, S.L. Stürmer, J.B. Morton, and C. Walker. 2013. An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). Mycorrhiza 23(7): 515–531. doi: 10.1007/s00572-013-0486-y.

Rillig, M.C., S.F. Wright, and V.T. Eviner. 2002. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. Plant Soil 238: 325–333.

Rovira, A. 1969. Plant root exudates. Bot. Rev. 35(1): 35–57. doi: 10.1007/BF02859887.

Ruiz-Lozano, J.M., R. Azcón, and M. Gómez. 1996. Alleviation of salt stress by arbuscular-mycorrhizal Glomus species in Lactuca sativa plants. Physiol. Plant. 98(4): 767–772. doi: 10.1111/j.1399-3054.1996.tb06683.x.

Russell, W.A. 1972. Registration of B70 and B73 parental lines of maize. Crop Sci. 12(5): 721–721. doi: 10.2135/cropsci1972.0011183X001200050085x.

Ryan, M.H., H. Liao, and R.J. Simpson. 2017. Belowground solutions to global challenges: special issue from the 9th symposium of the International Society of Root Research. Plant Soil 412(1–2): 1–5. doi: 10.1007/s11104-017-3200-6.

Saeed, R., A. Bashir, M. Qasim, I. Mehmood, and K. Bakhsh. 2013. Does productivity matter in the adoption of hybrid rice? A comparative study. Eur. J. Bus. Econ. 8(3).

Schmidt, J.E., T.M. Bowles, and A.C.M. Gaudin. 2016. Using ancient traits to convert soil health into crop yield: Impact of selection on maize root and rhizosphere function. Front. Plant Sci. 7. doi: 10.3389/fpls.2016.00373.

Shinozaki, K., and K. Yamaguchi - Shinozaki. 2007. Gene networks involved in drought stress response and tolerance. J. Exp. Bot. 58(2): 221–227. doi: 10.1093/jxb/erl164.

Siciliano, S.D., C.M. Theoret, J.R. de Freitas, P.J. Hucl, and J.J. Germida. 1998. Differences in the microbial communities associated with the roots of different cultivars of canola and wheat. Can. J. Microbiol. 44(9): 844–851. doi: 10.1139/w98-075.

Singh, B.P., A.L. Cowie, and K.Y. Chan. 2011. Soil health and climate change. Springer, Heidelberg ; New York.

Smith, S.E. 1997. Mycorrhizal symbiosis. 2nd edition.. Academic Press, San Diego.

Smith, S.E., I. Jakobsen, M. Grønlund, and F.A. Smith. 2011. Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiol. 156(3): 1050–1057.

Smith, S.E., and D.J. Read. 2010. Mycorrhizal Symbiosis. Academic Press, San Diego.

Smith, F.A., and S.E. Smith. 2013. How useful is the mutualism-parasitism continuum of arbuscular mycorrhizal functioning? Plant Soil 363(1–2): 7–18. doi: 10.1007/s11104-012-1583-y.

Sojka, R.E., D.R. Upchurch, and N.E. Borlaug. 2003. Quality soil management or soil quality management: performance versus semantics. Adv. Agron. 79: 1–68. doi: 10.1016/S0065-2113(02)79001-9.

Stojaković, M., M. Ivanović, G. Bekavac, and S. Stojaković. 2010. Grain yield of B73 × Mo17-type maize hybrids from different periods of breeding. Cereal Res. Commun. 38(3): 440–448. doi: 10.1556/CRC.38.2010.3.14.

Stürmer, S.L., and J.O. Siqueira. 2011. Species richness and spore abundance of arbuscular mycorrhizal fungi across distinct land uses in Western Brazilian Amazon. Mycorrhiza 21(4): 255–267. doi: 10.1007/s00572-010-0330-6.

Szoboszlay, M., J. Lambers, J. Chappell, J.V. Kupper, L.A. Moe, and D.H. McNear Jr. 2015. Comparison of root system architecture and rhizosphere microbial communities of Balsas teosinte and domesticated corn cultivars. Soil Biol. Biochem. 80: 34–44. doi: 10.1016/j.soilbio.2014.09.001.

Tan, F., J. Wang, Z. Chen, Y. Feng, G. Chi, and S.U. Rehman. 2011. Assessment of the arbuscular mycorrhizal fungal community in roots and rhizosphere soils of Bt corn and their non-Bt isolines. Soil Biol. Biochem. 43(12): 2473–2479.

Taylor, H.M., and H.R. Gardner. 1963. Penetration of cotton seedling taproots as influenced by bulk density, moisture content, and strength of soil. Soil Sci. 96(3): 153–156.

Theodoulou, F.L. 2000. Plant ABC transporters. BBA - Biomembr. 1465(1): 79–103. doi: 10.1016/S0005-2736(00)00132-2.

Tian, H., R.A. Drijber, X. Li, D.N. Miller, and B.J. Wienhold. 2013. Arbuscular mycorrhizal fungi differ in their ability to regulate the expression of phosphate transporters in maize (Zea mays L.). Mycorrhiza 23(6): 507–14. doi: 10.1007/s00572-013-0491-1.

Troyer, A. 1999. Background of US hybrid corn. Crop Sci. 39(3): 601–626. doi: 10.2135/cropsci1999.0011183X003900020001x.

Turner, T.R., E.K. James, and P.S. Poole. 2013. The plant microbiome. Genome Biol. 14(6). doi: 10.1186/gb-2013-14-6-209.

Turrini, A., C. Sbrana, M. Nuti, B. Pietrangeli, and M. Giovannetti. 2005. Development of a model system to assess the impact of genetically modified corn and aubergine plants on arbuscular mycorrhizal fungi. Plant Soil 266(1): 69–75. doi: 10.1007/s11104-005-4892-6.

Umehara, M., A. Hanada, H. Magome, N. Takeda - Kamiya, and S. Yamaguchi. 2010. Contribution of strigolactones to the inhibition of tiller bud outgrowth under phosphate deficiency in rice. Plant Cell Physiol. 51(7): 1118–1126. doi: 10.1093/pcp/pcq084.

USDA ERS. 2016. United States Department of Agriculture.

Van Overbeek, L., and J.D. Van Elsas. 2008. Effects of plant genotype and growth stage on the structure of bacterial communities associated with potato (Solanum tuberosum L.). FEMS Microbiol. Ecol. 64(2): 283–296. doi: 10.1111/j.1574-6941.2008.00469.x.

de Vaufleury, A., P.E. Kramarz, P. Binet, J. Cortet, S. Caul, M.N. Andersen, E. Plumey, M. Coeurdassier, and P.H. Krogh. 2007. Exposure and effects assessments of Bt-maize on non-target organisms (gastropods, microarthropods, mycorrhizal fungi) in microcosms. Pedobiologia 51(3): 185–194. doi: 10.1016/j.pedobi.2007.04.005.

Velasco, A.G.V., G.A. Kowalchuk, F.J.G. Mañero, B. Ramos, E. Yergeau, and J.A.L. García. 2013. Increased microbial activity and nitrogen mineralization coupled to changes in microbial community structure in the rhizosphere of Bt corn. Appl. Soil Ecol. 68: 46–56. doi: 10.1016/j.apsoil.2013.03.010.

Verbruggen, E., E.E. Kuramae, R. Hillekens, M. De Hollander, E.T. Kiers, W.F.M. Röling, G.A. Kowalchuk, and M.G.A. Van Der Heijden. 2012. Testing potential effects of maize expressing the Bacillus thuringiensis Cry1Ab endotoxin (Bt maize) on mycorrhizal fungal communities via DNA- and RNA-based pyrosequencing and molecular fingerprinting. Appl. Environ. Microbiol. 78(20): 7384–92. doi: 10.1128/AEM.01372-12.

Walker, T., E. Grotewold, and J. Vivanco. 2003. Root exudation and rhizosphere biology. Plant Physiol. 132(1): 44–51. doi: 10.1104/pp.102.019661.

Walker, C., and A. Schüßler. 2004. Nomenclatural clarifications and new taxa in the Glomeromycota Pacispora. Mycol. Res. 108(9): 981–982. doi: 10.1017/S0953756204231173.

Ward, M. 1995. Corn crosses last hurdle for genetically modified crops. Nature 376(6541): 544–544. doi: 10.1038/376544b0.

Ward, D.M., R. Weller, and M.M. Bateson. 1990. 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. Nature 345(6270): 63–65. doi: 10.1038/345063a0.

Wright, S., J. Starr, and I. Paltineanu. 1999. Changes in aggregate stabililty and concentration of glomalin during tillage management transition. Soil Sci. Soc. Am. J. 63(6): 1825–1829. doi: 10.2136/sssaj1999.6361825x.

Wright, S.F., and A. Upadhyaya. 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Sci. 161(9): 575–586. doi: 10.1097/00010694-199609000-00003.

Wu, Q.-S., A.K. Srivastava, and Y.-N. Zou. 2013. AMF-induced tolerance to drought stress in citrus: A review. Sci. Hortic. 164: 77–87. doi: 10.1016/j.scienta.2013.09.010.

Yamato, M., S. Ikeda, and K. Iwase. 2009. Community of arbuscular mycorrhizal fungi in drought-resistant plants, Moringa spp., in semiarid regions in Madagascar and Uganda. Mycoscience 50(2): 100–105. doi: 10.1007/S10267-008-0459-8.

Yang, Y., X. Han, Y. Liang, A. Ghosh, J. Chen, and M. Tang. 2015. The combined effects of arbuscular mycorrhizal fungi (AMF) and lead (Pb) stress on Pb accumulation, plant growth parameters, photosynthesis, and antioxidant enzymes in Robinia pseudoacacia L. PLoS One 10(12): e0145726.

Yazaki, K., A. Sugiyama, M. Morita, and N. Shitan. 2008. Secondary transport as an efficient membrane transport mechanism for plant secondary metabolites. Phytochem. Rev. 7(3): 513–524. doi: 10.1007/s11101-007-9079-8.

Zhang, L., and Z. Xu. 2008. Assessing bacterial diversity in soil. J. Soils Sediments 8(6): 379–388. doi: 10.1007/s11368-008-0043-z.

Zuber, M.S. 1973. Registration of 20 Maize Parental Lines1 (Reg. Numbers PL 18 to 37). Crop Sci. 13(6): 779–780.

Chapter 2 - The Impact of Maize Cultivars on the Soil Microbiome surrounding the Roots

Abstract

Improving beneficial relationships between plants and their surrounding soil microbiome could reduce the need for expensive fertilizers and irrigation. These relationships will also mitigate crop losses associated with climate change. In order to utilize the beneficial relationships, a better understanding is necessary. This study compared four maize cultivars (B73, B73xMo17, DKC63-55RIB, and DKC64-69RIB) over three growth stages (V6, V12, and R1) to see how breeding has impacted maize's influence on the soil microbiome. Aboveground biomass production, aboveground biomass nutrient content (C, N, and P), percent arbuscular mycorrhizal fungi (AMF) root colonization at three depths (0-7.5cm, 7.5-15cm, and 15-30cm), root biomass at R1, and soil microbial community structures were measured. No significant differences were found for aboveground biomass, root biomass, or aboveground biomass nutrient content. Root scoring for AMF colonization showed an impact by cultivar and a significant decrease in colonization with increasing depth. The abundance of total PLFAs, gram-positive bacteria and gram-negative bacteria were highest in the control (mid-row), while no significant differences for these PLFAs were observed between the maize cultivars. Significance was observed between the different cultivars for soil AMF. Fungi showed no significant differences between cultivars but there was an impact by time. There was a significant interaction between treatment and time for actinomycete concentrations; however, there was no clear trend. These results indicate the soil microbiome is affected by maize cultivar as well as growth stage. Breeding seems to not have impaired the ability of maize to sustain beneficial relationships with surrounding microbes; neither did genetic manipulation have a negative effect on AMF.

Introduction

The increasing human population is placing growing demand on the agricultural sector. Especially while also dealing with changing climate conditions in addition to a decreasing amount of arable land and fresh water. Moreover, many current agricultural management practices are not sustainable and need to be adjusted while also reducing their environmental impact. Whether or not farmers can continue to increase crop yields to feed the increasing population is uncertain, as there will be challenges to maintain what is currently being produced. Not only is climate change bringing with it increasing temperatures and changes in precipitation patterns, but these factors will also facilitate increased crop stress through diseases and pests. This will lead to the need for greater chemical use and will increase the cost of crop production. In addition, phosphorus (P) fertilizer is a resource that is finite and non-renewable (Özbek et al., 2016). Based on this, it is imperative to adjust the current agricultural practices so that they are more sustainable, cost-effective, and achievable.

There has been an increasing interest in the usage of microorganisms as biofertilizers to mitigate some of the before mentioned issues. This is because certain soil microorganisms are known to benefit plant growth by increasing access to nutrients, improving plant water uptake, reducing soil pathogens, defending against herbivory, and more. As plants grow, their roots release exudates, mucilage, and root border cells, a process also known as rhizodeposition. Because of this process, soil microorganisms are drawn to the plant roots. The exudates make up the largest portion of the rhizodeposition (Shahzad et al., 2015) and the release of this photosynthetically fixed carbon allows plants to manipulate the microbes that surround them. This process can amount to 5-21% of the total photosynthetically fixed carbon (Walker et al., 2003). As exudation comes as a cost to the plant, the plant has systems in place to regulate this

process. Moreover, the compounds that are being exuded are not all the same, depend on plant species and genotype, and can be triggered by changes in the rhizosphere (Walker et al., 2003) that are leading to an abiotic or biotic stress to the plant. By signaling the surrounding soil microbes, the plant is able to attract or deter particular groups of microbial organisms. This interaction between plants and microbes is not a newfound concept; knowledge about symbiotic relationships, such as between legumes and nitrogen-fixing bacteria, has been established for a while. One important symbiotic relationship relevant to this study is that between plants and arbuscular mycorrhizal fungi (AMF). These fungi are capable of forming symbiosis with the vast majority of terrestrial plants (Smith et al., 2011) and can increase nutrient and water uptake of the plant while also protecting the plant from multiple types of stress (Jeong et al., 2006). This beneficial interaction has drawn the attention of agronomists in their quest to reduce the usage of chemical fertilizers and pesticides (Harrier and Watson, 2004) as well as looking for ways to produce crops in a changing climate.

This study focused on maize (*Zea mays* L.) as it is the largest crop produced in the USA (Kim et al., 2014) that was domesticated 9,000 years ago and has since been cultivated and manipulated to meet our current feed, food, and biofuel needs. The main objective of this study was to determine the impact of maize breeding on the soil microbiome by comparing cultivars released in the 1970s to those from 2016 (“old” vs. “new”). The second objective was to determine how long-term breeding has affected the cultivars responsiveness to arbuscular mycorrhizal fungi colonization.

The hypothesis was that breeding for aboveground traits has affected the belowground interactions between maize and the surrounding soil microorganisms. More specifically, improved cultivars, such as the hybrid B73xMo17 and the genetically modified hybrids DKC63-

55RIB and DKC64-69RIB are expected to have less dependency on the soil microbes and will likely have less AMF colonization compared to the inbred B73.

Materials and Methods

Site Description and Experimental Design

This study was conducted at the Kansas State University research farm in Manhattan, KS (39.20˚N, 96.59˚W), in a field that has been under continuous maize since 1990 and under no-till management since 2006. The soil was classified as moderately well drained Kennebec silt loam (fine-silty, mixed, superactive, mesic Cumulic Hapludolls) with soil characteristics (Table 2.1). Atrazine was applied at pre-plant and urea was manually applied at 168 kg N ha^{-1}.

The field experiment was a split-plot randomized complete block design (RCBD) with four replications and a total of five treatments. Treatments included four maize cultivars (B73, B73xMo17, DKC63-55RIB, and DKC64-69RIB) and a control (soil from between the middle two maize rows per plot). Blocks consisted of four 3 m x 3 m plots with 2 m alleyways. Each plot comprised of four rows of sterilized maize seeds. Sterilization of seeds consisted of surface-disinfecting the seeds for 10 min in 3% hydrogen peroxide (H_2O_2), followed by a rinse in distilled water, one wash in 70% ethanol, and three rinses with distilled water. Seeds were hand-planted on 7th May 2016 into the rain-fed no-till field, with seeds 18 cm apart, 4 cm deep, and with a row spacing of 76 cm. Plots were sampled at three growth stages (V6, V12, and R1). To eliminate border-effects, only the inside two rows were used for data collection. In addition, plots were manually weeded when necessary to avoid an impact of non-maize roots on soil microorganisms.

Soil Chemistry

Soil samples for general analyses were taken to a depth of 15 cm, placed in labeled Ziploc bags, and stored at 5˚C until processed. Available nitrate (NO_3^-) and ammonium (NH_4^+) were measured by weighing out 25 g moist soil into an Erlenmeyer flask and adding a 1 M potassium chloride (KCl) solution (1:4 ratio). Samples were then placed on an orbital shaker for 1 h and left to settle for 5 min. Once the soil was settled, the supernatant was decanted onto Whatman no. 42 filter paper and collected into labeled scintillation vials. The scintillation vials along with soil samples for Mehlich III phosphorus (P) (Mehlich, 1984), potassium (K) (Schollenberger and Simon, 1945), and pH (Tran and van Lierop, 1982) analyses were submitted to the K-State Research and Extension Soil Testing Lab, Manhattan, Kansas. Soil texture analysis was performed using the standard pipette method (Kilmer and Alexander, 1949).

Arbuscular Mycorrhizal Fungi

Volumetric root cores (8 cm dia.) were obtained using a hand-driven root auger (B.V. Eijkelkamp, Lathum, Netherlands) consisting of a 15 cm long cylindrical sampling tube with serrated edges. Core removal from the auger was facilitated using a hand-cranked mechanism that extrudes each root core. Root samples were up to a depth of 30 cm and subdivided into three depth increments: 0-7.5, 7.5-15, and 15-30 cm. After sampling, the root cores were stored at 5˚C until processed. Root biomass was separated from the soil by washing each soil root core in an automated root washer (Benjamin and Nielsen, 2006). To allow for easier washing, each obtained soil root core was carefully broken up and placed into individually labeled 300 μm stainless steel mesh cylinders. The cylinders were then loaded into the root washer and rotated under high-pressure water spray. After the soil was washed off, the live roots were manually picked from leftover debris, placed into labeled histosettes, and frozen.

Root AMF colonization was determined by root scoring. Maize roots were cleared and stained using a slightly modified Phillips and Hayman method (1970). First, frozen roots placed in histosettes were thawed and rinsed with tap water. Next, the roots were cleared in a heated 2.5% potassium hydroxide (KOH) solution at 90˚C for 20 min in a water bath. Once the coloration of the solution turned a light brown, the histosettes were removed, rinsed, and placed in a boiling solution of 3% hydrogen peroxide for 10-30 min to bleach the root pigments. Bleaching was complete when roots were white to yellow in color, after which the histosettes were rinsed with deionized water and acidified for 10 min in a 1% hydrochloric acid solution to allow the trypan blue stain to bind to the fungal structures. The histosettes were then placed in 0.05% trypan blue stain and placed in an oven at 90˚C for 30-60 min. The histosettes were removed from the trypan blue stain and placed in a de-staining solution containing a mixture of 250 mL glycerol, 225 mL distilled water, and 25 mL of 1% hydrochloric acid (HCl) for 2-3 days.

The estimation of AMF colonization in maize roots, also known as root scoring, was done using a method by McGonigle et al. (1990). Roots were washed in glycerol and cut into 1 cm segments. Next, 10 root segments were carefully placed onto a microscope slide using tweezers, making sure the roots did not cross. Roots were then carefully flattened with a coverslip. Using 200 to 400x magnification, the microscope lens passed each segment five times, allowing 50 intercepts in total. With the use of a crosshair, the eyepiece was moved until the root segment was 90˚ perpendicular to the root. The segment was then examined at each root intersection using the following four categories: no AMF structures, arbuscules, vesicles, or mycorrhizal hyphae. As mycorrhizal root colonization was expressed as the percent of total root intersections that had AMF present, the following formula was used:

% colonization = [(vesicles + arbuscules + hyphae) / n˚ of intersections] * 100

Aboveground Biomass

Maize biomass was collected during each of the three growth stages (V6, V12, R1) by randomly selecting two maize plants in each plot and cutting them at the soil surface. The cut plants were then individually placed into labeled paper bags and oven dried at 60˚C until a constant weight was achieved. After weighing, biomass samples were ground in a Wiley mill and then in a ball mill until the biomass was a fine powder. The ground material was weighed and analyzed for total C and N by dry combustion using a Thermo-Finnigan EA Flash 1112 (Waltham, MA). The K-State Research and Extension Soil Testing Lab, Manhattan, Kansas determined total P using a salicylic-peroxide acid digestion (Lindner and Harley, 1942).

Microbial Lipids

The soil microbial community structure was analyzed following a modified Bligh and Dyer (1959) method by White (1996). For each plot, 5 g of ground and lyophilized soil was weighed and incubated in a 2:1:0.8 chloroform-methanol-phosphate buffer mixture in order to extract the organic phase. Lipids were then fractionated using silicic acid chromatography into glycolipids, neutral lipids, and phospholipids (polar) by eluting them consecutively with chloroform (glycolipids), acetone (neutral lipids), and methanol (phospholipids) into separate test tubes. Only the phospholipids were kept and were subsequently evaporated in a nitrogen evaporation bath. These lipids were then saponified using potassium hydroxide (KOH) and methylated to form fatty acid methyl esters (FAME). The FAMEs were separated using a Thermo Scientific Trace GC-ISQ mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) using a DB5-MS column (30m x 250 µm i.d. x 0.25 µm film thickness; Agilent Technologies, Santa Clara, California, USA) with helium as a carrier gas. The individual FAME peaks were identified by comparing retention times to peaks of a known bacterial acid

methyl esters (BAME) mixture (Matreya 1114; Matreya LLC, Pleasant Gap, Pennsylvania, USA). FAME peaks that were not present in the BAMEs mixture were assigned by mass spectral interpretation. Concentrations were determined using the internal standard, methyl nonadecanoate, and were converted into nmol PLFA g^{-1} dry soil. Signature PLFAs were used as indicators for specific microbial groups: gram-positive bacteria (i15:0, a15:0, i16:0, i17:0, a17:0), gram-negative bacteria (2-OH 10:0, 2-OH 12:0, 3-OH 12:0, 2-OH 14:0, 3-OH 14:0, 16:1ω7c, cy17:0, cy19:0), arbuscular mycorrhizal fungi (AMF) (16:1ω5), and saprophytic fungi (18:2ω6,9c).

Statistical Analysis

Analysis of variance (ANOVA) was performed on both soil and biomass data using PROC GLIMMIX in SAS 9.4 (SAS Institute Inc., 2013). Results were considered statistically significant when $p<0.05$. The treatment and sampling time were considered fixed effects whereas the block and the interactions were random effects. Means separation was conducted using Fisher's LSD at $\alpha=0.05$.

Results

Air temperature and precipitation during the growing season of 2016 were recorded by a nearby meteorological station ("Kansas State University Mesonet," 2017) (Table 2.2). Total precipitation for that growing period was 86.8 cm, which was 14 cm above the 30-year average (1981-2010) of 72.7 cm (Arguez et al., 2012). Growing season monthly average temperatures were also compared to the 30-year averages. These showed slightly higher for the mean by 2-3°C for April, June, September, and October compared to the 30-year averages. Slightly lower temperatures were recorded for May, July, and August.

Plant Biomass

There was no significant effect ($p < 0.05$) on aboveground biomass due to differences in cultivar (Table 2.3). Cultivar did not significantly impact biomass total C, N, or P. Root biomass was not affected by cultivar.

Arbuscular Mycorrhizal Fungi

Colonization by AMF was significantly ($p < 0.05$) different among the four cultivars, over time, as well as by depth (Table 2.4). Among the cultivars, only DKC64-69RIB and DKC63-55RIB were significantly different with DKC63-55RIB having the least amount of AMF root colonization (Fig. 2.1, A.). Colonization of AMF decreased with depth where colonization at 0-7.5 cm depth was significantly greater to that of 15-30 cm (Fig. 2.1, B.). Colonization also decreased over time with the roots during the V6 growth stage having greater AMF colonization compared to those at V12 (Fig. 2.1, C.).

Microbial Lipids

No significant interaction was found between treatment and time for total PLFA, gram-positive bacteria, gram-negative bacteria, AMF, or fungi (Table 2.5). There was, however, a significant interaction ($p < 0.05$) between treatment and time for actinomycetes. The effect of treatment was significant for all of the biomarkers except for those of actinomycetes and fungi, while the effect of time was significant for all biomarkers except for those of gram-negative bacteria. For total PLFA, the effect of time was significant during the R1 growth stage, which showed a decrease in total PLFA compared to the V6 and V12 growth stages (Fig. 2.2, A.). There was significant difference observed for total PLFA between the different cultivars, however, the control treatment had significantly greater total PLFA compared to the cultivars (Fig. 2.3, A.). Gram-positive bacteria had less total concentration at the R1 growth stage

compared to V6 and V12 with no significant differences between the latter (Fig. 2.2, B.). As with total PLFA, and gram-positive were also most abundant in the control treatment compared to the different cultivars (Fig. 2.3, B.).

Treatment and time had a significant impact on the fungi to bacteria ratio (F:B) although the interaction between the two was not significant (Table 2.5). The F:B increased significantly by the R1 growth stage while no significant differences were observed between V6 and V12 (Fig. 2.4). Between treatments, B73 was significantly different from cultivars B73xMo17, DKC63-55RIB, and from the control but not from DKC64-69RIB. No significant differences were observed in F:B between the control and B73xMo17, DKC63-55RIB, and DKC64-69RIB (Fig. 2.5). Total fungi concentrations increased over time with R1 having a significantly greater fungi concentration compared to V6 and V12. There was no significant difference in fungi concentration between V6 and V12 (Fig. 2.2, D.). The soil AMF showed significant differences between cultivars with the control still having the greatest concentration but this was not significantly different from DKC63-55RIB. The greatest difference in soil AMF was between B73 and DKC63-55RIB, where B73 had the smallest concentrations. Both B73xMo17 and DKC64-69RIB were not statistically different (Fig 2.3, D.). Time also impacted total soil AMF with a decrease at V13 and R1 compared to V6 (Fig 2.2, C.). Although total actinomycetes showed a significant interaction between cultivar and time (Table 2.5), there was no trend aside from a decrease at R1 (Fig. 2.6).

Discussion

No significant differences were observed for shoot biomass among the four cultivars. These results contradict findings where hybrid maize cultivars tend to have greater plant biomass compared to their parental inbred lines due to the improved performance of the heterozygous

hybrids compared to that of the homozygous inbred parental lines. This phenomenon is also known as heterosis (Hoecker et al., 2008). In addition, DKC63-55RIB and DKC64-69RIB cultivars were expected to have greater dry biomass compared to both B73 and B73xMo17. Past trials comparing older hybrids to newer hybrids showed that the genetic improvement of newer hybrids resulted in greater dry biomass and heavier kernels. Especially in low yield environments, the newer hybrids would perform better (Duvick, 1984). In addition, plant vigor tends to increase when crossing maize lines that are more genetically different than when crossing isogenic lines (Hallauer et al., 2010). The reason for the lack of biomass heterosis in this study was unclear, although weather conditions may have inhibited responses. The wet weather resulted in the rotting of some emerging maize plants which were replanted as well as caused greensnap and lodging later in the growing period. Neither was it clear why the shoots were not significantly different in nutrient content despite having differences in AMF colonization between the cultivars.

The ANOVA of arbuscular mycorrhizal fungal colonization levels demonstrated that cultivar, depth, and time had significant effects on mycorrhizal colonization. Between cultivars, the greatest differences were seen between DKC64-69RIB and DKC63-55RIB with DKC64-69RIB having the greatest amount of AMF colonization. Neither Bt maize cultivars had significant differences from B73 or B73xMo17, supporting evidence that Bt toxins have no significant impact on AMF colonization in maize (Cheeke et al., 2013). Higher levels of AMF colonization did not result in increased biomass or increased P content. This supports previous research comparing Bt maize with parental lines in which no increase in biomass or chlorophyll content was found with greater AMF colonization (Cheeke et al., 2011).

With increasing depth, AMF colonization decreased. This is likely due to greater organic matter inputs at the surface of the soil, creating a richer and diverse microbiome. Previous research supports our findings and found that the amount of AMF spores and AMF species richness decreased with depth (Oehl et al., 2005; Jumpponen et al., 2010).

Interestingly, AMF colonization decreased over time, with the greatest colonization at the V6 growth stage compared to V12. Although there is seasonal variation in mycorrhizal root colonization during plant development, the colonization of AMF usually increases up to silking (R1), after which AMF colonization decreases (Lutgen et al., 2003). The high soil water might have impaired colonization. Poor aeration and extreme moisture conditions are known to impair AMF colonization (Reid and Bowen, 1979; Hayman, 1980; Shukla et al., 2013).

Contrary to previous studies we did not find greater microbial populations in the rhizosphere compared to the bulk soil. Soil surrounding roots is expected to have greater microbial biomass compared to the bulk soil as evident in studies by Kowalchuck et al. (2002) and Li et al. (2014). There was more crop residue from the previous year between the rows of maize, but as growing maize plants exude a continuous supply of nutrients via their roots, the soil surrounding the roots should have been a more favorable environment. The sampling may not have been at sufficient spatial resolution to capture differences in the rhizosphere.

Maize genotype appeared to have no significant impact on microbial groups such as gram-positive bacteria, gram-negative bacteria, and fungi. The control had greater concentrations of both gram-positive and gram-negative bacteria compared to the soil surrounding the maize roots while fungal concentrations were not statistically different between the treatments.

Genotype did have a significant effect on AMF and the F:B. While the total concentration of AMF was greatest in the control soil, a significant difference was observed between B73 and

DKC63-55RIB. This does not correlate with the root scoring results for AMF colonization, as B73 and DKC63-55RIB were not statistically different. No significant differences were observed between DKC63-55RIB and the other treatments: control, B73xMo17, and DKC64-69RIB. Cultivars B73xMo17 and DKC64-69RIB did have significantly lower concentrations of AMF in the soil compared to that of the control treatment. These lower concentrations of AMF biomarkers in their root environment did not impede root colonization. In fact, DKC64-69RIB had the greatest colonization percentage with B73xMo17 and B73 being statistically similar and DKC63-55RIB having significantly lower root colonization compared to DKC64-69RIB. Although the reason for differences in AMF biomarkers between the treatments is not well understood, it is clear that Bt maize hybrids have no adverse impact on AMF.

The F:B was also different between treatments, with B73 having a significantly lower ratio compared to all other treatments except DKC64-69RIB. No significant differences were observed in F:B between DKC64-69RIB, B73xMo17, DKC63-55RIB, and the control.

Conclusion

Differences in cultivar genotype did not influence plant or root biomass. The four cultivars did show differences in AMF root colonization; however, these differences did not impact biomass nutrients such as C, N, or P, despite previous research linking AMF to enhanced nutrient uptake. In addition, although previous research indicated that the expression of Bt in maize cultivars resulted in reduced AMF colonization, this study demonstrated no such negative impact. Interestingly, PLFA analyses indicated a reduction in total microbial biomass, AMF biomass, and gram-positive bacteria between V6 and R1. The reduction of AMF biomass correlated with the amount of AMF root colonization over time. These findings were unusual as AMF was expected to increase over time while greater root biomass was expected to result in

greater total microbial biomass surrounding them. Another unusual result was that the control had greater amounts of microbial biomass compared to the cultivars. These controls had maize residue left over from the previous year, which may have impacted the results. Nonetheless, actively growing roots were expected to have a greater microbial biomass surrounding them.

This study would suggest that maize breeding for particular traits had no significant impact on the soil microbial communities, except for on actinomycetes and AMF. Moreover, despite having an impact on AMF, this did not result in heterosis or in increased nutrient uptake.

References

Arguez, A., I. Durre, S. Applequist, R.S. Vose, M.F. Squires, X. Yin, R.R. Heim, and T.W. Owen. 2012. NOAA's 1981–2010 U.S. Climate Normals: An Overview. Bull. Am. Meteorol. Soc. 93(11): 1687–1697. doi: 10.1175/BAMS-D-11-00197.1.

Benjamin, J.G., and D.C. Nielsen. 2006. Water deficit effects on root distribution of soybean, field pea and chickpea. Field Crops Res. 97(2–3): 248–253. doi: 10.1016/j.fcr.2005.10.005.

Bligh, E.G., and W.J. Dyer. 1959. A rapid method of total lipid extraction. Can. J. Biochem. Physiol. 37: 911–917.

Cheeke, T.E., M.B. Cruzan, and T.N. Rosenstiel. 2013. Field evaluation of arbuscular mycorrhizal fungal colonization in Bacillus thuringiensis toxin-expressing (Bt) and non-Bt maize. Appl. Environ. Microbiol. 79(13): 4078–86. doi: 10.1128/AEM.00702-13.

Cheeke, T.E., B.A. Pace, T.N. Rosenstiel, and M.B. Cruzan. 2011. The influence of fertilizer level and spore density on arbuscular mycorrhizal colonization of transgenic Bt 11 maize (Zea mays) in experimental microcosms. FEMS Microbiol. Ecol. 75(2): 304–312. doi: 10.1111/j.1574-6941.2010.01013.x.

Duvick, D.N. 1984. Genetic contributions to yield gains of US hybrid maize, 1930 to 1980. Genet. Contrib. Yield Gains Five Major Crop Plants: 15–47.

Hallauer, A.R., M.J. Carena, and J.B. Miranda Filho. 2010. Quantitative Genetics in Maize Breeding. 3rd ed. Springer, New York ; London.

Harrier, L.A., and C.A. Watson. 2004. The potential role of arbuscular mycorrhizal (AM) fungi in the bioprotection of plants against soil-borne pathogens in organic and/or other sustainable farming systems. Pest Manag. Sci. 60(2): 149–157.

Hayman, D.S. 1980. Mycorrhiza and crop production. Nature 287(5782): 487–488. doi: 10.1038/287487a0.

Hoecker, N., B. Keller, N. Muthreich, D. Chollet, P. Descombes, H.-P. Piepho, and F. Hochholdinger. 2008. Comparison of maize (Zea mays L.) F1-hybrid and parental inbred line primary root transcriptomes suggests organ-specific patterns of nonadditive gene expression and conserved expression trends. Genetics 179(3): 1275–1283.

Jeong, H.-S., J. Lee, and A.-H. Eom. 2006. Effects of interspecific interactions of arbuscular mycorrhizal fungi on growth of soybean and corn. Mycobiology 34(1): 34–37.

Jumpponen, A., K.L. Jones, and J. Blair. 2010. Vertical distribution of fungal communities in tallgrass prairie soil. Mycologia 102(5): 1027–1041.

Kansas State University Mesonet. 2017. Hist. Weather. http://mesonet.k-state.edu/weather/historical/ (accessed 13 April 2018).

Kilmer, V.J., and L.T. Alexander. 1949. Methods of making mechanical analyses of soils. Soil Sci. 68(1): 15–24.

Kim, S., B.E. Dale, and P. Keck. 2014. Energy Requirements and Greenhouse Gas Emissions of Maize Production in the USA. BioEnergy Res. 7(2): 753–764. doi: http://dx.doi.org.er.lib.k-state.edu/10.1007/s12155-013-9399-z.

Kowalchuk, G.A., D.S. Buma, W. de Boer, P.G.L. Klinkhamer, and J.A. van Veen. 2002. Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. Antonie Van Leeuwenhoek 81(1–4): 509–520.

Li, X., J. Rui, J. Xiong, J. Li, Z. He, J. Zhou, A.C. Yannarell, and R.I. Mackie. 2014. Functional potential of soil microbial communities in the maize rhizosphere. PloS One 9(11): e112609.

Lindner, R.C., and C.P. Harley. 1942. A rapid method for the determination of nitrogen in plant tissue. Science 96(2503): 565–566.

Lutgen, E., D. Muir-Clairmont, J. Graham, and M. Rillig. 2003. Seasonality of arbuscular mycorrhizal hyphae and glomalin in a western Montana grassland. Plant Soil 257(1): 71–83. doi: 10.1023/A:1026224209597.

Mcgonigle, T.P., M.H. Miller, D.G. Evans, G.L. Fairchild, and J.A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol. 115(3): 495–501. doi: 10.1111/j.1469-8137.1990.tb00476.x.

Mehlich, A. 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Commun. Soil Sci. Plant Anal. 15(12): 1409–1416. doi: 10.1080/00103628409367568.

Oehl, F., E. Sieverding, K. Ineichen, E.-A. Ris, T. Boller, and A. Wiemken. 2005. Community structure of arbuscular mycorrhizal fungi at different soil depths in extensively and intensively managed agroecosystems. New Phytol. 165(1): 273–283. doi: 10.1111/j.1469-8137.2004.01235.x.

Özbek, F., A. Leip, and M. Velde. 2016. Phosphorous stock changes in agricultural soils: a case study in Turkey. Nutr. Cycl. Agroecosystems 105(1): 51–59. doi: 10.1007/s10705-016-9773-2.

Phillips, J.M., and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55(1): 158,IN16–161,IN18. doi: 10.1016/S0007-1536(70)80110-3.

Reid, C.P.P., and G.D. Bowen. 1979. Effect of Water Stress on Phosphorus Uptake by Mycorrhizas of Pinus radiata. New Phytol. 83(1): 103–107.

Schollenberger, C.J., and R.H. Simon. 1945. Determination of exchange capacity and exchangeable bases in soil-ammonium acetate method. Soil Sci. 59(1): 13–24.

Shahzad, T., C. Chenu, P. Genet, S. Barot, N. Perveen, C. Mougin, and S. Fontaine. 2015. Contribution of exudates, arbuscular mycorrhizal fungi and litter depositions to the rhizosphere priming effect induced by grassland species. Soil Biol. Biochem. 80: 146–155.

Shukla, A., A. Kumar, A. Jha, O. Salunkhe, and D. Vyas. 2013. Soil moisture levels affect mycorrhization during early stages of development of agroforestry plants. Biol. Fertil. Soils 49(5): 545–554.

Smith, S.E., I. Jakobsen, M. Grønlund, and F.A. Smith. 2011. Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiol. 156(3): 1050–1057.

Tran, T.S., and W. van Lierop. 1982. Lime requirement determination for attaining pH 5. 5 and 6. 0 of coarse-textured soils using buffer-pH methods. Soil Sci. Soc. Am. J. 46.

Walker, T., E. Grotewold, and J. Vivanco. 2003. Root exudation and rhizosphere biology. Plant Physiol. 132(1): 44–51. doi: 10.1104/pp.102.019661.

White, D.C. 1996. Signature lipid biomarker analysis for the quantitative analysis of environmental microbial ecology. Natl. Meet. - Am. Chem. Soc. Div. Environ. Chem. 36(2): 139–141.

Tables

Table 2.1 Selected soil properties for the Kennebec silt loam soil collected from the North Agronomy Research Farm, Kansas State University.

Depth	BD	pH	SOC	NO_3^--N	NH_4^--N	M3-P	M3-K	Sand	Silt	Clay
	--g cm^{-3}--		-g kg^{-1}-	----------μg g^{-1} soil----------				----------mg g^{-1}-------		
0-30 (cm)	1.2	6.9	0.129	0.95	0.18	22.4	240	40	43	17

BD = bulk density. SOC = soil organic carbon.

§ M3-P = Mehlich-3 phosphorus. M3-K = Mehlich-3 potassium.

Table 2.2 Precipitation and temperature information for Manhattan, KS from 2016 (Kansas State University Mesonet, 2017) and the 30-year average from 1981-2010 (Arguez et al., 2012).

		Year	
	Month	2016	30-yr. avg.
Average monthly air temperature (C°)	January	-0.7	-1.7
	February	4.9	1.1
	March	10.7	6.4
	April	14.6	12.5
	May	17.7	18.4
	June	26.7	23.7
	July	26.9	26.6
	August	25.1	25.6
	September	22.6	20.4
	October	16.9	13.6
	November	10.7	6.2
	December	-0.5	-0.4
Precipitation (mm)	January	14.0	16
	February	8.6	27
	March	9.1	63
	April	201.7	81
	May	150.9	129
	June	32.3	145
	July	168.4	112
	August	149.6	105
	September	157	87
	October	55.1	68
	November	10.9	44
	December	19.1	27

Table 2.3 ANOVA for shoot dry biomass, shoot total C, shoot total N, shoot total P, and root dry biomass for maize cultivars (B73, B73/Mo17, DKC63-55RIB, and DKC64-69RIB) grown under field conditions at the Agronomy North Research farm in 2016 and evaluated over three growth stages (V6, V12, and R1). There was insufficient root material to provide statistical data for root nutrient content and root biomass at V6 and V12.

Effect	Shoot Dry Biomass	Shoot Total C	Shoot Total N	Shoot Total P	Root Dry Biomass
Cultivar	0.8810	0.8483	0.7918	0.2110	0.2862
Time	< .0001	< .0001	< .0001	< .0001	N/A
Cultivar*Time	0.8888	0.7417	0.8332	0.0989	N/A

Table 2.4 ANOVA for percent AMF colonization from roots of four different maize cultivars (B73, B73/MO17, DKC63-55RIB, and DKC64-69RIB) evaluated over three different depths (0-7.5, 7.5-15, and 15-30cm) and three growth stages (V6, V12, and R1).

Effect	% AMF colonization
Cultivar	0.0433
Depth	0.0035
Cultivar*Depth	0.6959
Time	< .0001
Cultivar*Time	0.0826
Depth*Time	0.1525
Cultivar*Depth*Time	0.3391

Table 2.5 The ANOVA for total phospholipid fatty acids (PLFA), fungi:bacteria ratio, total gram-positive bacteria, total gram-negative bacteria, total actinomycetes, total fungi, and total arbuscular mycorrhizal fungi (AMF) from soil surrounding different maize cultivars (B73, B73/Mo17, DKC63-55RIB, and DKC64-69RIB) evaluated over three growth stages (V6, V12, and R1).

Effect	Total PLFA	Fungi/ Bacteria	Total $Gram^+$	Total $Gram^-$	Total Actinomycetes	Total Fungi	Total AMF
Cultivar	0.0489	0.0487	0.0369	0.0163	0.1789	0.0610	0.0034
Time	0.0073	< .0001	< .0001	0.1239	< .0001	0.0002	0.0002
Cultivar*Time	0.0738	0.6553	0.0977	0.2161	0.0041	0.3053	0.4520

Figures

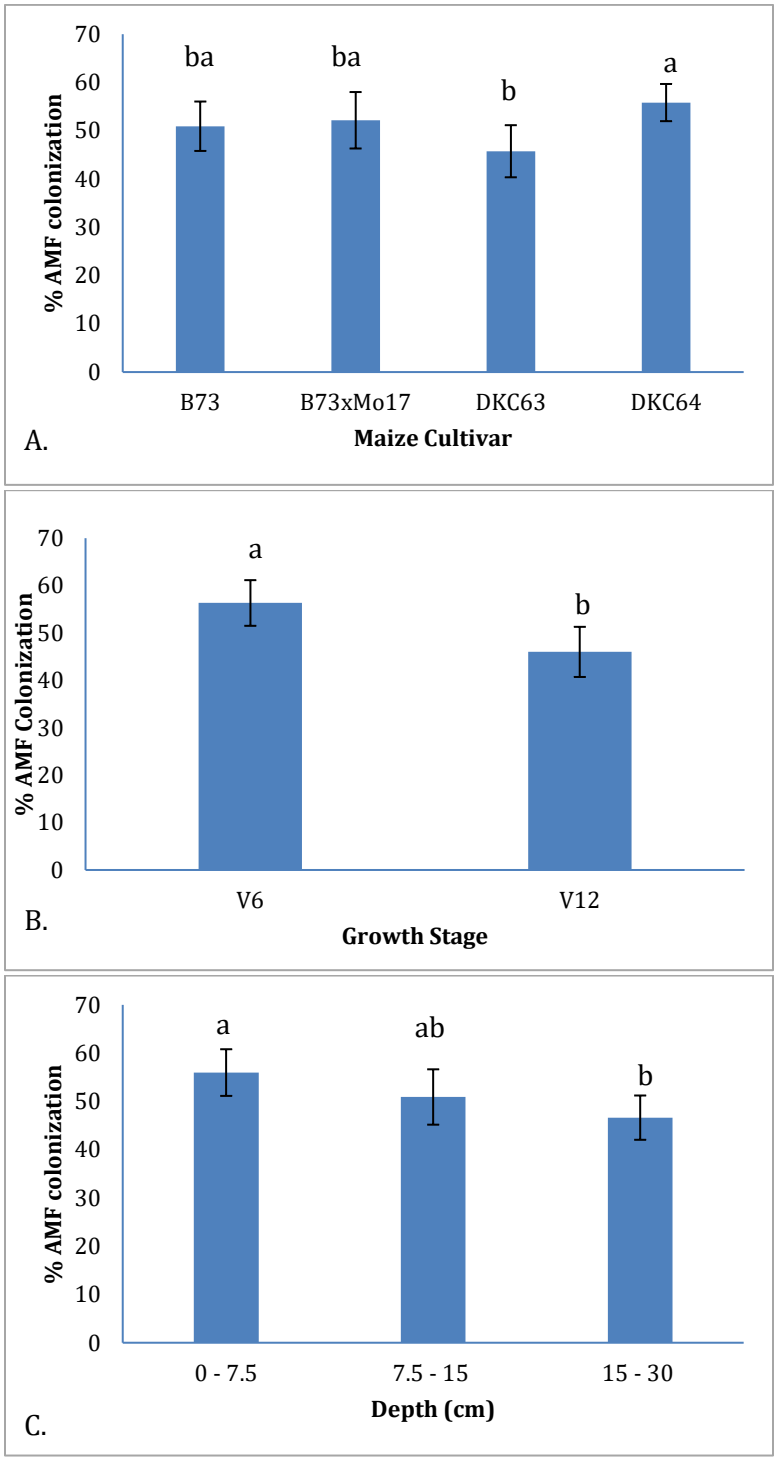

Figure 2.1. Percentages of arbuscular mycorrhizal fungi (AMF) root colonization as impacted by cultivar (B73, B73/Mo17, DKC63-55RIB, DKC64-69RIB) (A.), by growth stage (V6, V12) (B.) and by depth (0-7.5, 7.5-15, 15-30 cm) (C.). All cultivars were grown under field conditions at the Agronomy North Farm, Manhattan, Kansas in 2016. Bars with different letters show statistically significant differences ($p<0.050$).

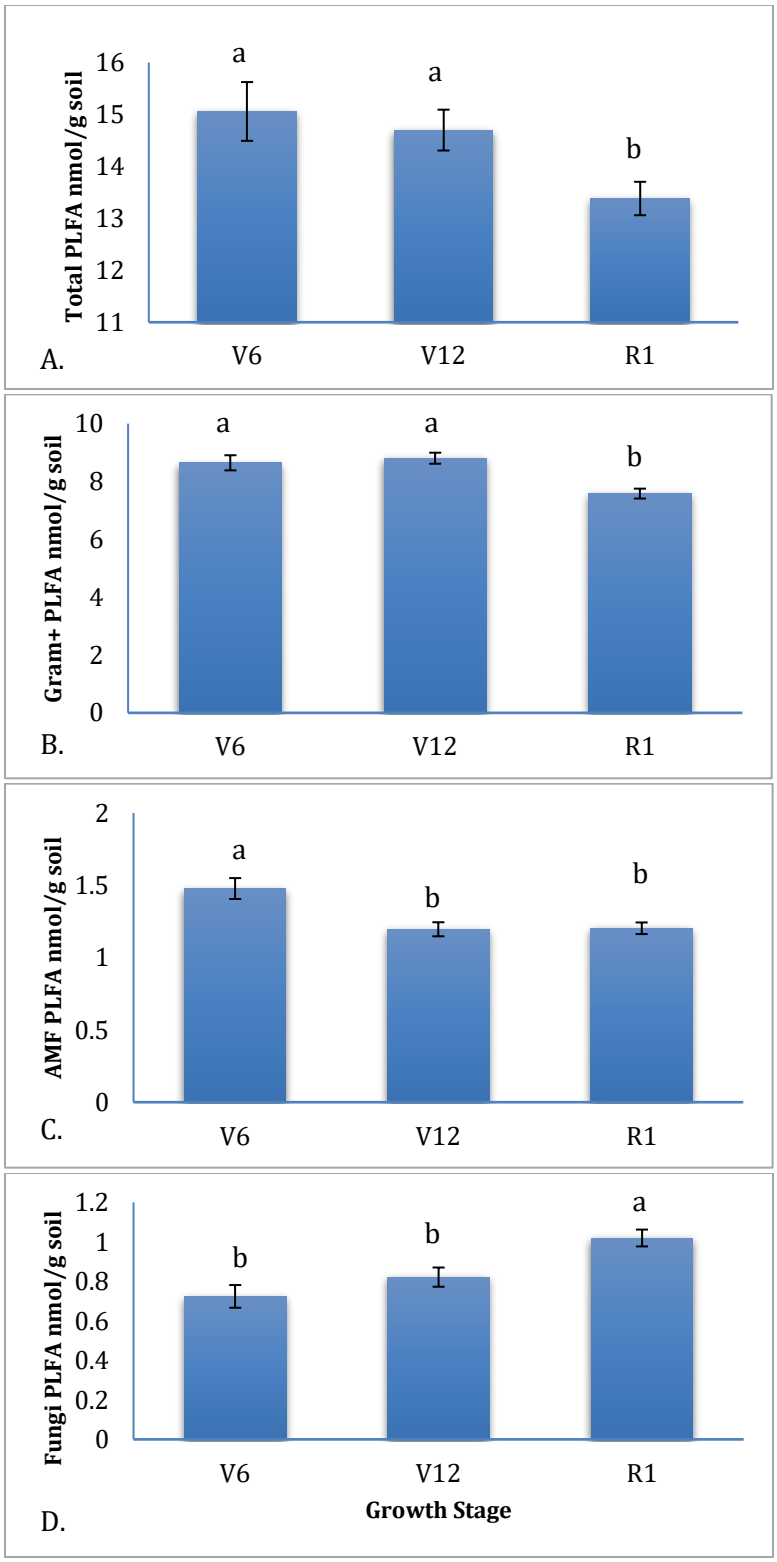

Figure 2.2 Total microbial biomass (A.), gram-positive bacteria (B.), AMF (C.), and fungi (D.) over three growth stages (V6, V12, and R1) for all four maize cultivars (B73, B73/Mo17, DKC63-55RIB, and DKC64-69RIB) and the control (mid-row soil). Bars with the same letter are not significantly different ($p<0.05$).

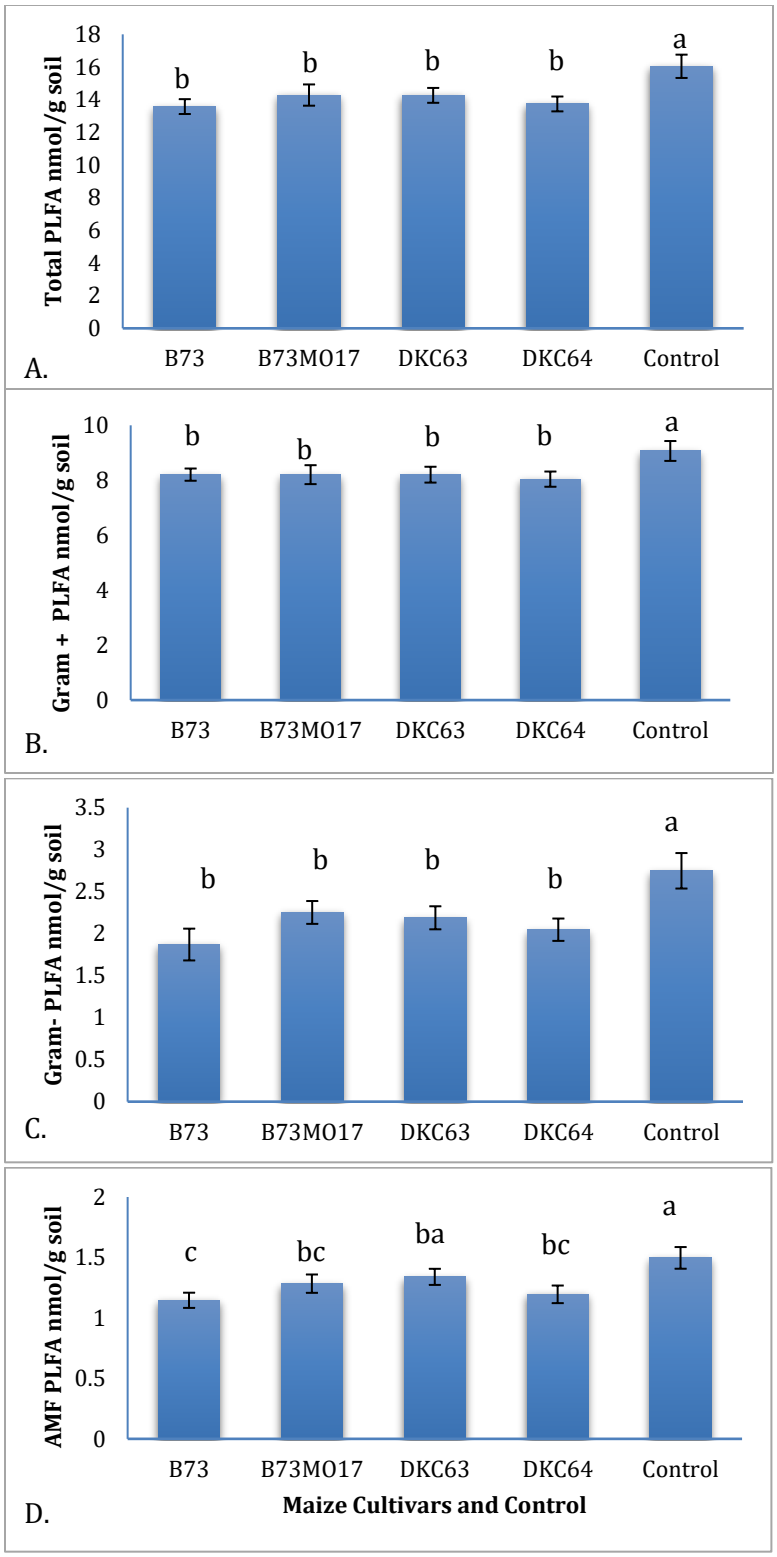

Figure 2.3 Total microbial biomass (A.) gram-positive (B.), gram-negative bacteria (C.), and arbuscular mycorrhizal fungi (AMF) (D.) as impacted by maize cultivar (B73, B73/Mo17, DKC63-55RIB, and DKC64-69RIB) and control (mid-row soil). All maize cultivars were grown under field conditions in 2016. Bars with the same letters are not significantly different ($p<0.05$).

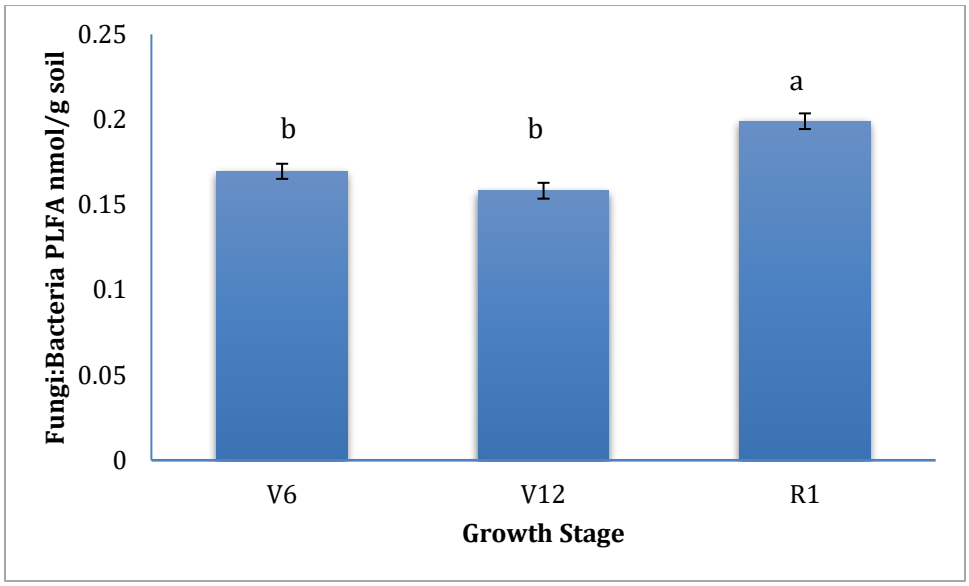

Figure 2.4 Fungi to bacteria ratio over three growth stages (V6, V12, and R1) for all four maize cultivars (B73, B73/Mo17, DKC63-55RIB, and DKC64-69RIB). Bars with different letters are significantly different ($p<0.05$).

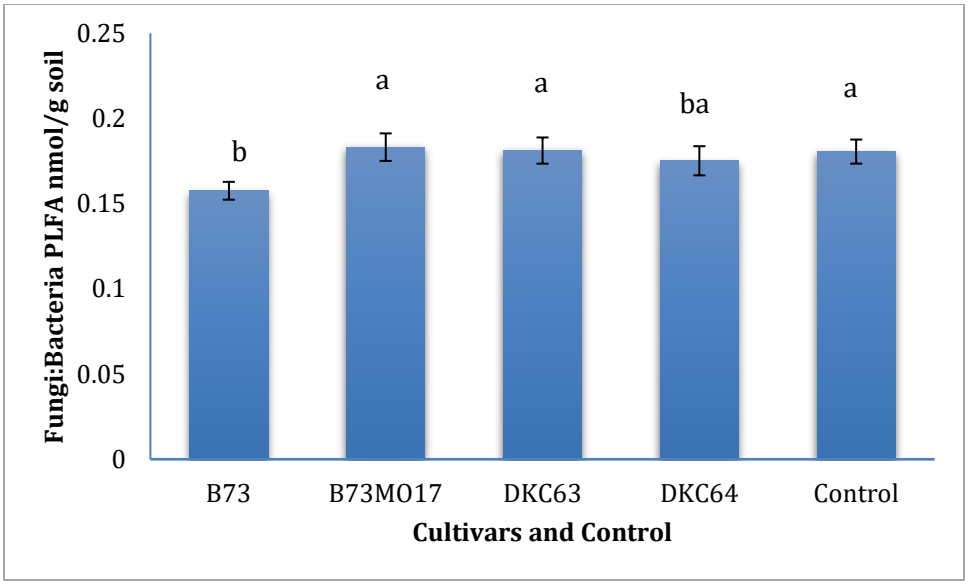

Figure 2.5 Fungi to bacteria ratios between the soils surrounding the roots of four different maize cultivars (B73, B73/Mo17, DKC63-55RIB, and DKC64-69RIB) and the control (mid-row soil). Cultivars were grown under field conditions in 2016. Bars with the same letters are not significantly different ($p<0.05$).

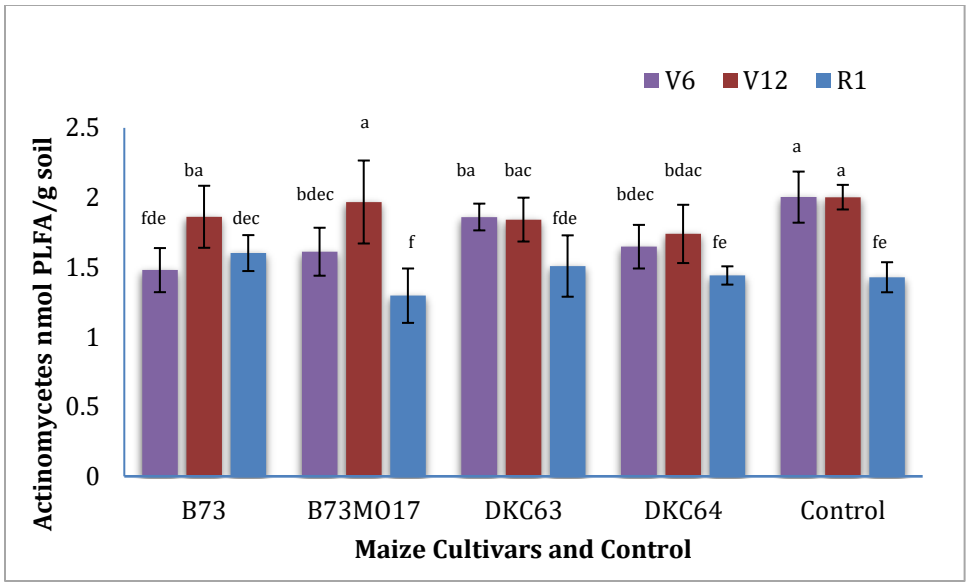

Figure 2.6 Total concentration of actinomycetes between the four cultivars (B73, B73/Mo17, DKC63-55RIB, and DKC64-69RIB) as well as the control (mid-row soil) over three different growth stages (V6, V12, and R1). Maize cultivars were grown under field conditions in 2016. Any bars with the same letter(s) are not significantly different ($p<0.05$).

Figure 2.7. Preparing the field experiment at the Agronomy North Farm, Kansas State University, Manhattan, Kansas.

Figure 2.8. Hand planting of four maize cultivars (B73, B73/Mo17, DKC63-55RIB, and DKC64-69RIB) at the Agronomy North Farm, Kansas State University, Manhattan, Kansas.

Figure 2.9. Sampling the field experiment at the Agronomy North Farm, Kansas State University, Manhattan, Kansas for phospholipid fatty acids (PLFAs) (top picture), root biomass (bottom left picture), and aboveground plant biomass (bottom right picture).

Chapter 3 - The Root-Soil Microbiome of Maize Cultivars grown in Different Soil P Concentrations

Abstract

There is a need for a more sustainable agriculture that meets the challenge of feeding a growing human population. Climate change and non-renewable resources have made this more difficult. One possible solution lies in belowground plant-microbial relationships, which could reduce fertilizer input and reduce the need for irrigation. Long-term breeding likely impacted these relationships and therefore an improved understanding is important. This study compared four maize cultivars (B73, B73xMo17, DKC63-55RIB, and DKC64-69RIB) and a control (unplanted) over three growth stages (V6, VT, and R1), and two P-levels grown under greenhouse conditions. Measured were: shoot biomass and nutrient content (C, N, and P); root biomass and nutrient content (C, N, and P); percent AMF root colonization, and the soil microbiome. Shoot biomass was affected by cultivar over time as well as the soil P over time. High P soil also enhanced nutrient uptake in the shoots, which increased over time. Roots, on the other hand, only saw enhanced uptake of P. Root biomass also showed a significant interaction between cultivar and time. For all treatments, the microbial biomass concentrations were lowest in the control and greatest in high P-level soils, with the only exception being AMF. No significant differences in microbial biomass concentrations were found between the cultivars, except for fungi. Percent colonization of AMF was very low in all cultivars and no significant differences were observed.

Introduction

Today's agricultural practices face a stiff challenge to provide food for projected future human populations. In addition, several practices still in use today are not sustainable as they result in degraded soil. With available arable land decreasing due to human expansion and the degradation of agricultural land, current practices must improve significantly. Adding to this are factors such as climate change and the need for non-renewable resources, such as P (Özbek et al., 2016). All of these will leave farmers with enhanced difficulties to maintain or increase crop production. While genetic modification has helped tremendously in battling many stresses that crops face daily, there is still a stigma associated with the concept in many areas around the world. Moreover, maize crops are still highly dependent on inputs of expensive fertilizers that can result in eutrophication of waters if not properly managed. One much neglected aspect of crop production has been the belowground aspect, yet this is where plant roots gather nutrients and water, while also communicating with the surrounding microbial environment. Each plant has the ability to modify their root-encompassing soil microbiome to its own advantage. This is an amazing evolutionary trait as the exudation process of plants can attract or deter specific microbial groups. The attraction of microbes usually benefits the plant in a multitude of ways depending on the microbial groups. For example, certain microbes can enhance nutrient uptake, water uptake, or relieve plant stresses such as drought. Despite these benefits, the optimization of these relationships has not yet been established in farming. This is largely due to the fact that research into root-microbial relationships is still relatively new and some parts of root research can prove to be challenging. Nonetheless, some basic understandings have been established. One of these is the fact that the soil microbiome surrounding roots can differ between cultivars of the

same species within the same soil. A better understanding of these differences may provide new insight into what exactly impacts the plant's alteration of the soil microbiome.

Certain soil microorganisms are known to benefit plant growth by increasing access to nutrients, improving plant water uptake, reducing soil pathogens, defending against herbivory, and more. As plants grow, their roots release exudates, mucilage, and root border cells, a process also known as rhizodeposition. Because of this process, soil microorganisms are drawn to the plant roots. The exudates make up the largest portion of the rhizodeposition (Shahzad et al., 2015) and the release of this photosynthetically fixed carbon allows plants to manipulate the microbes that surround them. This process can amount to 5-21% of the total photosynthetically fixed carbon (Walker et al., 2003). As exudation comes as a cost to the plant, the plant has systems in place to regulate this process. Moreover, the compounds that are being exuded are not all the same, depend on plant species and genotype, and can be triggered by changes in the rhizosphere (Walker et al., 2003) that are leading to an abiotic or biotic stress to the plant. By signaling the surrounding soil microbes, the plant is able to attract or deter particular groups of microbial organisms. This interaction between plants and microbes is not a newfound concept; knowledge about symbiotic relationships, such as between legumes and nitrogen-fixing bacteria, has been established for a while. One important symbiotic relationship relevant to this study is that between plants and arbuscular mycorrhizal fungi (AMF). These fungi are capable of forming symbiosis with the vast majority of terrestrial plants (Smith et al., 2011) and can increase nutrient and water uptake of the plant while also protecting the plant from multiple types of stress (Jeong et al., 2006). More recent research is showing that plant genotype within a plant species can alter the soil microbiome, especially as it relates to microbial function and microbial community structure (Mazzola et al., 2004).

To what extent genetic manipulation and long-term breeding for more desirable traits have altered the rhizosphere processes is not well known. However, microbial diversity is influenced by the physical and chemical properties of the soil which are influenced by the genetics of the plant (Peiffer et al., 2013).

This study was designed to test the hypothesis that breeding for aboveground traits in maize may have changed the plant's ability to manipulate the soil microbiome. More specifically, we hypothesize that older cultivars would be better at utilizing beneficial microbial groups compared to newer cultivars. The objectives of this study were designed to compare aboveground biomass yield; root yield; percent AMF root colonization; C, N, and P content of both aboveground biomass and root biomass; and PLFA concentrations for four genetically different maize cultivars grown under greenhouse conditions and under two soil P-levels.

Materials and Methods

Soil and Container Preparation

Containers consisted of labeled five-gallon buckets into which holes were drilled at the bottom to allow water to drain freely. Each container received 4 cm of gravel prior to adding soil. To assess differences between low and high P levels, low P soil was gathered from a maize field at the Agronomy North Farm, Kansas State University, Manhattan, KS. This field has been under monoculture maize since 1990 and under no-till management since 2006 where the soil is classified as moderately well drained Kennebec silt loam (fine-silty, mixed, superactive, mesic Cumulic Hapludolls) with soil characteristics (Table 3.1). The freshly collected soil was transported to the greenhouses at Kansas State University and laid out to dry onto a clean tarp. Once dry, the soil was ground and sieved using a handmade sieve (1.27 cm openings) to remove all organic residue and rocks. The soil was then mixed with washed sand (1:2 ratio) to improve

drainage. The soil mixture had a pH of 7.61 and a Mehlich III P content of 8.75-9.09 ppm. Containers that were considered for low-P soil received 22.5 kg of soil mixture each, while the containers that were considered for high-P soil received 22.5 kg of soil mixture that was additionally mixed with 13.65 g of ground triple superphosphate (TSP).

The containers were placed on two benches in blocks by sampling time (V6, VT, and R1) to avoid any effect of inter-pot spaces after sampling. Each row of containers consisted of alternating soil P levels (low versus high) and all containers were assigned a treatment (cultivar or control) at random so that all treatments were equal between the low and high P soil containers. All containers were well watered on 15 August 2016 and left to drain for three days. On 18 August 2016, each cultivar treatment had three sterilized seeds planted 4 cm deep and roughly 2 cm apart in a triangular fashion. Control treatments did not receive any seeds as to represent soil without root influence. Once all cultivar treatments were planted, the containers, including the control, received 9.04 g urea each. Five days after planting, the excess maize was removed until only one cultivar was left in each container. All containers were monitored for the duration of the experiment and watered when needed.

Soil Chemistry

Available nitrate (NO_3^-) and ammonium (NH_4^+) were measured by weighing out 25 g moist soil into an Erlenmeyer flask and adding a 1 M potassium chloride (KCl) solution (1:4 ratio). Samples were then placed on an orbital shaker for 1 h and left to settle for 5 min. Once the soil was settled, the supernatant was decanted onto Whatman no. 42 filter paper and collected into labeled scintillation vials. The scintillation vials along with soil samples for Mehlich III phosphorus (P) (Mehlich, 1984), potassium (K) (Schollenberger and Simon, 1945), and pH (Tran and van Lierop, 1982) analyses were submitted to the K-State Research and Extension Soil

Testing Lab, Manhattan, Kansas. Additional soil was used for soil texture analysis, which was performed using the standard pipette method (Kilmer and Alexander, 1949).

Root Stocks and Arbuscular Mycorrhizal Fungi

When all greenhouse soil samples were obtained, each container was individually emptied into a large tote after which roots were removed from the soil by hand and placed into labeled bags. Once all root samples were gathered, the roots were washed by hand over a fine-meshed sieve to avoid root loss and placed into labeled aluminum trays. A small mass of thin live roots were randomly selected, removed, placed into labeled histosettes, and frozen for future AMF analysis. The remaining root mass was oven dried at 60°C weighed, and analyzed for total C and N by dry combustion using a Thermo-Finnigan EA Flash 1112. The K-State Research and Extension Soil Testing Lab, Manhattan, Kansas, determined total P using a salicylic-sulfuric acid digest (Bremner, 1965).

Root AMF colonization was determined by root scoring. Maize roots were cleared and stained using a slightly modified Phillips and Hayman method (1970). First, frozen roots placed in histosettes were thawed and rinsed with tap water. Next, the roots were cleared in a heated 2.5% potassium hydroxide (KOH) solution at 90˚C for 20 minutes using a water bath. Once the coloration of the solution turned a light brown, the histosettes were removed, rinsed, and placed in a boiling solution of 3% hydrogen peroxide for 10-30 minutes to bleach the root pigments. Bleaching was complete when roots were white to yellow in color, after which the histosettes were rinsed with deionized water and acidified for 10 minutes in a 1% hydrochloric acid solution to allow the trypan blue stain to bind to the fungal structures. The histosettes were then placed in 0.05% trypan blue stain and placed in an oven at 90˚C for 30-60 minutes. Next, the histosettes

were removed from the trypan blue stain and placed in a de-staining solution containing a mixture of 250 ml glycerol, 225 ml distilled water, and 25 ml of 1% HCl for 2-3 days.

The estimation of AMF colonization in maize roots, also known as root scoring, was done using a method by McGonigle et al. (1990). First, roots were washed in glycerol and cut into 1 cm segments. Next, 10 root segments were carefully placed on a microscope slide using forceps while ensuring the roots did not cross. Roots were then carefully flattened with a coverslip. Using 200 to 400x magnification, the microscope lens was passed over each segment five times, allowing 50 intercepts in total. With the use of a crosshair, the eyepiece was moved until the root segment was 90˚ perpendicular to the root. The segment was then examined at each root intersection using the following four categories: no AMF structures, arbuscules, vesicles, or mycorrhizal hyphae. As mycorrhizal root colonization is expressed as the percent of total root intersections that have AMF present, the following formula was used:

% colonization = [(vesicles + arbuscules + hyphae) / n˚ of intersections] * 100

Aboveground Biomass

Biomass was removed during each selected growth stage (V6, VT, and R1) by cutting the maize plant at the soil surface. Each plant was carefully placed in labeled paper bags and oven dried at 60°C until constant weight was achieved. Biomass was then ground in a Wiley mill followed by a ball mill until the biomass was a fine powder. The ground biomass was weighed and analyzed for total C and N by dry combustion using a Thermo-Finnigan EA Flash 1112. The K-State Research and Extension Soil Testing Lab, Manhattan, Kansas determined total P using a salicylic-sulfuric acid digest (Bremner, 1965).

Microbial Lipids

The soil microbial community structure was analyzed following a modified Bligh and Dyer (1959) method by White (1996). For each plot, 5 g of ground, lyophilized soil was weighed and incubated in a 2:1:0.8 chloroform-methanol-phosphate buffer mixture in order to extract the organic phase. Lipids were then fractionated using silicic acid chromatography into glycolipids, neutral lipids, and phospholipids (polar) by eluting them consecutively with chloroform (glycolipids), acetone (neutral lipids), and methanol (phospholipids) into separate test tubes. Only the phospholipids were kept and subsequently evaporated in a nitrogen evaporation bath. These lipids were then saponified using potassium hydroxide (KOH) and methylated to form fatty acid methyl esters (FAME). The FAMEs were separated using a Thermo Scientific Trace GC-ISQ mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) using a DB5-MS column (30m x 250 µm i.d. x 0.25 µm film thickness; Agilent Technologies, Santa Clara, California, USA) with helium as a carrier gas. Individual FAME peaks were identified by comparing retention times to peaks of a known bacterial acid methyl esters (BAME) mixture (Matreya 1114; Matreya LLC, Pleasant Gap, Pennsylvania, USA). FAME peaks that were not present in the BAMEs mixture were assigned by mass spectral interpretation. Concentrations were determined using the internal standard, methyl nonadecanoate, and were converted into nmol PLFA g^{-1} dry soil. Signature PLFAs were used as indicators for specific microbial groups: gram-positive bacteria (i15:0, a15:0, i16:0, i17:0, a17:0), gram-negative bacteria (2-OH 10:0, 2-OH 12:0, 3-OH 12:0, 2-OH 14:0, 3-OH 14:0, 16:1ω7c, cy17:0, cy19:0), arbuscular mycorrhizal fungi (AMF) (16:1ω5), and saprophytic fungi (18:2ω6,9c).

Statistical Analysis

Analysis of variance (ANOVA) was performed on both soil and biomass data using PROC GLIMMIX in SAS 9.4 (SAS Institute Inc., 2013). Results were considered statistically significant when $p < 0.05$. The treatment and sampling time were considered fixed effects whereas the block and the interactions were random effects. Means separation was conducted using Fisher's LSD at $\alpha = 0.05$.

Results

Plant Biomass

There was a significant interaction between cultivar and time for the shoot biomass (Table 3.2). Although there were no significant differences between the cultivars at growth stages V6 and VT, a significant difference occurred at R1 between the more modern cultivars and the older cultivars. The modern, genetically modified cultivars DKC63-55RIB and DKC64-69RIB had greater plant biomass at R1 than B73 and B73xMo17 (Fig. 3.1).

There was also a significant interaction between soil test P and time. Shoot dry biomass increased over time but was significantly greater in soil that had a greater P content than in soil with low P (Fig. 3.2). There was no significant difference observed at the V6 growth stage.

Shoot C behaved similarly under the different P treatments to that of the biomass, except, a significant difference was also observed at V6 in which cultivars grown in high P soil had greater shoot C content than those grown in low P soil (Fig. 3.3, C.). Treatment also had a significant impact on shoot C. The cultivar B73 had significantly less shoot C compared to the other three cultivars, while the other cultivars were not significantly different in shoot C content (Fig. 3.4). Shoot N was similar to shoot biomass in that no differences were observed at V6. Differences in shoot N become enhanced at VT and R1 with B73 having the least shoot N

content and DKC63-55-RIB and DKC64-69RIB having the greatest shoot N content (Fig. 3.5) There was also a significant interaction between growth stage and soil P content for shoot N. At V6, no significant differences were observed. At VT and R1, the high P soil produced greater shoot N compared to the low P soil (Fig. 3.3, A.).

Root Stocks and Arbuscular Mycorrhizal Fungi

A significant interaction ($p < 0.05$) was observed between treatment and time for root biomass (Table 3.2). Although no significant differences were observed between the first two growth stages (V6 and VT), there was a significant increase in root biomass at R1 as well as differentiation between the cultivars. Greatest root biomass at R1 was found in DKC63-55RIB, followed by statistically lower but similar values for B73xMo17 and DKC64-69RIB. The lowest root biomass at the R1 growth stage was observed in B73 (Fig. 3.6).

No significant differences were found for root C content. However, a significant interaction was observed for root N between treatment and time (Table 3.2, Fig. 3.7). Similar to root biomass, no significance was observed for root N between cultivars during the first two growth stages. However, at R1, root N significantly increased compared to the previous growth stages and was significantly greater in DKC63-55RIB. For root P, a significant interaction was observed between soil P content and time. While no significant differences were seen for root P between high P and low P soil during V6, differentiation between the two becomes clear at VT and R1, with roots grown in high P soils having significantly greater P content compared to roots grown in low P soils (Fig. 3.8). Maize takes up the majority of P (about 60%) during the reproductive period (Karlen et al., 1988), which could explain greater differentiation at that time.

There was no significance observed for percent AMF root colonization concerning time, cultivar, and P content (Table 3.4). The differences in observed root colonization of the maize

cultivars were not significant. In addition, colonization was minimal and had great variation between replications. There was also no increasing or decreasing trend observed in AMF colonization between roots at the different growth stages and the variability between the two growth stages was large.

Microbial Lipids

There was a significant effect ($p < 0.05$) of treatment, time, and soil test P on the total PLFA, gram-positive bacteria, AMF, and fungi concentrations (Table 3.3) Among treatments, the control had significantly less total PLFA, gram-positive bacteria, gram-negative bacteria, and AMF compared to the cultivars, which were not significantly different from one another (Fig. 3.9, A.; B.; C.; D.). Total PLFA, gram-positive bacteria, and AMF concentrations also significantly increased over time with V6 having the least while VT and R1 had the greatest total concentrations. The growth stages VT and R1 were also not significantly different from one another in total PLFA, gram-positive bacteria, and AMF concentrations (Fig. 3.10, A.; B.; D.; E.). Soil P also had an impact in that high soil P content had significantly more total PLFA, gram-positive bacteria, and AMF concentrations compared to soils with low P (Fig. 3.11, A.; B.; C.). The control treatment resulted in significantly less fungal concentrations, however, significant differences were also found among the cultivars with B73xMo17 having greater fungal concentrations compared to that of B73. The cultivars DKC63-55RIB and DKC64-69RIB were not significantly different from one another or the other cultivars. Fungal concentrations also differed from the others in that there was a significant increase in concentration at VT, which then decreased at R1. The V6 and R1 growth stage were not significantly different from one another in fungal concentrations (Fig. 3.10, F.). As with the other PLFA biomarkers, the increased soil P content resulted in significantly greater fungal concentrations (Fig. 3.11, D.).

Actinomycetes were not significantly impacted by treatment or soil P content. Time did have an impact on actinomycete concentration with VT and R1 having significantly greater actinomycete concentrations compared to that of V6 (Fig. 3.12). There was no significant difference observed between VT and R1.

Gram-negative bacteria were significantly impacted by treatment and time, however, there was no significant impact by soil P level. Between treatments, the control had significantly less gram-negative bacteria compared to the cultivar treatments (Fig. 3.10, C.). There was no significant difference found in gram-negative bacteria concentrations between the cultivars. (Fig. 3.9, C.)

The fungi to bacteria ratio (F:B) showed a significant interaction between treatment, time, and soil P content (Table 3.3). Control treatment had the least F:B during all three sampling times. At the V6 growth stage, it appears that B73xMo17 (in low P), DKC63-55RIB (in low P), B73 (in low P), and B73 (in high P) behaved alike and had lower F:B ratio similar to that of the control. Cultivars DKC64-69RIB (in high P), DKC63-55RIB (in high P), and DKC64-69RIB (in low P) also behaved similar to one another and had significantly greater F:B ratios to the aforementioned cultivars. The cultivar B73xMo17 (in high P) had significantly greater F:B compared to all other cultivars during V6. By the VT growth stage, all cultivars have similar F:B ratios that are significantly greater than that of the controls. This remains into the R1 growth stage although the F:B for both controls decreases (Fig. 3.12).

Discussion

Correlating with previous research (Araus et al., 2010; Dahal et al., 2012), heterosis led to shoot biomass differences during the VT stage where the inbred line B73 had significantly less biomass compared to all other cultivars. During the R1 growth stage, B73xMo17 and B73 had

the lowest dry biomass with the more modern cultivars DKC63-55RIB and DKC64-69RIB having the greatest. As these are genetically modified drought-resistant cultivars, there was speculation that these cultivars would have more vigor, however, these differences could also be due to the high natural variability among maize varieties (Batista and Oliveira, 2010; Balsamo et al., 2011). In addition, drought-resistant transgenic cultivars can overexpress the *Asr1* gene, which can result in increased leaf biomass (Virlouvet et al., 2011).

Phosphorus level had an impact on shoot biomass, with greater biomass accumulation in soil that had sufficient P and lower biomass in P deficient soil. This was expected, as P is an important nutrient for both plant growth and reproductive processes (Havlin, 2013). Interestingly, during the V6 stage, the biomass in the high P soil was slightly higher, however, not significantly different from the biomass in the low P soil. This was unexpected because the effects of P deficiency usually show symptoms such as reduced growth at early growth stages. Moreover, as the shoots were reduced in size, the plant's energy gets relocated to the roots resulting in a greater root to shoot ratio (Hermans et al., 2006). Not only does a low root P environment tend to affect the root to shoot ratio, it also affects root morphological changes such as total root length, a larger number of lateral roots (Liu et al., 2004), and enhances AMF symbiosis (Schweiger et al., 2007). Interestingly, this study does not correlate with these expectations, because soil test P had no significant ($p = 0.0932$) impact on root dry biomass. There were differences between the cultivars at R1 with DKC63-55RIB having the greatest biomass and B73 having the smallest. The other two cultivars (B73xMo17 and DKC64-69RIB) had greater root biomass than B73 and less than DKC63-55RIB but they were not significantly different from one another. B73 was expected to have less vigor compared to the other cultivars; however, it is interesting that DKC63-55RIB had the greatest root biomass. This could be related

to having the greatest drought tolerance of all four cultivars potentially due to the ability to grow a larger root biomass.

Root nutrients indicated that DKC63-55RIB was better at acquiring nutrients from the soil. While there was no statistical significance for root C content, differences among cultivars did lean toward significance ($p = 0.0652$) with DKC63-55RIB having the greatest C content, followed by B73xMo17, DKC64-69RIB and B73 respectively. This corresponds with the root dry biomass for these cultivars and research indicates that root morphology can play an important role in nutrient acquisition (Liu et al., 2009; Deng et al., 2014). Nitrogen content in roots was significantly affected by the interaction between cultivars and time. However, there were no significant differences until the cultivars reached R1. During this growth stage, the roots of DKC63-55RIB obtain significantly greater N compared to the other cultivars. Why the differences only appeared at the R1 growth stage is unclear, although previous studies by Sayre (1948) (and Hanway (1962) have reported changes in N uptake at different growth stages. More specifically, they saw greatest N accumulation before the VT stage in older cultivars. Higher-yielding cultivars, on the other hand, were found to accumulate N rapidly between V12 and V18 and also near the end of the grain-filling period (Karlen et al., 1988). The roots of the other cultivars remained statistically similar in N content. Root P content was significantly affected by the interaction between soil P and time. While not significant during V6, differentiation of root P content between the two soil P levels became significant at VT and R1. A reason that root P content was not significant between at V6 between the two soils may be the fact that most of the P was translocated towards the shoot as shoot P content was significant at V6 between low and high P soil.

Arbuscular mycorrhizal fungi colonization also affects root P content as the AM hyphae can explore a greater soil area and obtain nutrients such as P in return for plant C. No AMF colonization was observed in the roots of the maize for both investigated growth stages V6 and VT (R1 roots were not scored). As the drying of the field soil should not have affected the AMF since they can withstand several months in dry soil (Braunberger et al., 1996), the lack of colonization could have been due to mixing the soil with washed sand. Inoculation with AMF would likely have alleviated this problem.

Microbial biomass analyses indicated an influence of plant roots on the microbial abundances in the soil. This was evident by the lower total microbial concentrations in the control soil containing no plants and the increasing total microbial concentrations with time as roots obtain greater biomass. Interestingly, there was a slightly greater total microbial abundance at VT compared to R1. This slight difference was, however, not statistically significant. Microbial biomass concentrations were also found to be significantly greater in high P soils. Previous research by Liu et al. (2013) and Tang et al. (2016) supports these findings as they found that addition of P stimulates the soil microbial biomass. Gram-positive bacteria are the only microbial group that follows the same trend as the total microbial biomass concentration. Gram-negative bacteria, on the other hand, were not significantly affected by the differences in soil P concentration.

The exudates produced by maize roots in P deficient soil contain strigolactones. This compound is found to stimulate AMF colonization (Badri and Vivanco, 2009). Maize roots obtained from the greenhouse for both low and high P soil did not show any AMF colonization, however, PLFA analyses did show greater AMF concentrations in low P soil. Also, it is clear that all roots (whether in low or high P soil) produced exudates that stimulated AMF as the

control had significantly less AMF. Despite the fact that some studies have discovered that a plant's genotype influences the response it has to AMF (Chu et al., 2013), this study was unable to provide evidence to support that.

Analyses of total soil fungi concentration showed a significant effect by variety, time, and soil P level. This is not surprising as there are several factors that can influence the distribution and function of soil fungi such as soil type, soil moisture, plant age, plant species, and plant genotype (Hannula et al., 2012). In addition, the soil's available nutrients are known to impact soil microbes (Beauregard et al., 2010) as well as the growth of plants, which in turn influences their exudation processes. Unlike AMF, total soil fungi had greater concentrations in the high P soil than in the low P soil. It is likely that the low P soil constrained fungal biomass growth as it did the gram-positive bacterial biomass. Soil fungal concentrations were also significantly different among the cultivar treatments and between the cultivars and the control.

Differences in fungal concentration between bulk and rhizosphere soil have been established in previous research in which the rhizosphere tends to enhance fungal biomass concentrations (Gomes et al., 2001). This correlates with the results of this study, as the control was significantly lower in fungal biomass compared to any of the cultivars. A number of recent studies have also established that a plant's genotype affects the soil microbiome. For maize, the greatest microbial community differences were found when the genotypes were distinctly different from one another such as with an inbred versus a hybrid (Picard and Bosco, 2006). This study's results for soil fungal concentrations confirm that, as there were significant differences between the inbred line (B73) and the hybrid (B73xMo17). There were, however, no significant differences in soil fungal biomass between the two modern lines (DKC63-55RIB and DKC64-69RIB) and the other cultivars. These maize lines may have more subtle genetic variations

between themselves and the other two cultivars, resulting in a lack of differential impacts on the soil fungal biomass. Some studies into the impact of maize genotype on soil microbial communities have resulted in similar findings where subtle to no significant differences were found (Dohrmann et al., 2013; Bakker et al., 2015). As for time, the VT growth stage had greater fungal biomass surrounding the maize roots compared to V6 and R1. This is different from all the other microbial groups that were analyzed in that the fungal biomass decreased to a concentration similar to that at V6. Changes in fungal concentrations between different growth stages are likely due to the specific needs of the maize plants at that time. That is because at different plant developmental stages, the root exudation process changes to select for particular microbes (De-la-Peña et al., 2010).

Conclusions

Plant roots are known to influence the microbial communities that surround them through a process called exudation. This study reflects that as a rhizosphere effect was found at all three growth stages for each cultivar. Although there is no direct evidence that the exudates were different among the cultivars, the difference in soil fungal concentrations between them would suggest a cultivar effect. Contrary to the hypothesis, the cultivar effect was limited to soil fungal concentrations and was not impacted by soil P concentration. In addition, growth stage also had an influence on microbial biomass, although this was likely due to the root biomass and not the influence of the plant. There was, however, a difference in soil fungal concentrations between the VT and R1 growth stage.

References

Araus, J.L., C. Sánchez, and L. Cabrera-Bosquet. 2010. Is heterosis in maize mediated through better water use? New Phytol. 187(2): 392–406. doi: 10.1111/j.1469-8137.2010.03276.x.

Badri, D.V., and J.M. Vivanco. 2009. Regulation and function of root exudates. Plant Cell Environ. 32(6): 666–681. doi: 10.1111/j.1365-3040.2009.01926.x.

Bakker, M., J. Chaparro, D. Manter, and J. Vivanco. 2015. Impacts of bulk soil microbial community structure on rhizosphere microbiomes of Zea mays. Plant Soil 392(1): 115–126. doi: 10.1007/s11104-015-2446-0.

Balsamo, G.M., G.C. Cangahuala-Inocente, J.B. Bertoldo, H. Terenzi, and A.C.M. Arisi. 2011. Proteomic analysis of four Brazilian MON810 maize varieties and their four non-genetically-modified isogenic varieties. J. Agric. Food Chem. 59(21): 11553–9. doi: 10.1021/jf202635r.

Batista, R., and M. Oliveira. 2010. Plant natural variability may affect safety assessment data. Regul. Toxicol. Pharmacol. 58(3): S8–S12. doi: 10.1016/j.yrtph.2010.08.019.

Beauregard, M., C. Hamel, M. Atul-Nayyar, and M. St-Arnaud. 2010. Long-Term phosphorus fertilization impacts soil fungal and bacterial diversity but not AM fungal community in alfalfa. Microb. Ecol. 59(2): 379–389. doi: 10.1007/s00248-009-9583-z.

Bligh, E.G., and W.J. Dyer. 1959. A rapid method of total lipid extraction. Can. J. Biochem. Physiol. 37: 911–917.

Braunberger, P.G., L.K. Abbott, and A.D. Robson. 1996. Infectivity of arbuscular mycorrhizal fungi after wetting and drying. New Phytol. 134(4): 673–684. doi: 10.1111/j.1469-8137.1996.tb04933.x.

Bremner, J.M. 1965. Total nitrogen. Methods Soil Anal. Part 2 Chem. Microbiol. Prop.: 1149–1178. doi: 10.2134/agronmonogr9.2.c32.

Chu, Q., X. Wang, Y. Yang, F. Chen, F. Zhang, and G. Feng. 2013. Mycorrhizal responsiveness of maize (Zea mays L.) genotypes as related to releasing date and available P content in soil. Mycorrhiza 23(6): 497–505. doi: 10.1007/s00572-013-0492-0.

Dahal, D., B.P. Mooney, and K.J. Newton. 2012. Specific changes in total and mitochondrial proteomes are associated with higher levels of heterosis in maize hybrids. Plant J. Cell Mol. Biol. 72(1): 70–83. doi: 10.1111/j.1365-313X.2012.05056.x.

De-la-Peña, C., D.V. Badri, Z. Lei, B.S. Watson, M.M. Brandão, M.C. Silva-Filho, L.W. Sumner, and J.M. Vivanco. 2010. Root secretion of defense-related proteins is development-dependent and correlated with flowering time. J. Biol. Chem. 285(40): 30654–30665. doi: 10.1074/jbc.M110.119040.

Deng, Y., K. Chen, W. Teng, A. Zhan, Y. Tong, G. Feng, Z. Cui, F. Zhang, and X. Chen. 2014. Is the inherent potential of maize roots efficient for soil phosphorus acquisition? PLOS ONE 9(3): e90287. doi: 10.1371/journal.pone.0090287.

Dohrmann, A.B., M. Küting, S. Jünemann, S. Jaenicke, A. Schlüter, and C.C. Tebbe. 2013. Importance of rare taxa for bacterial diversity in the rhizosphere of Bt- and conventional maize varieties. ISME J. 7(1): 37–49. doi: 10.1038/ismej.2012.77.

Gomes, N.C.M., H. Heuer, J. Schönfeld, R. Costa, L. Mendonça-Hagler, and K. Smalla. 2001. Bacterial diversity of the rhizosphere of maize (Zea mays) grown in tropical soil studied by temperature gradient gel electrophoresis. Plant Soil 232(1–2): 167–180. doi: 10.1023/A:1010350406708.

Hannula, S.E., W. de Boer, and J. van Veen. 2012. A 3-year study reveals that plant growth stage, season and field site affect soil fungal communities while cultivar and GM-trait have minor effects. PloS One 7(4): e33819. doi: 10.1371/journal.pone.0033819.

Hanway, J.J. 1962. Corn growth and composition in relation to soil fertility: II. Uptake of N, P, and K and their distribution in different plant parts during the growing season. Agron. J. 54(3): 217. doi: 10.2134/agronj1962.00021962005400030011x.

Havlin, J.L. 2013. Soil Fertility and Fertilizers: an Introduction to Nutrient Management. Pearson Prentice Hall, Upper Saddle River, N.J.

Hermans, C., J.P. Hammond, P.J. White, and N. Verbruggen. 2006. How do plants respond to nutrient shortage by biomass allocation? Trends Plant Sci. 11(12): 610–617. doi: 10.1016/j.tplants.2006.10.007.

Jeong, H.-S., J. Lee, and A.-H. Eom. 2006. Effects of interspecific interactions of arbuscular mycorrhizal fungi on growth of soybean and corn. Mycobiology 34(1): 34–37.

Karlen, D.L., R.L. Flannery, and E.J. Sadler. 1988. Aerial accumulation and partitioning of nutrients by corn. Agron. J. 80(2): 232. doi: 10.2134/agronj1988.00021962008000020018x.

Kilmer, V.J., and L.T. Alexander. 1949. Methods of making mechanical analyses of soils. Soil Sci. 68(1): 15–24.

Liu, J., F. Chen, C. Olokhnuud, A.D.M. Glass, Y. Tong, F. Zhang, and G. Mi. 2009. Root size and nitrogen-uptake activity in two maize (Zea mays) inbred lines differing in nitrogen-use efficiency. J. Plant Nutr. Soil Sci. 172(2): 230–236. doi: 10.1002/jpln.200800028.

Liu, Y., G. Mi, F. Chen, J. Zhang, and F. Zhang. 2004. Rhizosphere effect and root growth of two maize (Zea mays L.) genotypes with contrasting P efficiency at low P availability. Plant Sci. 167(2): 217–223. doi: 10.1016/j.plantsci.2004.02.026.

Liu, L., T. Zhang, F.S. Gilliam, P. Gundersen, W. Zhang, H. Chen, and J. Mo. 2013. Interactive effects of nitrogen and phosphorus on soil microbial communities in a tropical forest. PLOS ONE 8(4): e61188. doi: 10.1371/journal.pone.0061188.

Mazzola, M., D.L. Funnell, and J.M. Raaijmakers. 2004. Wheat cultivar-specific selection of 2,4-Diacetylphloroglucinol-producing fluorescent Pseudomonas species from resident soil populations. Microb. Ecol. 48(3): 338–348. doi: 10.1007/s00248-003-1067-y.

Mcgonigle, T.P., M.H. Miller, D.G. Evans, G.L. Fairchild, and J.A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol. 115(3): 495–501. doi: 10.1111/j.1469-8137.1990.tb00476.x.

Mehlich, A. 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Commun. Soil Sci. Plant Anal. 15(12): 1409–1416. doi: 10.1080/00103628409367568.

Özbek, F., A. Leip, and M. Velde. 2016. Phosphorous stock changes in agricultural soils: a case study in Turkey. Nutr. Cycl. Agroecosystems 105(1): 51–59. doi: 10.1007/s10705-016-9773-2.

Peiffer, J.A., A. Spor, O. Koren, Z. Jin, S.G. Tringe, J.L. Dangl, E.S. Buckler, and R.E. Ley. 2013. Diversity and heritability of the maize rhizosphere microbiome under field conditions. Proc. Natl. Acad. Sci. U. S. 110(16): 6548–6553. doi: 10.1073/pnas.1302837110.

Phillips, J.M., and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55(1): 158,IN16–161,IN18. doi: 10.1016/S0007-1536(70)80110-3.

Picard, C., and M. Bosco. 2006. Heterozygosis drives maize hybrids to select elite 2,4-diacethylphloroglucinol-producing Pseudomonas strains among resident soil populations. FEMS Microbiol. Ecol. 58(2): 193–204. doi: 10.1111/j.1574-6941.2006.00151.x.

Sayre, J.D. 1948. Mineral accumulation in corn. Plant Physiol. 23(3): 267–81. doi: 10.1104/pp.23.3.267.

Schollenberger, C.J., and R.H. Simon. 1945. Determination of exchange capacity and exchangeable bases in soil-ammonium acetate method. Soil Sci. 59(1): 13–24.

Schweiger, P., A. Robson, N. Barrow, and L. Abbott. 2007. Arbuscular mycorrhizal fungi from three genera induce two-phase plant growth responses on a high P-fixing soil. Plant Soil 292(1): 181–192. doi: 10.1007/s11104-007-9214-8.

Shahzad, T., C. Chenu, P. Genet, S. Barot, N. Perveen, C. Mougin, and S. Fontaine. 2015. Contribution of exudates, arbuscular mycorrhizal fungi and litter depositions to the rhizosphere priming effect induced by grassland species. Soil Biol. Biochem. 80: 146–155.

Smith, S.E., I. Jakobsen, M. Grønlund, and F.A. Smith. 2011. Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiol. 156(3): 1050–1057.

Tang, X., S.A. Placella, F. Dayde, L. Bernard, A. Robin, E.-P. Journet, E. Justes, and P. Hinsinger. 2016. Phosphorus availability and microbial community in the rhizosphere of intercropped cereal and legume along a P-fertilizer gradient.(Regular Article)(Report). Plant Soil 407(1–2): 119. doi: 10.1007/s11104-016-2949-3.

Tran, T.S., and W. van Lierop. 1982. Lime requirement determination for attaining pH 5. 5 and 6. 0 of coarse-textured soils using buffer-pH methods. Soil Sci. Soc. Am. J. 46.

Virlouvet, L., M.-P. Jacquemot, D. Gerentes, H. Corti, S. Bouton, F. Gilard, B. Valot, J. Trouverie, G. Tcherkez, M. Falque, C. Damerval, P. Rogowsky, P. Perez, G. Noctor, M. Zivy, and S. Coursol. 2011. The ZmASR1 protein influences branched-chain amino acid biosynthesis and maintains kernel yield in maize under water-limited conditions. Plant Physiol. 157(2): 917–36. doi: 10.1104/pp.111.176818.

Walker, T., E. Grotewold, and J. Vivanco. 2003. Root exudation and rhizosphere biology. Plant Physiol. 132(1): 44–51. doi: 10.1104/pp.102.019661.

White, D.C. 1996. Signature lipid biomarker analysis for the quantitative analysis of environmental microbial ecology. Natl. Meet. - Am. Chem. Soc. Div. Environ. Chem. 36(2): 139–141.

Tables

Table 3.1. Soil properties for Kennebec silt loam soil mixed with washed sand (purchased from Hartford Sand and Gravel, Manhattan, Kansas) in a 2:1 sand/soil ratio. The Kennebec silt loam soil was obtained from the Agronomy North Farm, Kansas State University.

Soil-P	pH	NO_3^--N	NH_4^--N	M3-P	M3-K	Sand	Silt	Clay
		----------μg g^{-1} soil----------				----------mg g^{-1}----------		
High P	7.25	7.3	9.9	183	101	82	11	7
Low P	7.70	11.3	9.2	9	91			

M3-P = Mehlich-3 phosphorus. M3-K = Mehlich-3 potassium.

Table 3.2. The ANOVA for root biomass, root C, root N, root P, shoot biomass, shoot C, shoot N, and shoot P for four maize cultivars (B73, B73xMo17, DKC63-55RIB, and DKC64-69RIB) evaluated over three growth stages (V6, VT, R1) and two soil-P levels (high and low). All maize cultivars were grown under controlled greenhouse conditions.

Effect	Root Biomass	Root C	Root N	Root P	Shoot Biomass	Shoot C	Shoot N	Shoot P
Cultivar	0.0081	0.0652	0.0158	0.2413	< .0001	0.0008	< .0001	0.0793
Soil-P	0.0932	0.1137	0.2116	< .0001	< .0001	< .0001	< .0001	< .0001
Cultivar*Soil-P	0.3929	0.6258	0.6283	0.5655	0.1929	0.2982	0.1581	0.6030
Time	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001
Cultivar*Time	< .0001	0.1145	0.0173	0.7093	0.0171	0.5430	0.0073	0.9433
Soil-P*Time	0.5298	0.5107	0.7967	< .0001	< .0001	0.0009	< .0001	< .0001
Cultivar*Soil-P*Time	0.5750	0.6123	0.8045	0.8332	0.5969	0.9539	0.6095	0.7317

Table 3.3. The ANOVA for the fungi to bacteria ratio, total PLFA, total gram-positive bacteria, total gram-negative bacteria, total actinomycetes, total arbuscular mycorrhizal fungi (AMF), and total fungi from soil collected from four maize cultivars (B73, B73/Mo17, DKC63-55RIB, and DKC64-69RIB) and a control (unplanted soil), two different P-levels (high and low P), and evaluated over three growth stages (V6, VT, and R1).

Effect	Fungi/ Bacteria	Total PLFA	Total $Gram^+$	Total $Gram^-$	Total Actinomycetes	Total AMF	Total Fungi
Treatment	< .0001	0.0071	0.0112	0.0046	0.1505	0.0033	< .0001
Time	0.4517	< .0001	0.0004	0.0015	0.0086	0.0139	0.0251
Treatment*Time	0.4589	0.1660	0.2873	0.2291	0.6365	0.7930	0.3848
P-Level	0.6208	0.0273	0.0241	0.0864	0.4983	0.0022	< .0001
Treatment*P-Level	0.1672	0.3706	0.5311	0.1032	0.4910	0.4035	0.3554
Time*P-Level	0.0087	0.4545	0.5184	0.5770	0.5902	0.3109	0.2452
Treatment*Time* P-Level	0.0472	0.4608	0.5132	0.3786	0.6505	0.7652	0.3118

Table 3.4. The ANOVA for percent root AMF colonization for all maize cultivars (B73, B73xMo17, DKC63-55RIB, and DKC64-69RIB) evaluated over two growth stages (V6 and VT) and between two soil P-levels (high and low). All maize cultivars were grown under greenhouse conditions.

Effect	% Root AMF colonization
Cultivar	0.8510
Soil P	0.2313
Cultivar*Soil P	0.2817
Time	0.8345
Cultivar*Time	0.5725
Soil P*Time	0.1388
Cultivar*Soil P*Time	0.3858

Figures

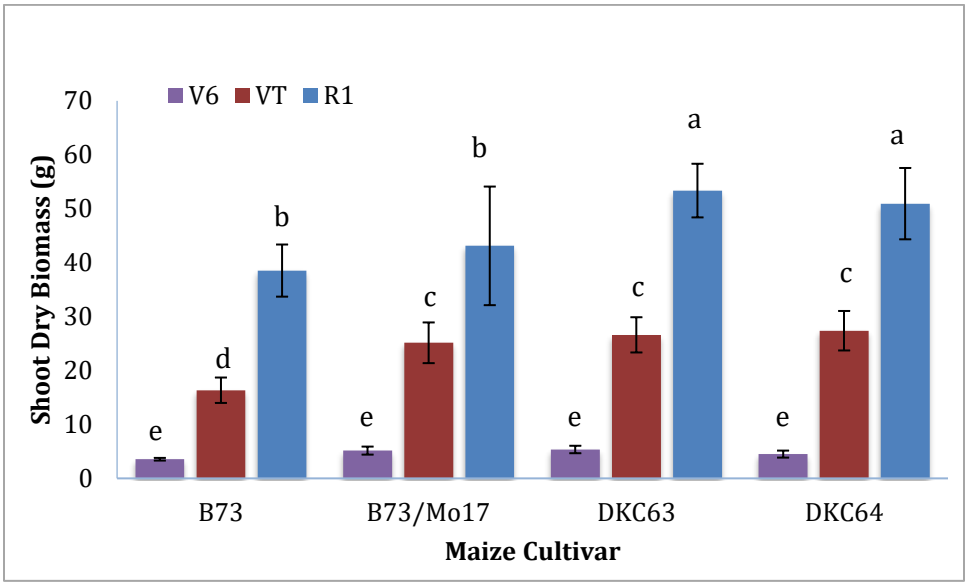

Figure 3.1 Shoot dry biomass between maize cultivars (B73, B73/Mo17, DKC63-55RIB, and DKC64-69RIB) over three growth stages (V6, VT, and R1). Maize cultivars were grown under greenhouse conditions. Means with different letters are significantly ($p<0.05$) different from each other.

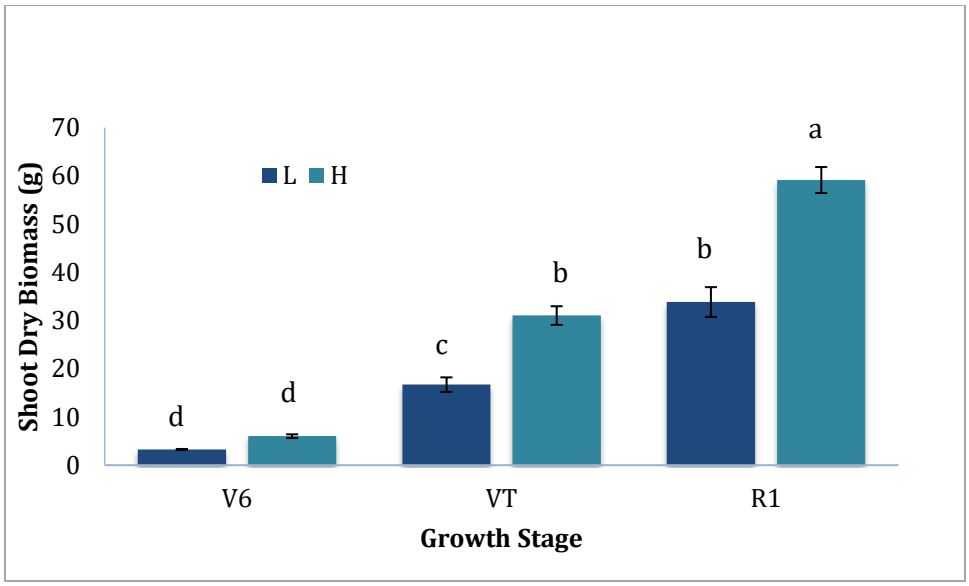

Figure 3.2. Shoot dry biomass for all maize cultivars (B73, B73/Mo17, DKC63-55RIB, and DKC64-69RIB) between two P treatments (L = low P; H = high P) over three growth stages (V6, VT, and R1). The maize cultivars were grown under greenhouse conditions. Means with different letters denote significant differences ($p<0.05$) between them.

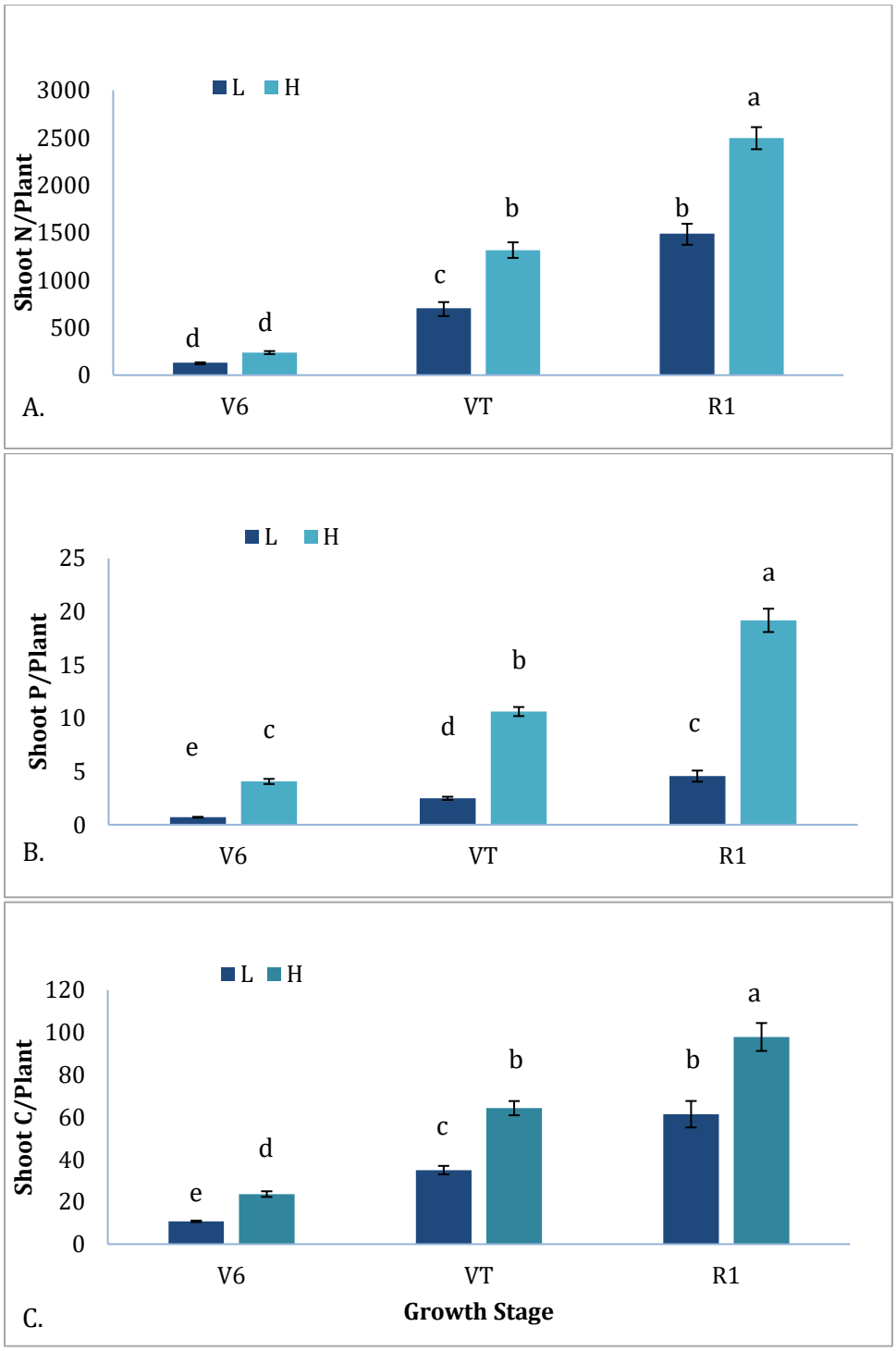

Figure 3.3 Shoot nitrogen (A.), phosphorus (B.), and carbon (C.) for all maize cultivars (B73, B73/Mo17, DKC63-55RIB, and DKC64-69RIB) between two P treatments (L = low P; H = high P) over three growth stages (V6, VT, and R1). Maize cultivars were grown under greenhouse conditions. Any means with different letters indicate statistically significant differences ($p<0.05$).

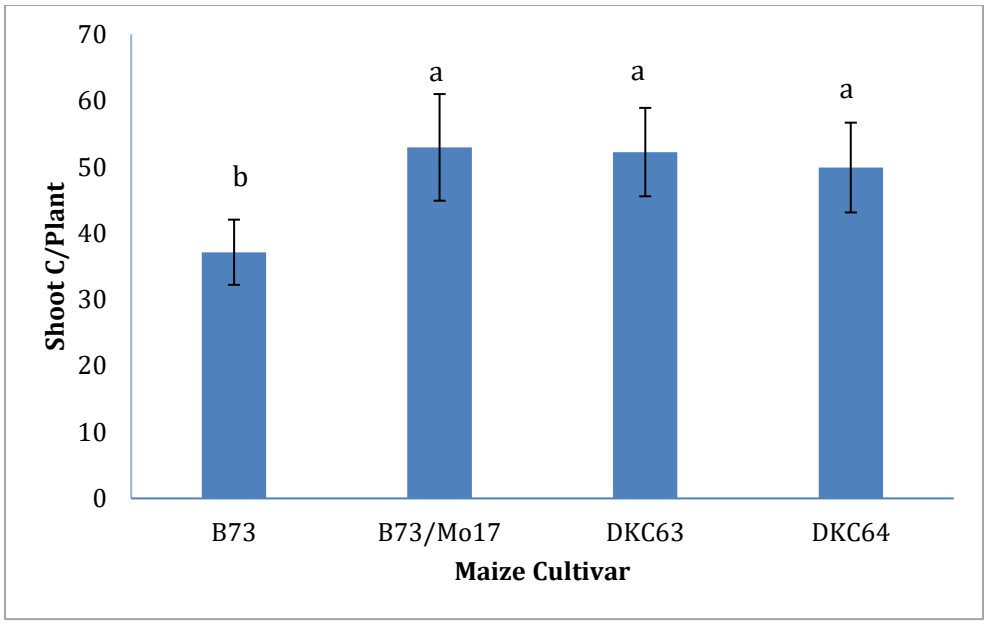

Figure 3.4. Shoot carbon between four maize cultivars (B73, B73/Mo17, DKC63-55RIB, and DKC64-69RIB) grown under greenhouse conditions. Means with different letters indicate significant ($p<0.05$) differences between them.

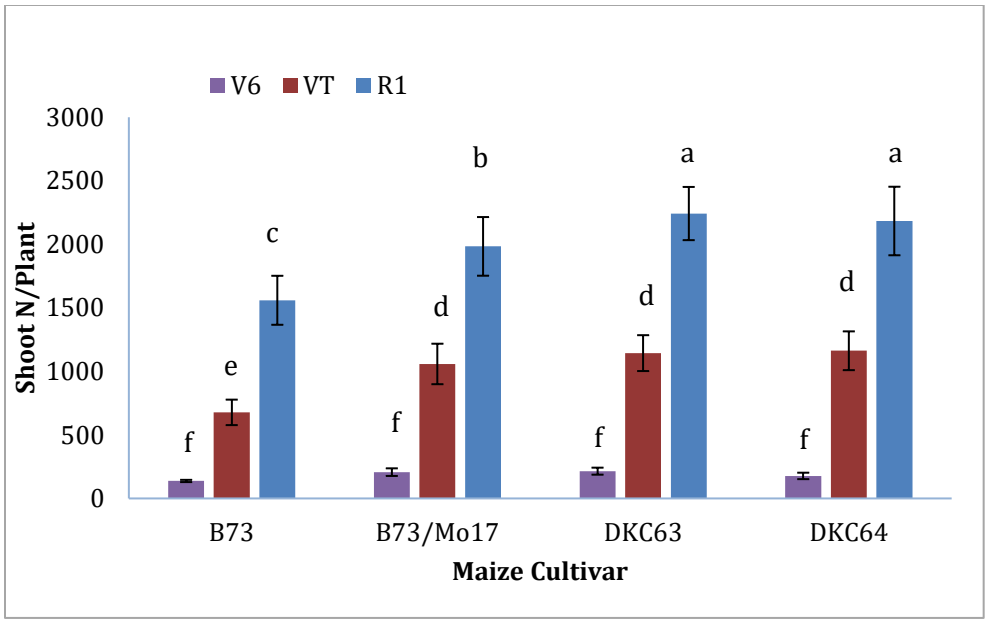

Figure 3.5. Shoot nitrogen between four maize cultivars (B73, B73/Mo17, DKC63-55RIB, and DKC64-69RIB) over three growth stages (V6, VT, and R1). The maize cultivars were grown under greenhouse conditions. Means with different letters indicate a significant ($p<0.05$)

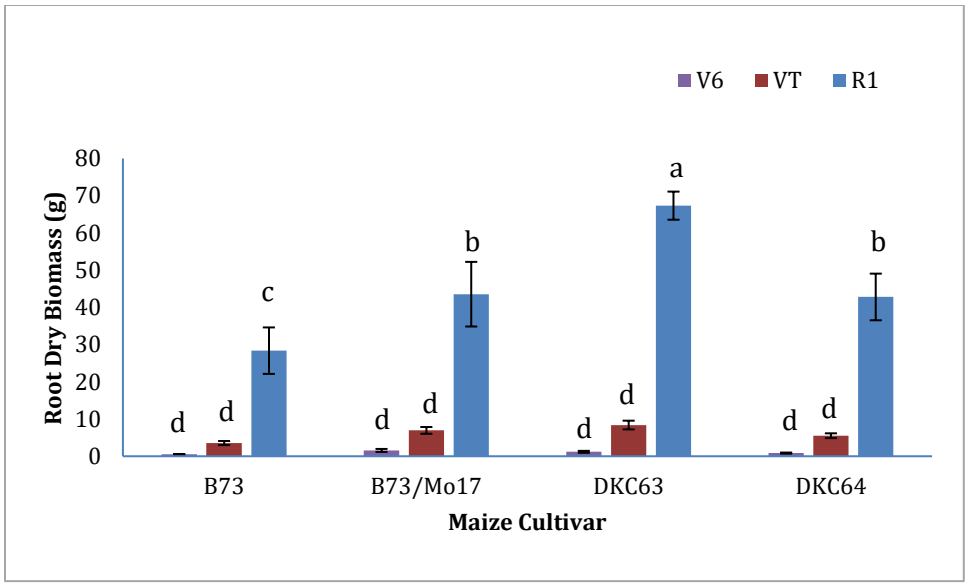

Figure 3.6 Root biomass differences between maize cultivars (B73, B73/Mo17, DKC63-55RIB, and DKC64-69RIB) over three growth stages (V6, VT, and R1). Maize cultivars were grown under greenhouse conditions. Means with the different letters indicate significant differences ($p<0.05$).

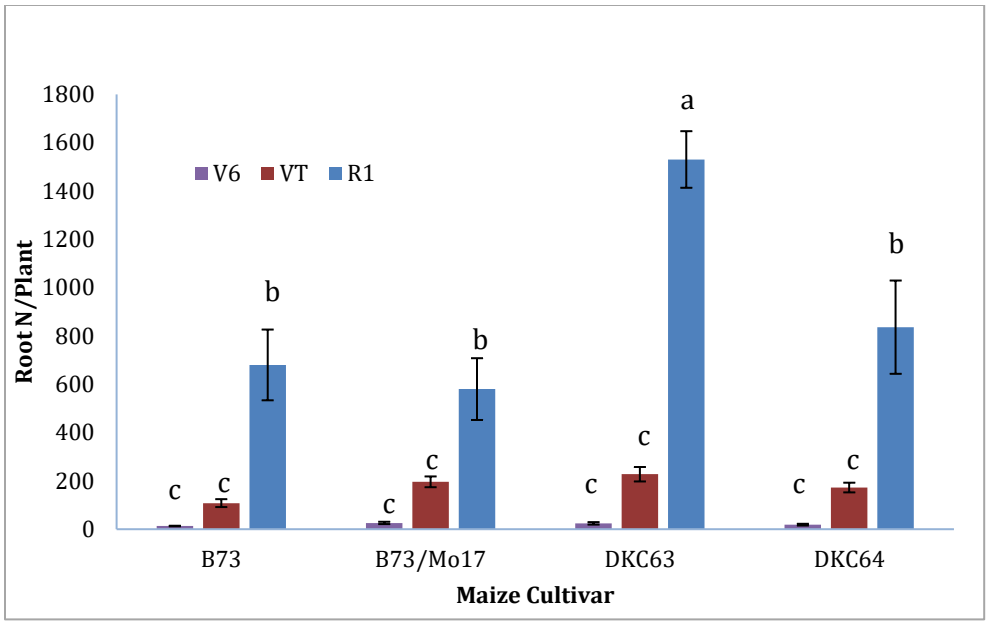

Figure 3.7. Root nitrogen between maize cultivars (B73, B73/Mo17, DKC63-55RIB, and DKC64-69RIB) over three growth stages (V6, VT, and R1). Maize cultivars were grown under greenhouse conditions. Means with different letters indicate statistically significant differences ($p<0.05$).

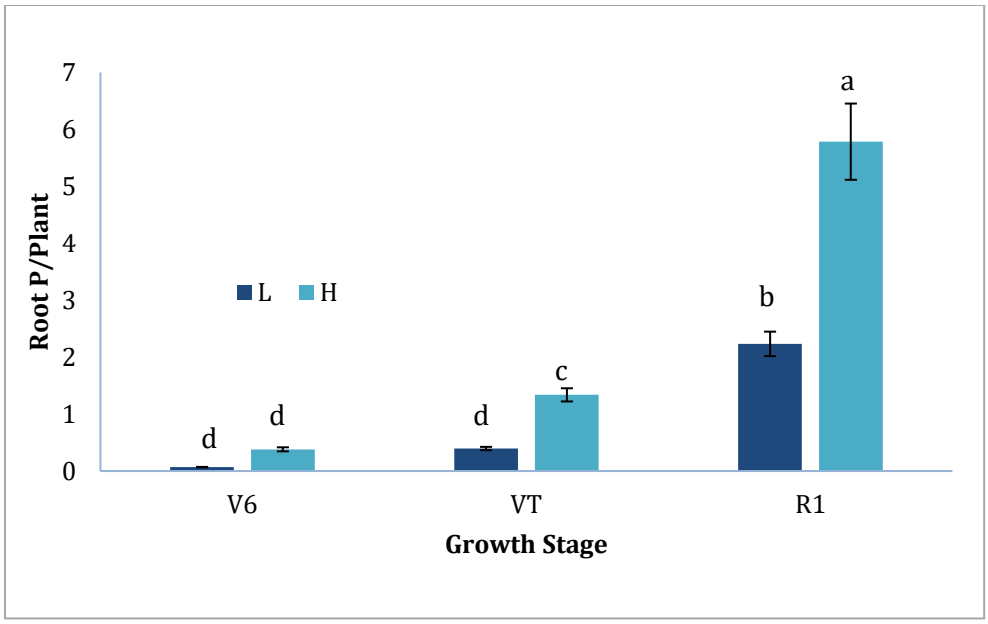

Figure 3.8. Root phosphorus for all maize cultivars (B73, B73/Mo17, DKC63-55RIB, and DKC64-69RIB) between two P treatments (L = low P; H = high P) over three growth stages (V6, VT, and R1). Maize cultivars were grown under greenhouse conditions. Means with different letters indicate statistically significant differences ($p<0.05$).

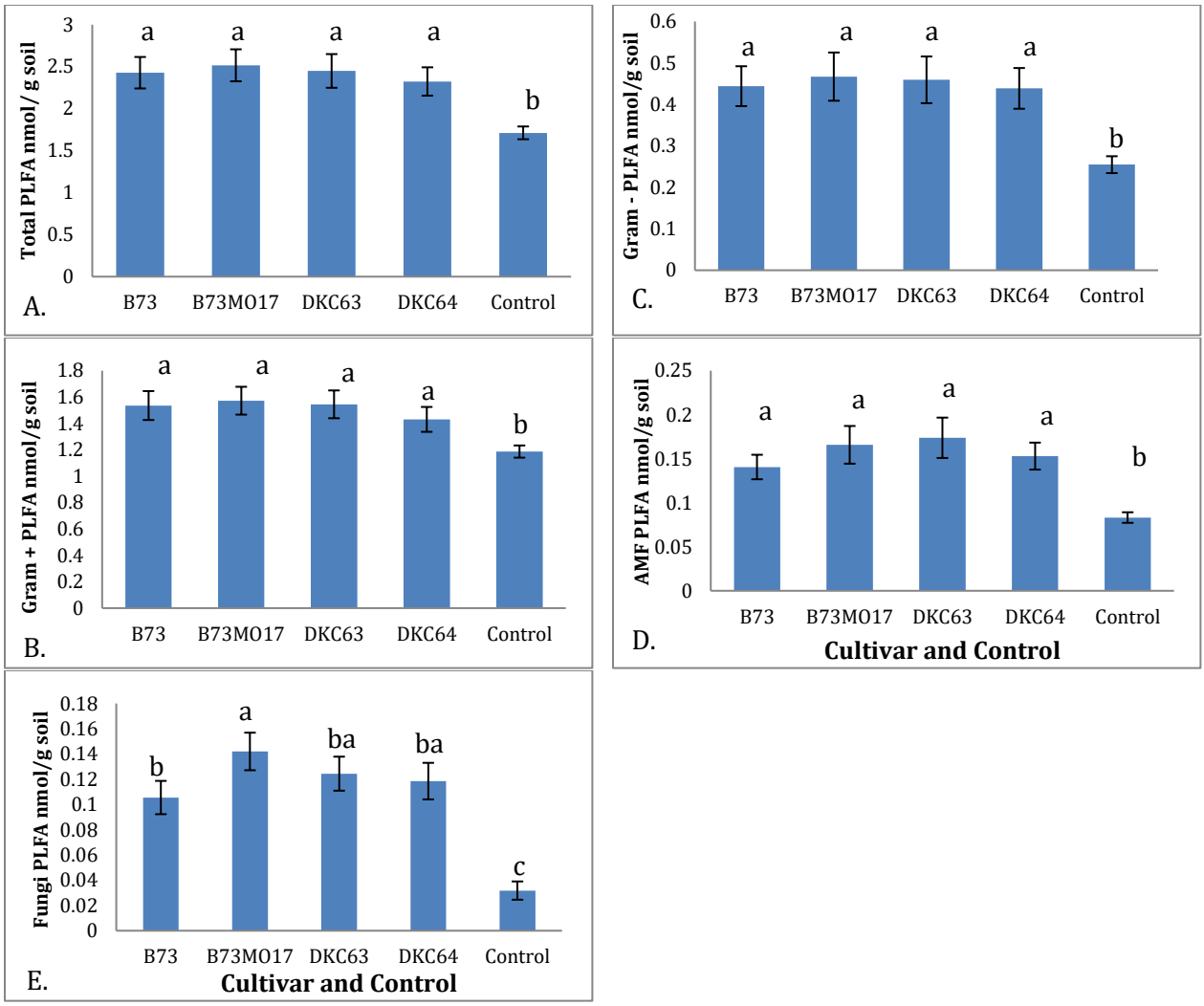

Figure 3.9 Total phospholipid fatty acids (PLFAs) (A.), gram-positive bacteria (B.), gram-negative bacteria (C.), arbuscular mycorrhizal fungi (AMF) (D.), and fungi (E.) for the soils surrounding the roots of four maize cultivars (B73, B73/Mo17, DKC63-55RIB, and DKC64-69RIB), in addition to the control (unplanted soil). Maize cultivars were grown under greenhouse conditions. Means with different letter notation indicate statistically significant differences ($p<0.05$).

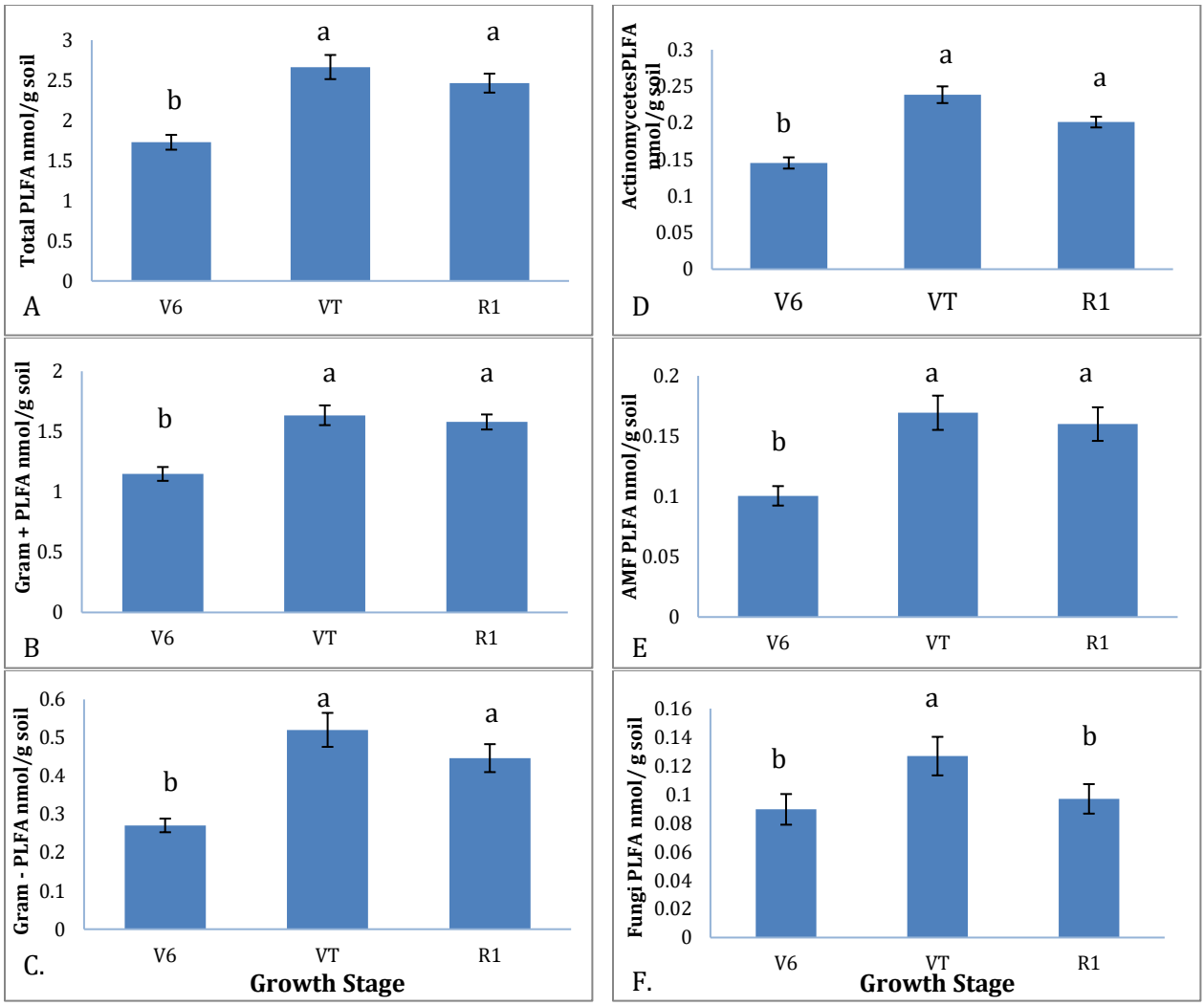

Figure 3.10. Total phospholipid fatty acids (PLFAs) (A.), gram-positive bacteria (B.), gram-negative bacteria (C.), actinomycetes (D.), arbuscular mycorrhizal fungi (AMF) (E.), fungi (F.) for the soil of all maize cultivars (B73, B73/Mo17, DKC63-55RIB, and DKC64-69RIB) including the control (unplanted soil) over three different growth stages (V6, VT, and R1). All maize cultivars, including the control were under greenhouse conditions. Means with a different letter indicate statistically significant differences ($p<0.05$).

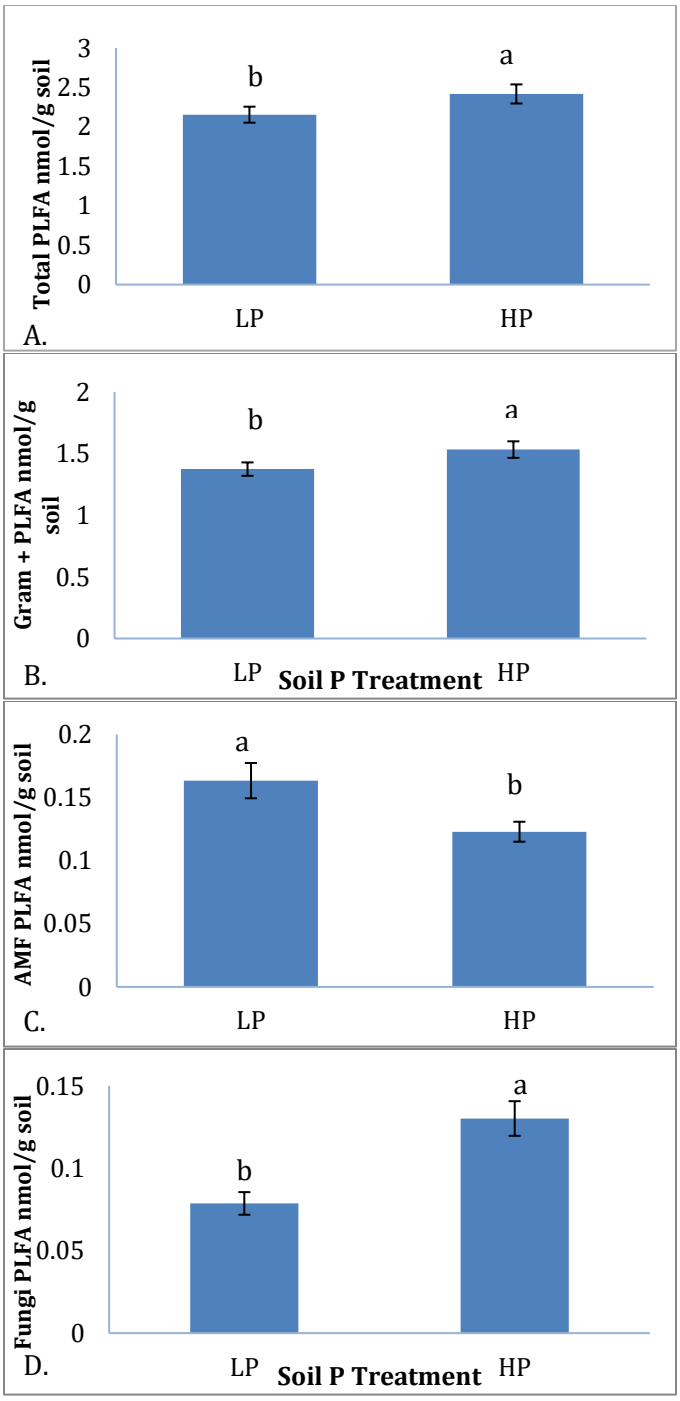

Figure 3.11. Total phospholipid fatty acids (PLFAs) (A.), gram-positive bacteria (B.), arbuscular mycorrhizal fungi (AMF) (C.), and fungi (D.) between two soil P-treatments (LP = low P; HP = high P) for all maize cultivars grown under greenhouse conditions (B73, B73/Mo17, DKC63-55RIB, and DKC64-69RIB) as well as for the control (unplanted soil). Means with different letters indicate a statistically significant difference ($p<0.05$).

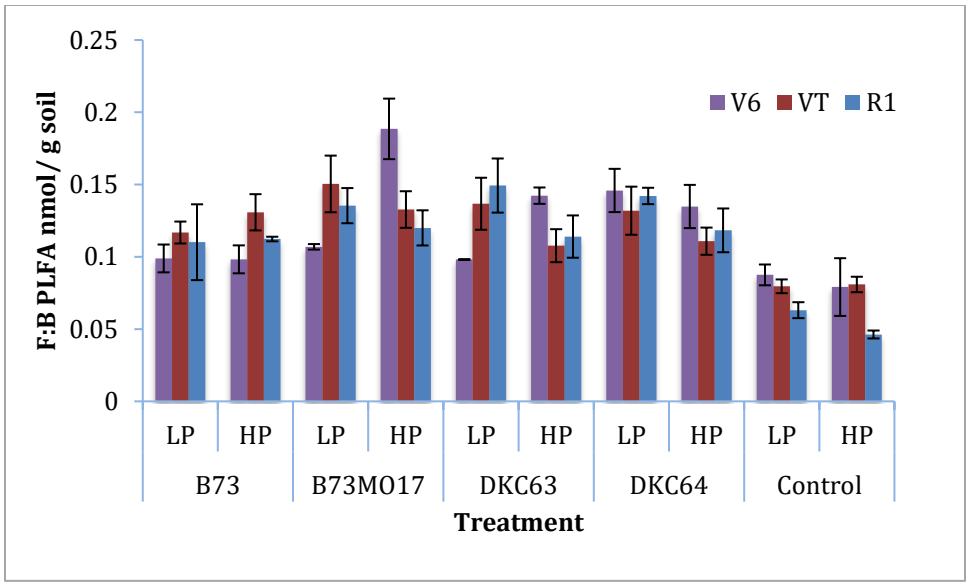

Figure 3.12. Soil fungi to bacteria ratio as impacted by the interaction between maize cultivars (B73, B73/Mo17, DKC63-55RIB, and DKC64-69RIB), P-treatments (L = low P; H = high P), and growth stages (V6, VT, and R1).

Figure 3.13 Removal of extra maize upon emergence.

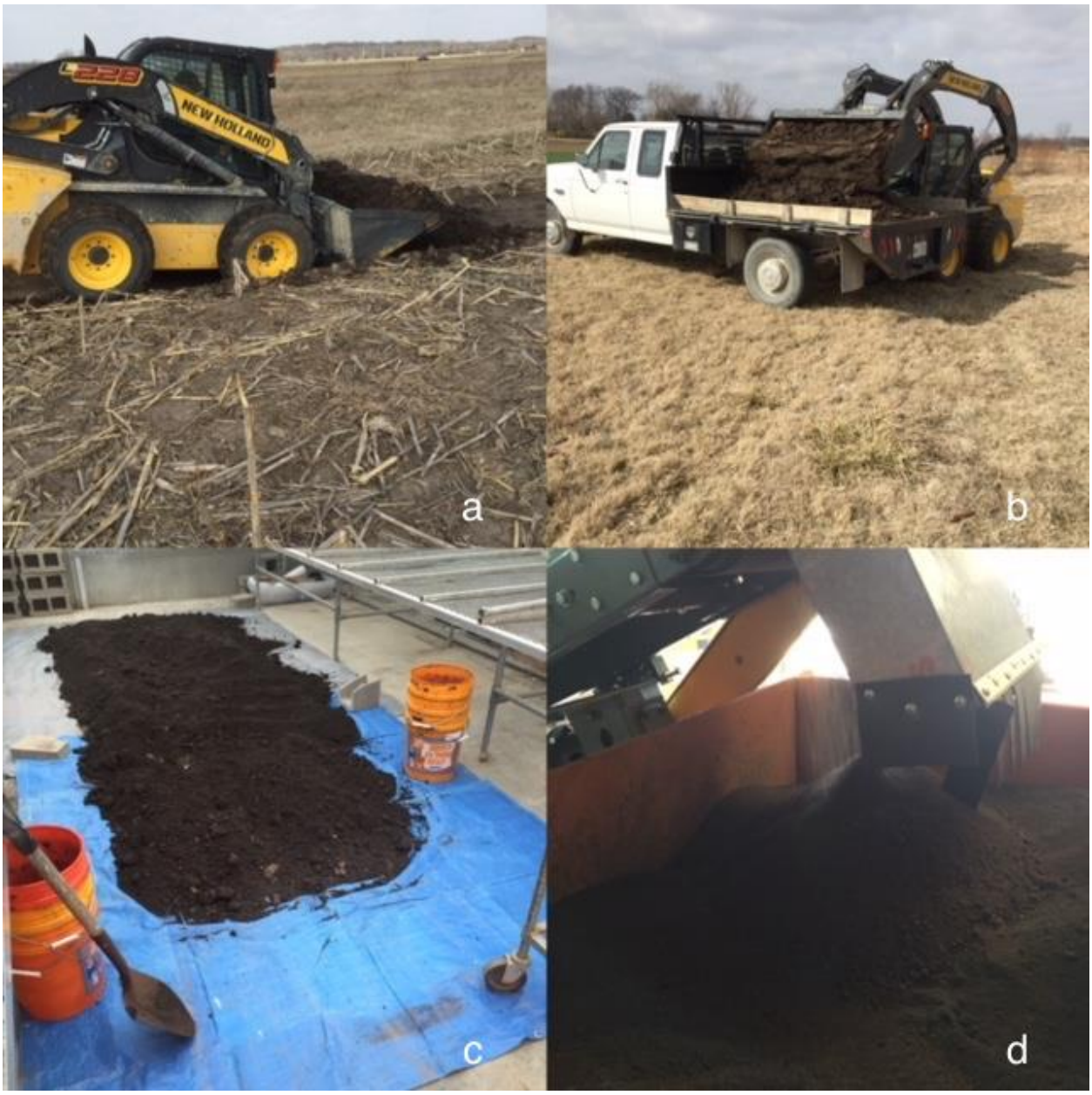

Figure 3.14 Preparation of soil mixture (a. removal of top residue, b. collection of soil, c. laying soil out to dry, d. mixing soil with washed sand (1:2 ratio).)

Chapter 4 - Summary

The combination of an exponentially growing human population with climate change, soil erosion, and non-renewable resources is bringing forth concerns about the ability to continue providing sufficient food. This study focused on potential solutions in the much-neglected belowground aspect of agriculture. Microbial associations with plants are known to provide several advantages to the plant as well as the soil. Agricultural practices are known to affect these relationships, however, how long-term breeding for beneficial traits has impacted these relationships is not well understood.

Results for both the field and greenhouse study show that maize genotype had an effect on the surrounding soil microbiome. However, each study provided somewhat different results from the other.

Differences in genotype between the maize cultivars grown under field conditions showed no significant impact on either shoot or root biomass, nor did they impact shoot C, N, and P content. The cultivars did show differences in symbiotic relationships with AMF, with DKC64-69RIB having greater root AMF present compared to DKC63-55RIB. Interestingly, both of these cultivars were not significantly different from B73 or B73xMo17 in root AMF concentrations. For all of the cultivars, the root concentrations of AMF decreased with depth and over time. While little is known about how genotypes affect AMF colonization, previous research has revealed that AMF tends to increase over time up to silking (Lutgen et al., 2003). The decline in AMF colonization in this experiment, however, might be explained by the extreme weather that the maize endured for the duration of the experiment. Multiple year observation of the field experiment would be advisable to avoid weather bias. The analysis of AMF colonization in the roots was also difficult due to the limited amount of utilizable roots that

were obtained. A greater amount of root cores or a different root obtaining method would aid in minimizing this experimental error. Another aspect that could be improved upon is the number of seeds planted per row as excessive rain resulted in some green snap and lodging. These plants were not utilized when sampling. While certain seeds are hard to come by, it may be best to determine how many seeds would be needed before choosing a specific maize cultivar.

Microbial biomass was greater in the control soil in the field, while differences between cultivars appeared to have no impact on the total microbial biomass. This was unexpected and might be due to the fact that the mid-row had leftover residue from last year's maize. Planting the maize between the residues from last year's maize may also have an impact on the root microbial community; therefore, a solution toward finding a better control would be advisable. The controls in this experiment may have been compromised. Another unexpected factor was that total microbial concentrations decreased over time with a significant decrease at R1. Gram-positive and gram-negative bacterial concentrations showed a similar pattern as that of total microbial concentrations, however, there was no significant decrease in gram-negative concentrations over time. With increasing root mass, root exudation was expected to increase as well and therefore would have provided a greater nutrient pool for microbes. The previously mentioned weather conditions prior to sampling may have caused the microbial decreases. Experimentation over more than one year may help determine this. Actinomycete concentrations were impacted by the interaction between treatment (cultivars and control) and time. All treatments had a significant decrease in total actinomycetes between V12 and R1, while only B73 and B73xMo17 saw a significant increase in actinomycetes between V6 and V12. Treatment appeared to have an impact on total AMF concentrations where B73 had significantly less AMF in the soil compared to that of DKC63-55RIB and the control. The control also had significantly

greater AMF present compared to B73xMo17 and DKC64-69RIB but was not statistically different from DKC63-55RIB. Soil AMF concentrations also decreased over time with a significant decrease between V6 and V12. There was no treatment effect on soil fungi. Fungal concentrations increased over time, with a significant increase between V12 and R1. The increase in fungal concentrations is a clear result of increased soil moisture. To see how the soil microbial groups under field conditions change over time without extreme weather effects would be beneficial, however, with current climate changes, this may prove difficult. Potentially, human manipulation using rain covers along with rainwater irrigation could improve experimentation of this particular kind by reducing soil saturation or heat and water stress.

Greenhouse data showed an interaction between maize cultivar and time on root and shoot biomass. At V6, all cultivars had similar root and shoot biomasses. Differences became more enhanced at VT, where B73 had significantly lower shoot biomass compared to the other cultivars. By R1, DKC63-55RIB and DKC64-69RIB had gained most shoot biomass with both B73 and B73xMo17 having significantly less shoot biomass compared to these but similar to one another. These results were somewhat expected as DKC63-55RIB and DKC64-69RIB were bred and genetically modified to perform well. Interestingly, B73xMo17 ended up having a similar biomass as B73 at R1. With heterosis in hybrids usually resulting in greater biomass than that of the parent plant, this was unexpected. Root dry biomass showed an impact by cultivar at R1 with B73 having the lowest root biomass and DKC63-55RIB having the greatest. Both B73xMo17 and DKC64-69RIB had root biomasses that were greater than B73 and less than that of DKC63-55RIB but they did not differ in root biomass from one another. Perhaps the reduction in shoot biomass for B73xMo17 can be explained by the increased root biomass found at R1. The question remains, however, why this cultivar chose to enhance root biomass at this particular

growth stage. Moreover, why did DKC63-55RIB have the greatest biomass? Phosphorus level in the soil appeared to have no impact on root biomass production but shoot biomass was significantly increased when P was readily available.

Nutrient analyses of the roots showed that N was significantly different between the cultivars over time. This means that the cultivar may impact N uptake in the roots. Greatest differences were observed at R1 with DKC63-55RIB having the greatest root N content, however, there appeared to be no impact of maize cultivar on root C and P content. The enhanced N in DKC63-55RIB might explain the increased root biomass in that cultivar and not the others. Root P was impacted by soil P levels, which was not yet evident at V6 but became very clear at VT and R1. Shoot nutrient analyses also indicated an impact by cultivar. B73 had significantly lower C compared to the other cultivars. Shoot N was also significantly lower in B73, but only at VT and R1. Greatest shoot N differences were found at R1 with DKC63-55RIB and DKC64-69RIB having the greatest N, followed by B73xMo17. No differences in shoot N were found between the hybrids during V6 or VT. Shoot P differences depended on soil P-levels in that shoot P was greater in soils with higher P concentrations.

Microbial analyses using the PLFA method indicated no impact of cultivar on total, gram-positive, gram-negative, actinomycete, and AMF microbial concentrations. The only group that was affected by differences in cultivar was that of fungi, where B73 had the lowest fungal concentration and B73xMo17 the greatest. DKC63-55RIB and DKC64-69RIB had similar fungal concentrations that were statistically no different from either B73 or B73xMo17. For all of the microbial groups, the control had the lowest concentrations. Insufficient soil P-level also impacted microbial groups by decreasing the total microbial concentrations, gram-positive bacterial concentrations, and total fungal concentrations. As expected, soil AMF concentrations

were greater in soil with low P-level. The actinomycetes and gram-negative bacterial groups were not impacted by soil P-levels. Total concentration of each microbial group increased between V6 and VT. This increased concentration stayed the same for all of the microbial groups during R1, except for fungi where there was a significant decrease towards concentrations similar to that at V6. The reason behind this decrease in fungi at R1 is not clear and ought to be further investigated.

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