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Manipulating the root mycobiome to improve plant performance and reduce pathogen pressure in corn (Zea mays)

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Abstract

Crop yield often varies within a field of a single genetically uniform crop plant, with the causes presumed to be a mix of both biotic and abiotic factors. Manipulating crop root mycobiomes could potentially increase yield by reducing pathogen impacts and improving access to soil water and nutrients. This study aimed to identify different fungal inoculation treatments that could increase the growth of corn seedlings sown in low productivity soils to that in high productivity soils and shift the root mycobiome composition. Fungal inoculation treatments did not have significantly different root mycobiome composition than seedlings grown in low yield control soils. However, indicator species varied across primary inoculation treatments and controls. Although corn grown in an autoclaved substrate showed growth promotion with the fungal inoculant *Fusarium oxysporum*, no fungal inoculant added to low productivity soils resulted in a similar yield to that of seedlings grown in high productivity soils.

Keywords

Root-associated fungi, plant-fungal interactions, soil communities, applied mycology, agroecology, metabarcoding

Summary for Lay Audience

Methods for maximizing crop yield, without negatively affecting the environment, are important with the growing food demand in a world facing climate change. However, some current methods of maximizing crop yield, such as the application of agricultural chemicals (such as insecticides, fungicides, and fertilizers) can harm the environment through processes such as run-off and leaching. To pursue more sustainable agriculture and retain high yield, researchers are exploring how to manipulate the microbial composition of soil instead of the application of agricultural chemicals. Research surrounding the microbial composition of soil must consider the microbiome of crop roots since the root is the crop's means of interaction with the soil. The root microbiome consists of all root-associated microorganisms (such as bacteria, fungi, and nematodes). Related to the microbiome is the root mycobiome which specifically refers to root-associated fungi. Manipulating the root myco- and microbiome of crops could increase yield by reducing pathogen pressure and improving access to soil water and nutrients. However, understanding how specific fungi impact the root mycobiome and crop yield has not been fully explored. My objective is to investigate how selected fungal isolates affect plant performance and the root mycobiome when applied to soil in which corn seedlings are grown under growth room conditions. In previous studies, A&L Biologicals observed major differences in crop yield in various sites growing corn. When compared, the sites revealed significant differences in root mycobiome of low- and high-yielding corn. Root-associated fungi from these sites were identified through analysis of genetic variations and were isolated in culture. Comparing the fungal communities in high- versus low-yielding sites may help identify key fungal candidates to improve crop health and productivity. Corn seeds were sown into the soil from low-yielding sites that were inoculated with potentially beneficial fungal isolates, with or without a co-inoculated soilborne fungal pathogen of corn. Although the fungal inoculant *Fusarium oxysporum* showed growth promotion when grown in sterile conditions, I did not observe this phenomenon in seedlings grown in inoculated field soils due to differences in soil composition or inadequate time for effective soil colonization by the inoculants. While I was not able to identify fungal inoculation treatments resulting in significantly different root mycobiome composition than seedlings grown in low yield control soils, there were indicator species that varied across treatments which could be explored as future fungal inoculants that drive changes in the root mycobiome.

Co-Authorship Statement

This research project was initially developed from ideas, contributions, and input from Dr. R. Greg Thorn, our industry partners Dr. George Lazarovits, Dr. Saveetha Kandasamy, Dr. Soledad Saldias, and Nimalka Weerasuriya of A&L Biologicals located in London, ON. This thesis will be revised for publication with their co-authorship.

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List of Abbreviations

% Ca = % calcium (expressed as a percentage saturation of the cation exchange capacity)

% K = % potassium saturation

% Mg = % magnesium saturation

% Na = % sodium saturation

Al = aluminum (ppm)

ASV = amplicon sequence variant

B = boron (ppm)

BCA = biological control agent

CEC = cation exchange capacity (mEq/100g)

Cl = chlorine (ppm)

DNA = deoxyribonucleic acid

FDR = false discovery rate

F. oxysporum = Fusarium oxysporum

Fe = iron (ppm)

HYC = high yield control

KMG = potassium/magnesium (K/Mg) ratio

LYC = low yield control

Mn = manganese (ppm)

Na = sodium (ppm)

NGS = next-generation sequencing

NO_3N = nitrate nitrogen (ppm)

OM = organic matter biomass

P. janthinellum = Penicillium janthinellum

PCR = polymerase chain reaction

S. terrestris = Setophoma terrestris

SALT = soluble salts (ms/cm)

SOC = soil organic carbon

T. atroviride = Trichoderma atroviride

T. koningii = Trichoderma koningii

Zn = zinc (ppm)

1 Introduction

1.1 Agriculture

1.1.1 Growing agricultural demands in the face of climate change

The human population is growing exponentially, as is the demand for agricultural output to support human and livestock consumption. Agricultural yield needs to increase 60–100% by 2050 to support these growing needs (Tilman et al. 2011; Ray et al. 2013). The rise of biofuels, such as ethanol derived from corn, further increases the demand of agricultural output (Vasile et al. 2016). Current methods of maximizing crop yield involve the application of agrochemicals such as synthetic fertilizers, insecticides, fungicides, and herbicides, used to artificially increase nutrition or reduce the persistence of various pests, weeds, or pathogens that may limit agricultural yield.

The application of agrochemicals, however, such as synthetic fertilizers and pesticides, can negatively affect the surrounding environment through processes such as run-off and leaching (Önder et al. 2011). Furthermore, the use of synthetic nitrogen fertilizers could potentially increase greenhouse gas emissions, as CO_2 emissions are released from the production of ammonia, as well as N_2O emissions from the denitrification of nitrogen inputs (Kahrl et al. 2010). The application of agrochemicals, particularly insecticides and fungicides, results in adverse effects on biodiversity and the natural potential for biological pest control (Geiger et al. 2010). As such, there are many public concerns about the application of agrochemicals due to their negative impacts on human health and the environment (Massart and Jijakli 2007). Future agricultural methods and management practices for maximizing crop yield must reduce these negative environmental impacts often associated with modern agriculture.

1.1.2 Agroecosystem management

Ecosystems are complex biological networks involving many species and their interactions with one another and their physical environment. Ecosystems can involve natural, undisturbed habitats and their inhabitants but, in the context of agriculture, agroecosystems are defined as communities of plants and animals interacting with their physical and chemical environments where there is a modification by humans to produce food, fibre, fuel, or other products for consumption (Maes, 2013; Al-Kaisi et al. 2017). For example, the interactions of crop plants, other significant biota such as soil microbes, and the surrounding physical environment can be seen as an agroecosystem. Soil is an important physical and biotic component of agroecosystems.

1.1.2.1 The role of soils in agroecosystem contexts

Soil health is a state of soil meeting its necessary ecosystem functions for its given environment; within agroecosystems, this involves the continued capacity of soil to function as a vital, living ecosystem that can sustain plants, animals, and humans (Schlatter et al. 2022). Soil provides ecosystem services such as nutrient cycling, decomposition, biological control, and soil structure formation (Brady et al. 2015; Costa et al. 2018; Enebe and Babalola 2019; Cao et al. 2022). Brady and others (2015) found that soil-provided ecosystem services related to nutrient cycling cannot be fully replaced by mineral fertilizers. However, agricultural intensification has resulted in rapid degradation of soil quality globally. Soil organic carbon (SOC) is an important indicator of soil productivity and fertility, and it improves the structure, porosity, water retention, nutrient cycling and storage capabilities, and biological activity within the soil (Prăvălie et al. 2021). Prăvălie and others (2021) found that 21st century soil organic carbon (SOC) has declined in 79% of countries worldwide due to agricultural intensification. Rapid degradation threatens the soil's capacity to maintain healthy agroecosystem processes, including those essential to maximizing future crop yields to meet the growing population (Brady et al. 2015). Soil health management efforts must be made quickly to prevent further losses due to degradation.

1.1.2.2 Soil structure, aggregation, and microbes

The heterogeneous nature of the soil is important to understand when addressing soil management strategies. Soil is a mixture of solid particulates (sand, silt, clay, and organic matter), and space (air or water) (Wood 1995). Soil organic matter is made of living components (roots, macro-fauna, and microorganisms) and their non-living remains in various stages of decomposition (particulate and dissolved organic) (Wood 1995). Numerous abiotic (structural and chemical) and biotic (microorganisms and their abundances) components affect aspects of the soil that affect agricultural output.

Soil aggregates are secondary particles formed from primary soil particulates (sand, silt, and clay) and organic matter bound together (Papadopoulos 2011). Soil aggregates are classified by size as microaggregates (< 0.21 mm), small macroaggregates (0.21 – 2 mm), and large macroaggregates (> 2 mm) (Al-Kaisi et al. 2014; Šantrůčková et al. 1993). Stable soil aggregates can resist disruption from forces such as erosion, tillage, or water. Aggregate stability is an important indicator of healthy soil (Papadopoulos 2011). Microbes, particularly filamentous fungi, use hyphal structures to entangle soil particles and release extracellular polymeric substances that facilitate soil aggregation (Costa et al. 2018). A study conducted by Rillig and others (2002) showed that arbuscular mycorrhizal fungi and glomalin were positively associated with soil aggregate stability. In a meta-analysis by Lehmann and others (2017), soil microbes consistently show positive effects on soil aggregation, with bacteria and fungi playing more significant roles in aggregation than other soil taxa.

There are many land use or cultivation practices that disrupt soil microbes that aid in aggregation, such as breaking up fungal mycelial networks, reduce aggregate stability (Rillig and Mummey 2006). A study by Gupta and Germida (2015) found that soil after 69 years of cultivation showed decreased aggregate stability, microbial biomass, respiration, and enzyme activity. Agricultural intensification results in disturbances that negatively affect aggregate stability and mineralization, in turn decreasing water use efficiencies and reducing crop growth (Zhang et al. 2014; Gupta and Germida 2015). In contrast, amendments to soils that increase soil organic matter (which is often associated

with higher microbial biomass), such as adding agricultural straw, can increase soil aggregate stability, aiding in water use efficiency and higher crop yields (Zhang et al. 2014). Merino-Marin and colleagues (2021) investigated the relationship between land use, microbes, and plant traits in relation to soil aggregation and found microbial community composition influenced soil aggregate stability, but aggregation was also highly influenced by land use.

1.1.2.3 Root-soil interactions

Plant roots interact most closely with the soil. The rhizosphere refers to the soil and related biota near a plant's roots ("rhiza" in Greek) (Hartmann et al. 2008). Bulk soil refers to all other soil in crop systems (Whalley et al. 2005). Root systems dramatically impact soil characteristics related to soil-crop interactions, thus making features of the rhizosphere highly influential to plant growth, as compared to bulk soil. Root exudates, for instance, are a nutrient source for rapidly proliferating microorganisms, thus making the rhizosphere soil rich in microbes, some of which can aid in plant growth (Zhang et al. 2017). Bulk soil becomes the rhizosphere as it is penetrated by root growth and is altered by microbial activity. The introduction of root activity and associated microbes creates many beneficial associations between the soil microbes and plants, such as nutrient exchange, increased water uptake, or reductions in pathogen pressure (Shi et al. 2016; Zhang et al. 2017). Thus, investigating the microorganisms of the rhizosphere and root area may provide valuable insights for soil management.

1.2 Role of fungi in agriculture

1.2.1 Benefits and costs of plant-fungal interactions

Fungi are a predominant taxon within soil ecosystems. The evolutionary relationship between plants and fungi is over 400 million years old, with fossil records showing fungi as symbionts of plants as early as the establishment of plants on land (Rai and Agarkar 2016). Plants and associated fungi are often described as co-evolving units based on relationships between the plant and fungus where microbial diversity and interaction are fundamental in keeping host plants healthy and productive (Vandenkoornhuyse et al.

2015; Schiro et al. 2019). Most studies focus on mycorrhizal fungi, but there is evidence that other fungi play significant roles in plant fitness too (Mommer et al. 2018). Almario and others (2017) found non-mycorrhizal fungi, such as *Helotiales*, isolated from the roots of wild *Arabis alpine* growing in phosphorus (P) limited soil improved plant growth and P uptake, showing mycorrhiza-like traits including the colonization of the root endosphere. All plants are thought to interact with the fungi residing within plant tissues (Petrini 1996; Southworth 2012), and these interactions fall into three main categories: parasitism, mutualism, and commensalism.

The nature of these fungal-plant interactions is dynamic; there are instances where commensal or mutualistic fungal endophytes become pathogenic under specific conditions (alterations to nutrient availability and other abiotic stresses) (Schulz and Boyle 2005; Rai and Agarkar 2016). Many factors affect the strength and ecological nature of plant-fungal interactions, including host range, host-specificity, tissue-specificity, and nutrient imbalances (Rai and Agarkar 2016). Nutrient imbalances can lead to reactive oxidative stress and can change endophytes from neutral or mutualistic to pathogenic (Rai and Agarkar 2016). However, environmental conditions such as temperature and humidity may also make the host plant more susceptible to the transition of an associated fungus from one life mode to another (Freeman and Rodriguez 1993; Rai and Agarkar 2016).

Additionally, evolutionary genetics also contribute to the virulence or beneficial attributes of fungal associates of plants, in a strain-specific manner. *Colletotrichum magnum,* a fungal pathogen, causes anthracnose in cucurbits (Cucurbitaceae – gourds) and can grow asymptomatically as a commensal endophyte in many non-cucurbit species (Freeman and Rodriguez 1993). However, when the virulent strain (CmL2.5) was mutated under UV mutagenesis, the mutated strain asymptomatically colonized cucurbit host plants and conferred many fitness benefits such as disease and drought resistance and growth enhancements (Freeman and Rodriguez 1993). Mutation or alteration of a fungal pathogen or endophyte may result in changes in life mode.

1.2.1.1 Mutualism & Commensalism

Despite the disadvantages fungal pathogens pose to plant health, there are many beneficial fungal-plant interactions. Beneficial fungi positively affect a plant's fitness through processes such as aiding in biotic and abiotic stress tolerance, accelerating growth, and controlling reproduction (Petrini 1996; Southworth 2012). Beneficial fungi often take the form of mycorrhiza, endophytes, or are free living. An example of a mutualistic endophyte is *Fusarium oxysporum* (*F. oxysporum*) strain Fo162, which can act as a biological control against infection by root-parasitic nematodes (Rai and Agarkar 2016). Some mutualistic fungal endophytes emit beneficial compounds such as phytohormones that stimulate the growth of antimicrobial secondary metabolites that result in disease suppression (Meena et al. 2017).

1.2.1.2 Pathogenesis

With fungal-plant interactions, there are many examples of pathogenic fungi infecting host cells, resulting in damage or death. Fungal biotrophs are fungi which cannot live without a host plant and thus do not have a saprotrophic independent life stage (Pawlowski and Hartman 2016). Many of these fungal biotrophs are also plant pathogens. Biotrophic fungal plant pathogens often have highly developed infection structures called appressoria which are specialized cells for entering the host plant’s cells. Necrotrophic fungal plant pathogens rely on dead tissue to derive their nutrition (Pawlowski and Hartman 2016). Necrotrophic fungal plant pathogens produce secondary metabolites with toxic properties. Plants can become infected through host- or non-host-specific fungal toxins, such as deoxynivalenol (DON) toxin that induces cell death (Pusztahelyi et al., 2015). Necrotrophic fungal plant pathogens use various secretory pathways to cause localized cell death, resulting in localized necrosis, lesions, and in some cases, plant death (Pawlowski and Hartman 2016). Some external conditions can magnify the effects of fungal pathogens. For example, nutrient limitations, such as nitrogen limitation, may act as stimulants to induce infection by phytopathogenic fungi (Pusztahelyi et al. 2015).

1.2.2 Agricultural benefits to root-associated fungi

1.2.2.1 Nutrient acquisition & growth promotion

Root-associated fungi can help with nutrient acquisition, such as those in a mutualistic interaction, which in turn promotes plant growth. The rhizosphere and root area are enzymatic hotspots that strongly regulate nutrient cycling and plant growth (Cao et al. 2022). Fungi can aid in plant nutrient acquisition since the branching mycelium has a higher surface-area-to-volume ratio for nutrient absorption as compared to plant root systems (Chibucos and Tyler 2009). Afterwards, nutrients are released from the beneficial fungi to the host plant, either in the rhizosphere or from fungal hyphae that penetrate the roots of the host plant. Fungi may also produce enzymes or other compounds that break down forms of essential plant nutrients, such as nitrogen, that are inaccessible to plants. Ectomycorrhizal and ericoid mycorrhizal fungal species produce degradative enzymes that aid in decomposing organic compounds that contain nitrogen and help in translocating the bioavailable nitrogen (Moreau et al. 2019). Additionally, fungal saprotrophs are responsible for the decomposition of organic materials and act as nutrient “miners” that degrade complex polymeric organic substances within the litter, whereas arbuscular mycorrhizal fungi (AMFs) play a more indirect and stimulatory role in nutrient cycling (Cao et al. 2022).

1.2.2.2 Pathogen Defense

Growth-promoting fungi help prime the plant’s immune system to detect and evade pathogens through the production of elicitors such as volatile organic compounds, antimicrobials, and competition (Enebe and Babalola 2019). Fungal and bacterial species can help suppress pathogen growth through direct antagonism with pathogens for space and nutrients by producing antimicrobial metabolites through induction of systemic resistance or increasing resistance against pathogens via upregulation of the host plant’s defence genes (White et al. 2019). The interaction of multiple species of microbes may amplify these effects. Liu and others (2021b) investigated the role of dominant microbes in wheat-associated microbiomes in reducing the virulence of *Fusarium*

pseudograminearum. They found high numbers of a dominant bacterium, *Stenotrophomonas rhizosphila,* helped increase plant growth while reducing the virulence of *F. pseudograminearum*. Here modulation of the plant immune system occurred using microbiome manipulation.

1.2.2.3 Stress reduction and tolerance

Plant growth-promoting fungi can aid in a plant's ability to tolerate environmental and biological stressors (Ray et al. 2020). Fungal endophytes help plants become more tolerant to abiotic stressors such as drought, salinity, and temperature (Redman et al. 2002; Márquez et al. 2007; Rodriguez et al. 2008; Southworth 2012). The ability of fungi to help plants tolerate drought stress was especially important during the initial colonization of plants onto land since symbiotic fungi aided in drought tolerance of many plants (Pirozynski and Malloch 1975). An extreme example of fungal endophytes co-evolving with plants involves the grass species *Dichanthelium lanuginosum,* which thrives in hot geothermal soils with root zone temperatures of 57°C. An analysis of 100 *D. lanuginosum* plants revealed that all were colonized by one dominant fungal endophyte, *Curvularia protuberata* but when grass plants were grown without *C. protuberata* in simulated geothermal conditions, the plants died (Redman et al. 2002). This extreme thermotolerance associated with this endophyte-plant symbiosis could be attributed to the fungal endophyte producing cell wall melanin that helps dissipate heat along the hyphae or form a complex with oxygen radicals generated during heat stress (Redman et al. 2002; Verghese et al. 2012). Other soil fungi, such as *Trichoderma virens*, have been shown to enhance plant growth and help plants become more tolerant to extreme environmental conditions, such as heavy metal stress through processes such as changes in valence and intracellular localization (Babu et al. 2014). Similarly, Ikram and others (2018) found the inoculation of heavy metal-rich soil with *Penicillium roqueforti* resulted in heavy metal tolerance and increasing nutrient uptake resulting in higher plant growth than wheat grown in control soil rich in heavy metals. Thus, fungi, particularly endophytes use intracellular processes, nutrient acquisition, and reductions in reactive oxygen species to aid in a plant's ability to tolerate abiotic stresses.

1.3 Mycobiomes

1.3.1 Investigating and engineering mycobiomes

Microbiomes are the many microorganisms within a particular environment. The microbiome is highly dynamic and can involve ecological communities of particular microorganisms, their interactions with one another, and their interactions with the surrounding environment (Berg et al. 2020); a mycobiome is a fungal community and its associated functions (Fernandes et al. 2022). Studying crop microbiomes can allow for more targeted and predictive management in agriculture when considering the unique conditions and interactions within each agricultural system (Berg et al. 2020). Crop microbiomes involve microorganisms across a diverse set of taxa. Manipulation of crop microbiomes is a potential agricultural management practice. Determining which taxa within the microbiome to investigate and manipulate is integral to management efficacy. Schlatter and colleagues (2022) found that fungal communities were more predictive of spring wheat yield than bacterial communities, with some fungal taxa more strongly correlated with grain yields, including Ascomycete and Basidiomycete decomposers. These fungal groups were also indicative of no-till and upper soil depths (Schlatter et al. 2022). Specific fungal taxa are often associated with high yield, but these can be dependent on farming practices, location, and depth (Schlatter et al. 2022). The introduction of these beneficial isolates may aid in the soil's capacity to maximize crop yield.

Identifying which groups of fungi contribute to beneficial fungal-plant interactions, that increase plant growth through processes such as nutrient acquisition, stress tolerance, or soil stability, can aid in more effective microbiome management. Most studies focus on mycorrhizal fungi, but there is evidence that other fungi play significant roles in plant fitness too (Mommer et al. 2018). Almario and others (2017) found non-mycorrhizal fungi, such as *Helotiales*, isolated from the roots of wild *Arabis alpina* growing in phosphorus (P) limited soil improved plant growth and P uptake, showing mycorrhiza-like traits including the colonization of the root endosphere. If the isolates or groups of microbes which elicit positive effects in the microbiome can be identified, then

manipulation of the soil microbiome via inoculation with these isolates could be a promising agricultural management practice. Bacterial isolates have been successfully used as inoculants in historically low-yielding soil and were shown to increase the soil potential of low productivity soils to function more similarly to high-yielding soils (Kandasamy et al. 2019). However, using fungal isolates to increase the growth potential of low productivity soils, is still to be investigated.

1.3.1.1 Next-generation sequencing tools in microbiome investigation

Greater access to high-throughput, next-generation sequencing (NGS) and the development of effective bioinformatics analyses allow for accurate studies of microbial community structure (Goodrich et al. 2014). Next-generation sequencing allows effective identification of important microorganisms within the microbiome which could influence agricultural management practices (Esposito et al. 2016). But targeted primers are essential in the precision use of NGS in microbiome investigation. Ineffective primer use could render inaccurate results if the primers do not target the correct genetic region needed for a successful identification. Metabarcoding of fungal communities often makes use of the ITS region, made up of 3 subregions (ITS1, 5.8S, and ITS2). The ITS2 region has lower length variation than ITS1 and there are primer sites targeting fungi in the flanking conserved 3'-end of the 5.8S and 5'-end of the large subunit (28S) regions that reduce taxonomic bias (Nilsson et al. 2019).

1.4 Root mycobiome of corn

1.4.1 Importance of corn

Corn (*Zea mays*) is a monoecious grass that has been selectively bred from wild teosinte from Central Mexico approximately 9,000 years ago (Kistler et al. 2020). It is a significant crop globally for human and livestock consumption, biofuels, organic materials, and other uses. It accounts for up to 30% of the total caloric intake of some developing countries (Watson 2017). There is great concern about large-scale crop losses

due to disease. However, large yield variations are often observed in seemingly healthy fields of corn (Kandasamy et al. 2021). Understanding what drives the variation within fields and improving productivity in previously low-yielding sites may help in tackling larger-scale crop losses.

1.4.2 Previous work

My work is preceded by a study conducted by Kandasamy and colleagues (2021), which investigated the relationship between the root mycobiome, high versus low yielding corn sites, and physicochemical properties of soil. In 2017, A&L Biologicals Inc. (London, ON) sampled 10 farms across Southwestern Ontario. Each field historically rotates corn, soybean, and wheat. To identify high and low yield patches of corn in the field, aerial drones were flown over each of the fields, capturing infrared images. These were converted into Normalized Difference Vegetation Index (NDVI) maps, which are commonly used in agriculture to detect plant health (based on chlorophyll density) as a predictor of crop yield (Kandasamy et al. 2021). Based on NDVI maps, high yield (good) and low yield (bad) patches were identified in each of the 10 fields. Yield at each site was confirmed upon harvest, and it was found that some had as much as a fourfold difference between high and low yielding sites (Kandasamy et al. 2021). Fungal communities varied greatly between different locations, showing that site-specific conditions such as soil texture and chemistry largely affected the mycobiome composition. Many soil physicochemical properties contributed to the yield differences and mycobiome diversity between different sites, such as the proportion of clay or sand, moisture, organic matter content, cation exchange capacity, pH, % phosphorus saturation, aluminum, iron, potassium, and chlorine. Despite site-specific drivers, there were 35 Operational Taxonomic Units (OTUs) shared across the root mycobiome of high-yielding corn, and 31 OTUs shared across the low-yield sites (Kandasamy et al. 2021). Sequences identified as *F. oxysporum, Chalara fungorum, Talaromyces* sp., *T. diversus, Penicillium ochrochloron, P. janthinellum, P. paneum/chrysogenum, Gibellulopsis* sp., *Neonectria fuckeliana,* and *Mucor hiemalis* were found in high yield sites across multiple farms. Fungi associated with low yielding sites were less consistent. I investigated whether these

taxa do provide beneficial yield effects, and also if their benefits alleviate the negative effects on plant growth caused by pathogenic fungi.

1.5 Research Question, Objectives, and Predictions

I chose some of these fungal taxa that were found to be consistently associated with high or low yield in corn (Kandasamy et al. 2021) for use as inoculants to see if they could alter predicted corn yield (as estimated by dry biomass at 21 days) in pot culture experiments.

1.5.1 Research Question

Using the current knowledge of root-associated corn mycobiomes, can fungal isolates from high yield soils improve corn growth in low yield soils in growth room trials or when co-inoculated with known soil-borne pathogens?

1.5.2 Objectives

My goal is to investigate how selected fungal isolates affect plant performance and the root mycobiome when applied to soil in which corn seedlings are grown under growth room conditions. More specifically, my research objectives are to:

1) Screen and rank fungal isolates predominant in high and low yielding sites as potential inocula by conducting preliminary experiments to quantify whether a given fungal isolate increases or decreases plant performance.
2) Investigate and quantify changes in plant growth associated with various fungal inocula (selected from objective 1) by measuring shoot height, root length, and dry biomass of roots and shoots.
3) Investigate changes in the mycobiome of washed roots from each treatment group (selected from objective 1), using metabarcoding of the ITS2 region, and identify patterns in community composition associated with specific treatment types, soil chemistry values, and biometric values predicting plant growth or vigour (objective 2).

1.5.3 Predictions

Based on the underlying hypothesis that certain fungi predominant in high yield sites will provide protective or growth-promoting benefits to the crop, I predict that one or more fungal inocula will act to improve crop performance in soils from low yield sites or when co-inoculated with known soil-borne pathogens.

2 Methods

2.1 Soil selection

Candidate sites for collection of low-yielding and high-yielding soils were selected from 10 farms studied in 2017 by Kandasamy and colleagues (2021), based on the largest yield differences predicted by Normalized Difference Vegetation Index (NDVI) imaging (Figure 2.1), and confirmed using harvest data (Table 2.1). I collected soils from Farm 10 and Farm 9. However, I selected Farm 10 for subsequent growth trials because Farm 10 had the largest difference in yield (bu/ac) between high and low yield sites (Table 2.1). I collected soils from F10B2 and F10G2 on September 22, 2020, and from F10B1, F9G1, F9B1, F9B2, sites within and bordering F10G1, and sites within and bordering F10G2 on November 10, 2020 (Figure 2.1). The soils from F9B1 and F9B2 were mixed to form F9BadMixed. The soil from within the plot of F10G1 was called F10G1A, and the site just south of the south-eastern border was called F10G1B. The soil from within the plot of F10G2 was called F10G2A, and the site just east of the border was called F10G2B. These soils were evenly mixed into their respective groups. Once mixed, soil chemical and particle size analyses were conducted by A&L Canada Laboratories Inc. for cation exchange capacity (Allen 1974), sodium (Soil and Plant Analysis Council 1999), pH (Anderson and Ingram 1993), nitrogen (Baird 2017), chloride (Baird 2017), phosphorous (Olsen 1954), as well as various metals and metalloids (Soil and Plant Analysis Council 1999) (some listed in Table 2.2).

Three growth experiments were conducted to confirm candidate low yield soil and candidate high yield soil. However, the first two growth experiments were unsuccessful, the first due to nutrient limitation since pots were not fertilized and the second due to issues with seed germination. In the third growth trial, I grew plants in soils F10G2A, F10G1, and F10B2. There were 8 replicates per treatment, a new seed source (Pioneer PO9998 AMXT) was used, and there were both unfertilized treatments and N-fertilized

treatments. For the latter, 15 mL of a 1.08% (w/v) aqueous solution of 46% urea fertilizer (Brussels Agromart Limited) was added to each pot to yield 25 ppm N based on the dry weight of the soil. Mid-sized to large seeds were used, weighing between 0.26 g and 0.32 g (Kandasamy et al. 2020). Plants were grown for 21 days and fertilized on days 8 and 15.

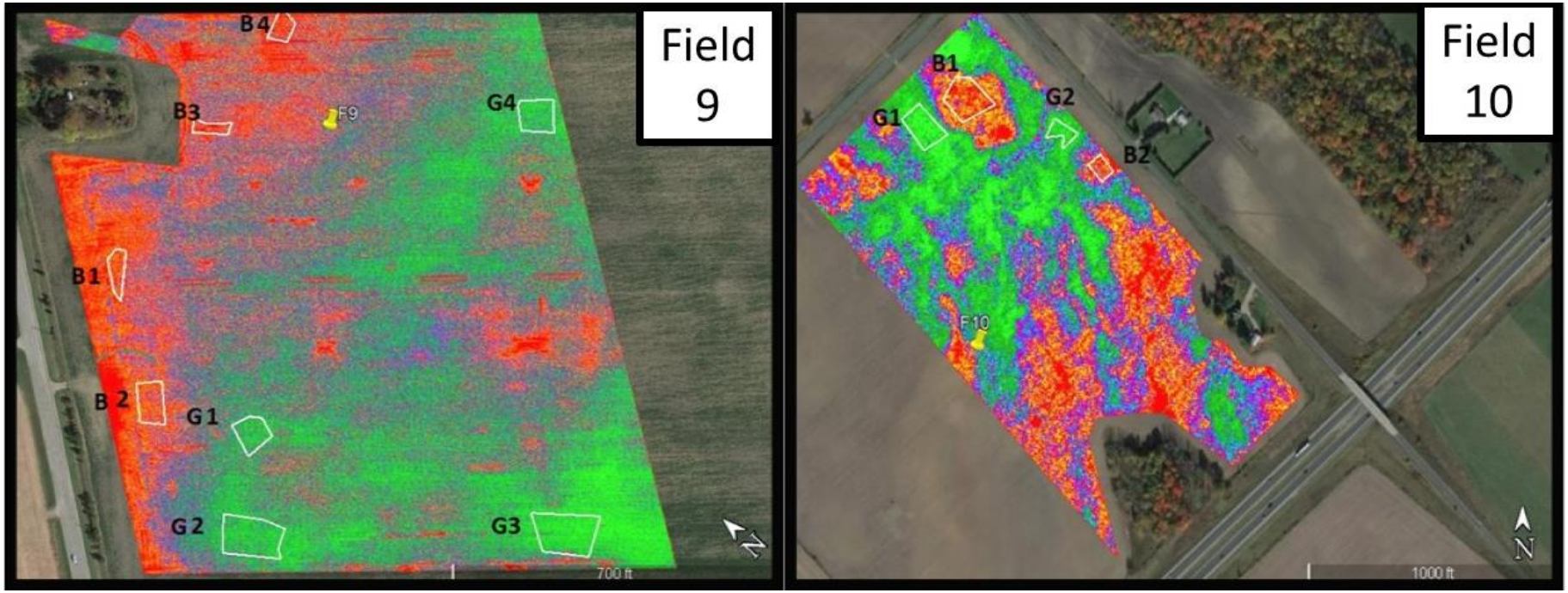

Figure 2.1: Aerial Normalized Difference Vegetation Index (NDVI) map of Field 9 (left) and Field 10 (right) growing corn at the V10 growth stage. The photo was taken using infra-red aerial imaging taken by an Unmanned Aerial Vehicle (UAV). White polygons outline associated yield sites with low yield appearing red/orange, and high yield appearing blue/green (Kandasamy et al., 2021).

Table 2.1: Descriptions of farms studied. From Kandasamy and colleagues (2021): field sites, location, corn variety, sampling dates, cropping treatments, and yield (bu/ac) at harvest per lowest yielding site (L) and yield per highest yielding site (H). Farm 10 is highlighted because soils from this site were chosen for experimentation due to the large yield differences observed upon harvest.

Site	Location (Lat/Long)	Variety	Planting date	Sampling date	Cropping regimes	Yield (bu/ac)
Farm 1	43.656327, –80.534160	Dekalb 45–65	11-May-17	17-July-17	Corn monocropping	L: 173 (11.63 t/ha) H: 284 (19.1 t/ha)
Farm 2	43.638370, –80.399300	DKC 39-97RIB	18-May-17	17-July-17	Corn monocropping with fall cover cropping	L: 169 (11.37 t/ha) H: 186 (12.51 t/ha)
Farm 3	43.052629, –81.449286	PO496AMXT	16-May-17	19-July-17	Corn monocropping	L: 262 (17.62 t/ha) H: 307 (20.65 t/ha)
Farm 4	43.335260, –80.792026	DKC48-56RIB	17-May-17	24-July-17	Corn-Soybean rotation	L: 137 (9.21 t/ha) H: 228 (15.33 t/ha)
Farm 5	43.139408, –80.837053	DKC50-78RIB	18-May-17	24-July-17	Corn-Soybean-Wheat	L: 239 (16.07 t/ha) H: 320 (21.52 t/ha)
Farm 6	43.090045, –81.346837	PO496AMXT	14-May-17	24-July-17	Corn monocropping	L: 273 (18.36 t/ha) H: 322 (21.65 t/ha)
Farm 7	42.836740, –81.101202	Dekalb 48-56	17-May-17	25-July-17	Corn-Soybeans-Wheat	L: 247 (16.61 t/ha) H: 292 (19.64 t/ha)
Farm 8	43.289837, –80.545299	P9526YXR	20-May-17	26-July-17	Corn-Soybeans-Wheat	L: 223 (15.00 t/ha) H: 262 (17.62 t/ha)
Farm 9	42.705807, –81.316867	DKC 48-56RIB	09-Jun-17	01-August-17	Corn-Edible beans-Wheat	L: 172 (11.57 t/ha) H: 266 (15.2 t/ha)
Farm 10	42.736850, –81.443932	P0157AMX	02-Jun-17	08-August-17	Corn-Soybean rotation with fall cover cropping	L: 171 (11.50 t/ha) H: 299 (20.11 t/ha)

Table 2.2: Particle size and selected soil chemical properties for candidate soil source sites. Particle size and soil chemical analysis were conducted by A&L Canada Laboratories Inc. (London, ON). Additional soil chemical properties for soils used in main growth experiments (Experiment 2) are listed in Appendix III.

Sample	Rep	Sand%	Silt%	Clay%	Soil Textural Class	pH	Organic Matter%	K (ppm)	NO_3N (ppm)
F9 G2	1	43	35	22	Loam	7.6	3.2	188	11
	2	43	37	20	Loam	7.8	3.2	197	11
F9 Bad Mix	1	36	37	28	Clay Loam	7.0	3.1	350	25
	2	37	36	27	Clay Loam	6.9	3.1	356	27
F10G1A	1	61	24	15	Sandy Loam	6.2	3.9	166	7
	2	66	20	14	Sandy Loam	6.0	4.0	157	6
F10G1B	1	62	24	14	Sandy Loam	5.9	3.8	153	4
	2	64	23	13	Sandy Loam	6.0	3.8	140	5
F10B1	1	58	25	18	Sandy Loam	6.5	4.9	271	9
	2	73	16	11	Sandy Loam	6.6	5.1	289	9
F10G2A	1	51	25	24	Sandy Clay	6.6	5.4	274	9
	2	44	28	27	Clay Loam	6.6	4.9	295	8
F10G2B	1	46	28	27	Sandy Clay	7.4	2.4	190	3
	2	44	28	28	Clay Loam	7.5	2.4	197	3
F10B2	1	44	29	28	Clay Loam	7.5	3.0	180	5
	2	44	28	28	Clay Loam	7.5	3.0	164	5

2.2 Fungal Isolates

From the paper by Kandasamy and colleagues (2021), fungal operational taxonomic units (OTUs) were derived from metabarcoding the ITS2 and D1 variable region of the LSU regions of corn roots and rhizosphere obtained from the field. These OTUs were analyzed using the *ALDEx2* package (Fernandes et al. 2013) to determine OTUs whose relative abundance of sequence reads was significantly different in high versus low yielding sites in each of the ten fields. Additionally, OTUs were analyzed with the *IndicSpecies* package in R which is an alternative to *ALDEx* (De Cáceres and Legendre, 2009) to determine potential indicator species for the high and low-yielding sites in each field of the ten fields. Candidate beneficial fungal isolates (from high yield sites) and detrimental isolates (from low yield sites) were determined using these analyses, but the selection of potential inoculum (using site-specific OTUs) was limited by the availability of fungal cultures in the culture collection at A&L Biologicals, with additional cultures requested from the Canadian Collection of Fungal Cultures (isolate sources listed in Table 2.3). I plated glycerol stocks containing each fungal isolate onto Petri dishes with potato dextrose agar with chloramphenicol (PDA-C). After 7-10 days of growing on Petri dishes with PDA-C in a fungal incubator at 25 °C, five 5 mm x 5 mm squares of mycelium on agar were added to magenta boxes (~ 8 cm x 8 cm x 10 cm) filled with autoclaved substrate. The substrate was either a mixture of ground corn kernel and coarse vermiculite or barley grain. The magenta boxes were wrapped with parafilm along the lid and placed in the incubator at 25 °C for 10-14 days until the entirety of the substrate was colonized by mycelium. All fungal cultures used in experiments were grown for the same duration.

Table 2.3: Codes, source, and identifications (confirmed by previous ITS sequencing) for all fungal isolates tested in Experiment 1 and Experiment 2 where CCFC = Canadian Collection of Fungal Cultures, GRI = Growth Room Isolate, and AIP = Agricultural Innovation Program.

Code	Origin	Source	Sequence ID
CM001	A&L: 2015 AIP F63	Field 11, Corn Sap	*Fusarium oxysporum*
CM002	A&L: 2017 GRI M12	Growth Room Corn	*Fusarium oxysporum*
CM003	A&L: 2018 Isolate	Tomato	*Fusarium oxysporum*
CM004	A&L: 2017 GRI K9	Growth Room Corn	*Fusarium oxysporum*
CM005	A&L: 2017 GRI M12	Growth Room Corn	*Fusarium oxysporum*
CM006	A&L: 2017 GRI K8	Growth Room Corn	*Fusarium oxysporum*
CM007	A&L: 2017 AIP AL004	AIP Corn	*Trichoderma atroviride*
CM008	A&L: 2015 AIP F17	Field 2, Corn roots	*Fusarium oxysporum*
CM009	A&L: 2017 GRI M11	Growth Room Corn	*Fusarium oxysporum*
CM010	A&L: 2015 AIP F48	Field 5, Corn sap	*Alternaria solani*
CM011	A&L: 2015 AIP F49	Field 5, Corn sap	*Alternaria alternata*
CM012	A&L: 2015 AIP F65	Field 11, Corn Sap	*Clonostachys rosea*
CM013	A&L: 2015 AIP F66	Field 12, Corn Sap	*Clonostachys rosea*
CM014	A&L: 2017 AIP AL091	AIP Corn (?)	*Penicillium janthinellum*
CM015	A&L: 2017 GRI K11	Growth Room Corn	*Fusarium chlamydosporum*
CM016	A&L: 2017 GRI M9	Growth Room Corn	*Mucor hiemalis*
CM017	A&L: 2015 AIP F40	Field 3, Corn sap	*Sarocladium zeae*
CM018	A&L: 2017 GRI K4	Growth Room Corn	*Penicillium janthinellum*
CM019	CCFC: DAOMC 241253	Corn Root	*Setophoma terrestris*
CM020	CCFC: DAOMC 241255	Corn Root	*Setophoma terrestris*
CM021	CCFC: DAOMC 222124	Casing	*Trichoderma koningii*
CM022	CCFC: DAOMC 222180	Floor	*Trichoderma koningii*
CM023	A&L: Fg2	Infected corn cobs	*Fusarium graminearum*
CM024	A&L: Fg9	Infected corn cobs	*Fusarium graminearum*
CM025	A&L: Fg10	Infected corn cobs	*Fusarium graminearum*

2.3 Experiment 1: Inoculum screening trials

Experiment 1 was conducted to screen candidate fungal isolates to be used in the design of Experiment 2 by inoculating low yield soils. Experiment 1 also identified a suitable soil source for high and low yield controls for Experiment 2. Additionally, Experiment 1 was conducted to determine which type of substrate (mix of ground corn kernels and coarse vermiculite or barley grain) and concentrations were ideal for soil inoculation.

2.3.1 Substrate used

As a substrate for fungal inocula, I initially used a mixture of autoclaved ground corn kernels (blended in a food processor to an even consistency) mixed with coarse vermiculite (Grade 3) as substrate. While dry, I added vermiculite and ground corn kernels by weight in a 1:1.33 ratio of vermiculite to ground corn kernels. Once evenly mixed, 45 g of the mixture and 50 mL of deionized water were added to each magenta box and mixed, so the substrate was fully saturated by the water. Magenta boxes were autoclaved for 25 minutes at 121 °C. Later trials used pot barley as inoculum substrate. The barley was rinsed thoroughly in deionized water until the water ran clear. Then, I soaked it in deionized water for 24-48 h. Excess water was drained and 1 mL of water was added per gram of soaked barley. Barley was brought to a boil and cooked for 5 minutes, strained with cheesecloth, and 60–70 g of barley was added per magenta box and autoclaved for 25 minutes at 121 °C. In different trials, as explained in Appendix I, inoculated or uninoculated substrate was added to soils at concentrations ranging from 1% to 5% on a dry weight basis. Inoculated substrate was tested to test its efficacy as a method of delivery for fungal inoculum into the soil. Uninoculated substrate was used to determine if it effected plant growth as compared to controls without substrate.

Before planting, soils were broken up, passed through a sieve of 1 cm diameter and inoculants or substrates added based on fresh weight to dry weight of soil. 220 g of soil or soil mixed with inoculant/substrate were placed in a 10 oz ULINE paper cup with 3 drainage holes pre-punched in the bottom. I added 15-20 mL of water to the 220 g of soil using a spray bottle and placed 5 seeds with the radicle pointed down. Then, 80 g of soil

or soil mixed with inoculant/substrate was placed on top and another 10 mL of water was added to allow adequate moisture for germination. All pots were covered to maintain adequate moisture for germination and placed in the growth room. Covers were lifted from pots, exposing the plants to the growth room lights once seedlings emerged from the soil. Seedling shoots were thinned at day 5-6 of growth, so there were only 3 plants per pot to reduce competition for nutrients between seedlings in the same pot. The plants were grown for 3 weeks until seedlings were at the V3 stage (Nleya et al. 2019). Three to five replicates were used per treatment, with more replicates used in control groups. Power analysis calculations were not completed for the replications needed for this project as probabilities related to each treatment group are difficult to generate from the currently available literature (Jones et al. 2003). Pots were fertilized with nitrogen on days 8 and 15.

2.3.2 Harvest process

Soil for chemical analysis was removed from the pot without disrupting the corn roots. All soil chemical analysis was conducted by A&L Canada Laboratories Inc. I dipped the contents of each pot in room temperature tap water, then rinsed the roots under running tap water until all debris was removed. The following biometrics were measured: root length (cm), shoot length (cm), root dry weight (g), and shoot dry weight (g). Dry weights were taken after 5–7 days of drying in the oven at 60 °C in paper bags. The biometric data was pooled per pot: meaning the three plants per pot were treated as a single replicate and the measurements per plant were averaged.

2.4 Vermiculite and fungal isolate growth experiment

A growth experiment using autoclaved vermiculite (a hydrous phyllosilicate mineral) inoculated with barley substrate containing each fungal isolate was conducted to determine the effect each isolate had on seedling growth in the absence of any microbial, textural, or nutritional components present in the soil. The vermiculite used as potting material was a 1:1 ratio by weight of super-fine vermiculite (Grade 1) to coarse vermiculite (Grade 3). Approximately 1.33 mL of deionized water was added for every 1

g of vermiculite mixture and mixed evenly so the vermiculite was saturated throughout. The vermiculite mixture was then autoclaved in Pyrex trays for 25 minutes at 121 °C. The barley substrate was inoculated in the same method described in section 2.2. Inoculation of the autoclaved vermiculite mixture was done by adding the same weight of inoculated or uninoculated barley that was used for 300 g of dry low-yield soil from F10B2 in Experiment 2.

Each pot contained a total of 90 g of potting material (inoculated or uninoculated autoclaved vermiculite mixture): 65 g of potting material was initially added to each pot and sprayed with 15-20 mL of water, 5 seeds added with radicle facing down, and 25 g of potting material added to the top with an addition 10 mL of water to maintain adequate moisture for germination. Pots were placed on a covered greenhouse tray to maintain adequate moisture for germination. The cover was removed once seedlings emerged, exposing them to the growth room lights. On day 5, pots were thinned to 3 seedlings per pot to reduce competition. Seedlings were fertilized using 15mL of 2% w/v of 20-20-20 fertilizer in deionized water every 4 days. Seedlings were grown for 3 weeks in growth room conditions until plants reached the V3 growth stage. The plants were harvested using the same protocol outlined in section 2.3.3.

2.5 Experiment 2: Main growth experiment

Experiment 2 was the main growth experiment where corn seedlings were grown in soils with or without various inoculation treatments. Pot barley grain was used as a substrate, following the same preparation as described in section 2.2, and the measurement of concentrations followed the same protocol outlined in section 2.3.1. The same planting and growth protocol described in section 2.3.2 was used for Experiment 2 aside from replication. Experiment 2 had 6 replicates per treatment. High- (F10G2A) and low-yielding (F10B2) soils were used for the control groups: high yield soil only, high yield soil with amendments of 2% autoclaved barley substrate, low yield soil only, and low yield soil with amendments of 2% autoclaved barley substrate. The amended controls (2% barley) were used as the substrate (method of inoculant delivery) had effected plant growth in the inoculum screening trials. There were also 12 treatments of low-yielding

(F10B2) soil inoculated with various fungal isolates. Each candidate fungal isolate was tested independently at 1% inoculated barley (autoclaved prior to adding the fungal isolate), with 1% autoclaved barley substrate to match the concentrations of substrate or inoculant of the amended controls and co-inoculated treatments. Each pairwise combination of beneficial candidate fungal isolate and pathogenic candidate fungal isolate was tested at 1% of inoculant per isolate present (Table 2.4). The total concentration of inoculated or uninoculated substrate for each treatment was 2%. This experiment was repeated in its entirety three times. The harvest protocol outline in section 2.3.3 was used, except that fine root tissue sub-samples (less than 0.5 g fresh weight) were harvested and frozen at -20 °C for future molecular work.

Table 2.4: Experimental design for main growth experiment (Experiment 2). Each of the 18 treatments had 6 replicate pots, with three corn plants grown for three weeks. Inoculants followed by (B) were predicted to be beneficial, whereas (P) were putatively pathogenic.

Treatment	Soil	Inoculant 1	Inoculant 2
T1	F10G2A	N/A	N/A
T2	F10G2A	2% Barley	N/A
T3	F10B2	N/A	N/A
T4	F10B2	2% Barley	N/A
T5	F10B2	1% CM003 *Fusarium oxysporum.* (B)	1% barley
T6	F10B2	1% CM007 *Trichoderma atroviride* (B)	1% barley
T7	F10B2	1%CM018 *Penicillium janthinellum* (B)	1% barley
T8	F10B2	1%CM022 *Trichoderma koningii* (B)	1% barley
T9	F10B2	1%CM004 *Fusarium oxysporum* (P)	1% barley
T10	F10B2	1%CM019 *Setophoma terrestris* (P)	1% barley
T11	F10B2	1% CM003 *Fusarium oxysporum* (B)	1%CM004 *Fusarium oxysporum* (P)
T12	F10B2	1% CM003 *Fusarium oxysporum* (B)	1%CM019 *Setophoma terrestris* (P)
T13	F10B2	1% CM007 *Trichoderma atroviride* (B)	1%CM004 *Fusarium oxysporum* (P)
T14	F10B2	1% CM007 *Trichoderma atroviride* (B)	1%CM019 *Setophoma terrestris* (P)
T15	F10B2	1% CM018 *Penicillium janthinellum* (B)	1%CM004 *Fusarium oxysporum* (P)
T16	F10B2	1% CM018 *Penicillium janthinellum* (B)	1%CM019 *Setophoma terrestris* (P)
T17	F10B2	1%CM022 *Trichoderma koningii* (B)	1%CM004 *Fusarium oxysporum* (P)
T18	F10B2	1%CM022 *Trichoderma koningii* (B)	1%CM019 *Setophoma terrestris* (P)

2.6 Molecular procedures

Only the roots of seedlings grown during the main growth experiment underwent molecular processing. Approximately 0.5 g of roots were taken from three replicates in a treatment and combined to create a sample composite. Each treatment in each repeated trial had 2 associated sample composites, for a total of 108 samples. Roots were chopped finely (each piece less than 2 mm), and 0.17 g was placed in a bead beating tube for DNA isolation using Norgen Biotek Soil DNA Isolation Plus Kit (Norgen Biotek Corporation) following the manufacturer's instructions with slight modification, using a FastPrep®-24 bead beating system (MP Biomedicals™). One negative control was used (sterile molecular grade water) and two positive controls in PCRs used DNA from *Agaricus bisporus* and *Saccharomyces cerevisiae*. All DNA extraction products were stored at -20 °C. Concentrations of the extracted DNA were measured using the SpectraMax QuickDrop Micro-Volume Spectrophotometer (Molecular Devices). Extracted DNA with suitable concentration and absorbance values were PCR-amplified using a T100™ - Thermal Cycler (Bio-Rad Laboratories) with the following cycle: lid temperature of 105°C with. 94 °C for 2 minutes, 30 cycles of 94 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 30 seconds, and after cycling, holding at 4 °C. Each PCR tube was filled with 13 µL of 2 × ToughMix (Quanta Biosciences - VWR International), 1 µL of forward primer, 1 µL of reverse primer, template DNA (ranging from 1 µL to 4 µL), and the remaining volume of molecular grade water bringing the total volume to 25 µL. Root-extracted DNA samples were amplified using fungal primers 5.8S-Fun (5′-AACTTTYRRCAAYGGATCWCT-3′) and ITS4-Fun (5′-CCTCCGCTTATTGATATGCTTAART-3′) that amplify the internal transcribed spacer (ITS2) region of nuclear ribosomal DNA (Taylor et al. 2016). PCR primers were modified for Illumina sequencing by including a forward or reverse Illumina adapter, a 4 base pair linker (NNNN), and an 8 base index barcode that allows sequences to be assigned to sample origin after multiplexing. PCR products were assessed using capillary electrophoresis in a QiAxcel Advanced (QIAGEN) with alignment markers ranging from 15 to 3000 base pairs. Positive amplification products, including the *A. bisporus* and *S.*

cerevisiae positive controls, were sent for paired-end (2x300 kit) sequencing in an Illumina MiSeq at Robarts Research Institute, located at the University of Western Ontario in London, ON.

2.7 Illumina sequencing and sequence processing

Amplicon reads received from Robarts as Illumina MiSeq FASTQ files were initially demultiplexed to separate reads by their primer pairs using a custom BASH script (Weerasuriya 2021). Demultiplexed files were quality filtered using quality plots to determine the necessary parameters to denoise the reads; low quality, and chimeric sequences were removed using DADA2 (Callahan et al. 2016). Sequences with 100% similarity after error correction were grouped together into amplicon sequence variants (ASVs). Taxonomy was assigned to ASVs using the UNITE ITS sequence database via the DADA2 pipeline (Kõljalg et al. 2020). ITS2 data were additionally filtered with a minimum relative abundance threshold of ≥1.12% in each sample to completely minimize sample bleeding using the positive control (*S. cerevisiae* and *A. bisporus*) as a guide.

2.8 Statistical analysis

2.8.1 Analysis of biometric data

Experiment 1 was analyzed using nested two-way ANOVA, Tukey post hoc testing in R (version 4.1.2) using *lme4* and *multcomp* packages. Data analysis was outputted from R and data visualization was done with Excel (Microsoft Office Suite 2016). Levels of inoculant (fungal isolate) and concentration (% of inoculant or substrate use) were tested for each trial, as nested components. For Experiment 2, principal component analysis (PCA) was done using the package *ade4* (Thioulouse et al. 2018) to determine whether

specific soil nutrient parameters or harvest biometrics were correlated in explaining the variation in the parameters among the samples. Suitable, non-correlated variables (shoot dry mass and root length) were assessed using one-way ANOVA to identify differences between treatments for each variable, with Dunnett's post hoc testing used to detect which specific treatments were significantly different from the main comparison groups. Dunnett's post-hoc testing was conducted using the *DescTools* (Signorell 2021). The data was separated into subsets of data, then a series of ANOVAs were used to reduce the risk of false positives and maintain adequate power in the tests (Greenland et al. 2016). Data analysis was outputted from R and data visualization was done with Excel (Microsoft Office Suite 2016). A preliminary analysis to explore correlated soil chemistry values was conducted using Principal Component Analysis using the *factoextra* package on R (Kassambara and Mundt 2017).

2.8.2 Mycobiome analysis

The mycobiome analysis aimed to detect any patterns or changes in mycobiome composition between the different treatments, The *phyloseq* package in R was used to create bar plots of the most abundant genera in the root mycobiome data to visualize differences in community structure between primary inoculation treatments and controls (McMurdie et al. 2013). PERMANOVA (using *adonis*) was used to identify significant differences in community composition (based on beta diversity values using Bray-Curtis dissimilarity values, determined using *vegan*) between different inoculation treatments and controls (Frey et al. 2021; Oksanen et al. 2019). The R package *microeco* was used to visualize the community composition of various treatment groups by conducting principal coordinate analysis using Bray-dissimilarities of ASVs. Additionally, *microeco* was used to conduct Mantel's tests using Pearson correlation testing each physicochemical properties against the ASV distance matrix to determine significant correlations between soil physicochemical properties and mycobiome composition (Liu et al. 2021a). Redundancy analysis was used to visualize these correlations and compositional differences between the primary inoculation treatments and control groups

(Liu et al. 2021a). The *Indicspecies* package was used to identify significant ASVs with adjusted p-values using the FDR method, based on primary inoculation treatments and soil types (De Cáceres and Legendre 2009).

3 Results

The results for experiment 1 helped identify suitable substrate, soil sources, and candidate fungal isolates using in subsequent experiments. These results are reported in Appendix I. In summary, CM003 *Fusarium oxysporum* and CM018 *Penicillium janthinellum* significantly increase seedling growth and were therefore identified as potential beneficial inocula. CM007 *Trichoderma atroviride* and CM022 *Trichoderma koningii* were selected as beneficial inoculants for subsequent experiments due to their positive effects on plant growth in previous studies (Esparza-Reynoso et al. 2021; Tripathi et al. 2021). In contrast, CM004 *Fusarium oxysporum* significantly reduced seedling growth and was predicted to be putatively pathogenic. CM019 *Setophoma terrestris* showed a slight reduction in seedling growth as compared to controls and has been identified in the literature as a known soil-borne pathogen (Yoshida 2022), so was therefore used as a putatively pathogenic isolate.

3.1 Vermiculite and fungal isolate growth experiment: Biometric Data

The results of the vermiculite and fungal isolate growth experiment identified how each isolate affected plant growth in the absence of other microbes. Principal component analysis of the vermiculite and fungal growth experiment biometric data indicated that shoot length, root dry mass, and shoot dry mass were correlated with one another (Figure 3.1). Root length did not undergo further statistical investigation as pot-bound roots impeded consistent results.

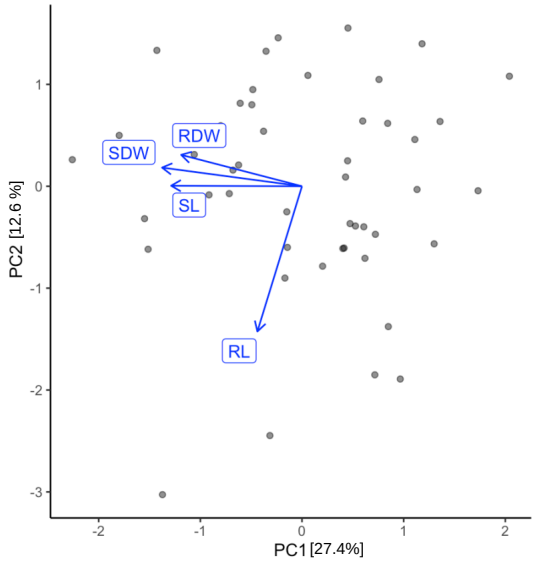

Figure 3.1: Principal component analysis of the biometric data using a correlational biplot. (SDW = shoot dry mass (g), RDW = root dry mass (g), SL = shoot length (cm), RL = root length (cm)).

Shoot dry mass of corn plants grown with *F. oxysporum* (CM003) was significantly greater than plants grown with autoclaved barley substrate but no inoculum ("amended control") or with any of the other isolates tested (Figure 3.2). Putatively pathogenic isolates *F. oxysporum* (CM004) and *S. terrestris* (CM019) did not reduce shoot dry mass compared to the amended control treatment, whereas isolates anticipated to be beneficial (*P. janthinellum* CM018 and *T. koningii* CM022) resulted in significantly reduced growth compared to the non-amended control, although not significantly compared to the amended control (Figure 3.2). Inoculation with *T. atroviride* (CM007), anticipated to be beneficial, did not affect growth significantly compared to the controls.

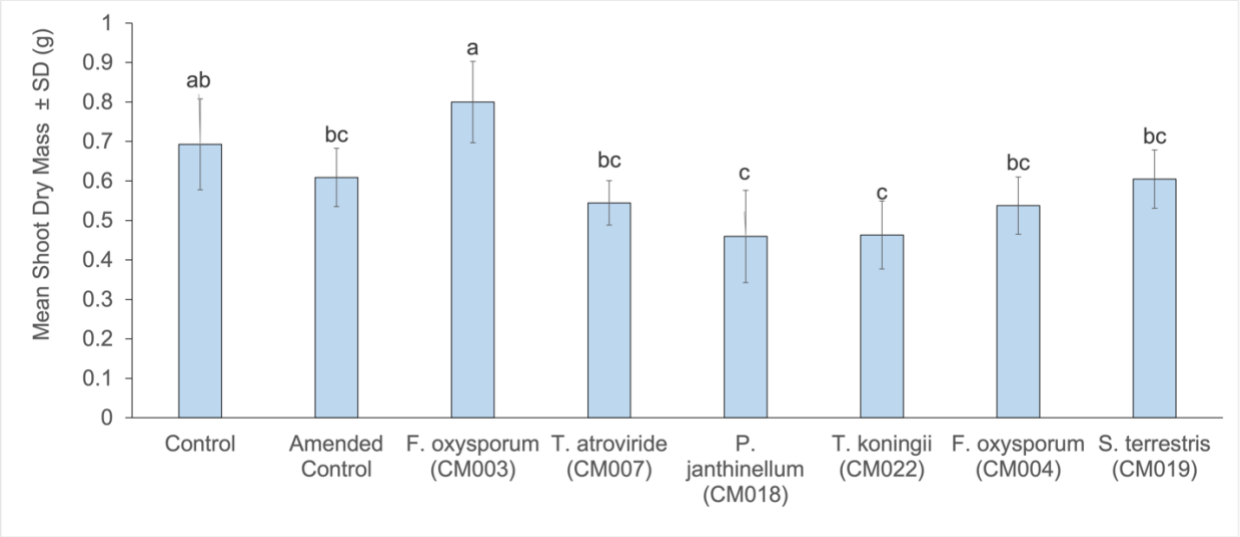

Figure 3.2: Mean shoot dry mass ± SD (g) for treatments in the vermiculite and fungal isolate growth experiment (n=6). Data were analyzed using one-way ANOVA (F [7, 40] = 9.94, $p < 0.001$), and Tukey's post-hoc testing (α=0.05). Significance is denoted by lowercase letters above each bar. If letters are different, then treatments are significantly different.

3.2 Experiment 2: Biometric Data

Principal component analysis was conducted on the biometric data (root length, shoot length, shoot dry mass, and root dry mass) from all treatment pooled together in Experiment 2, and results showed that root dry mass, shoot dry mass, and shoot length were approximately correlated with one another (Figure 3.3). Thus, root dry mass and shoot length did not undergo further statistical analyses, and root length did not indicate any significant differences between beneficial or pathogenic treatments and control groups (data not shown).

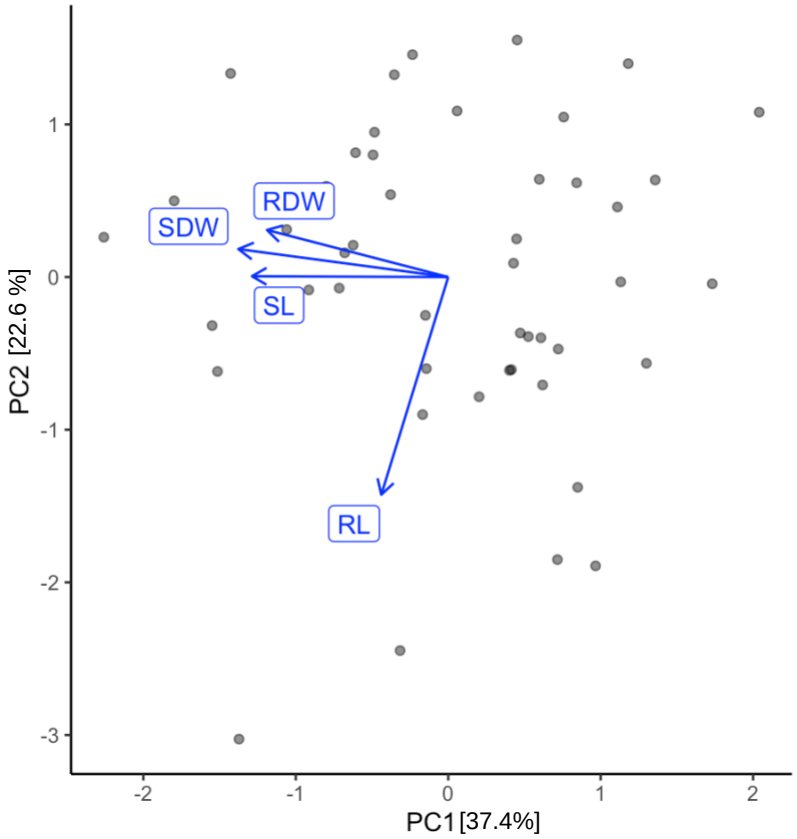

Figure 3.3: Principal component analysis of the biometric values from Experiment 2, using a correlational biplot. (SW = shoot dry mass (g), RW = root dry mass (g), SL = shoot length (cm), RL = root length (cm)).

Subsequent analysis of trial repeats/replicates were done separately as preliminary investigations of the data identified the trial repeats as a significant driver of variation across the biometric data.

3.2.1 Pathogenic Isolates

The mono-inoculated treatments involving the putatively pathogenic isolates *F. oxysporum* (CM004) and *S. terrestris* (CM019) did not result in growth reductions, as neither treatment resulted in significantly different dry mass than the amended low yield control (Figure 3.4). The shoot dry mass of seedlings grown in high yield soil only was significantly greater than seedlings grown in amended low yield soil, across all trial repeats (Figure 3.4). Seedlings grown in amended low yield soil had significantly reduced shoot dry mass compared to those grown in low yield soil only, in Trial Repeat 1.1 (Figure 3.4).

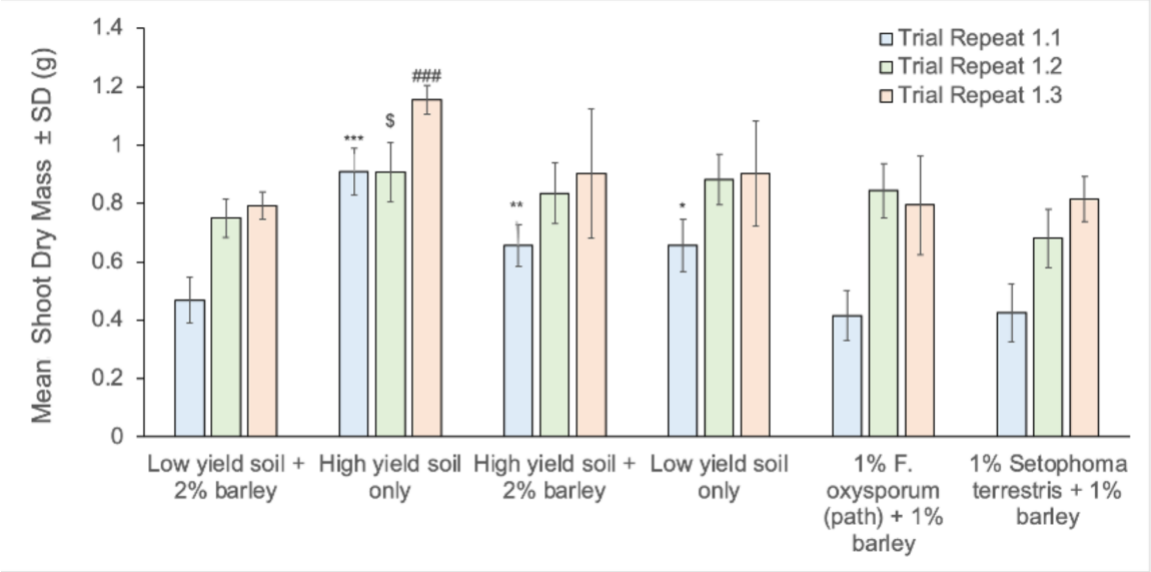

Figure 3.4: Mean shoot dry mass ± SD (g) for treatments involving all control groups and putatively pathogenic inoculants, where n=6 per bar. Each Repeat trial is depicted by different colours, with statistical significance determined within trials. Trial Repeat 1.1 is represented by blue, was analyzed via one-way ANOVA (F[5, 30] = 29.93, $p < 0.001$), and significance determined by Dunnett's post-hoc results are depicted using "*". Trial Repeat 1.2 is represented by green, was analyzed via one-way ANOVA (F[5, 27] = 3.02, $p < 0.05$), and significance determined by Dunnett's post-hoc results are depicted using "$". Trial Repeat 1.3 is represented by pink, was analyzed via one-way ANOVA (F[5, 30] = 5.81, $p < 0.001$), and significance determined by Dunnett's post-hoc results are depicted using "#". Higher significance is denoted by more symbols (*, $, #): where 1 symbol (ex. "*") $0.05 > p > 0.01$, where 2 symbols (ex. "**") $0.01 > p > 0.001$, and where 3 symbols (ex. "***") $p < 0.001$.

3.2.2 *Fusarium oxysporum* (CM003)

The anticipated beneficial isolate, *F. oxysporum* (CM003) did not significantly increase crop growth of seedlings grown in inoculated low yield soil to levels comparable to seedlings grown in high yield soil. When the mono-inoculated treatment, 1% *F. oxysporum* (CM003) + 1% barley, is the primary comparison group: 1% *F. oxysporum* (CM003) + 1% barley resulted in a significantly smaller shoot dry mass than the seedlings grown in non-amended high yield soil, in Trial Repeat 1.1 and 1.3 (Figure 3.5). Compared to low yield controls, seedlings grown in soils with 1% *F. oxysporum* (CM003) + 1% barley had significantly greater growth than those grown in amended low yield soil for Trial Repeat 1.1, but there were no significant differences in other trial Repeats (Figure 3.6). For Trial Repeat 1.1, the co-inoculation of 1% *F. oxysporum* (CM003) + 1% *S. terrestris* (CM019) resulted in a significantly greater shoot dry mass than the mono-inoculated treatment (Figure 3.5).

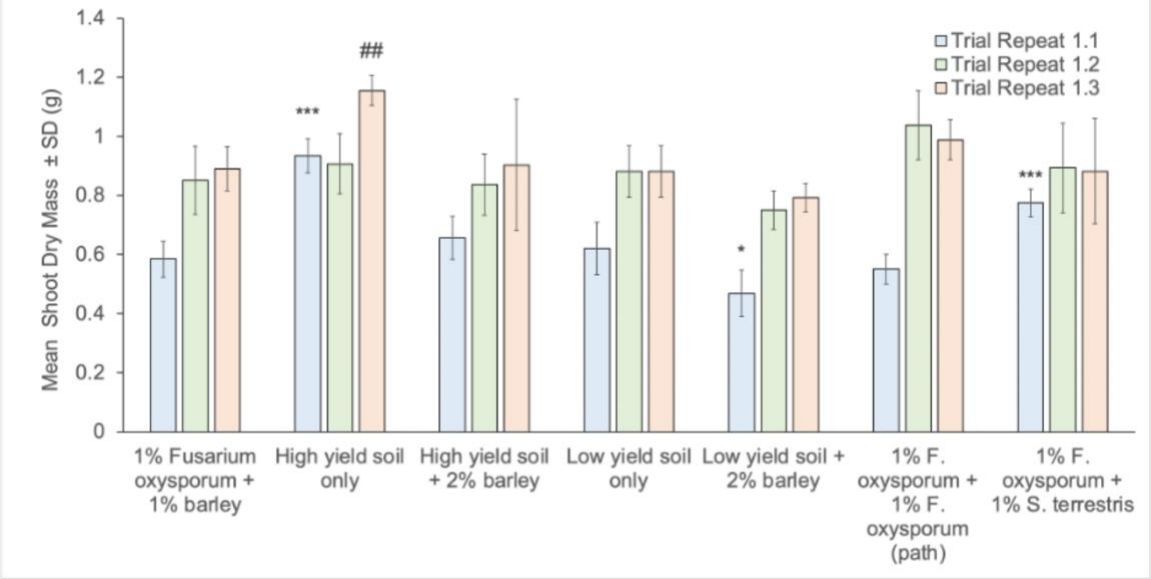

Figure 3.5: Mean shoot dry mass ± SD (g) for treatments involving *F. oxysporum* (CM003) and all control groups where n=6 per bar. Each Repeat trial is depicted by different colours, with statistical significance determined within trials. Trial Repeat 1.1 is represented by blue, was analyzed via one-way ANOVA (F [6, 35] = 26.63, $p < 0.001$), and significance determined by Dunnett's post-hoc results are depicted using "*". Trial Repeat 1.2 is represented by green, was analyzed via one-way ANOVA (F [6, 32] = 3.61, $p < 0.01$), and significance determined by Dunnett's post-hoc results are depicted using "$". Trial Repeat 1.3 is represented by pink, was analyzed via one-way ANOVA (F [6, 35] = 4.71, $p < 0.001$), and significance determined by Dunnett's post-hoc results are depicted using "#". Higher significance is denoted by more symbols (*, $, #): where 1 symbol (ex. "*") $0.05 > p > 0.01$, where 2 symbols (ex. "**") $0.01 > p > 0.001$, and where 3 symbols (ex. "***") $p < 0.001$.

3.2.3 *Trichoderma atroviride* (CM007)

The proposed beneficial isolate *T. atroviride* (CM007) did not improve seedling growth. The mono-inoculation of *T. atroviride* (CM007) significantly reduced shoot dry mass of seedlings, as compared to high yield soil only in Trial Repeat 1.1 and 1.3 (Figure 3.6). In Trial Repeat 1.1, the mono-inoculation of *T. atroviride* (CM007) resulted in a significant reduction in seedling shoot dry mass as compared to seedlings grown in amended high yield soil, low yield soil only, and seedlings grown in soil co-inoculated with 1% *T. atroviride* (CM007) + 1% *S. terrestris* (CM019). In Trial Repeat 1.2, the co-inoculated treatments with *F. oxysporum* (CM004) and *S. terrestris* (CM019) both significantly increased shoot dry mass, as compared to the mono-inoculated soil (Figure 3.6).

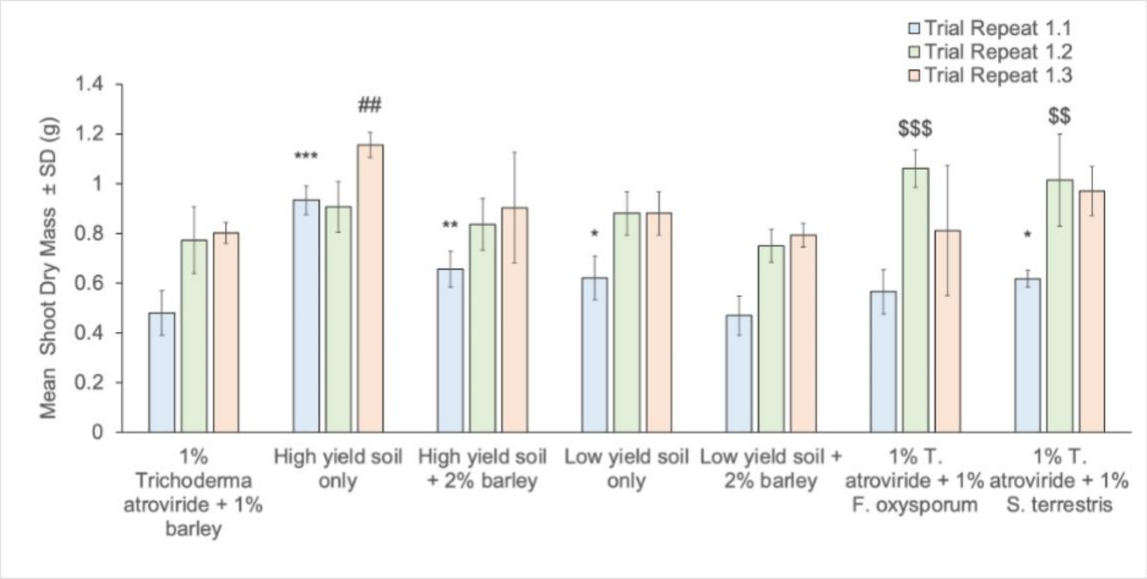

Figure 3.6: Mean shoot dry mass ± SD (g) for treatments involving *Trichoderma atroviride* (CM007) and all control groups where n=6 per bar. Each Repeat trial is depicted by different colours, with statistical significance determined within trials. Trial Repeat 1.1 is represented by blue, was analyzed via one-way ANOVA (F [6, 35] = 21.11, $p < 0.001$), and significance determined by Dunnett's post-hoc results are depicted using "*". Trial Repeat 1.2 is represented by green, was analyzed via one-way ANOVA (F [6, 33] = 5.87, $p < 0.001$), and significance determined by Dunnett's post-hoc results are depicted using "$". Trial Repeat 1.3 is represented by pink, was analyzed via one-way ANOVA (F [6, 35] = 4.35, $p < 0.01$), and significance determined by Dunnett's post-hoc results are depicted using "#". Higher significance is denoted by more symbols (*, $, #): where 1 symbol (ex. "*") $0.05 > p > 0.01$, where 2 symbols (ex. "**") $0.01 > p > 0.001$, and where 3 symbols (ex. "***") $p < 0.001$.

3.2.4 *Penicillium janthinellum* (CM018)

P. janthinellum (CM018) showed increases in plant growth in certain trial repeats. Although the mono-inoculated treatment of *P. janthinellum* (CM018) had significantly reduced shoot dry mass compared to seedlings grown in high yield soil only in Trial Repeats 1.1 and 1.3, it significantly increase growth compared to amended low yield soil in Trial Repeat 1.1 (Figure 3.7). The co-inoculation of 1% *F. oxysporum* (CM004) (path) and 1% *P. janthinellum* (CM018) resulted in a higher shoot dry mass than the mono-inoculation using *P. janthinellum* (CM018) in Trial Repeat 1.2 (Figure 3.7).

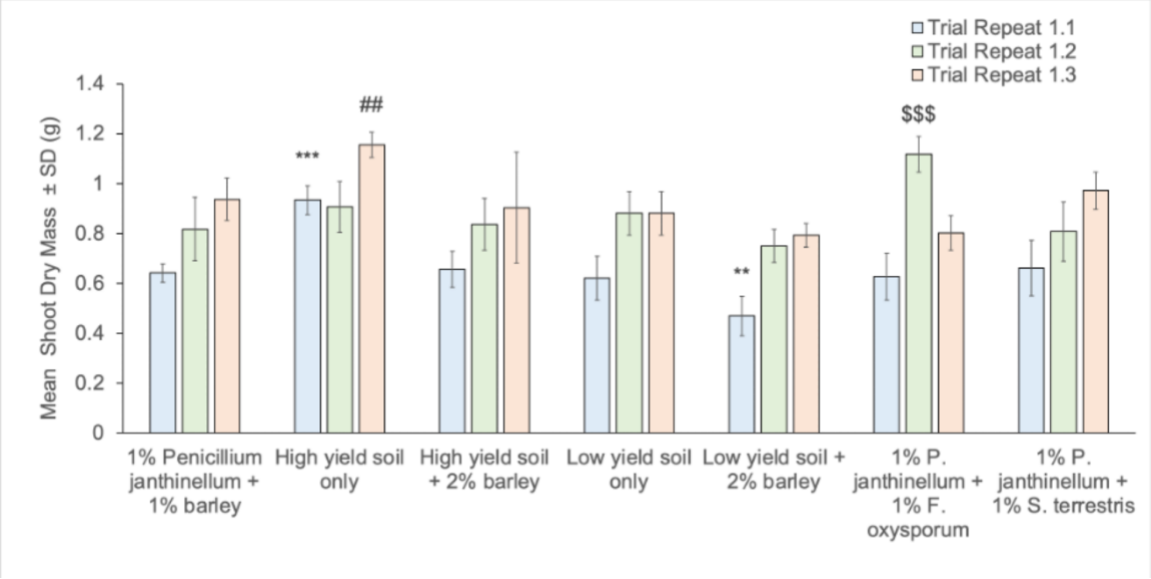

Figure 3.7: Mean shoot dry mass ± SD (g) for treatments involving *Penicillium janthinellum* (CM018) and all control groups where n=6 per bar. Each Repeat trial is depicted by different colours, with statistical significance determined within trials. Trial Repeat 1.1 is represented by blue, was analyzed via one-way ANOVA (F [6, 35] = 14.75, $p < 0.001$), and significance determined by Dunnett's post-hoc results are depicted using "*". Trial Repeat 1.2 is represented by green, was analyzed via one-way ANOVA (F [6, 32] = 7.99, $p < 0.001$), and significance determined by Dunnett's post-hoc results are depicted using "$". Trial Repeat 1.3 is represented by pink, was analyzed via one-way ANOVA (F [6, 35] = 6.45, $p < 0.001$), and significance determined by Dunnett's post-hoc results are depicted using "#". Higher significance is denoted by more symbols (*, $, #): where 1 symbol (ex. "*") $0.05 > p > 0.01$, where 2 symbols (ex. "**") $0.01 > p > 0.001$, and where 3 symbols (ex. "***") $p < 0.001$.

3.2.5 *Trichoderma koningii* (CM022)

T. koningii (CM022) was anticipated to be beneficial but did not significantly increase the crop growth of seedlings. In Trial Repeat 1.1, the mono-inoculated treatment, 1% *T. koningii* (CM022) + 1% barley, resulted in a significant reduction of shoot dry mass than

the non-amended high yield control (Figure 3.8). The mono-inoculated *T. koningii* (CM022) treatment resulted in a significantly lower mean shoot dry mass than the co-inoculation of 1% *T. koningii* (CM022) + 1% *F. oxysporum* (CM004) (path) in Trial Repeat 1.2 (Figure 3.8)

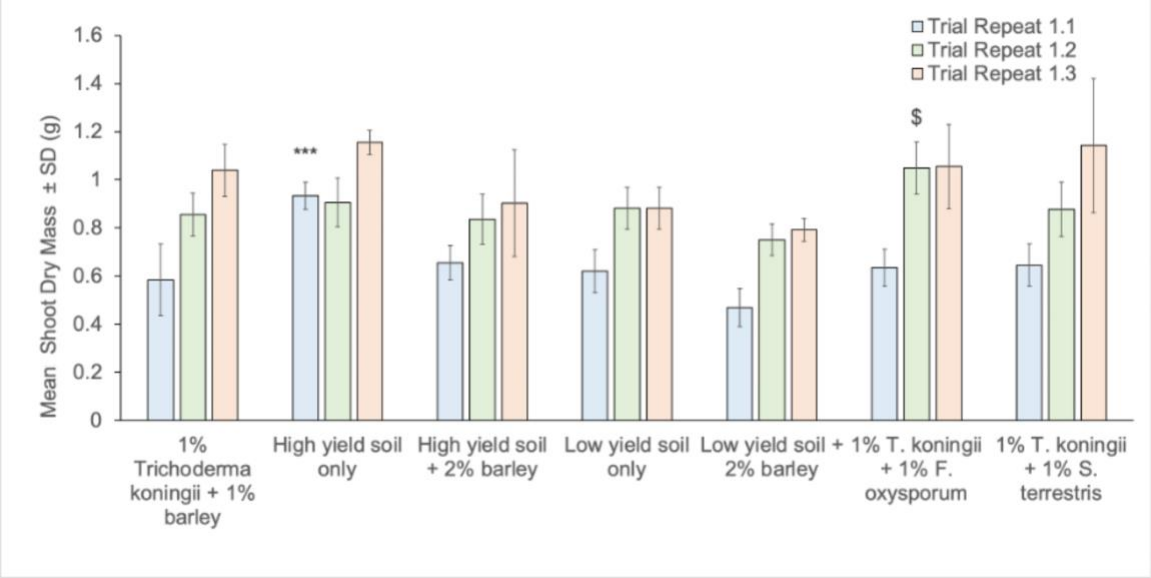

Figure 3.8: Mean shoot dry mass ± SD (g) for treatments involving *Trichoderma koningii (CM022)* and all control groups where n=6 per bar. Each Repeat trial is depicted by different colours, with statistical significance determined within trials. Trial Repeat 1.1 is represented by blue, was analyzed via one-way ANOVA (F [6, 35] = 11.90, $p < 0.001$), and significance determined by Dunnett's post-hoc results are depicted using "*". Trial Repeat 1.2 is represented by green, was analyzed via one-way ANOVA (F [6, 33] = 4.91, $p < 0.01$), and significance determined by Dunnett's post-hoc results are depicted using "$". Trial Repeat 1.3 is represented by pink, was analyzed via one-way ANOVA (F [6, 35] = 4.35, $p < 0.01$), and significance determined by Dunnett's post-hoc results are depicted using "#". Higher significance is denoted by more symbols (*, $, #): where 1 symbol (ex. "*") $0.05 > p > 0.01$, where 2 symbols (ex. "**") $0.01 > p > 0.001$, and where 3 symbols (ex. "***") $p < 0.001$.

3.3 Analysis of corn root mycobiome

The sample set was comprised of 2 root sample composites derived from 3 replicates within 18 different treatments across 3 trial repeats, resulting in a total of 108 samples (Table 3.1). The PCR amplicons from these samples and positive control samples were pooled into one Illumina MiSeq run, resulting in 7,281,536 reads (Table 3.2). After filtering out low-quality reads, all samples had 3,595,709 reads. However, after denoising and removing chimeras, there were a total of 1,476,807 reads across all treatments before threshold analysis.

Table 3.1: Root samples from three trial repeats collected from 18 identical treatments.

Trial	# of Treatments	# of Root Composites per Treatment	Total # of Samples
Repeat 1.1	18	2	36
Repeat 1.2	18	2	36
Repeat 1.3	18	2	36
Total	**54**	**6**	**108**

Table 3.2: Summary of Illumina MiSeq reads processing using quality control plugin DADA2 prior to threshold analysis. These reads were derived from 108 root samples, excluding positive control samples.

	Input Sequences	**Filtered Sequences**	**Denoised Sequences**	**Non-Chimeric Sequences**
Sum	7,281,536	3,595,709	1,517,878	1,476,807

There were no non-fungal ASVs but removal of low-quality reads, removal of zero-sum ASVs, and threshold analysis to address bleeding across samples using positive control abundances as a baseline resulted in 202 ASVs and 1,435,151 reads across all treatments (108 samples) (Table 3.3A: Appendix II). Biometric results did not result in any

subsequent patterns for co-inoculated treatments. Thus, co-inoculated treatments were removed from the mycobiome analysis, resulting in 102 ASVs and 874,253 sequence reads across 60 samples (Table 3.3B).

Table 3.3: Summary of retained ASVs and reads per phylum. A) Phylum-level classification of 202 ASVs identified from 1,435,151 retained sequence reads across all treatments. Summary of retained reads after threshold analysis and the removal of low abundance ASVs, low-quality ASVs, and non-fungal ASVs from 108 samples of corn roots from various inoculation treatments and controls. B) Kingdom and phylum-level classification of 102 ASVs identified from 874,253 retained sequence reads from primary inoculant treatments and controls only. Summary of retained reads after threshold analysis and the removal of low abundance ASVs, low-quality ASVs, and non-fungal ASVs from 60 samples of corn roots from primary inoculation treatments and controls only (T1-T10).

A)

Phylum	Retained ASVs	% of Retained ASVs	Total Reads Associated	% of Total Relative Reads
Ascomycota	123	60.89	1133648	78.99
Basidiomycota	15	7.43	23319	1.62
Glomeromycota	31	15.35	23933	1.67
Mortierellomycota	5	2.48	10812	0.75
Mucoromycota	16	7.92	177009	12.33
Olpidiomycota	4	1.98	45910	3.20
Unknown	8	3.96	20520	1.43
Total	**202**	**100**	**1435151**	**100**

B)

Phylum	Retained ASVs	% of Retained OTUs	Total Reads Associated	% of Total Relative Reads
Ascomycota	73	71.57	683,517	78.18
Basidiomycota	1	0.98	12,633	1.45
Glomeromycota	6	5.88	3891	0.45
Mortierellomycota	3	2.94	5430	0.62
Mucoromycota	12	11.76	143,576	16.42
Olpidiomycota	3	2.94	17,062	1.95
Unknown	4	3.92	8144	0.93
Total	**102**	**100**	**874,253**	**100**

Analysis of the top 10 genera and unknown genera in relation to relative abundances depicts common patterns in composition between primary inoculation treatments and low yield controls (amended and soil only); whereas high yield controls (amended and soil only) are similar to each other but differ from all other treatments involving low yielding soil (inoculated and controls) (Figure 3.9). Generally, all treatments involving low-yielding soil had high abundance of *Gibberella.* The primary inoculation isolates did not result in an increase of the isolate's genus in the top relative abundances; for example, CM007 *T. atroviride*, CM018 *P. janthinellum*, and CM022 *T. koningii* do not have their associated genus in the top 10 genera (Figure 3.9). Additionally, despite *Setophoma* being the primary inoculant isolate in CM019 *S. terrestris* treatment, other treatments have higher abundances of *Setophoma*. Primary inoculation with CM003 *F. oxysporum* or CM007 *T. atroviride*, as well as the addition of 2% barley substrate to low yield soiling increased the relative abundance of *Actinomucor* as compared to other inoculation treatments, and the non-amended control. Amendments to both the high and low yield controls seemed to change the relative abundance of some genera substantially (Figure 3.9).

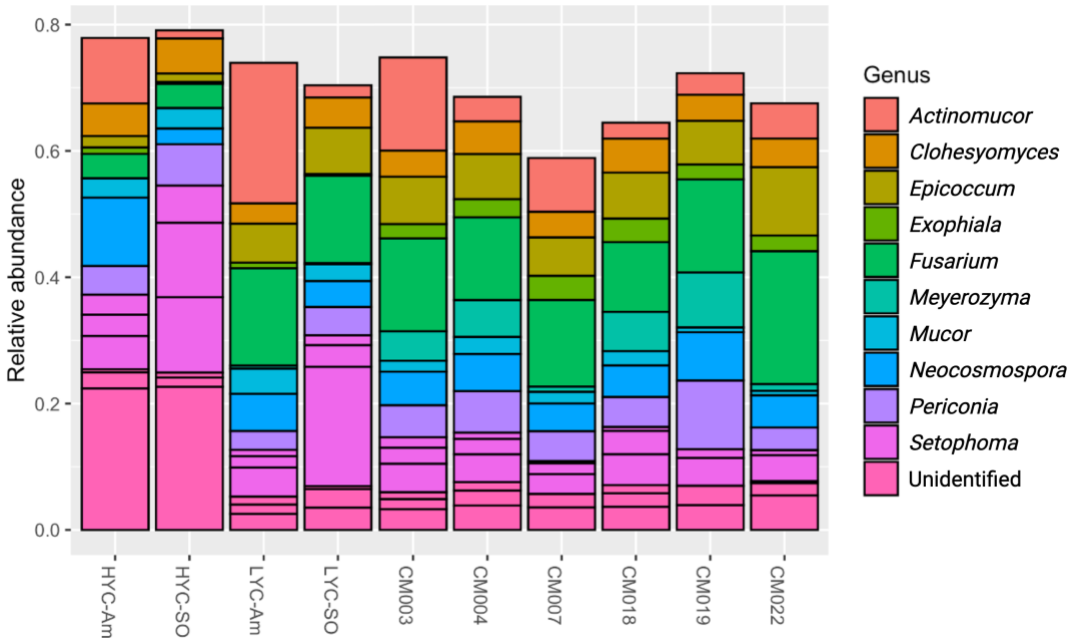

Figure 3.9: Relative abundances of genera in corn roots across the primary inoculation treatments and two control types. Analysis of the relative abundances of the top 10 most abundant genera, and the abundance of unknown genera for primary inoculation treatments or controls (n=6). The primary inoculation groups involve the following inoculants: CM003 is *Fusarium oxysporum* (beneficial strain), CM004 is *Fusarium oxysporum* (pathogenic strain), CM007 is *Trichoderma atroviride,* CM018 is *Penicillium janthinellum*, and CM019 is *Setophoma terrestris*, CM022 is *Trichoderma koningii*. Controls are outlined as: HYC-Am is high yielding control with amendment, HYC-SO is high yielding control soil only, LYC-Am is low yielding control with amendment, and LYC-SO is low yielding control soil.

Ordinations were used to visualize patterns of relationships among specific metrics, treatments, and associated ASVs. Bray-Curtis non-metric multidimensional scaling (NMDS) shows ASVs associated with lower shoot mass (<0.6g) cluster together (Figure 3.10). However, there are no other clear patterns shown across shoot dry mass values higher than 0.6 g.

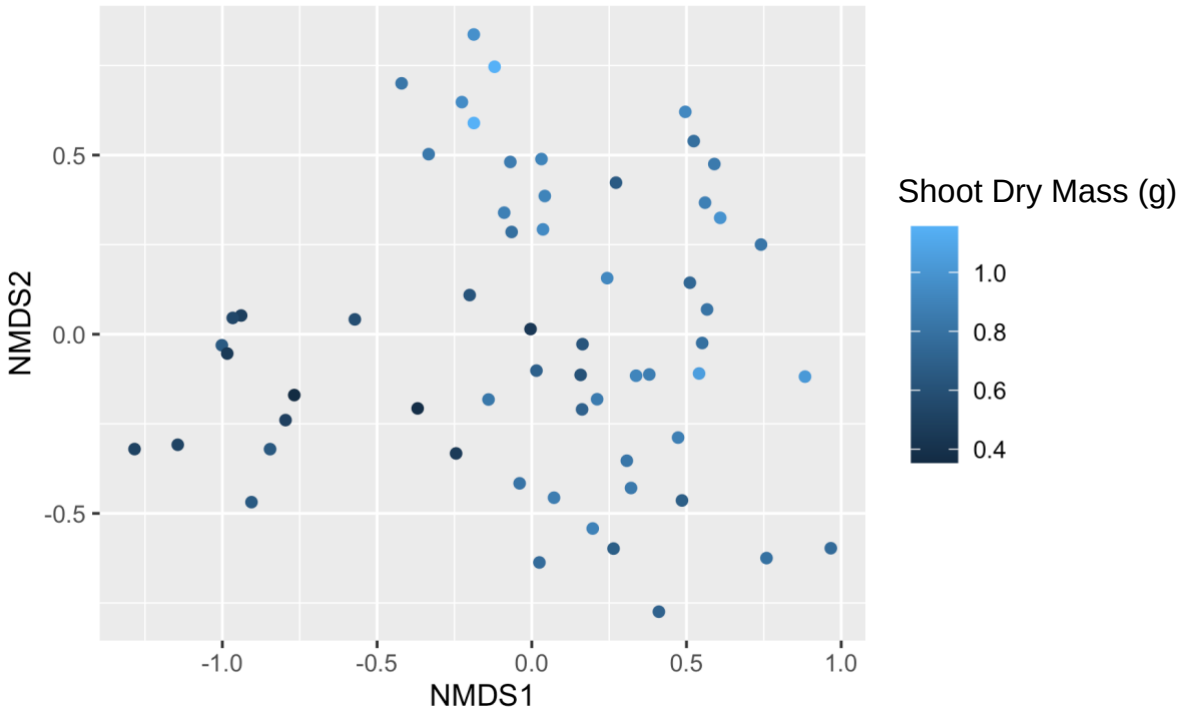

Figure 3.10: Non-metric multidimensional scaling (NMDS) plot of Bray-Curtis dissimilarity indices derived from fungal communities detected in roots of primary inoculation treatments and controls. Samples are depicted using a colour gradient representing a scale of shoot dry mass (g).

Principal coordinate analysis using Bray-Curtis dissimilarity indices between the control groups and different primary inoculation treatments depicts the fungal community in roots grown in high yield control soil only (HYC-SO) is distinct, but close in composition to the amended high yield control (HYC-Am) (Figure 3.11). Both high yield control groups are distinct from all other primary inoculation treatments, and low yield control groups. All primary inoculant groups share great overlap in their composition, with the high yield controls remaining distinct.

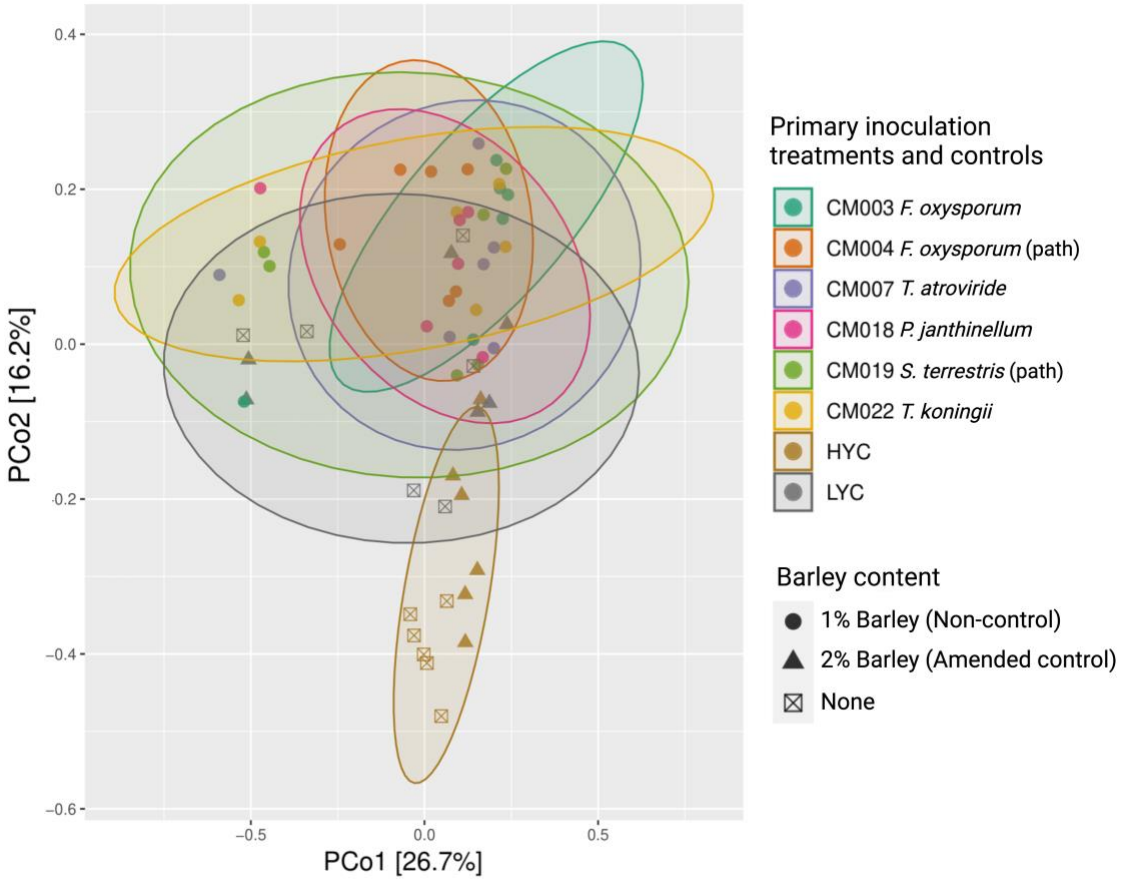

Figure 3.11: Principal coordinate analysis (PCoA) grouped by primary inoculation types and controls. There is confidence ellipse around ordination points with a confidence level of 90% for each primary inoculation treatment or control group. There were 6 replicates per treatments: CM003 is *Fusarium oxysporum* (beneficial strain), CM004 is *Fusarium oxysporum* (pathogenic strain) CM007 is *Trichoderma atroviride*, CM018 is *Penicillium janthinellum*, CM019 is *Setophoma terrestris*, CM022 is *Trichoderma koningii*, HYC is high yielding soil controls (amended and non-amended), and LYC is low yielding soil controls (amended and non-amended). Barley content refers to the additional barley substrate added to primary inoculation treatments (1_barley), or to amended controls (2_barley); or no barley added to LYC and HYC (None).

PERMANOVA (Adonis) analysis assessing the differences in fungal communities of roots grown in various inoculant and control treatments revealed that the different

treatments had significantly different fungal community composition (F [9,50] = 2.306, p = 0.001) (Table 3.4A). Pairwise comparisons of *Adonis* PERMANOVA revealed all primary inoculation treatments and the low yield control types had significantly different fungal community composition as compared to the high yield controls (amended and soil only) (p<0.05) (Table 3.4B). All primary inoculation treatments and low yield controls (amended and non-amended) do not have significantly different fungal community composition (p>0.05).

Table 3.4: PERMANOVA (Adonis) analysis of fungal communities of roots from various treatments, based on Bray-Curtis dissimilarity values. A) PERMANOVA summary statistic, with primary inoculation treatments and controls as the primary comparison group. B) Semi-matrix of pairwise combinations of PERMANOVA (Adonis) organized by different first comparisons with FDR-adjusted *p* values

A)	*df*	Sums Of Sqs	Mean Sqs	*F value*	R^2	*p*
Primary inoculation treatments and controls	9	4.055	0.451	2.306	0.293	0.001
Residuals	50	9.767	0.195	NA	0.707	NA
Total	59	13.822	NA	NA	1	NA

B)	High yield control - soil only	Amended high yield control	Low yield control - soil only	Amended low yield control	CM003 *F. oxysporum*	CM007 *T. atroviride*	CM018 *P. janthinellum*	CM022 *T. koningii*	CM004 *F. oxysporum*	CM019 *S. terrestris*
High yield control - soil only										
Amended high yield control	**0.021***									
Low yield control - soil only	**0.014***	**0.014***								
Amended low yield control	**0.014***	**0.021***	0.303							
CM003 *F. oxysporum*	**0.014***	**0.021***	0.126	0.307						
CM007 *T. atroviride*	**0.017***	**0.017***	0.303	0.364	0.574					
CM018 *P. janthinellum*	**0.014***	**0.016***	0.303	0.297	0.312	0.497				
CM022 *T. koningii*	**0.014***	**0.014***	0.312	0.396	0.361	0.668	0.693			
CM004 *F. oxysporum*	**0.014***	**0.021***	0.182	0.123	0.303	0.340	0.731	0.415		
CM019 *S. terrestris*	**0.014***	**0.014***	0.214	0.312	0.321	0.415	0.685	0.693	0.497	

Mantel's test using Pearson's correlation investigated the soil physicochemical properties correlated with the distance matrix of ASVs/fungal community composition (Table 3.5). CEC, Na, Mn, B, and Al ppm have the most significant correlation with fungal community composition ($p \leq 0.001$) (Table 3.5). The associated Redundancy Analysis (RDA) plot depicts soil physicochemical properties with $R \geq 0.1$ and/or $p \leq 0.01$ (Figure 3.12).

Table 3.5: Results of Mantel's test of physicochemical properties. Correlation values and significance of soil physicochemical properties and the distance matrix associated with fungal community composition. Abbreviations: CEC = cation exchange capacity (meg/100g), Na = sodium (ppm), Mn = manganese (ppm), B = boron (ppm), AlM_3= aluminum ppm, pH = pH, % K = % potassium saturation, % Ca = % calcium saturation, % Na = % Sodium saturation, Zn = zinc (ppm), K/Mg = potassium/magnesium (K/Mg) ratio, OM = organic matter biomass, Fe = iron (ppm), % Mg = % magnesium saturation, NO_3N = nitrate nitrogen (ppm), Cl = chlorine. Based on soil physicochemical data from Appendix IIIA.

Soil Physicochemical Property	*R*	*p*
CEC	0.140	**0.001**
Na	0.208	**0.001**
Mn	0.256	**0.001**
B	0.315	**0.001**
ALM_3	0.302	**0.001**
pH	0.159	**0.002**
% K	0.154	**0.002**
% Ca	0.201	**0.002**
% Na	0.135	**0.003**
Zn	0.101	**0.010**
K/Mg	0.110	0.012
OM	0.087	0.049
Fe	0.061	0.131
% Mg	0.037	0.239
NO_3N	0.013	0.394
Cl	-0.001	0.490

Redundancy analysis reveals that *Setophoma,* unknown genera (NA), and *Clohesyomyces* are associated with high yield control groups (non-amended and amended with 2% barley substrate) (Figure 3.12). *Rhizoctonia*, *Epicoccum*, and *Gibberella* are negatively associated with the community composition of high yield control groups and % sodium saturation and zinc are associated with the high yield control groups. In contrast, % calcium saturation, pH, and manganese are associated with primary inoculation treatments and controls involving low yielding soil. *Actinomucor* and *Meyerozyma* are associated with the composition of primary inoculant groups and low yield control groups, as well as cation exchange capacity and boron levels.

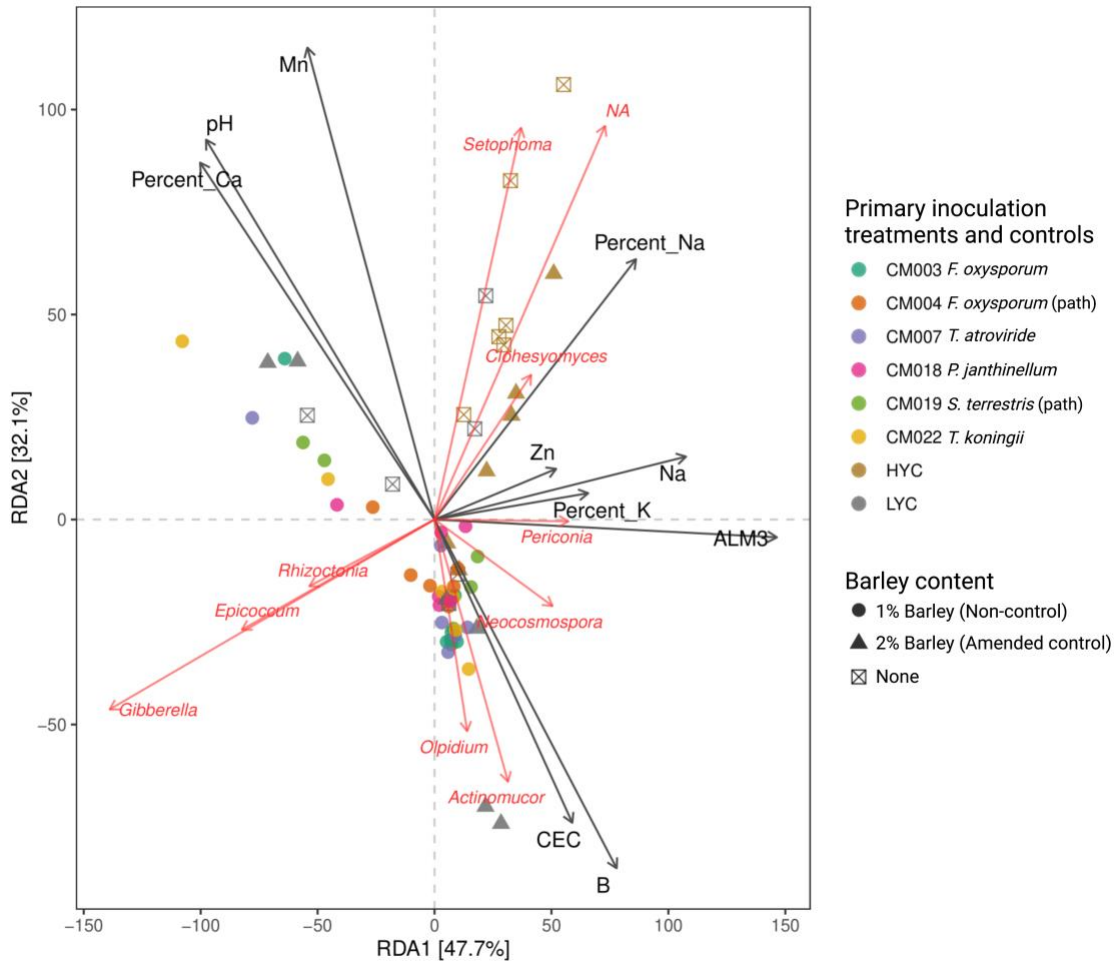

Figure 3.12: Redundancy analysis (RDA) of fungal communities by treatment, with correlated soil physicochemical factors. The most abundant genera are overlayed to show how variation in fungal community composition may be influenced by changed in the relative abundance of these taxa. There were 6 replicates per treatments; inoculants as in Fig. 3.13, abbreviations of soil physicochemical factors as in Fig. 3.9.

Indicator species analysis was used to identify ASVs that are strong predictors of the community compositions of primary inoculation treatments and controls (Table 3.6). Indicators of high yield controls (HYC-SO and HYC-Am) were *Helotiales*, *S. terrestris*, *Actinomucor elegans*, and *Ophiosphaerella* sp. Indicators of low yielding controls were

Exserohilum pedicellatum. *Actinomucor elegans*, *Ophiosphaerella* sp., and *Actinomucor elegans*. *Meyerozyma carribica* and *Exserohilum pedicellatum* are indicators of primary inoculation with CM003 *Fusarium oxysporum*, CM018 *P. janthinellum*, CM004 *Fusarium oxysporum*, and CM019 *S. terrestris*. A shared indicator species of inoculation with CM007 *T. atroviride* or CM022 *T. koningii* is *Exserohilum pedicellatum* (Table 3.6).

Table 3.6: Indicator species analysis of primary inoculant treatments and controls. Significant ASVs ($p<0.05$) depicted based on the FDR-adjusted *p*-value. IndVal is the indicator value of the species in parts per unit. *P*-values are based on 9999 permutations and adjusted using the FDR method. A 0 indicates that the ASV was not an indicator species, whereas a 1 indicates the ASV was an indicator species within that particular treatment.

ASV	Taxonomic ID	HYC-SO	HYC-Am	LYC-SO	LYC-Am	CM003	CM007	CM018	CM022	CM004	CM019	IndVal	*p*
ASV_3	*Meyerozyma carribica*	0	0	0	0	1	0	1	0	1	1	0.91	0.02
ASV_5	*Helotiales*	1	1	0	0	0	0	0	0	0	0	0.94	0.02
ASV_16	*Exserohilum pedicellatum*	0	0	1	0	1	1	1	1	1	1	0.86	0.02
ASV_17	*Setophoma terrestris*	1	1	0	0	0	0	0	0	0	0	0.88	0.02
ASV_26	*Actinomucor elegans*	0	1	0	1	1	1	0	0	0	0	0.82	0.02
ASV_27	*Ophiosphaerella sp*	1	0	1	0	0	1	0	0	0	0	0.73	0.02
ASV_49	*Actinomucor elegans*	0	0	0	1	1	0	0	0	0	0	0.71	0.04

4 Discussion

4.1 Impacts of inoculants on corn seedling growth

Initial inoculum screening trials (experiment 1; Appendix I) revealed some putatively beneficial and two pathogenic fungal isolates to inform the design of the main growth experiment (experiment 2). However, despite some isolates resulting in significantly higher shoot dry mass as compared to the low yield controls in the main growth experiment, none of the isolates improved plant performance to that of the high yield controls. If there is a protective mycobiome in high yield controls, it may be more complex than the presence of one particular, beneficial fungal species. Additionally, the duration of this experiment may not have been long enough for chosen inoculants to effectively colonize the roots.

4.1.1 Putative pathogens

There was no evidence of pathogenic effects or signs of disease on corn seedlings inoculated with CM004 *F. oxysporum* and CM019 *S. terrestris* in the vermiculite growth experiment or main growth experiment, despite evidence of growth reduction in experiment 1. Additionally, there were no clear signs of disease at any growth stage throughout experiment 2. This may be an indication that the conditions and duration of the main growth experiment were insufficient for the infection of corn seedlings by the putatively pathogenic inoculants.

4.1.1.1 *Fusarium oxysporum* (CM004)

CM004 *Fusarium oxysporum* (pathogenic) resulted in a significant reduction in corn growth at 1% concentration within the soil during the inoculum screening trials. However, there was no evidence of corn growth reduction evident in the subsequent experiments. Pathogenic strains of *F. oxysporum* produce jasmonic acids, a fungal toxin, using the lipoxygenase enzyme related to that in plants (Pusztahelyi et al. 2015). Bakker

and colleagues (2016) assessed the effect of fungal pathogens isolated from cereal rye on corn seedling growth by growing corn seedlings in inoculated medium with *F. oxysporum* (Foxy12). Koch's postulates were completed and showed corn seedlings grown in the inoculated medium had necrotic lesions that often killed the root tips. However, these lesions were limited to the root tissue that came into direct contact with or grew through the inoculum. Additionally, the root length was not significantly reduced as compared to controls. Thus, the progression of disease caused by *F. oxysporum* (Foxy12) was limited to areas of direct contact (Bakker et al. 2016). A similar phenomenon may have occurred in the main growth experiment, where root tissue did not make effective contact with the inoculated barley grain to induce infection.

4.1.1.2 *Setophoma terrestris* (CM019)

Setophoma terrestris is a known pathogen of corn but during the inoculum screening trials, there were no apparent reductions in above-ground growth of corn seedlings sown in soils inoculated with CM019 *Setophoma terrestris*. However, the below-ground dry mass was reduced (Appendix I). Kandasamy and colleagues (2021) found sequences identified as *S. terrestris* in similar abundances in high or low yielding sites within F10. Across all sites, over 30% of relative abundance belonged to *S. terrestris* or *Chalara fungorum* (Kandasamy et al. 2021). Thus, it's unsurprising that *S. terrestris* appears across many root samples within the main growth trials. *S. terrestris* causes pink root rot in many crops, but its virulence and presence are exacerbated by the soil depth, monoculture, and host (Yoshida 2022). Additionally, the severity of infection caused by *S. terrestris* in the roots of inoculated onion seedlings was inconsistent between individuals of the same variety and stage of growth (Yoshida 2022). The isolate may be pathogenic in some instances but did not have ideal conditions for diseases induction in the main growth experiment indicated by no reduction in growth. Putatively pathogenic fungi found predominantly in high yielding controls may also be non-pathogenic strains of known fungal pathogens (Chulze et al. 2015) or have alternative ecological roles that are yet to be explored. Additionally, it has been documented that the biotic and abiotic conditions within the soil can change the ecological role of isolates of this species (Minerdi et al. 2011).

4.1.2 Putatively Beneficial isolates

Beneficial isolates were selected based on significant increases in growth of corn seedlings as compared to low yield controls, or reference to the current literature. In the inoculum screening trials, CM003 *Fusarium oxysporum* (beneficial) and CM018 *Penicillium janthinellum* resulted in significant improvements of corn seedling growth as compared to low yield controls. Whereas CM007 *Trichoderma atroviride* and CM022 *Trichoderma koningii* were selected based on the documented use of this genus as either growth-promoters or biological control agents (Baldi et al. 2016; Whipps et al. 1988; Lewis et al. 1990). In successive experiments, a majority of putatively beneficial isolates failed to increase corn seedling growth to be comparable to seedlings grown in high-yielding soil, with the exception of growth promotion of corn seedlings inoculated with CM003 *F. oxysporum* (beneficial) in the vermiculite growth experiment.

4.1.2.1 *Fusarium oxysporum* (CM003)

Fusarium oxysporum was significantly associated with high yielding sites in cornfields and occurred in lower abundances in low yielding sites (Kandasamy et al. 2021). The agricultural benefit of non-pathogenic *F. oxysporum* lies within its potential to reduce pathogen pressure. Non-pathogenic strains of *F. oxysporum* have been shown to control pathogens using antagonism (Alabouvette and Olivain 2002). Bolwerk and others (2005) demonstrated *F. oxysporum* F047 can control the soilborne pathogen *F. oxysporum* f. sp. *radicis-lycopersici*, which causes tomato foot and root rot. However, my results did not indicate any evidence of pathogen reduction with CM003 *Fusarium oxysporum* (putative beneficial). The presence of both pathogenic and non-pathogenic strains of *F. oxysporum* can activate defense mechanisms in the host plants, where the pathogenic strain colonization is inhibited by the non-pathogenic strain, causing locally induced resistance (Alabouvette and Olivain 2002; Sajeena et al. 2020). However, CM004 *F. oxysporum* (putative pathogen) did not result in signs of disease, so the assessment of the co-inoculation with CM003 *Fusarium oxysporum* (putative beneficial) and CM004 *F. oxysporum* (putative pathogen) cannot be related to this phenomenon.

The life mode and ecological role of *F. oxysporum* can vary greatly depending on the strain, conditions, and host. A study by Minerdi and others (2011) found a non-pathogenic strain of *F. oxysporum* (strain MSA 35) exhibits antagonistic activity towards pathogenic *F. oxysporum* isolates and creates volatiles, such as beta-caryophyllene, which stimulate plant growth. The WT strain of MSA 35 *F. oxysporum* works in association with ecto-symbiotic bacteria. In the absence of the bacteria, the cured (CU) form of MSA 35 *F. oxysporum* is pathogenic, causes wilt symptoms, and has reduced production of beneficial volatiles typically produced by the wildtype (Minerdi et al. 2011). The environmental conditions and microbiota of F10B2 (low yielding soil from main growth experiment) may not have stimulated a positive effect from *F. oxysporum* CM003 (putative beneficial), whereas the conditions of F10B1 (low yielding soil from inoculum screen trials) did.

4.1.2.2 *Trichoderma atroviride (CM007)*

Trichoderma atroviride in agricultural soils has been documented to aid in disease suppression and plant performance. *T. atroviride* is a known mycoparasite, and therefore biological control agent to pathogens such as *Cronartium ribicola* (Li at al. 2014). *T. atroviride* produces hydrolytic enzymes, such as chitinase and proteases, to degrade the cell wall of other pathogens (Li et al. 2022; Simkovic et al. 2011). Additionally, *T. atroviride* has been shown to aid in healthy soil composition, and plant growth promotion. Longa and others (2009) found that a long and stable persistence of *T. atroviride* SC1 under field conditions resulted in higher abundances of beneficial fungi and lower incidence of disease. This may be due to secondary compounds emitted by the fungus. Esparza-Reynosos and colleagues (2020) found *T. atroviride*-emitted volatiles applied to *Arabidopsis* seedlings increased endogenous sugar levels in shoots, roots, and roots exudates, which improved root growth and branching, and strengthened the symbiosis in vitro (Esparza-Reynoso et al. 2021). Despite the agriculture benefits outlined in the literature, CM007 *T. atroviride* did not show any growth promotion in the main growth experiment.

4.1.2.3 *Trichoderma koningii* (CM022)

Trichoderma koningii has been shown to assist in virus suppression and stress tolerance in many crops, but inoculation with *T. koningii* (CM022) did not result in any growth promotion during the main growth experiment or the vermiculite growth experiment. In a study by Taha and others (2021), 6-pentyl-α-pyrone (6PP) isolates from *T. koningii* induced systemic resistance to the tobacco mosaic virus when applied to tobacco plants. Tripathi et al. (2021) found that *T. koningii* treated tomato plants had growth promotion under heat stress through the increased production of antioxidants. *T. koningii's* predominance in high yielding sites may have contributed to a protective mycobiome for corn in these regions (Kandasamy et al. 2021). In contrary to these documented benefits, Harris (1999) investigated the ability of two isolates of *T. koningii* to act as biological control agents for damping-off diseases in seedlings of *Capsicum annum* under greenhouse conditions. Isolates of *T. koningii* did reduce seedling death caused by *Rhizoctonia solani* but did not consistently prevent seedling death caused by *Pythium ultimum* var. *sporangiiferum*. Harris (1999) concluded the use of fungicides more effective in controlling damping-off diseases. Similarly, CM022 *Trichoderma koningii* did not increase soil productivity of low yielding soils, apparent by the lack of seedling growth promotion in the main growth experiment.

4.1.2.4 *Penicillium janthine*llum (CM018)

Kandasamy and others (2021) found *P. janthinellum* was associated with high yield sites in farm 10. In a study by Khan and others (2013), the application of *P. janthinellum* LK5 increased the shoot length of abscisic acid (ABA)-deficient tomato mutants under salinity stress. Some fungi only elicit benefits to crops in stressful conditions. Thus, *P. janthinellum*-stimulated growth promotion of crops might only occur during stress events, which were not emulated in a controlled environment such as my growth chamber experiments. Thus, CM018 *P. janthinellum* did not show any growth promotion to corn seedlings in the main growth experiment, despite increasing seedling growth that of seedlings grown in high yield soils during the inoculum screening trials. There were no ASVs identified as *Penicillium* in the main experiment, thus the fungus did not

effectively colonize any of the roots of corn. This may be due to insufficient time for root colonization. Khan and others (2013) saw results of seed inoculation using *P. janthinellum,* when tomato plants were grown for 3 weeks. In another study by Ikram and others (2018), wheat plants were inoculated with another *Penicillium* species, *P. roqueforti,* and grown for 4 weeks. Thus, more time may be needed to allow for root colonization of *P. janthinellum* when applied as a soil inoculant.

4.2 Soil heterogeneity

Soil heterogeneity is a challenge for precise crop management. Similarly, it can induce problems with treatment consistency with growth experiments using field soil. Aerial field maps, conventional soil analysis, and censoring technology are costly and often inadequate when trying to incorporate soil heterogeneity analysis into field management practices (Patzold et al. 2008). In-field soil heterogeneity results in inconsistent yield and crop quality within the same field (Habib-ur-Rahman et al. 2022). Heterogeneity in soil can affect plant productivity, pest abundances, and pathogen pressure (Dordas 2008; Patzold et al 2008; Veresoglou et al. 2013). Farming practices such as tillage, fertilization, and proper irrigation can mitigate the effects of soil heterogeneity (Patzold et al. 2008). Processes to create more uniform soils can be adapted to controlled growth experiments by ensuring adequate breakdown of large clods of clay, mixing, and sieving. Soils within this study were sieved and mixed, however the soil composition and textural profile of the low yield control soil (F10B2) may have resulted in heterogeneous soil conditions within the main growth experiment.

4.3 Soil physicochemical properties and productivity

Sodium content and % Na saturation were correlated with the high yielding controls. Na^+ (sodium) is essential for C_4 plants, where plants require trace amounts of Na^+ for the essential uptake of pyruvate into chloroplasts by using a Na^+-pyruvate co-transporter (Furumoto et al. 2011). High salinity (Na ppm) can impair plant growth and photosynthesis due to impaired cell wall anatomy, stomatal closure, and reduced root nutrient uptake (Cocozza et al. 2019). However, the levels of sodium in high yielding

sites do not depict salinity stress as high yield soils resulted in increased corn seedling growth as compared to other controls and treatments.

Cation exchange capacity (CEC) and boron (B) content were correlated with ASVs associated with low yield controls and inoculated low yield soils. The application of boron has been shown to increase plant productivity by aiding in nutrient uptake (Hossain et al. 2020). Boron fertilization resulted in increased stem diameter and grain yield for corn plants regardless of the concentration used (Silva and Bosu 2020). CEC has a strong association with water vapour sorption, which can be beneficial for crops in agricultural soils (Arthur et al. 2020). Higher cation exchange capacity is associated with higher clay content (Arthur et al. 2020). In a study by Nunes and others (2021), root length was reduced in corn seedlings grown in clay soils. The low yield soil in the main growth experiments, F10B2, was found to be Clay Loam. Although high B and CEC can increase soil productivity, the texture of the soil may have limited plant growth in treatments involving low yield soils (control and inoculated).

4.4 Mycobiome composition

4.4.1 Differences between treatments

None of the primary inoculation treatments had significant differences in the root mycobiome composition as compared to the low yield controls, and some inoculation treatments did not effectively result in root colonization of the fungal isolate. The high yield controls had significantly different mycobiome composition from all other primary inoculation treatments and low yield control, likely due the differences in source soil.

4.4.2 Indicator species

Identifying species that are associated with particular habitats, conditions, or treatments can assist in assessing the differences between or among macro- and micro- habitats; such species are often referred to as indicators (Bakker 2008). Indicator species across primary inoculation treatments and control groups were identified. The indicators of high

yielding controls varied from those of low yield controls, and primary inoculation treatments.

Some of the identified indicator species of the primary inoculation treatments and low yield controls have been shown to be pathogenic. *Ophiosphaerella* sp. was found was across high yield controls, low yield controls, and primary inoculation with CM007 *T. atroviride*. *Ophiosphaerella* species are associated with spring dead leaf spot in other plants in the Poaceae family, particularly Bermuda grass (Wetzel et al. 2007). Pathogens in high-yielding control soils may be suppressed by other beneficial microbiota and thus do not reduce growth. Whereas the pathogens are not in high enough abundance to result in apparent signs of infection in low yielding soils but are frequent enough to contribute to the reduced growth. *Exserohilum pedicellatum* was an indicator all low yield controls and primary inoculation treatments, but not the high yield controls. *Exserohilum pedicellatum* has been associated with rot in corn roots, specifically, it causes mesocotyl necrosis of corn root tissue (Isakeit et al. 2007). *E. pedicellatum* may be a key pathogen in the low yielding soil and finding a biological control to mitigate its abundance may increase soil productivity. *Setophoma terrestris* was an indicator for the high yield controls only, which may be due to the presence of a non-pathogenic strain of this known pathogen.

There were non-pathogenic indicators, as well. An ASV identified as Helotiales was an indicator only found in high yield controls, so this ASV may play an important role in soil productivity which elicit positive effect exclusively in the high yield soil. The order Helotiales contains many soil saprotrophs, as well as ericoid mycorrhizal fungi and aquatic hyphomycetes (Krauss et al. 2011; Wang et al. 2006). *Meyerozyma carribica* was an indicator across all primary inoculation treatments, except those containing *Trichoderma atroviride* or *T. koningii.* Research by Bautista-Rosales and others (2013) indicates the yeast *M. carribica* (strain L6A2) acts as a biological control of phytopathogenic *Colletotrichum gloeosporioides* on mango fruits through antagonistic mechanisms such as competition, production of hydrolytic enzymes, biofilm production, and parasitism. *Meyerozyma carribica* may provide some pathogen control, but not enough to elicit positive effects across low yield treatments. *Actinomucor elegans* (ASVs

26 and 49) were found across many different treatments and controls. Nicola and others (2021) identified *Actinomucor elegans* in the association with the soil of pears. It is also a biological control for insect pests; *Actinomucor elegans* was detected in the tissue of dead chafer beetles and caused 100% mortality in experimental *Anisoplia austricaca* larvae (Karimi et al. 2015). It is difficult to extrapolate *A. elegans*' role in the corn mycobiome of this study as it was an indicator across a variety of treatments with no evident pattern in their performance.

4.5 Future considerations

Adequate time for inoculants to establish root association with the corn seedlings must be assessed. In many primary inoculation treatments, it was found that the fungal inoculants failed to colonize the root tissue. Thus, additional growth time may be needed to induce beneficial or pathogenic effects within the corn seedlings. Additionally, methods to reduce variability within treatments should be incorporated into future growth room studies designs using field soils to rule out potential confounding effects due to soil heterogeneity. Future trials using field-sourced soils should have effective systems to prevent heterogeneity. Increasing the number of replicates used for soil testing that assesses particle size and soil physicochemical properties of soil post-mixing may help prevent heterogeneity across treatment soils.

Soil collection should occur prior to the growth season because this study used soil collected in the fall, which resulted in nitrogen limitation in the soil as the season's crops had depleted the soil nitrogen level. Since soil nitrogen levels were low upon collection, the pots in the growth experiments were fertilized with nitrogen. However, nitrogen fertilization increased disease severity from increased foliar nitrogen concentrations (Veresoglou et al. 2013). Although nitrogen concentrations during fertilizing was relatively low (25ppm per soil weight), this could have muted beneficial mycobiome species by increasing pathogen load. Assessing the effect different nitrogen concentrations have on fungal persistence in growth room conditions can help rule out any confounding effects from fertilizing if soil must be collected post-crop maturation.

I selected fungal isolates with high relative abundances from fungal communities associated with high productivity sites investigated by Kandasamy et al (2021). In contrast, Xiong and colleagues (2021) investigated whether abundant taxa (in high quantities and often widespread) or rare taxa (lower abundances and generally habitat-specific) were more influential on ecosystem functions. They found rare taxa to be associated with crop yield, soil C and N cycling, and other soil enzymatic functions, as compared to abundant taxa (Xiong et al. 2021). Investigation of rare taxa within high productivity soils could reveal other potential isolates to use as inoculum in low yielding soils. However, this was not possible within this study as threshold analysis was conducted to address barcode bleeding across NGS samples, which eliminated rare ASVs (< 1.1% relative abundance).

The main growth experiments investigated inoculation using specific fungal isolates and co-inoculation of beneficial strains and pathogenic strains. In a model created by Xu and Jeger (2013), biological control efficacy was tested using two mycoparasitic biological control agents (BCAs), two competitive BCAs, and a mycoparasitic and a competitive BCA with heterogenous conditions with foliar pathogen infections. The model revealed two competitive BCAs or a combination of mycoparasitic and competitive BCAs had high biocontrol efficacy (Xu and Jeger 2013). This model indicates a promising potential to increase biocontrol efficacy by synergism when using combinations of two BCAs in heterogenous conditions. Future studies focusing on co-inoculation with two or more beneficial strains, where mechanisms of biological control are predetermined, may identify strategies of mycobiome engineering that involve synergism of multiple BCAs to boost biological control efficiency.

4.6 Conclusions

This study aimed to identify one or more fungal isolates that would improve plant performance for seedlings sown in inoculated low yield soil, and measured shifts in the mycobiome composition that were induced by inoculation with several putatively beneficial or harmful inoculants. The initial predictions of this study were not supported, due to other factors such as time required for effective root colonization, and soil

heterogeneity. Although there were promising effects on plant performance in some inoculum screening experiments, the results of main growth experiment and vermiculite trial do not identify a fungal isolate that improved plant performance of corn seedlings grown in low yielding soil to that of seedlings grown in high yielding soil. The high yield control soils had significantly different mycobiome composition than all other treatments, likely due to the differences in source soil. However, none of the primary inoculation treatments resulted in significantly changes in mycobiome composition of low yield soils. An experimental design that controls for the limiting factors identified in this thesis may allow for higher efficacy of these inoculation treatments in the future. Indicator species and correlated physicochemical properties may reveal important characters to explore when predicting soil health and productivity. The results of this study can help inform best practices for study design in future investigations involving mycobiome engineering and effective inoculation methods in growth room trials.

5 References

Al-Kaisi, M.M., Douelle, A., and Kwaw-Mensah, D. 2014. Soil microaggregate and macroaggregate decay over time and soil carbon change as influenced by different tillage systems. Journal of Soil and Water Conservation, **69**(6): 574–580. https://doi.org/10.2489/jswc.69.6.574.

Al-Kaisi, M.M., Lal, R., Olson, K.R., and Lowery, B. 2017. Fundamentals and functions of soil environment. *In* Soil Health and Intensification of Agroecosystems. Elsevier Inc. pp. 1–23. doi:10.1016/B978-0-12-805317-1.00001-4.

Alabouvette, C., and Olivain, C. 2002. Modes of action of non-pathogenic strains of *Fusarium oxysporum* in controlling Fusarium wilts. Plant Protection Science, **38**: 195–199.

Allen, S.E. (ed.). 1974. Chemical Analysis of Ecological Materials. Blackwell Scientific Publications, Oxford. pp. 252.

Almario, J., Jeena, G., Wunder, J., Langen, G., Zuccaro, A., Coupland, G., and Bucher, M. 2017. Root-associated fungal microbiota of nonmycorrhizal *Arabis alpina* and its contribution to plant phosphorus nutrition. Proceedings of the National Academy of Sciences (USA), **114**(44): E9403–E9412. https://doi.org/10.1073/pnas.1710455114.

Anderson, J.M., and Ingram, J.S.I. (ed.) 1993. Chemical analyses. *In* Tropical Soil Biology and Fertility: A Handbook of Methods. *2nd edition*. C.A.B. International. pp. 57–93.

Arthur, E., Tuller, M., Moldrup, P., & Jonge, L. W. 2020. Clay content and mineralogy, organic carbon and cation exchange capacity affect water vapour sorption hysteresis of soil. European Journal of Soil Science, **71**(2): 204–214. https://doi.org/10.1111/ejss.12853.

Babu, A.G., Shim, J., Bang, K.S., Shea, P.J., and Oh, B.T. 2014. *Trichoderma virens* PDR-28: A heavy metal-tolerant and plant growth-promoting fungus for remediation and bioenergy crop production on mine tailing soil. Journal of Environmental Management, **132**: 129–134. doi:10.1016/j.jenvman.2013.10.009.

Baird, R.B. 2017. Standard methods for the examination of water and wastewater. *23rd Edition*. American Water Works Association.

Bakker, J. 2008. Increasing the utility of Indicator Species Analysis. Journal of Applied Ecology, **45**: 1829-1835. doi:10.1111/j.1365-2664.2008.01571.x

Bakker, M.G., Acharya, J. Moorman, T.B., Robertson, A.E., and Kaspar, T.C. 2016. The potential for cereal rye cover crops to host corn seedling pathogens. Phytopathology, **106**(6): 591-601. https://doi.org/10.1094/PHYTO-09-15-0214-R.

Baldi, E., Amadei, P., Pelliconi, F., and Tosell, M. 2016. Use of *Trichoderma* spp. and arbuscular mycorrhizal fungi to increase soil beneficial population of bacteria in a nectarine commercial orchard: effect on root growth, nutrient acquisition and replanting disease. Journal of Plant Nutrition, **39**(8): 1147–1155. doi:10.1080/01904167.2015.1109111.

Bautista-Rosales, P.U., Calderon-Santoyo, M., Servín-Villegas, R., Ochoa-Álvarez, N.A., Arturo, Ragazzo-Sánchez, J.A. 2013. Action mechanisms of the yeast *Meyerozyma caribbica* for the control of the phytopathogen *Colletotrichum gloeosporioides* in mangoes. Biological Control, **65**(3): 293-301. https://doi.org/10.1016/j.biocontrol.2013.03.010

Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M.C.C., Charles, T., Chen, X., Cocolin, L., Eversole, K., Corral, G.H., Kazou, M., Kinkel, L., Lange, L., Lima, N., Loy, A., Macklin, J.A., Maguin, E., Mauchline, T., McClure, R., Mitter, B., Ryan, M., Sarand, I., Smidt, H., Schelkle, B., Roume, H., Kiran, G.S., Selvin, J., Souza, R.S.C. de, van Overbeek, L., Singh, B.K., Wagner, M., Walsh, A., Sessitsch, A., and Schloter, M. 2020. Microbiome definition re-visited: old concepts and new challenges. Microbiome, **8**(103): 1-22. doi:10.1186/s40168-020-00875-0.

Bolwerk, A., Lagopodi, A.L., Lugtenberg, B.J.J., and Bloemberg, G.V. 2005. Visualization of interactions between a pathogenic and a beneficial *Fusarium* strain during biocontrol of tomato foot and root rot. Molecular Plant-Microbe Interactions, **18**(7): 710–721. doi:10.1094/MPMI-18-0710.

Brady, M.V., Hedlund, K., Cong, R.G., Hemerik, L., Hotes, S., Machado, S., Mattsson, L., Schulz, E., and Thomsen, I.K. 2015. Valuing supporting soil ecosystem services in agriculture: a natural capital approach. American Society of Agronomy Journal, **107**(5): 1809–1821. doi:10.2134/agronj14.0597.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., and Holmes, S.P. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods, **13**(7): 581–583. doi:10.1038/nmeth.3869.

Cao, T., Fang, Y., Chen, Y., Kong, X., Yang, J., Alharbi, H., Kuzyakov, Y., and Tian, X. 2022. Synergy of saprotrophs with mycorrhiza for litter decomposition and hotspot formation depends on nutrient availability in the rhizosphere. Geoderma, **410**: 115662. doi:10.1016/j.geoderma.2021.115662.

Chibucos, M.C., and Tyler, B.M. 2009. Common themes in nutrient acquisition by plant symbiotic microbes, described by the gene ontology. BMC Microbiology, **9**(Suppl. 1): S6. doi:10.1186/1471-2180-9-S1-S6.

Chulze, S.N., Palazzini, J.M., Torres, A. M., Barros, G., Ponsone, M.L., Geisen, R., Schmidt-Heydt, M., and & Köhl, J. 2015. Biological control as a strategy to reduce the impact of mycotoxins in peanuts, grapes and cereals in Argentina. Food Additives & Contaminants, **32**(4): 471-479. DOI:10.1080/19440049.2014.984245.

Cocozza, C., Brilli, F., Miozzi, L., Pignattelli, S., Rotunno, S., Brunetti, C., Ciordano, C., Pollastri, S., Centritto, M., Accotto, G.P., Tognetti, R., and Loreto, F. 2019. Impact of high or low levels of phosphorus and high sodium in soils on productivity and stress tolerance of *Arundo donax* plants. Plant Science, **289**:110260–110260. https://doi.org/10.1016/j.plantsci.2019.110260.

Costa, O.Y.A., Raaijmakers, J.M., and Kuramae, E.E. 2018. Microbial extracellular polymeric substances: ecological function and impact on soil aggregation. Frontiers in Microbiology, **9**: 1636. doi:10.3389/fmicb.2018.01636.

De Cáceres, M., and Legendre, P. 2009. Associations between species and groups of sites: indices and statistical inference. Ecology, **90**(12): 3566–3574. doi:10.1890/08-1823.1.

Dordas, C. (2008). Role of nutrients in controlling plant diseases in sustainable agriculture. A review. Agronomy for Sustainable Development, **28**: 33–46. doi: 10.1051/agro:2007051.

Enebe, M.C., and Babalola, O.O. 2019. The impact of microbes in the orchestration of plants' resistance to biotic stress: a disease management approach. Applied Microbiology and Biotechnology, **103**: 9–25. doi:10.1007/s00253-018-9433-3.

Esparza-Reynoso, S., Ruíz-Herrera, L.F., Pelagio-Flores, R., Macías-Rodríguez, L.I., Martínez-Trujillo, M., López-Coria, M., Sánchez-Nieto, S., Herrera-Estrella, A., and

López-Bucio, J. 2021. *Trichoderma atroviride*-emitted volatiles improve growth of *Arabidopsis* seedlings through modulation of sucrose transport and metabolism. Plant Cell and Environment, **44**(6): 1961–1976. doi:10.1111/pce.14014.

Esposito, A., Colantuono, C., Ruggieri, V., and Chiusano, M.L. 2016. Bioinformatics for agriculture in the next-generation sequencing era. Chemical and Biological Technologies in Agriculture, **3**: 9. DOI: 10.1186/s40538-016-0054-8.

Fernandes, M.L.P., Bastida, F., Jehmlich, N., Martinović, T., Větrovský, T., Baldrian, P., Delgado-Baquerizo, M., and Starke, R. 2022. Functional soil mycobiome across ecosystems. Journal of Proteomics, **252**: 104428. https://doi.org/10.1016/j.jprot.2021.104428.

Freeman, S., and Rodriguez, R.J. 1993. Genetic conversion of a fungal plant pathogen to a non-pathogenic, endophytic mutualist. Science, **260**(5104): 75–78. doi: 10.1126/science.260.5104.75.

Frey, B., Walthert, L., Perez-Mon, C., Stierli, B., Köchli, R., Dharmarajah, A., and Brunner, I. 2021. Deep soil layers of drought-exposed forests harbor poorly known bacterial and fungal communities. Frontiers in Microbiology, **12**. doi:10.3389/fmicb.2021.674160.

Furumoto, T., Yamaguchi, T., Ohshima-Ichie, Y., Nakamura, M., Tsuchida-Iwata, Y., Dhimamura, M., Ohnishi, J., Hata, S., Gowik, U., Westhoff, P., Bräutigam, A., Weber, A.P., and Katsura, I. 2011. A plastidial sodium-dependent pyruvate transporter. Nature, **478**(7368): 472–476. https://doi.org/10.1038/nature10518.

Geiger, F., Bengtsson, J., Berendse, F., Weisser, W.W., Emmerson, M., Morales, M.B., Ceryngier, P., Liira, J., Tscharntke, T., Winqvist, C., Eggers, S., Bommarco, R., Pärt, T., Bretagnolle, V., Plantegenest, M., Clement, L.W., Dennis, C., Palmer, C., Oñate, J.J., Guerrero, I., Hawro, V., Aavik, T., Thies, C., Flohre, A., Hänke, S., Fischer, C., Goedhart, P.W., and Inchausti, P. 2010. Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. Basic and Applied Ecology, **11**(2): 97–105. doi:10.1016/j.baae.2009.12.001.

Goodrich, J.K., Waters, J.L., Poole, A.C., Sutter, J.L., Koren, O., Blekhman, R., Beaumont, M., Van Treuren, W., Knight, R., Bell, J.T., Spector, T.D., Clark, A.G., and Ley, R.E. 2014. Human genetics shape the gut microbiome. Cell, **159**(4): 789–799. doi:10.1016/j.cell.2014.09.053.

Greenland, S., Senn, S.J., Rothman, K.J., Carlin, J.B., Poole, C., Goodman, S.N., and Altman, D.G. 2016. Statistical tests, p values, confidence intervals, and power: a guide to misinterpretations. European Journal of Epidemiology, **31**(4): 337–350. doi:10.1007/s10654-016-0149-3.

Gupta, V.V.S.R., and Germida, J.J. 2015. Soil aggregation: Influence on microbial biomass and implications for biological processes. Soil Biology and Biochemistry, **80**: A3–A9. doi:10.1016/j.soilbio.2014.09.002.

Habib-ur-Rahman, M., Raza, A., Ahrends, H.E., Huging, H., and Gaiser, T. 2022. Impact of in-field soil heterogeneity on biomass and yield of winter triticale in an intensively cropped hummocky landscape under temperate climate conditions. Precision Agriculture**, 23:** 912–938. https://doi.org/10.1007/s11119-021-09868-x.

Harris, A. 1999. Biocontrol of *Rhizoctonia solan*i and *Pythium ultimum* on *Capsicum* by *Trichoderma koningii* in potting medium. Microbiological Research, **154**(2): 131–135. https://doi.org/10.1016/S0944-5013(99)80005-6.

Hartmann, A., Rothballer, M., and Schmid, M. 2008. Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. Plant and Soil, **312**(1–2): 7–14. doi:10.1007/s11104-007-9514-z.

Hossain, Q. A., Alam, K., Naser, H. M., Anwar, B., Khatun, F., Rashid, H., Khatun, F., and Siddiky, A. 2020. Application of zinc, boron, and molybdenum in soil increases lentil productivity, nutrient uptake, and apparent balance. Canadian Journal of Soil Science, **101**(1): 113–124. https://doi.org/10.1139/cjss-2019-014.

Ikram, M., Ali, N.I., Jan, G., Farzana, G.J., Rahman, I.U., Iqbal, A., and Hamayun, M., 2018. IAA producing fungal endophyte *Penicillium roqueforti* Thom., enhances stress tolerance and nutrients uptake in wheat plants grown on heavy metal contaminated soils. PLoS One, **13**(11):e0208150. doi:10.1371/journal.pone.0208150.

Isakeit, T., Gao, X., and Kolomiets, M. 2007. Increased resistance of a maize mutant lacking the 9-lipoxygenase gene, ZmLOX3, to root rot caused by *Exserohilum pedicellatum*. Journal of Phytopathology, **155**(11): 758–760. https://doi.org/10.1111/j.1439-0434.2007.01301.x

Jones, S.R., Carley, S., and Harrison. M. 2003. An introduction to power and sample size estimation. Emergency Medicine Journal, **20**(5): 453–458. doi:10.1136/emj.20.5.453.

Kahrl, F., Li, Y., Su, Y., Tennigkeit, T., Wilkes, A., and Xu, J. 2010. Greenhouse gas emissions from nitrogen fertilizer use in China. Environmental Science and Policy, **13**(8): 688–694. doi:10.1016/j.envsci.2010.07.006.

Kandasamy, S., Liu, E.Y.R., Patterson, G., Saldias, S., Ali, S., and Lazarovits, G. 2019. Introducing key microbes from high productive soil transforms native soil microbial community of low productive soil. MicrobiologyOpen, **8**(10). doi:10.1002/mbo3.895.

Kandasamy, S., Weerasuriya, N., Gritsiouk, D., Patterson, G., Saldias, S., Ali, S., and Lazarovits, G. 2020. Size variability in seed lot impact seed nutritional balance, seedling vigor, microbial composition and plant performance of common corn hybrids. Agronomy, **10**(2). doi:10.3390/agronomy10020157.

Kandasamy, S., Weerasuriya, N., Subramanian, G., Thorn, R.G., Patterson, G., Ali, S., and Lazarovits, G. 2021. Disentangling the association of corn root mycobiome with plant productivity and the importance of soil physicochemical balance in shaping their relationship. Frontiers in Sustainable Food Systems, **5**. doi:10.3389/fsufs.2021.617332.

Karimi, K., Arzanlou, M., Ahari, A.B., and Ghazi, M.M. 2015. Phenotypic and molecular characterization of the causal agent of chafer beetle mortality in the wheat fields of the Kurdistan province, Iran. Journal of Plant Protection Research, **55**(3): 227–234. https://doi.org/10.1515/jppr-2015-0031

Kassambara, A., and Mundt, F. (2017). *Factoextra extract and visualize the results of multivariate* data analysis. Available online at: https://cran.r-project.org/web/packages/factoextra/index.html

Khan, A.L., Waqas, M., Rahim Khan, A., Hussain, J., Kang, S.-M., Abdullah Gilani, S., Hamayun, M., Shin, J.-H., Kamran, M., Al-Harrasi, A., Yun, B.-W., Adnan, M., and Lee, I.-J. 2013. Fungal endophyte *Penicillium janthinellum* LK5 improves growth of ABA-deficient tomato under salinity. World Journal of Microbiology and Biotechnology, **29**: 2133–2144. doi:10.1007/s11274-013-1378-1.

Kistler, L., Thakar, H.B., Vanderwarker, A.M., Domic, A., Bergström, A., George, R.J., Harper, T.K., Allaby, R.G., Hirth, K., and Kennett, D.J. 2020. Archaeological Central American maize genomes suggest ancient gene flow from South America. Proceedings of

the National Academy of Sciences (USA), **117**(52): 33124–33129. doi:10.1073/pnas.2015560117/-/DCSupplemental.

Kõljalg, U., Nilsson, H.R., Schigel, D., Tedersoo, L., Larsson, K.-H., May, T.W., Taylor, A.F.S., Jeppesen, T.S., Frøslev, T.G., Lindahl, B.D., Põldmaa, K., Saar, I., Suija, A., Savchenko, A., Yatsiuk, I., Adojaan, K., Ivanov, F., Piirmann, T., Pöhönen, R., Zirk, A., and Abarenkov, K. 2020. The taxon hypothesis paradigm—on the unambiguous detection and communication of taxa. Microorganisms, **8**(12): 1910. doi:10.3390/microorganisms8121910.

Krauss, G.J., Solé, M., Krauss, G., Schlosser, D., Wesenberg, D., and Bärlocher, F. 2011 Fungi in freshwaters: ecology, physiology and biochemical potential. FEMS Microbiology Review**, 35**(4):620-651. doi:10.1111/j.1574-6976.2011.00266.x

Lehmann, A., Zheng, W., Rillig, M. 2017. Soil biota contributions to soil aggregation. Nature Ecology & Evolution, **1**(12): 1828-1835. doi: 10.1038/s41559-017-0344-y.

Lewis, K., Whipps, J.M., and Cooke, R.C. 1990. Mechanisms of biological disease control with special reference to the case study of *Pythium oligandrum* as an antagonist. *In* Biotechnology of Fungi for Improving Plant Growth. *Edited by* J.M. Whipps and R.D. Lumsden. Cambridge University Press, Cambridge. pp. 191–217.

Li, J., Kong, L., Ma, C., Bao, Z., Zhang, D., Chen, Y., and Wang, C. 2022. A chitinase involved in the mycoparasitism of *Trichoderma atroviride* on aeciospores of *Cronartium ribicola*. Journal of Phytopathology, **170**(4): 209–213. https://doi.org/10.1111/jph.13070.

Li, J., Yang, Y.H., Zhou, L., Cheng, L.J., and Chen, Y.H. 2014. Destructive Effects of a mycoparasite *Trichoderma atroviride* SS003 on aeciospores of *Cronartium ribicola*. Journal of Phytopathology, **162**(6): 396–401. https://doi.org/10.1111/jph.12202.

Liu, C., Cui, Y., Li, X., and Yao, M. 2021a. microeco: an R package for data mining in microbial community ecology. FEMS Microbiology Ecology **97**(2). doi:10.1093/femsec/fiaa255.

Liu, H., Li, J., Carvalhais, L.C., Percy, C.D., Prakash Verma, J., Schenk, M., and Singh, B.K. 2021b. Evidence for the plant recruitment of beneficial microbes to suppress soil-borne pathogens. New Phytologist, **229**: 2873–2885. doi:10.1111/nph.17057.

Longa, C.M.O., Savazzini, F., Tosi, S., Elad, Y., and Pertot, I. 2009. Evaluating the survival and environmental fate of the biocontrol agent *Trichoderma atroviride* SC1 in vineyards in northern Italy. Journal of Applied Microbiology, **106**(5): 1549–1557. doi:10.1111/j.1365-2672.2008.04117.x.

Maes, J., Teller, A., Erhard, M., Liquete, C., Braat, L., Berry, P. Egoh, B., Puydarrieux, P., Fiorina, C., Santos-Martin, F., Paracchini, M.L., Keune, H., Wittmer, H., Hauck, J., Fiala, I., Verburg, P., Condé, S., Schägner, J.P., San-Miguel-Ayanz, J., and Bidoglio, G. 2013. Mapping and assessment of ecosystems and their services: An analytical framework for ecosystem assessments under Action 5 of the EU Biodiversity Strategy to 2020. https:/doi.org/10.2779/12398.

Márquez, L.M., Redman, R.S., Rodriguez, R.J., and Roossinck, M.J. 2007. A virus in a fungus in a plant: three-way symbiosis required for thermal tolerance. Science, **315**(5811): 509–513. doi:10.1126/science.1137195.

Massart, S., and Jijakli, H.M. 2007. Use of molecular techniques to elucidate the mechanisms of action of fungal biocontrol agents: a review. Journal of Microbiological Methods, **69**(2), 229–241. https://doi.org/10.1016/j.mimet.2006.09.010doi:10.1016/j.mimet.2006.09.010.

McMurdie, P.J., Holmes, S., and Watson, M. 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE **8**(4): e61217. doi:10.1371/journal.pone.0061217.

Meena, M., Swapnil, P., Zehra, A., Aamir, M., Dubey, M.K., Goutam, J., and Upadyay, R.S. 2017. Beneficial microbes for disease suppression and growth promotion. "Beneficial microbes for disease suppression and plant growth promotion," in Plant-Microbe Interactions in Agro-Ecological Perspectives, eds D. P., Singh, H. B., Singh, and R. Prabha (Singapore: Springer), 395–432. doi: 10.1007/978-981-10-6593-4_16.

Merino-Martín, L., Stokes, A., Gweon, H.S., Moragues-Saitua, L., Staunton, S., Plassard, C., Olover A., Le Bissonnais, Y., and Griffiths, R. I. 2021. Interacting effects of land use type, microbes and plant traits on soil aggregate stability. Soil Biology & Biochemistry, **154**: 108072–. https://doi.org/10.1016/j.soilbio.2020.108072.

Minerdi, D., Bossi. S., Maffei, M.E., Gullino, M.L., Garibaldi, A. 2011. *Fusarium oxysporum* and its bacterial consortium promote lettuce growth and expansion A5 gene expression through microbial volatile organic compound (MVOC) emission, FEMS Microbiology Ecology **76**(2): 342–351. https://doi.org/10.1111/j.1574-6941.2011.01051.x

Mommer, L., Cotton, T.E.A., Raaijmakers, J.M., Termorshuizen, A.J., van Ruijven, J., Hendriks, M., van Rijssel, S.Q., van de Mortel, J.E., van der Paauw, J.W., Schijlen, E.G.W.M., Smit-Tiekstra, A.E., Berendse, F., de Kroon, H., and Dumbrell, A.J. 2018. Lost in diversity: the interactions between soil-borne fungi, biodiversity and plant productivity. New Phytologist, **218**: 542–553. doi:10.1111/nph.15036.

Moreau, D., Bardgett, R.D., Finlay, R.D., Johns, D.L., and Philippot, L. 2019. A plant perspective on nitrogen cycling in the rhizosphere. Functional Ecology, **33**: 540–552. doi:10.1111/1365-2435.13303.

Nicola, L., Landinez-Torres, A. G., Zambuto, F., Capelli, E., and Solveig, T. 2021. The mycobiota of high attitude pear orchards soil in Columbia. Biology, **10**(10): 1002. https://doi.org/10.3390/biology10101002.

Nilsson, R.H., Anslan, S., Bahram, M., Wurzbacher, C., Baldrian, P., and Tedersoo, L. 2019. Mycobiome diversity: high-throughput sequencing and identification of fungi. Microbiology, **17**(2): 95-109. doi:10.1038/s41579-018-0116-y.

Nleya, T., Chungu, C., and Kleinjan, J. 2019. Corn growth and development. *In* iGrow Corn: Best Management Practices. San Diego State University. pp. 5–8.

Nunes, M.R., de Lima, R.P., Tormena, C.A., and Karlen, D.L. 2021. Corn seedling root growth response to soil physical quality. Agronomy Journal, **113**(4): 3135–3146. https://doi.org/10.1002/agj2.20705.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P., O'Hara, R.B., et al. 2019. vegan: Community ecology package. R package version 2.5-5. Available online at: https://cran.r-project.org/package=vegan

Olsen, S.R., C.V. Cole, F.S. Watanabe, and L.A Dean. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular **939**:1-19. Government Printing Office Washington D.C.

Önder, M., Ceyhan, E., and Kahraman, A. 2011. Effects of agricultural practices on environment. International Conference on Biology, Environment and Chemistry (IPCBEE), **24**: 28–32.

Papadopoulos, A. 2011. Soil aggregates, structure, and stability. *In* Encyclopedia of Agrophysics. *Edited by* J. and L.J. Gliński Jan and Horabik. Springer Netherlands, Dordrecht. pp. 736–740. doi:10.1007/978-90-481-3585-1_142.

Patzold, S., Mertens, F. M., Bornemann, L., Koleczek, B., Franke, J., Feilhauer, H., et al. (2008). Soil heterogeneity at the field scale: a challenge for precision crop protection. Precision Agriculture, **9**: 367–390. doi: 10.1007/s11119-008-9077-x.

Pawlowski, M.L., and Hartman, G.L. 2016. Infection mechanisms and colonization patterns of fungi associated with soybean. *In* Fungal Pathogenicity. *Edited by* S. Sultan. InTech. doi:10.5772/62305.

Petrini, O. 1996. Ecological and physiological aspects of host specificity in endophytic fungi. Endophytic fungi in grasses and woody plants: systematics, ecology, and evolution. APS Press. Available from http://ci.nii.ac.jp/naid/10016569626/en/ [accessed 15 December 2021].

Pirozynski, K., and Malloch, D. 1975. The origin of land plants: a matter of mycotrophism. Biosystems, **6**(3): 153-164. DOI: 10.1016/0303-2647(75)90023-4.

Prăvălie, R., Nita, I.A., Patriche, C., Niculiță, M., Birsan, M.V., Roșca, B., and Bandoc, G. 2021. Global changes in soil organic carbon and implications for land degradation neutrality and climate stability. Environmental Research, **201**. doi:10.1016/j.envres.2021.111580.

Pusztahelyi, T., Holb, I.J., and Pócsi, I. 2015. Secondary metabolites in fungus-plant interactions. Frontiers in Plant Science, **6**: 573. doi:10.3389/fpls.2015.00573.

Rai, M., and Agarkar, G. 2016. Plant-fungal interactions: What triggers the fungi to switch among lifestyles? Critical Reviews in Microbiology, **42**(3), 428–438.

Ray, D.K., Mueller, N.D., West, P.C., and Foley, J.A. 2013. Yield trends are insufficient to double global crop-production by 2050. PLoS ONE, **8**(6): e66428. doi: 10.1371/journal.pone.0066428.

Ray, P., Lakshmanan, V., Labbé, J.L., and Craven, K.D. 2020. Microbe to microbiome: a paradigm shift in the application of microorganisms for sustainable agriculture. Frontiers in Microbiology, **11**: 622926. doi:10.3389/fmicb.2020.622926.

Redman, R.S., Sheehan, K.B., Stout, R.G., Rodriguez, R.J., and Henson, J.M. 2002. Thermotolerance generated by plant/fungal symbiosis. Science, **298**(5598), 1581–1581. doi:10.1126/science.1072191.

Rillig, M.C., and Mummey, D.L. 2006. Mycorrhizas and soil structure. New Phytologist **171**(1): 41-53. https://doi.org/10.1111/j.1469-8137.2006.01750.x.

Rillig, M.C., Wright, S.F., and Eviner, V.T. 2002. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. Plant and Soil, **238**(2): 325–333. doi:10.1023/A:1014483303813.

Rodriguez, R.J., Henson, J., van Volkenburgh, E., Hoy, M., Wright, L., Beckwith, F., Kim, Y.O., and Redman, R.S. 2008. Stress tolerance in plants via habitat-adapted symbiosis. ISME Journal, **2**(4): 404–416. doi:10.1038/ismej.2007.106.

Sajeena, A., Nair, D.S., and Sreepavan, K. 2020. Non-pathogenic *Fusarium oxysporum* as a biocontrol agent. Indian Phytopathology, **73**(2): 177–183. doi:10.1007/s42360-020-00226-x.

Šantrůčková, H., Heinemeyer, O., and Kaiser, E.A. 1993. The influence of soil compaction on microbial biomass and organic carbon turnover in micro-and macroaggregates. Geoderma, **56**(1): 587–598. doi:10.1016/0016-7061(93)90137-A.

Schiro, G., Colangeli, P., and Müller, M.E.H. 2019. A metabarcoding analysis of the mycobiome of wheat ears across a topographically heterogeneous field. Frontiers in Microbiology, **10:** 2095. doi:10.3389/fmicb.2019.02095.

Schlatter, D.C., Hansen, J., Carlson, B., Leslie, I.N., Huggins, D.R., and Paulitz, T.C. 2022. Are microbial communities indicators of soil health in a dryland wheat cropping system? Applied Soil Ecology, **170**: 104302. doi:10.1016/j.apsoil.2021.104302.

Schulz, B., and Boyle, C. 2005. The endophytic continuum. Mycology Research, **109**(6): 661-686. doi:10.1017/S095375620500273X.

Shi, S., Nuccio, E.E., Shi, Z.J., He, Z., Zhou, J., and Firestone, M.K. 2016. The interconnected rhizosphere: High network complexity dominates rhizosphere assemblages. Ecology Letters, **19**(8): 926-936. doi:10.1111/ele.12630.

Signorell, A. 2021. DescTools: tools for descriptive statistics. Available online at: https://cran.r-project.org/web/packages/DescTools

Silva, A.O. and Bosu, W.H.D. 2020. Application of different sources and doses of boron in the corn crop. Revista de Agricultura Neotropical, **7**(4): 23–28. https://doi.org/10.32404/rean.v7i4.4464.

Simkovic, M., Gdovinova, A., Zemkova, Z., and Varecka, L. 2011. Properties of secreted protease from vegetative *Trichoderma atroviride* mycelia cultivated with protein inducer reveal a complex protein-recognition mechanism. Antonie van Leeuwenhoek, **101**(2), 253–265. https://doi.org/10.1007/s10482-011-9629-3.

Soil and Plant Analysis Council. 1999. Soil Analysis Handbook of Reference Method. *1st edition.* St. Lucie Press.

Southworth, D. 2012. Defining complex interaction between plants and fungi. *In* Biocomplexity of plant-fungal interactions. Wiley-Blackwell Oxford, UK. pp 205-213. https://doi.org/10.1002/9781118314364.ch10.

Taha, M.A., Ismaiel, A.A., and Ahmed, R.M. 2021. 6-pentyl-α-pyrone from *Trichoderma koningii* induces systemic resistance in tobacco against tobacco mosaic virus. European Journal of Plant Pathology, **159**: 81–93. doi:10.1007/s10658-020-02142-2.

Taylor, D.L., Walters, W.A., Lennon, N.J., Bochicchio, J., Krohn, A., Caporaso, J.G., Pennanen, T., and Cullen, D. 2016. Accurate estimation of fungal diversity and abundance through improved lineage-specific primers optimized for Illumina amplicon sequencing. Applied and Environmental Microbiology **82**(24): 7217–7226. doi:10.1128/AEM.02576-16.

Thioulouse, J., Dray, S., Dufour, A.-B., Siberchicot, A., Jombart, T., and Pavoine, S. 2018. Multivariate analysis of ecological data with ade4. *1st edition*. Springer Science.

Tilman, D., Balzer, C., Hill, J., and Befort, B.L. 2011. Global food demand and the sustainable intensification of agriculture. Proceedings of the National Academy of Sciences (USA), **108**(50): 20260–20264. doi:10.1073/pnas.1116437108.

Tripathi, R., Keswani, C., and Tewari, R. 2021. *Trichoderma koningii* enhances tolerance against thermal stress by regulating ROS metabolism in tomato (*Solanum lycopersicum* L.) plants. Journal of Plant Interactions, **16**(1): 116–126. doi:10.1080/17429145.2021.1908634/FORMAT/EPUB.

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., le Van, A., and Dufresne, A. 2015. The importance of the microbiome of the plant holobiont. New Phytologist, **206**(4): 1196–1206. doi:10.1111/nph.13312.

Vasile, A.J., Raluca Andreea, I., Popescu, G.H., Elvira, N., and Marian, Z. 2016. Implications of agricultural bioenergy crop production and prices in changing the land use paradigm: the case of Romania. Land Use Policy, **50**: 399–407. doi:10.1016/j.landusepol.2015.10.011.

Veresoglou, S. D., Barto, E. K., Menexes, G., and Rillig, M. C. (2013). Fertilization affects severity of disease caused by fungal plant pathogens. Plant Pathology, **62**: 961–969. doi: 10.1111/ppa.12014.

Verghese, J., Abrams, J., Wang, Y., and Morano, K.A. 2012. Biology of the heat shock response and protein chaperones: budding yeast (*Saccharomyces cerevisiae*) as a model system. Microbiology and Molecular Biology Reviews, **76**(2): 115–158. doi:10.1128/MMBR.05018-11/FORMAT/EPUB.

Wang, Z., Johnston, P.R., Takamatsu, S., Spatafora, J.W. and Hibbett, D.S. 2006. Toward a phylogenetic classification of the Leotiomycetes based on rDNA data. Mycologia, **98**(6):1065-1075. doi:10.1080/15572536.2006.11832634

Watson, D., ed. 2017. Achieving Sustainable Cultivation of Maize. Burleigh Dodds Science Publishing Cambridge, UK.

Weerasuriya, N.M. 2021. primer_pull.sh for Demultiplexing Primers in a MiSEQ Run, https://github.com/nweerasu/primer_pull

Wetzel, H.C., Skinner, D.Z., and Tisserat, N.A. 2007. Geographic distribution and genetic diversity of three *Ophiosphaerella* species that cause spring dead spot of bermudagrass. Plant Disease, **83**(12): 1160-1166.

Whalley, W.R., Riseley, B., Leeds-Harrison, P.B., Bird, N.R.A., Leech, P.K., and Adderley, W.P. 2005. Structural differences between bulk and rhizosphere soil. European Journal of Soil Science, **56**(3): 353–360. doi:10.1111/j.1365-2389.2004.00670.x.

Whipps, J.M., Lewis, K., and Cooke, R.C. 1988. Mycoparasitism and plant disease control. *In* Fungi in Biological Control Systems. *Edited by* M.N. Burge. Manchester University Press, Manchester. pp. 161–187.

White, J.F., Kingsley, K.L., Zhang, Q., Verma, R., Obi, N., Dvinskikh, S., Elmore, M.T., Verma, S.K., Gond, S.K., and Kowalski, K.P. 2019. Review: Endophytic microbes and their potential applications in crop management. Pest Management Science, **75**(10): 2558–2565. doi:10.1002/ps.5527.

Wickham, H. 2016. Programming with ggplot2. *In* ggplot2. Cham: Springer International Publishing. pp. 241–253. doi:10.1007/978-3-319-24277-4_1.

Wood, Martin. 1995. Environmental Soil Biology (2nd ed.). Blackie Academic & Professional London, UK.

Xiong, C., He, J.Z., Singh, B.K., Zhu, Y.G., Wang, J.T., Li, P.P., Zhang, Q.B., Han, L.L., Shen, J.P., Ge, A.H., Wu, C.F., and Zhang, L.M. 2021. Rare taxa maintain the stability of crop mycobiomes and ecosystem functions. Environmental Microbiology, **23**(4): 1907–1924. Blackwell Publishing Ltd. doi:10.1111/1462-2920.15262.

Xu, X.M., and Jeger, M.J. 2013. Theoretical modeling suggests that synergy may result from combined use of two biocontrol agents for controlling foliar pathogens under spatial heterogeneous conditions. Phytopathology, 103(8), 768–775. https://doi.org/10.1094/PHYTO-10-12-0266-R

Yoshida, N. 2022. Seasonal dynamics of the pink root fungus (*Setophoma terrestris*) in rhizosphere soil: Effect of crop species and rotation. Plant Pathology, **71**(2): 361–372. https://doi.org/10.1111/ppa.13464.

Zhang, P., Wei, T., Jia, Z., Han, Q., and Ren, X. 2014. Soil aggregate and crop yield changes with different rates of straw incorporation in semiarid areas of northwest China. Geoderma, **230:** 41-49. doi:10.1016/j.geoderma.2014.04.007.

Zhang, R., Vivanco, J.M., and Shen, Q. 2017. The unseen rhizosphere root–soil–microbe interactions for crop production. Current Opinion in Microbiology, **17:** 8-14. doi:10.1016/j.mib.2017.03.008.

Appendices

Appendix I: Results of soil selection and experiment 1

1 Soil Selection Experiment

Addition of nitrogen fertilizer ~~r~~esulted in greater dry weights (g) between all soil sources (Figure 1). Among the fertilized treatments, seedlings grown in F10B2 soil a) had significantly lower above-ground biomass (g dry weight) than plants grown in both F10G1 and F10G2A (Figure 1), b) had significantly lower root dry weight than those grown in F10G2A (Figure 1), and c) were significantly shorter than plants grown in F10G1 (Figure 2).

2 Inoculant Effect

Different fungal isolates as inoculants had a significant effect on shoot dry weight (g), with CM003 *F. oxysporum* having a significantly higher dry weight than any other treatment except controls, and CM002 *F. oxysporum* and CM004 *F. oxysporum* resulted in the lowest weights (Figure 3). More specifically, plants inoculated with 1% CM004 *F. oxysporum* had significantly shorter shoots than all other treatments (Figure 4). Plants inoculated CM018 *P. janthinellum* and high yield controls had significantly taller shoot lengths than the low yield controls (Figure 5). Plants inoculated with CM018 *P. janthinellum* had greater shoot dry weights compared to controls, but the differences were not significant (data not shown). Seedlings grown in soils inoculated with CM003 *F. oxysporum* were significantly taller than all other treatments, including controls (Figure 6). Seedlings inoculated with CM003 *F. oxysporum* and the high yield controls had significantly greater shoot dry weights than the controls, and CM019 *S. terrestris* produced the lowest shoot dry weight (Figure 7).

3 Concentration Effect

I found that seedlings grown in soils with high concentrations (3-5%) of substrate or inoculant were significantly shorter and had lower dry weights than those grown in non-amended soils or low concentrations (1-1.67%) (Figures 8-9). Low concentrations of substrate did sometimes result in the highest dry weights, but not significantly higher than non-amended controls (Figure 10). In one instance, seedlings grown in soils with concentrations of 1.67% had the shortest shoot length compared to non-amended soils and soils with high concentrations of substrate, but this reduction was not significant (Figure 11). Barley as an inoculum substrate did not reduce seedling shoot dry weight as dramatically as the substrate of ground corn kernels and vermiculite (Figure 12).

4 Summary

As a result of these preliminary studies, I chose to use lower concentrations (1-2%) of barley substrate inoculated with CM003 *F. oxysporum,* CM007 *Trichoderma atroviride,* CM018 *P. janthinellum,* and CM022 *T. koningii* as candidate fungi to elicit beneficial responses, and CM004 *F. oxysporum* and CM019 *S. terrestris* as potential pathogens in my main research experimental design, in growth trials using F10B2 and F10G2A soil and 25 ppm N initial fertilization.

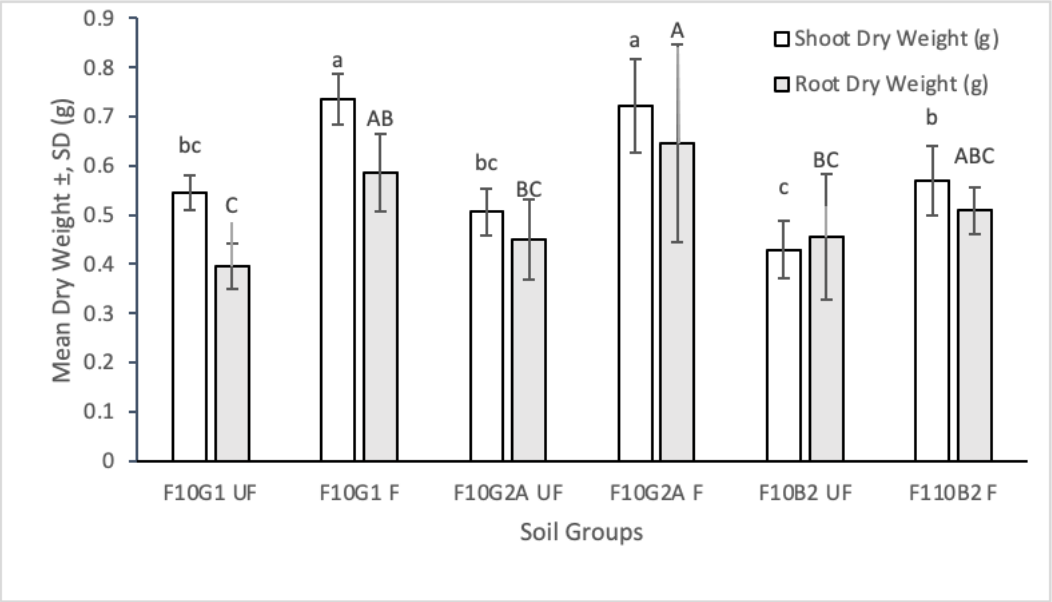

Figure 1: Mean ± SD shoot and root dry weight (g) for various soil sources (F10G1, F10G2A, and F10B2) and fertilizing regimes (UF = unfertilized; F = fertilized) (n=8). Significance is denoted by lower-case letters for shoot dry weight (p<0.05, F = 3.95, df = 7, two-way ANOVA) and by capital letters for root dry weight (p<0.05, F = 5.13, df = 8, two-way ANOVA). Data were analyzed using two-way ANOVA and Tukey post-hoc testing (α = 0.05).

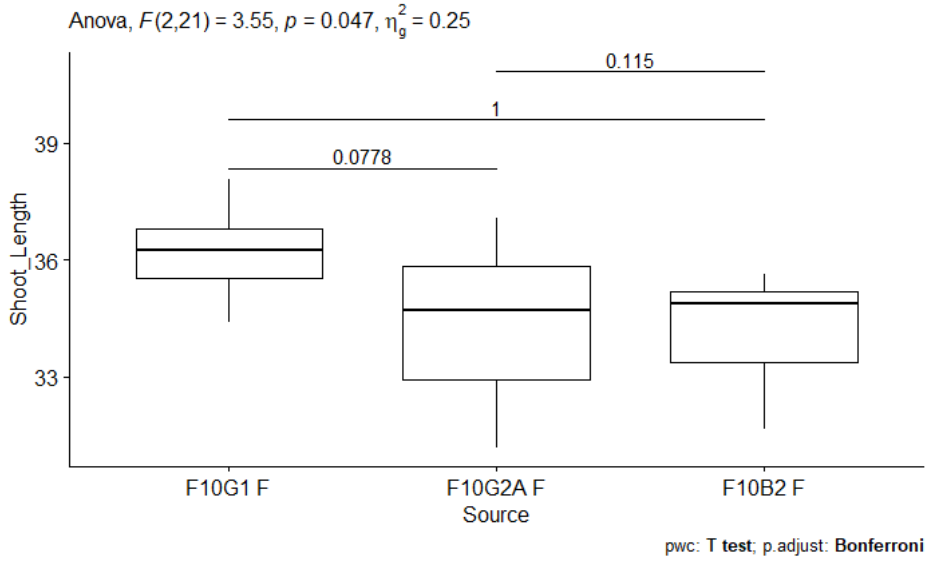

Figure 2: Boxplots of fertilized groups (n = 8) for shoot length (cm). Significance is denoted by horizontal p-value bars where p < 0.05. (p = 0.026).

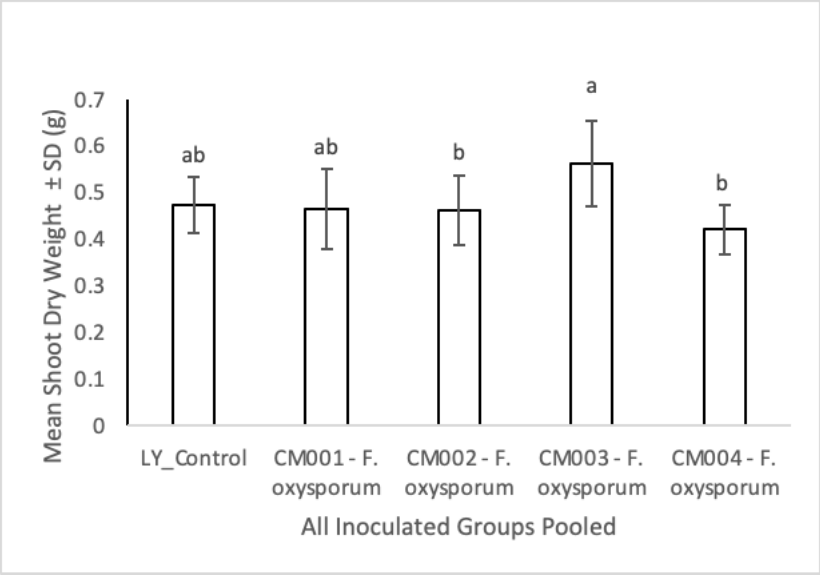

Figure 3: Mean ± SD shoot dry weight (g) for various fungal inoculants and controls (n=3). Significance is denoted by lower-case letters. Data were analyzed using two-way ANOVA (p<0.05, F = 5.45, df = 4) and Tukey post-hoc testing (α = 0.05).

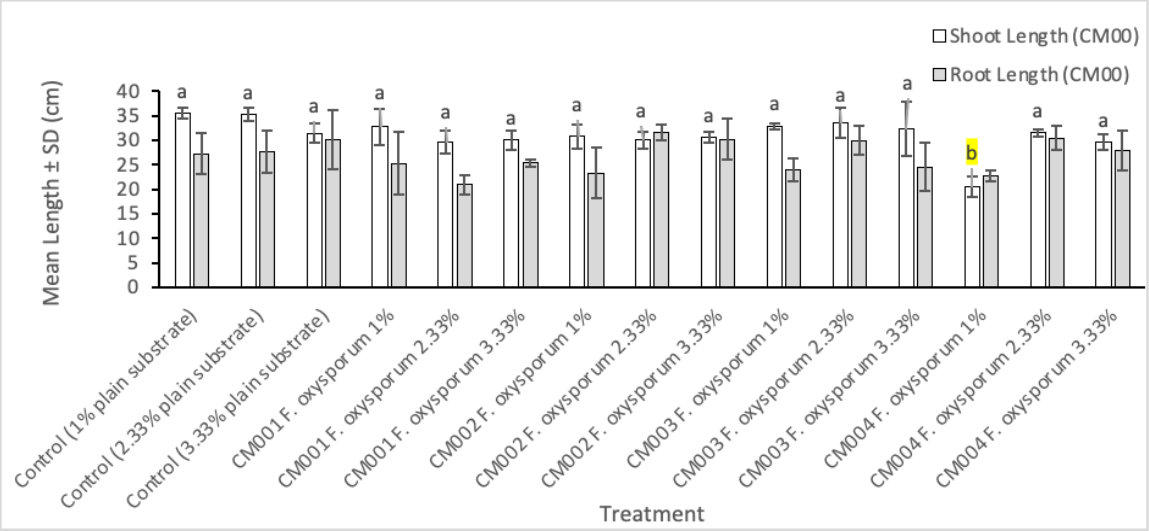

Figure 4: Mean ± SD shoot and root lengths (cm) of various fungal inoculants and varying concentrations (n=3). For shoot length, significance is denoted by lower-case letters. No significance is denoted on the root length values. Data were analyzed using two-way ANOVA (p < 0.05, F = 5.24, df = 8) and Tukey post-hoc testing (α = 0.05). One significantly different treatment is highlighted.

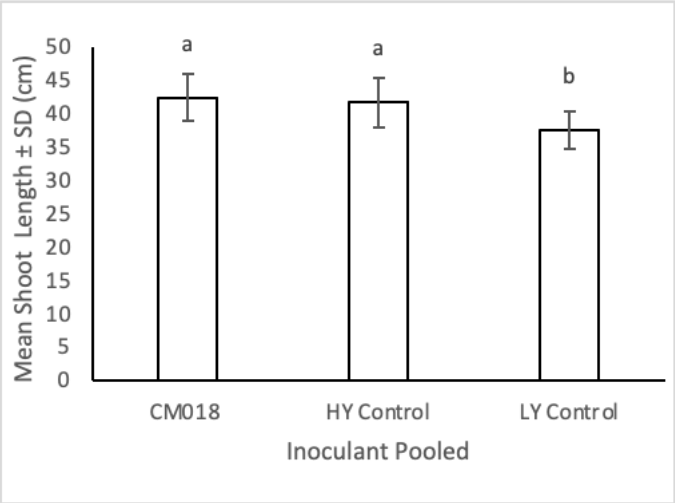

Figure 5: Mean ± SD shoot length (cm) for various inoculants and controls. Data were analyzed using two-way ANOVA (p<0.05, F=216.36, df=2,), and Tukey post-hoc testing ($\alpha = 0.05$).

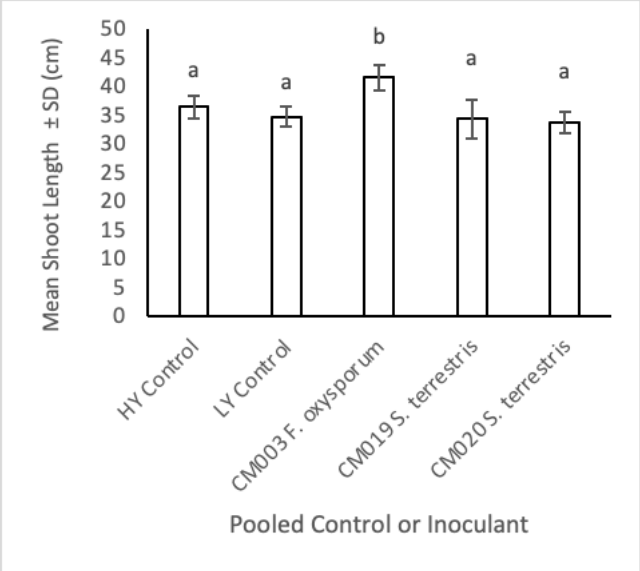

Figure 6: Mean ± SD shoot length (cm) for various inoculants and controls. Significance is denoted by lower-case letters. Data were analyzed using two-way ANOVA (p<0.05, F=16.16, df=4) and Tukey post-hoc testing ($\alpha = 0.05$).

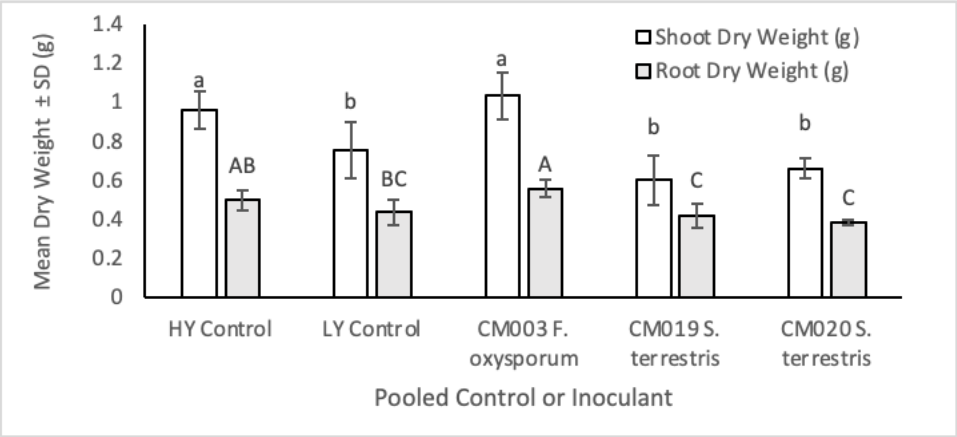

Figure 7: Mean ± SD shoot and root dry weight (g) for various concentrations of substrate or inoculant and controls (n=3). Significance is denoted by lower-case letters for shoot dry weight (p<0.05, F=14.66, df = 4, two-way ANOVA) and by capital letters for root dry weight (p<0.05, F=29.30, df = 4, two-way ANOVA). Data were analyzed using two-way ANOVA and Tukey post-hoc testing (α = 0.05).

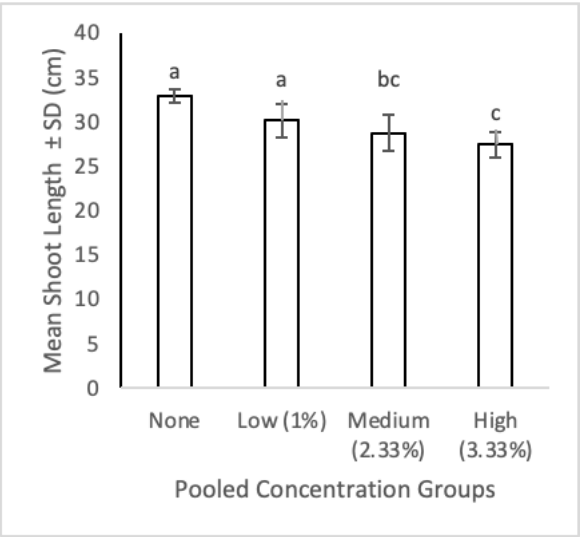

Figure 8: Mean ± SD shoot length (cm) for various concentrations of substrate or inoculant and controls (n=3). Significance is denoted by lower-case letters. Data were analyzed using two-way ANOVA (p<0.05, F=9.77, df = 2) and Tukey post-hoc testing (α = 0.05).

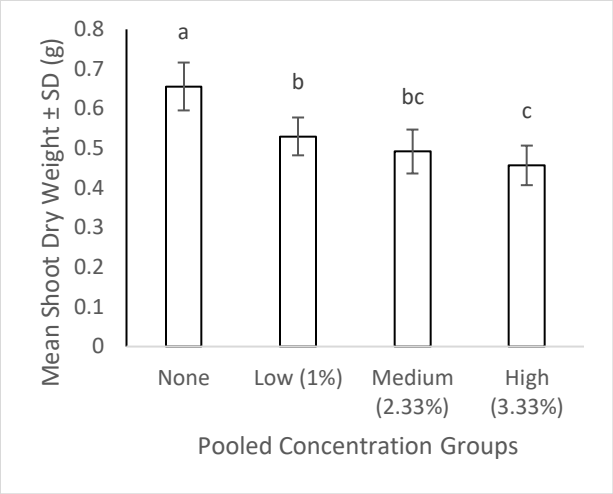

Figure 9: Mean ± SD shoot dry weight (g) for various concentrations of substrate or inoculant and controls (n=3). Significance is denoted by lower-case letters. Data were analyzed using two-way ANOVA (p<0.05, F = 11.77, df = 3) and Tukey post-hoc testing ($\alpha = 0.05$).

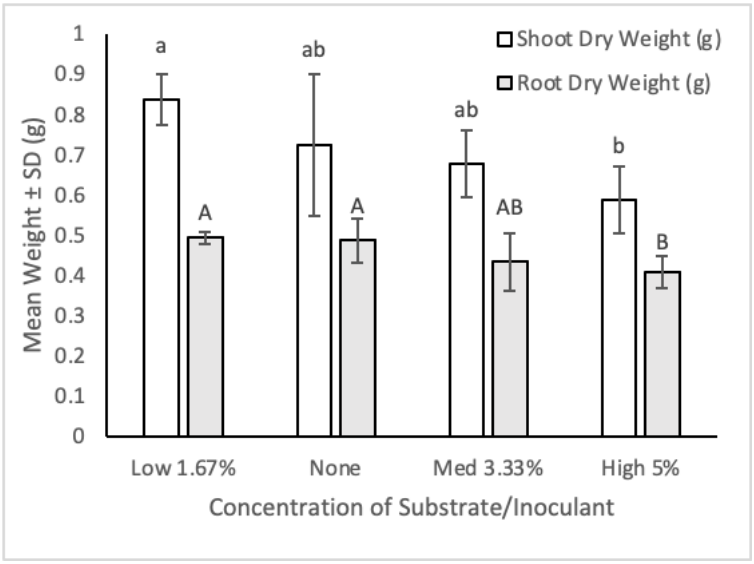

Figure 10: Mean ± SD shoot and root dry weight (g) for various concentrations of substrate or inoculant and controls (n=3). Significance is denoted by lower-case letters for shoot dry weight (p<0.05, F = 4.66, df = 3, two-way ANOVA) and by capital letters for root dry weight (p<0.05, F = 6.36, df = 3, two-way ANOVA). Data were analyzed using two-way ANOVA and Tukey post-hoc testing ($\alpha = 0.05$).

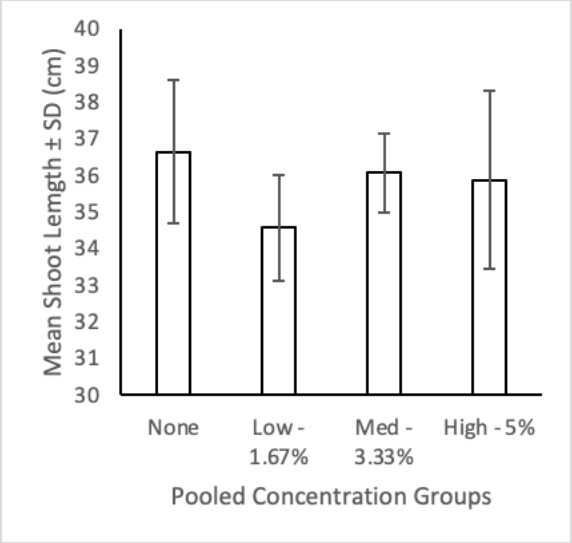

Figure 11: Mean ± SD shoot length (cm) for various concentrations of substrate or inoculant and controls. Data were analyzed using two-way ANOVA (p<0.05, F=3.67, df=3,) and Tukey post-hoc testing, with no significant differences detected (α = 0.05).

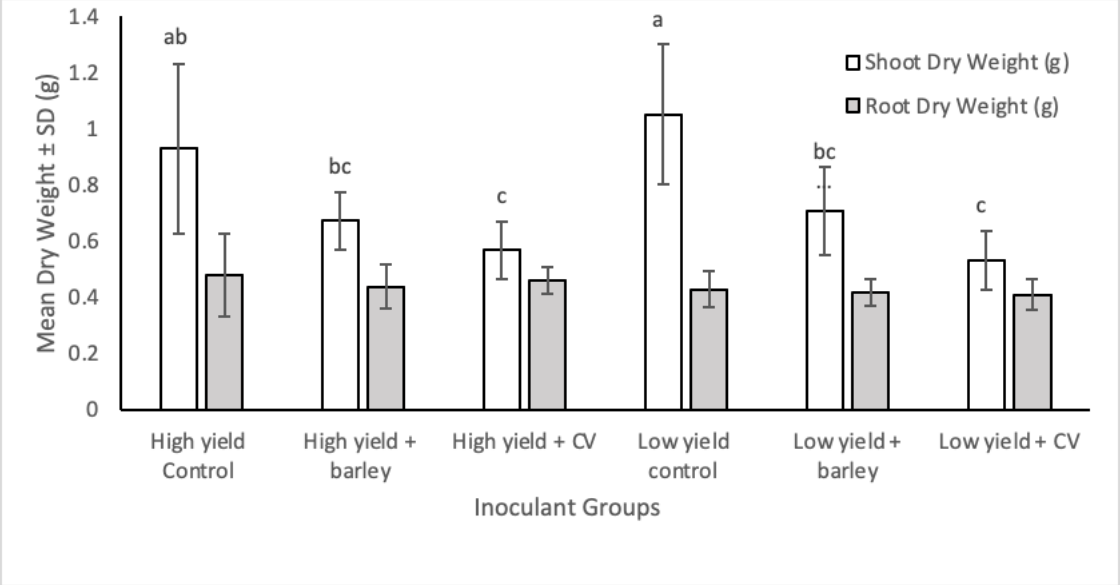

Figure 12: Mean ± SD shoot and root dry weights (g) of various substrates (CV = corn and vermiculite) and soils, and varying concentrations (n=5). For shoot dry mass (g), significance is denoted by lower-case letters I<0.05, F = 8.20, df = 11, two-way ANOVA. No significance was detected in the root dry weight values (p<0.05, F = 2.27, df = 11, two-way ANOVA). Data were analyzed using two-way ANOVA (p < 0.05, F = 5.24, df = 8) and Tukey post-hoc testing (α = 0.05).

Appendix II: List of ASV numbers and taxonomic identifications

ASV	Kingdom	Phylum	Class	Order	Family	Genus	Species
0	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Gibberella*	*intricans*
1	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Neocosmospora*	*solani*
2	Fungi	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Actinomucor*	*elegans*
3	Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Debaryomycetaceae	*Meyerozyma*	*carribica*
4	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	*Setophoma*	*terrestris*
5	Fungi	Ascomycota	Leotiomycetes	Helotiales			
6	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Periconiaceae	*Periconia*	
7	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Lindgomycetaceae	*Clohesyomyces*	
8	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae	*Epicoccum*	
9	Fungi	Ascomycota	Sordariomycetes				
10	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	*Setophoma*	*terrestris*
12	Fungi	Olpidiomycota	Olpidiomycetes	Olpidiales	Olpidiaceae	*Olpidium*	

13	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Exophiala*	*equina*
14	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae		
15	Fungi	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Mucor*	*circinelloides*
16	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae		
17	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	*Setophoma*	*terrestris*
18	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Dactylonectria*	*macrodidyma*
19	Fungi	Ascomycota	Sordariomycetes	Xylariales	Microdochiaceae	*Microdochium*	*bolleyi*
20	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	*Setophoma*	*terrestris*
21	Fungi	Mucoromycota	Mucoromycetes	Mucorales	Rhizopodaceae	*Rhizopus*	*arrhizus*
22	Fungi	Basidiomycota	Agaricomycetes	Cantharellales	Ceratobasidiaceae	*Rhizoctonia*	*solani*
23	Fungi	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	*Schizothecium*	
24	Fungi						
25	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Bionectriaceae	*Clonostachys*	
26	Fungi	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Actinomucor*	*elegans*

27	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	*Ophiosphaerella*	
28	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Alternaria*	*cerealis*
29	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Periconiaceae	*Periconia*	
30	Fungi	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Mucor*	*nidicola*
31	Fungi	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Mucor*	*circinelloides*
32	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Neocosmospora*	*solani*
33	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium*	*concentricum*
34	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Linnemannia*	*elongata*
35	Fungi	Ascomycota	Pezizomycetes	Pezizales	Pezizaceae	*Pezizacaea*	
36	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Periconiaceae	*Periconia*	
37	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	*Paraphoma*	*radicina*
38	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Rhizophagus*	*custos*
39	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*viride*
40	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Lindgomycetaceae	*Clohesyomyces*	

41	Fungi	Mortierellomycota					
42	Fungi	Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae	*Hyalorbilia*	
43	Fungi	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Mucor*	*circinelloides*
44	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium*	*pseudocircinatum-ramigenum*
45	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae		
46	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Funneliformis*	*mosseae*
47	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*viride*
48	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Neocosmospora*	*perseae*
49	Fungi	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Actinomucor*	*elegans*
50	Fungi						
51	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Exophiala*	*pisciphila*
52	Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Talaromyces*	*purpureogenus*
53	Fungi						
54	Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Scytalidium*	*circinatum*

55	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	*Clitopilus*	*hobsonii*
56	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Pyrenophora*	*dematioidea*
57	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Lindgomycetaceae	*Clohesyomyces*	
58	Fungi	Ascomycota	Sordariomycetes	Myrmecridiales	Myrmecridiaceae	*Myrmecridium*	
59	Fungi	Ascomycota	Sordariomycetes	Glomerellales	Plectosphaerellaceae	*Plectosphaerella*	*cucumerina*
60	Fungi	Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae	*Hyalorbilia*	
61	Fungi						
62	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium*	*graminearum*
63	Fungi	Ascomycota	Pezizomycetes	Pezizales	Pezizaceae	*Terfezia*	
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65	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	*Metacordyceps*	*chlamydosporia*
66	Fungi	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Mucor*	*hiemalis*
67	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Lindgomycetaceae	*Clohesyomyces*	
68	Fungi	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Mucor*	*circinelloides*

69	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	
70	Fungi	Ascomycota	Sordariomycetes				
71	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	*Paraphoma*	*radicina*
72	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Lindgomycetaceae	*Clohesyomyces*	
73	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Linnemannia*	
74	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusicolla*	*aquaeductuum*
76	Fungi	Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae	*Orbilia*	
77	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Linnemannia*	
78	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Rhizophagus*	*irregularis*
80	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Melanommataceae	*Camposporium*	
81	Fungi						
82	Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Debaryomycetaceae	*Meyerozyma*	*carribica*
83	Fungi	Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae	*Hyalorbilia*	
84	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Morosphaeriaceae	*Acrocalymma*	*vagum*

85	Fungi	Ascomycota	Pezizomycetes	Pezizales	Pezizaceae		
86	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Neocosmospora*	*perseae*
87	Fungi	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Actinomucor*	*elegans*
88	Fungi	Ascomycota	Pezizomycetes	Pezizales	Pezizaceae		
89	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Lindgomycetaceae	*Clohesyomyces*	
90	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium*	*equiseti*
91	Fungi	Ascomycota	Dothideomycetes	Pleosporales:g	Apiosporaceae		
92	Fungi	Ascomycota	Dothideomycetes	Capnodiales	Cladosporiaceae	*Cladosporium*	*ramotenellum*
93	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Lindgomycetaceae	*Clohesyomyces*	
94	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Microdominikia*	*irregularis*
96	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Rhizophagus*	*irregularis*
98	Fungi	Olpidiomycota	Olpidiomycetes	Olpidiales	Olpidiaceae	*Olpidium*	*brassicae*
99	Fungi	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae		
100	Fungi	Ascomycota	Laboulbeniomycetes	Pyxidiophorales	Pyxidiophoraceae	*Pyxidiophora*	*arvernensis*

101	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Funneliformis*	*mosseae*
102	Fungi	Ascomycota	Sordariomycetes	Xylariales	Microdochiaceae	*Microdochium*	*seminicola*
103	Fungi	Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae	*Arthrobotrys*	*conoides*
105	Fungi	Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	*Macrophomina*	*phaseolina*
106	Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Talaromyces*	*tumuli*
107	Fungi						
109	Fungi	Olpidiomycota	Olpidiomycetes	Olpidiales	Olpidiaceae	*Olpidium*	*brassicae*
110	Fungi						
111	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Rhizophagus*	*irregularis*
112	Fungi	Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae		
113	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Sexserohilum*	*pedicellatum*
114	Fungi	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Mucor*	*circinelloides*
116	Fungi	Basidiomycota	Agaricomycetes	Cantharellales	Ceratobasidiaceae	*Waitea*	*circinata*
117	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	*Metacordyceps*	*chlamydosporia*

118	Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Hymenoscyphus*	*menthae*
120	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Funneliformis*	*mosseae*
122	Fungi	Ascomycota	Dothideomycetes	Pleosporales			
124	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Exophiala*	
125	Fungi	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae		
126	Fungi	Ascomycota	Sordariomycetes	Sordariales			
127	Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Hymenoscyphus*	*menthae*
129	Fungi	Ascomycota	Orbiliomycetes	Orbiliales			
131	Fungi	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	*Schizothecium*	
133	Fungi	Ascomycota	Sordariomycetes	Sordariales			
135	Fungi	Ascomycota	Pezizomycetes	Pezizales	Pezizaceae	*Terfezia*	
136	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Rhizophagus*	*irregularis*
137	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Rhizophagus*	*custos*
138	Fungi	Ascomycota	Laboulbeniomycetes				

140	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Rhizophagus*	*irregularis*
141	Fungi	Basidiomycota	Agaricomycetes	Auriculariales		*Oliveonia*	
142	Fungi	Ascomycota					
143	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	*Clitopilus*	*hobsonii*
146	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Rhizophagus*	*custos*
147	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Rhizophagus*	*irregularis*
151	Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Phaffomycetaceae	*Wickerhamomyces*	*anomalus*
152	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Bipolaris*	
153	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium*	*algeriense*
154	Fungi	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Mucor*	*circinelloides*
158	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Rhizophagus*	*fasciculatus*
159	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Amniculicolaceae	*Murispora*	
160	Fungi	Basidiomycota	Agaricomycetes	Sebacinales	Serendipitaceae		
163	Fungi	Basidiomycota	Microbotryomycetes	Sporidiobolales	Sporidiobolaceae	*Rhodotorula*	*mucilaginosa*

164	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Rhizophagus*	*irregularis*
166	Fungi	Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae	*Arthrobotrys*	*conoides*
168	Fungi	Olpidiomycota	Olpidiomycetes	Olpidiales	Olpidiaceae	*Olpidium*	*brassicae*
169	Fungi	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Mucor*	*circinelloides*
170	Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Talaromyces*	
171	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Rhizophagus*	*custos*
174	Fungi	Ascomycota	Sordariomycetes	Sordariales			
175	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Rhizophagus*	*irregularis*
177	Fungi	Basidiomycota	Agaricomycetes	Cantharellales	Ceratobasidiaceae	*Waitea*	*circinata*
178	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae		
179	Fungi	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae		
183	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusicolla*	*aquaeductuum*
185	Fungi	Basidiomycota	Agaricomycetes	Cantharellales	Ceratobasidiaceae	*Waitea*	*circinata*
186	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Stachybotryaceae	*Albifimbria*	*verrucaria*

188	Fungi	Ascomycota	Sordariomycetes	Sordariales	Cephalothecaceae	*Phialemonium*	
189	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Lindgomycetaceae		
192	Fungi	Basidiomycota	Agaricomycetes	Auriculariales	Auriculariales	*Incertae*	
193	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Rhizophagus*	*custos*
196	Fungi	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	*Scutellinia*	
197	Fungi	Ascomycota	Sordariomycetes	Sordariales			
202	Fungi						
204	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Funneliformis*	*mosseae*
209	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Rhizophagus*	*irregularis*
210	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Delitschiaceae	*Delitschia*	*chaetomioides*
213	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Amniculicolaceae		
215	Fungi	Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	*Lachnum*	
217	Fungi	Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	*Lachnum*	
224	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Alternaria*	

225	Fungi	Mortierellomycota					
229	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	*Metacordyceps*	*chlamydosporia*
232	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Funneliformis*	*mosseae*
236	Fungi	Ascomycota	Sordariomycetes	Sordariales			
240	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Exophiala*	*opportunistica*
242	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Nidulariaceae	*Cyathus*	*stercoreus*
243	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreales	*Incertae*	
244	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Lindgomycetaceae		
245	Fungi	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae		
246	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae		
272	Fungi	Ascomycota	Dothideomycetes	Pleosporales			
276	Fungi	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Mucor*	*hiemalis*
279	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Funneliformis*	*mosseae*
280	Fungi	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Mucor*	*irregularis*

281	Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Hymenoscyphus*	*menthae*
290	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Funneliformis*	*mosseae*
303	Fungi	Ascomycota	Dothideomycetes	Pleosporales			
306	Fungi	Basidiomycota	Agaricomycetes	Phallales	Phallaceae	*Phallus*	*rugulosus*
307	Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Aspergillus*	
310	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae		
316	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Alternaria*	*betae-kenyensis*
320	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Funneliformis*	*mosseae*
321	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Funneliformis*	*mosseae*
323	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Funneliformis*	*mosseae*
332	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Rhizophagus*	
338	Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Meliniomyces*	
342	Fungi	Ascomycota	Sordariomycetes				
352	Fungi	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae		

363	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Funneliformis*	*mosseae*
375	Fungi	Ascomycota	Dothideomycetes	Pleosporales			
391	Fungi	Basidiomycota	Agaricomycetes	Auriculariales	Auriculariales	*Incertae*	
405	Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	*Piskurozyma*	*capsuligena*
410	Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	*Piskurozyma*	*capsuligena*
430	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Funneliformis*	*mosseae*
451	Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Tetracladium*	
468	Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Funneliformis*	*mosseae*

Appendix III: Mean ± SD of soil physicochemical values across main growth experiments A) Controls and primary inoculation treatments, B) co-inoculated treatments

A)

	High yield soil only	High yield amended	Low yield soil only	Low yield amended	CM003 *F. oxysporum*	CM007 *T. atroviride*	CM018 *P. janthinellum*	CM022 *T. koningii*	CM004 *F. oxysporum*	CM019 *S. terrestris*
OM	4.50 ± 0.97	4.90 ± 1.16	2.83 ± 0.27	3.23 ± 0.10	3.30 ± 0.09	3.33 ± 0.05	3.40 ± 0.09	3.47 ± 0.05	3.43 ± 0.14	3.38 ± 0.10
CEC	12.67 ± 1.66	12.90 ± 2.50	13.40 ± 1.76	13.70 ± 1.63	14.10 ± 0.63	14.23 ± 0.90	14.57 ± 0.81	14.80 ± 0.85	14.03 ± 1.74	14.10 ± 1.66
pH	6.80 ± 0.39	6.73 ± 0.19	7.63 ± 0.05	7.57 ± 0.05	7.43 ± 0.05	7.47 ± 0.05	7.37 ± 0.05	7.43 ± 0.05	7.50 ± 0.09	7.50 ± 0.00
Salt	0.34 ± 0.11	0.31 ± 0.07	0.25 ± 0.04	0.32 ± 0.04	0.43 ± 0.05	0.40 ± 0.02	0.41 ± 0.12	0.39 ± 0.03	0.32 ± 0.03	0.32 ± 0.03
Na	29.67 ± 9.31	30.33 ± 10.93	20.00 ± 2.37	23.00 ± 3.10	23.00 ± 1.79	25.00 ± 0.89	25.00 ± 3.22	25.00 ± 4.65	23.33 ± 5.09	24.33 ± 4.93
Zn	4.73 ± 0.42	4.87 ± 0.72	4.90 ± 0.97	4.20 ± 0.27	4.50 ± 0.24	4.67 ± 0.36	4.63 ± 0.29	4.27 ± 0.49	4.50 ± 0.41	4.20 ± 0.41
Mn	14.00 ± 0.89	18.33 ± 5.96	61.00 ± 14.06	52.33 ± 5.82	50.67 ± 4.93	53.33 ± 6.28	54.67 ± 6.09	53.00 ± 8.53	52.00 ± 10.32	54.00 ± 9.34
Fe	78.00 ± 6.26	93.00 ± 16.71	73.00 ± 6.20	69.67 ± 3.72	67.67 ± 2.25	68.00 ± 3.10	71.00 ± 4.10	67.33 ± 4.50	67.67 ± 4.03	70.00 ± 5.87
B	0.53 ± 0.14	0.60 ± 0.24	0.70 ± 0.18	0.70 ± 0.18	0.73 ± 0.14	0.72 ± 0.20	0.80 ± 0.18	0.73 ± 0.23	0.77 ± 0.23	0.73 ± 0.19
NO_3N	11.67 ± 2.73	11.33 ± 4.03	8.67 ± 5.75	13.33 ± 2.58	24.67 ± 8.02	21.00 ± 6.26	19.33 ± 14.26	17.33 ± 3.61	15.33 ± 1.37	16.00 ± 7.32
Cl	14.33 ± 5.24	23.33 ± 10.67	19.33 ± 12.91	17.00 ± 3.22	23.00 ± 10.88	25.00 ± 10.99	15.00 ± 0.89	16.00 ± 0.89	14.67 ± 1.37	16.00 ± 2.37
AlM_3	736.00 ± 94.27	773.67 ± 64.96	653.33 ± 76.20	659.00 ± 52.47	673.33 ± 33.67	673.33 ± 58.42	696.00 ± 45.25	661.33 ± 76.27	666.67 ± 74.24	681.33 ± 55.32
% K	3.43 ± 1.12	3.87 ± 1.30	2.80 ± 0.36	3.10 ± 0.47	2.97 ± 0.26	3.03 ± 0.36	3.07 ± 0.34	2.93 ± 0.51	2.97 ± 0.36	3.07 ± 0.19
% Mg	12.80 ± 0.47	13.00 ± 0.32	11.60 ± 1.09	13.13 ± 0.55	12.63 ± 0.19	12.83 ± 0.31	12.80 ± 0.15	12.70 ± 0.27	12.57 ± 0.19	12.80 ± 0.09
% Ca	72.80 ± 0.77	72.73 ± 0.83	85.10 ± 1.03	83.17 ± 0.58	83.83 ± 0.14	83.53 ± 0.23	83.57 ± 0.40	83.80 ± 0.50	83.90 ± 0.56	83.57 ± 0.31
% Na	0.97 ± 0.21	0.97 ± 0.21	0.67 ± 0.05	0.70 ± 0.00	0.70 ± 0.00	0.77 ± 0.05	0.73 ± 0.05	0.73 ± 0.10	0.70 ± 0.09	0.77 ± 0.05
KMG	0.27 ± 0.09	0.30 ± 0.10	0.24 ± 0.04	0.24 ± 0.04	0.24 ± 0.03	0.24 ± 0.03	0.24 ± 0.03	0.23 ± 0.04	0.24 ± 0.03	0.24 ± 0.01

B)

	CM003 + CM004	CM003 + CM019	CM007 + CM004	CM007 + CM019	CM018 + CM004	CM018 + CM019	CM022 + CM004	CM022 + CM019
OM	3.40 ± 0.09	3.27 ± 0.10	3.27 ± 0.29	3.20 ± 0.24	3.30 ± 0.09	3.33 ± 0.19	3.33 ± 0.26	3.30 ± 0.18
CEC	14.27 ± 1.29	14.37 ± 1.21	13.10 ± 1.44	13.33 ± 1.56	12.83 ± 1.49	11.80 ± 0.63	11.93 ± 1.06	13.20 ± 1.49
pH	7.53 ± 0.19	7.50 ± 0.09	7.43 ± 0.10	7.43 ± 0.10	7.40 ± 0.09	7.43 ± 0.14	7.43 ± 0.05	7.30 ± 0.00
Salt	0.37 ± 0.08	0.37 ± 0.06	0.35 ± 0.02	0.43 ± 0.09	0.40 ± 0.08	0.39 ± 0.09	0.36 ± 0.05	0.36 ± 0.06
Na	25.67 ± 2.07	24.67 ± 2.25	22.33 ± 2.07	22.67 ± 4.23	23.00 ± 2.37	20.67 ± 2.73	21.00 ± 2.37	22.33 ± 3.61
Zn	4.67 ± 0.29	5.93 ± 2.01	4.83 ± 0.76	5.07 ± 1.18	4.83 ± 0.49	4.30 ± 0.63	4.57 ± 1.18	5.07 ± 0.65
Mn	57.67 ± 2.73	59.00 ± 4.47	55.67 ± 0.52	57.33 ± 2.25	49.67 ± 5.39	45.33 ± 5.47	46.00 ± 11.63	53.33 ± 1.86
Fe	70.33 ± 1.03	71.00 ± 4.73	70.00 ± 3.58	69.67 ± 5.47	67.33 ± 1.37	64.33 ± 1.37	62.67 ± 5.82	66.33 ± 3.39
B	0.77 ± 0.19	0.80 ± 0.09	0.77 ± 0.19	0.77 ± 0.14	0.73 ± 0.05	0.63 ± 0.05	0.63 ± 0.05	0.70 ± 0.09
NO_3N	13.67 ± 3.61	16.67 ± 10.37	20.00 ± 4.10	25.67 ± 14.54	23.67 ± 10.05	19.67 ± 12.18	26.33 ± 5.75	24.67 ± 9.85
Cl	21.67 ± 3.61	48.33 ± 41.15	23.00 ± 14.89	24.33 ± 5.82	20.33 ± 1.37	16.33 ± 1.86	17.33 ± 2.88	32.00 ± 8.53
AlM_3	704.00 ± 6.75	708.33 ± 23.26	694.67 ± 15.03	676.33 ± 46.52	648.00 ± 23.66	611.00 ± 36.70	576.33 ± 85.23	656.33 ± 45.38
% K	3.40 ± 0.54	3.33 ± 0.49	3.30 ± 0.54	3.27 ± 0.49	3.20 ± 0.46	3.13 ± 0.31	3.13 ± 0.44	3.23 ± 0.49
% Mg	12.47 ± 0.37	12.57 ± 0.26	12.43 ± 0.57	12.07 ± 0.60	12.43 ± 0.37	12.43 ± 0.42	12.37 ± 0.61	12.30 ± 0.85
% Ca	83.53 ± 0.90	83.53 ± 0.67	83.63 ± 1.03	84.10 ± 1.03	83.77 ± 0.83	83.83 ± 0.75	83.93 ± 0.94	83.87 ± 1.28
% Na	0.80 ± 0.00	0.73 ± 0.05	0.73 ± 0.05	0.73 ± 0.05	0.77 ± 0.05	0.77 ± 0.10	0.77 ± 0.05	0.73 ± 0.05
KMG	0.27 ± 0.04	0.27 ± 0.04	0.26 ± 0.04	0.27 ± 0.03	0.26 ± 0.03	0.25 ± 0.02	0.25 ± 0.03	0.26 ± 0.03

Curriculum Vitae

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