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The Role of Arbuscular Mycorrhizal Fungi in Urban Brownfield Soils

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Abstract

AMF are symbionts to a majority of terrestrial plants and can improve plant nutrient uptake, water relations, and stress tolerance. This study evaluated the effects of AMF in heavy metal contaminated soils via a growth chamber experiment to determine the interactions between soil and arbuscular mycorrhizal fungi (AMF) affecting plant growth. Rye grass was grown in two contaminated soils from Liberty State Park, an urban brownfield, and one non-contaminated commercial soil, to which half of the treatments received AMF inoculum. Dried plant biomass, root:shoot ratio, and soil phosphatase activity were measured at the completion of the experiment. Soil contamination was seen to decrease plant biomass. Across all soil types, AMF facilitated plant growth. Furthermore, a significant interaction between AMF and soil type was seen in average shoot mass. Contaminated soil led to an increase in root AMF colonization compared to non-contaminated soil. Root:shoot ratio and soil phosphatase activity were affected by soil type but not AMF. These results emphasize the degree to which soil type affects plant primary production and soil functioning, as well as the role of AMF in facilitating plant growth in urban brownfield soils.

MONTCLAIR STATE UNIVERSITY

The Role of Arbuscular Mycorrhizal Fungi in Urban Brownfield Soils

by

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Introduction:

Developing a better understanding of the microbial and plant interactions of contaminated soils is vital in the modern industrial world. Global trends and practices in manufacturing and environmental regulations have produced a modern landscape severely shaped by anthropogenic factors (Franklin et al., 2016). Despite recognition and efforts to enforce "green" environmentally conscious regulations, there is still much concern for ongoing and past environmental damage (Percival et al., 2017). In 2016, the Environmental Protection Agency found that within the United States alone, there are over 450,000 brownfield sites (EPA, 2016). Brownfields are land left with hazardous or toxic pollutants from past industrial practices (Small Business Liability Relief and Brownfields Revitalization Act, 2002). Toxic contamination of soils, especially in heavily populated regions, poses a risk to public health (Lars et al., 2003). Additionally, brownfield sites decrease the available land space for agriculture, city development, and public parks.

Overall, plant-soil feedbacks of non-contaminated soils are well documented and show that both aboveground and belowground factors act to drive the combined ecology of terrestrial ecosystems (Bever et al., 1997; Kulmatiski et al., 2008; van de Voorde et al., 2011). Soil abiotic composition and biotic communities largely impact aboveground plant communities (Reynolds et al., 2003; Wardle et al., 2004). Microbes can produce and increase availability of vital enzymes and nutrients for plant growth (Reynold et al., 2003; Caldwell, 2004). Mycorrhizal fungal activity can also influence bacterial populations and soil enzymes (Vázquez et al., 2000), as well as shaping plant communities (Hartnett & Wilson, 1999). In return, plants influence the structure and

health of soil microbial communities (Berg & Smalla, 2009). For example, plant root exudates can shape rhizosphere bacterial communities (el Zahar Haichar et al., 2008) and provide nutrients in low fertility soils (Bais et al., 2006).

Brownfield soils offer an extreme condition to study the complex interactions between soil organisms, plants, and industrial contaminants. The stress of high concentrations of heavy metals affects plant-soil dynamics. Although trace amounts of some heavy metals are beneficial to plant functioning, higher amounts can be toxic to growth (Munzuroglu & Geckil, 2002; Li et al., 2005; Nagajyoti et al., 2010). Additionally, contaminants can limit the diversity and functionality of soil microbial communities (Brookes & McGrath, 1984; Kandeler et al., 2996). This can result in lowered soil enzymatic levels (Giller et al., 1998; Garcia-Gil et al., 2000). Despite these adverse effects, it is possible that these communities may adapt and flourish over time (Krumins et al., 2015). Some plants have mechanisms adapted for acclimation to heavy metals (Hall, 2002). Protective adaptations can also be seen in microbial communities affected by contaminants (Pennanen et al., 1996). Within an urban brownfield, Hagmann et al., (2015) identified one region with high metal load and high enzymatic activities, suggesting a functioning soil microbiota. Investigation of the ectomycorrhizal fungi of this brownfield found fungal community composition was affected by soil metal contamination (Evans et al., 2015). These findings led us to investigate the role of arbuscular mycorrhizae in contaminated soils.

Arbuscular mycorrhizal fungi (AMF) are an important component of vegetated terrestrial ecosystems. AMF are obligate symbionts to a wide variety of plant host species (Smith & Read, 2010). The majority of land plants have some form of mycorrhizal

association, with the most common type falling into the category of AMF (Cairney, 2000). Evolutionarily, AMF are also the most ancient form of mycorrhizae and are associated with the movement of plants from aquatic to terrestrial ecosystems (Redecker et al., 2000). Additionally, evidence supports the theory that genetic pathways for symbiosis in AMF were precursors to bacterial intracellular plant relations (Parniscke, 2008). These fungi of the glomeromycota kingdom grow into plant root cortical tissue and form arbuscules (Smith & Read, 2010). Within the AMF group, a subgroup forming vesicle structures are also referred to as VAM fungi, or vesicular arbuscular mycorrhizae (Bever et al., 2001). Arbuscules are the main site of contact between the plant and fungus and act as the main area for exchanges (Bever et al., 2001).

AMF can increase plant nutrient uptake, including phosphate and nitrogen, and in exchange receive up to 20 % of carbon fixed by the host (Parniske, 2008). Phosphorous is the primary limiting nutrient AMF can help plants absorb from the soil, with lower soil phosphate levels resulting in increased AMF infection (Koide, 1991). Increased water stress tolerance can also be seen in plants with AMF (Allen & Boosalis, 1983), as well as decreased negative effects resulting from salt stress (Heikham et al., 2009). Furthermore, plant-pathogen studies have found AMF can increase resistance to some pathogens (Borowicz, 2001; Wehner et al., 2010). Because of the importance of AMF on plant soil interactions, many studies consider improving plant growth with fungal inoculation. Particularly with agricultural crops species, AMF inoculation has been shown to increase plant yield in both field and growth chamber studies (Jensen, 1984; Raju et al., 1990; Dodd et al., 1990). Additionally, AMF and soil bacteria may act synergistically to increase plant growth (Artursson et al., 2006). It is important to note that a wider

investigation of AMF interactions show that this plant-fungi relationship can range on the spectrum of mutualistic to parasitic, largely dependent upon species and local adaptations (Johnson et al., 1997; Klironomos, 2003).

Due to AMF's numerous roles and complexity of relationships, these fungi impact community level structure, diversity, and function (Hartnett & Wilson, 1999). Soil type can shape AMF communities and their functioning (Dodd et al., 1990). High rates of soil disturbance can decrease AMF presence (Jansa et al., 2003). Additionally, both field and lab studies show that the AMF community changes with increased soil nutrients from anthropogenic sources (Egerton-Warburton & Allen, 2000). Conversely, AMF affects soils through glomalin, a glycoprotein, produced by the fungi that acts as a stabilizer and aggregator (Wright & Upadhyaya, 1998; Miller & Jastrow, 2000). How soils and plants are affected by AMF depends on a wide range of functional diversity between and within AMF species (Munkvold et al., 2004). The plant-fungi relationship is also highly diverse depending on plant species (Van Der Heijden, 2002). For instance, the role of AMF in promoting plant growth is greater in plants with a higher AMF dependency (Van Der Heijden, 2002). This can lead to a decrease in plant diversity if certain species receive greater benefits from the mutualism. Alternatively, plant community diversity and productivity can be increased from this symbiosis (Vogelsang et al., 2006). The presence of AMF may also decrease plant competition (Wagg et al., 2011). Community stabilization and interplant interactions are also influenced by common mycelial networking linking different plants through their mycorrhizae (Hartnett & Wilson, 2002). Evidence supporting the transfer of carbon between trees via ectomycorrhizal mycelial linkages has even been shown (Simard et al., 1997) Mycorrhizal, and specifically AMF

diversity, shape plant community biodiversity, function, and stability in ways researchers are still working to understand (Van der Heijden et al., 1998; Hodge & Fitter, 2013).

An increase in anthropogenic soil contamination and understanding of AMF importance has led many to investigate the importance of AMF in toxic ecosystems. When exposed to high levels of heavy metals, an increase in stress acclimating genes was seen in *Glomus intraradices* (Hildebrandt et al., 2007). Some AMF species have the ability to bind and absorb soil metals (Joner et al., 2000). However, there is a wide range of responses to heavy metals across different species from sensitive to potentially well adapted (del Val et al., 1999; Pawlowska & Charvat, 2004). Due to these different responses, moderately contaminated soils may have slight increase in AMF diversity while highly contaminated conditions can result in sharp declines in diversity (del Val et al., 1999). With new technologies and phylogenetic studies uncovering high diversity levels, it is likely that the understanding of AMF responses to contamination and adapted species is far from comprehensive (Redecker & Rabb, 2006).

Not only are fungi affected in brownfield soils, but plants face many challenges to establishment and survival. AMF colonization has been seen to facilitate the survival and growth of plants in heavy metal conditions (Hildebrandt et al., 2007). High colonization rates of tolerant AMF species have been found under experimental high heavy metal conditions (Gildon & Tinker, 1983). Of the numerous plant adaptations to avoid toxicity, hyperaccumulation of heavy metals can be enhanced with AMF (Miransari, 2011). Arsenic resistance and accumulation have been seen to increase in plants with AMF (Gonzalez-Chavez et al., 2002; Chen et al., 2007; Jankong & Visoottiviseth, 2008). The same pattern with increased accumulation is also found with the chromium

hyperaccumulator, *Helianthus annuus*, when grown in chromium enriched soils with AMF (Davies Jr et al., 2001). Additionally, plants grown in lead contaminated soils show increased protection, accumulation, and growth with AMF present, particularly for plant species more dependent on the symbiosis (Chen et al., 2005; Yang et al., 2015). AMF can also help alleviate the stress of organic pollutants, such as polycyclic aromatic hydrocarbons (PAH's) (Leyval & Binet, 1998; Nwoko et al., 2013). Despite the evidence that AMF can help plants grow in contaminated soils, this facilitation is not always seen and varies with fungal and plant species (Stahl et al., 1988) and can also be affected by soil particle size (Nwoko et al., 2013).

The beneficial impacts of AMF in contaminated ecosystems and a growing need for management practices has led to an interest in using AMF in soil remediation. Largely, the conservation and functioning of AMF in general soil maintenance is vital for high function above and below ground (Jeffries et al., 2003). Additionally, AMF has been found to be beneficial in re-establishing plant communities in reclaimed nutrient poor anthropogenically disturbed soils (Dodd et al., 2002). Specifically, there is a growing interest in phytoremediation, a more natural and cost-efficient process that utilizes plants and soil microorganisms to stabilize, extract, and degrade contaminated soils (Cunningham & Ow, 1996; Mahar et al., 2016). For heavy metal contamination, hyperaccumulators are of primary interest for extraction of soil metals (Mahar et al., 2016). AMF can enhance and speed up this process by extending the capabilities of plants to reach and uptake metals (Göhre & Paszkowski, 2006). Fungal species with resistance to a diverse set of heavy metals, such as *Glomus mosseae*, are promising for remediation studies (Gaur & Adholeya, 2004). AMF may also play a role in the

degradation of PAH's, an additional growing concern for remediation (Leyval et al., 2002).

To contribute to the current understanding of the role of arbuscular mycorrhizae in facilitating plant growth and soil functioning, this thesis utilizes heavy metal contaminated soils from Liberty State Park (LSP), an urban brownfield. LSP offers a unique urban ecosystem to study the interactions between soil fungi, anthropogenic contaminants, and plants. Based on the contributions of AMF in alleviating stress in contaminated soil conditions, my hypothesis was that AMF will have a greater role in facilitation of primary production in soils with greater contamination. I tested this by conducting a growth chamber experiment and utilizing root staining. Additionally, I expected that enzymatic measurements of the experimental soils would be impacted by the presence of AMF. The results from this study are important for brownfield ecology and management, with specific interest in phytoremediation applications

Methods:

Liberty State Park

Soils were collected from Liberty State Park (LSP), Jersey City, New Jersey in July 2017. Once an estuary on the Hudson River, this land has seen substantial human impact as a railyard with development occurring from the mid 19th to mid 20th centuries, followed by abandonment in the late 1960s and subsequent natural forestation. Currently an urban brownfield, the portion of this park with restricted access offers a unique case study of urban contaminated soils. The 100-hectare region of non-remediated soils of LSP have been well mapped and shown to have varying levels of heavy metal

contamination between sites, including arsenic, chromium, lead, zinc and vanadium (Gallagher et al., 2008) (Fig. 1). Specifically, my experiment utilizes soils collected from LSP sites 146 and 25R. On opposing sides of the field site, these soils are heavily contaminated with metal loads above the surrounding threshold (Gallagher et al., 2008). LSP 146 is a densely vegetated soil, whereas LSP 25R is barren of vegetation. Additionally, LSP 146 soils have high enzymatic activity compared to surrounding soils despite high metal load (Hagmann et al., 2015). An investigation of the ectomycorrhizal communities between these sites shows a separation of fungal composition dependent upon heavy metal load gradient (Evans et al., 2015). The communities and role of arbuscular mycorrhizae at LSP have yet to be clearly established. For preliminary findings, I stained root samples from LSP 146 and found evidence of arbuscular mycorrhizal colonization (Fig. 2A-2B).

Experimental Design

I designed and conducted a potted growth chamber experiment to compare the role of AMF in plant growth in LSP soils. I established a factorial design with 3 soil types and the presence of AMF or without AMF (Table 1). Each experimental treatment was replicated six times for a total of 36 pots. The three soils used were Miracle Grow Potting Soil (PS) as a reference and control, LSP 146 as a highly contaminated but well vegetated soil, and LSP 25R as a highly contaminated but unvegetated soil. I prepared each soil by first passing them through a 2mm sieve, and then sterilizing by autoclaving two times at 121.5°C for 20 minutes. I potted each sterilized soil base in 700ml pots lined on the bottom with one coffee filer (Table 2). I inoculated half of the pots with 5 grams per pot

of Commercial AMF inoculum (advertised to contain *Glomus intraradices, Glomus mosseae, Glomus aggregatum,* and *Glomus etunicatum*). After soil setup, I sowed ten winter rye grass seeds (*Lolium sp.)* into each experimental pot. Three weeks post germination, I culled the plants to leave the largest remaining plant per pot. Over the course of the experiment, I watered each pot with equal tap water twice a week (20-40ml per pot). Pots were maintained in a growth chamber with diurnal settings of 12 hours day at 24°C and 65% moisture for 12 nights at 16°C and 55% moisture. Plants from one replicate each of PS AMF+ and LSP146 AMF+ did not germinate, resulting in 5 replicates of these treatments instead of six. To evaluate the effects of soil metal load and the presence of AMF, plant biomass, AMF colonization of roots and soil, and free enzyme activity were measured as an indicator of soil functioning.

Plant Biomass

At 105 days post setup, I harvested the plants and took representative samples of roots from each replicate and stored them at -20°C for DNA extraction. Then, I separated plant roots and shoot and dried them at 70°C for 7 days. To account for the wet root samples removed for DNA extraction, a wet weight to dry conversion was performed and calculated into the total root mass for each sample.

AMF Colonization

I performed root staining of field and experimental roots using the classical AMF root staining methodology using trypan blue stain and followed the gridline interest method to quantify percent AMF colonization (Phillips & Hayman, 1970, Vierheilig et al,

1998). I performed this procedure on roots collected from LSP site 146 to investigate the presence of AMF in field collected roots. Additionally, I stained and counted percent AMF colonization of roots collected from my growth chamber experimental pots. I rehydrated dried roots with water for one hour, then cleared them with 10% KOH at 60°C for 90 minutes until translucent, following by three rinses with tap water. Clearing of roots grown in PS followed the same procedure with time extended to two hours to account for greater thickness. Next, I acidified the roots with cold 2 N HCl for two minutes, then stained for 20 minutes at 60°C with the following stain: 0.05% Trypan Blue, 50% glycerol, 48% water, and 2% 2 N HCl. I destained the roots with 50 % glycerol, 48 % water, and 2 % 2 N HCl. After completion of staining, I first viewed roots with a dissecting microscope and then evaluated for positive or negative AMF colonization of 200 grids per root squash with a compound light microscope to determine percent of root length colonized.

Enzymatic Activity

In collaboration with a doctoral student in Environmental Management (Bhagyashree P. Vaidya), free phosphatase enzyme potential of the experimental soils was measured in all replicates (PS-, PS+, LSP 146-, LSP 146+, LSP 25R-, LSP 25R+) at the completion of the experiment. Following procedures of Hagmann et al. (2015), Bhagyashree P. Vaidya followed the fluorometric assay protocol developed by Marx et al., (2001), modified as needed (Hagmann et al., 2015) to measure the amount of 4-Methylumbelliferone product formed by the phosphatase enzymes present in each soil

sample. Moisture was analyzed with 2.0 grams of soil in a drying oven at 70°C for 24 hours and used to calculate phosphatase activity per hour times grams dry mass of soil.

Statistical Analysis

I used two-way Factorial ANOVA tests (JMP®, Version 13.2 PRO. SAS Institute Inc., Cary, NC, 1989-2007) in which soil type and AMF inoculation were the fixed factors used to determine significance of my treatments on the following response variables: plant growth (total, root, and shoot dry biomass and root:shoot ratio, and soil phosphatase) (Table 3), followed by Tukey HSD analysis to determine pair-wise significance. In the case of enzymatic activity, phosphatase levels were below detectable levels for LSP 25R soils and excluded from statistical analysis. Percent root colonization was analyzed using a t-Test.

Results

Plant Biomass

Visual differences in size and vigor of plants between soil types could be seen during the experiment, with the largest plants seen growing in non-contaminated soil and the smallest plants in LSP 25R (Fig. 3). Measurements of total dry plant mass (grams) showed significant differences in total plant biomass by two-way ANOVA between soil types ($F_{2,34} = 313$, $p < 0.0001$), with a Tukey HSD finding all comparisons between soil types to be significant ($p < 0.0001$) (Fig. 4). By soil type, average total mass was seen highest in PS and lowest in LSP 25R. Additionally, using a two-way ANOVA, I found average total plant mass was significantly higher in treatments with the AMF inoculum

than those without AMF ($F_{1,34}$= 14.3, p = 0.0007). Pairwise comparison found significantly greater total plant mass in PS+ compared to PS- (p < 0.0129). For both LSP 146 and LSP 25R soils, greater plant mass was seen in treatments with inoculum, but not to a significant degree (Tukey HSD, p > 0.05). Additionally, I did not find a significant interaction between soil type and presence or absence of AMF on total plant mass (two-way ANOVA, $F_{2,34}$ = 3.15, p > 0.05).

Average root biomass was found to be significantly different between soil types by two-way ANOVA analysis ($F_{2,34}$ = 225, p < .0001), with significant differences in root mass between pairwise comparisons of all soil types (p < .0001) (Fig. 5). By soil type, the greatest average root biomass was seen in PS soil, and the lowest was in LSP 25R soil. Additionally, average root biomass was greater in treatments inoculated with AMF (Two-way ANOVA, $F_{1,34}$ = 6.76, p = 0.0147). Within each soil type, greater average root mass was seen in inoculated treatments, but not to a significant degree (Tukey HSD, p > .05). No significant interaction between soil type and AMF was found on average root biomass ($F_{2,34}$ = 1.64, p > .05).

Comparison of average shoot biomass via Two-way ANOVA analysis revealed a significant two-way interaction between soil type and AMF ($F_{2,34}$ = 23.7, p < .0001) (Fig. 6). Average shoot mass was greater in PS compared to both LSP 146 and LSP 25R (Tukey HSD, p < .0001) and greater in LSP 146 compared to LSP 25R (Tukey HSD, p = .0008). Treatments with AMF had greater average shoot mass (Two-Way ANOVA, $F_{1,34}$ = 36.5, p < .0001) (Fig. 6), with a significantly greater average shoot mass in PS+ than PS- (Tukey HSD, p < .0001).

Root:shoot ratio was found to be significantly different via Two-Way ANOVA analysis depending upon soil type ($F_{2,34} = 27.5$, $p < .0001$) (Fig. 7). Root:shoot ratio was found to be significantly higher in LSP 146 compared to both PS and LSP 25R (Tukey HSD, $p < .0001$). Comparison of PS and LSP 25R did not find significantly different root:shoot ratios (Tukey HSD, $p > .05$). Overall, no significant differences in root:shoot ratio was found between treatments with and without AMF (Two-Way ANOVA, $F_{1,34} = 0.07$, $p > .05$).

AMF Colonization

Roots stained with Trypan blue from PS- and LSP 146- had no evidence of AMF colonization. Indication of AMF colonization in experimental roots grown in inoculated soils was found (Fig. 8). Quantification of AMF root colonization using the stained roots showed that roots from LSP 146+ had significantly greater average colonization than those from PS+ (t-Test, $t_8 = -2.19$, $p = .0297$) (Fig. 10). Roots from LSP 25R were not stained due to insufficient root tissue of plants.

Phosphatase

Soil phosphatase levels were found to be significantly greater in PS soils than LSP 146 soils (Two-way ANOVA, $F_{1,34} = 8.68$, $p < 0.0090$), with no significant impact from AMF inoculum ($F_{1,34} = 0.124$, $p > .05$). Phosphatase levels in LSP 25R+ and LSP 25- were lower than levels found in PS soils and 146 soils, but were below detectable levels.

Discussion

The goal of this study was to explore the role of AMF in the facilitation of plant growth in urban brownfield soils via a growth chamber experiment. This study used soils of different heavy metal contamination loads from LSP and non-contaminated commercial potting soil to compare plant and soil trends with and without AMF present. I found that soil type was a significant factor across all measured responses including total plant mass, root mass, shoot mass, root:shoot ratio, AMF root colonization, and soil phosphatase levels. Additionally, AMF was a significant factor affecting average total plant mass, average shoot mass, and average root mass. My hypothesis was that contamination levels of the soils would affect the degree to which AMF increased plant growth. This experiment found that soil type affected plant growth, and the presence of AMF inoculum increased plant growth. A significant interaction between soil and AMF was found in average shoot mass.

Soil type was found to be the primary factor affecting plant growth in this study. This may in part be due to the fact that plant growth is dependent upon numerous soil factors, including soil fertility levels (De Deyn et al., 2004). Plant growth can also be impacted by structural components of soils, which affect water holding capacity and nutrient availability (Passioura, 1991). Although nutrient levels were not measured, PS was assumed to have greater fertility than either brownfield soils due to fertilizers used commercially. Each of the three soils I used had different abiotic characteristics and structures, with LSP 25R having a uniquely coarse texture. Additionally, LSP 146 is a vegetated site, whereas LSP 25R is not vegetated, suggesting these soil bases may have different levels of organic matter. Soil microbial community can also impact

aboveground functioning (Kulmatiski et al., 2008), but because each soil base was sterilized during experimental setup, biotic communities prior to inoculation are not a considered factor for this experiment. The texture and water holding capacity of the soils may account for differences in plant growth.

Toxicity of heavy metals to plant growth is important for the central question of my thesis. High levels of heavy metals can limit plant growth (Nagajyoti et al., 2010). Because of the stress that heavy metals pose on plant growth, plants were expected to have lower mass in LSP soils compared to non-contaminated PS. Additionally, LSP 25R was expected to have lower plant mass than LSP 146 due to observed inability to sustain plant growth in the field. As expected, average total plant mass, average root mass, and average shoot mass were all greatest in PS soils and lowest in LSP 25R (Fig 4-6). My findings support the concept that heavy metal contaminated soils are less hospitable to plant growth than non-contaminated soils. Miniscule plant growth in 25R may be due to high heavy metal loads and porousness of the soil affecting soil moisture (Basso et al., 2013). Additionally, there may be additional components of 25R that deter plant growth that have yet to be documented.

Plant biomass across all soils types was positively affected by AMF inoculum. This finding supports the role of AMF as a facilitator of plant growth (Fig. 4, 5, 6). AMF acts to increase nutrient uptake, thereby promoting primary production (Parniske, 2008). However, comparing inoculated and non-inoculated treatments of the same soil type found that despite overall trends of increased plant growth with AMF, the only significant increase was in PS+ compared to PS- (Fig. 4, 6). The role of AMF is shown to depend upon soil conditions (Dodd et al., 1990). Significant interaction between soil and AMF,

and significant increases in plant mass only in one soil type may then be attributed to differences in the compositions of the three soils types used.

My findings support the hypothesis that plants grown in contaminated soils can experience facilitation from AMF, but do not show that this facilitation is greater in contaminated soils compared to non-contaminated soils. Because AMF act to increase nutrient uptake, I expected plants to rely less upon AMF when grown in nutrient rich PS. Conversely, I expected plants grown in contaminated soils to depend upon AMF more than those in non-contaminated soils because AMF has been shown to help alleviate stress (Hildebrandt et al., 2007). Numerous studies have shown that AMF can increase plant growth in heavy metal contaminated soils (Miransari, 2011; Gonzalez-Chavez et al., 2002; Chen et al., 2007; Chen et al., 2005; Yang et al., 2015). Additionally, I expected AMF to have a significant impact on plants grown in LSP 25R by improving water relations in the porous soil. For my findings, AMF may not have had a significant impact on plants grown in LSP soils due to the stress of heavy metal toxicity on the fungi themselves. Although some AMF species have been found to be heavy metal resistant, there is a wide response to heavy metal stress across AMF species (del Val et al., 1999). It is possible that the strains of AMF present in the commercial inoculum I used were sensitive to heavy metals, as well as other uncharacterized contaminants in LSP soils. Additionally, the contaminants of LSP soils may have stunted plant growth to a degree greater than the AMF inoculum could have compensated for. Further investigation into the composition of LSP soils may provide deeper insight into these findings.

Although AMF interacts with plant roots, root:shoot ratio was not significantly affected by AMF inoculum (Fig. 7). Because AMF helps to increase surface area for

nutrient acquisition from the soil (Parniske, 2008), one may expect plants to allocate less energy to root tissue with greater AMF colonization. My data show soil type as the only significant factor in root:shoot ratio, with a significantly higher ratio in LSP 146 compared to both other soils, and no significant difference in PS and 25R. This unexpected finding brings to question why plants in LSP 146 allocated more energy to root growth than those of the other treatments. LSP 146 plants may have had relatively greater root mass than PS plants because of an increased need to generate root surface area for nutrient and water uptake in the brownfield soil. Plants grown in nutrient rich PS may not have needed to allocate as much energy to an extensive root system (Güsewell, 2004). This does not account for why LSP 25R plants were not significantly different than those grown in PS. A lower root:shoot ratio in 25R suggests less stress on the roots grown in these conditions. Additionally, the texture of LPS 25R may impact the morphology of the roots. These results show the plasticity of rye grass tissue allocation depending upon soil composition.

Seemingly contrary to the finding that AMF was not a significant factor in promoting plant growth in LSP 146 soils, percent AMF root colonization was found to be significantly greater in roots from LSP+ compared to PS+ (Fig. 9). The degree of root colonization can vary depending upon form of inoculum used and AMF species present (Klironomos & Hart, 2002). Because I used a mixed inoculum, it is possible that the species that thrived in each soil condition may have been different. If so, different relative abundance of AMF species between PS and LSP 146 may be one possible explanation behind differing degrees of root colonization. Additionally, despite greater degree of root colonization, LSP 146 plant biomass was lower than that of PS. This suggests that the

greater presence of AMF in LSP 146 roots was not enough to offset the impact of the soil contaminants. Overall, root colonization was low compared to field study findings (Wearn & Gange, 2007), therefore the experimental conditions may not have been ideal for extensive AMF growth. Additionally, staining of the roots post oven dehydration may have changed the integrity of the tissue.

Measurement of soil phosphatase levels found that AMF was not a significant factor, suggesting the presence of AMF did not affect nutrient cycling in my experimental soils. Past studies have shown that AMF can increase soil enzyme activities, including phosphatase activity (Kumar et al., 2008; Wu et a., 2011). This increase was found to be positively related to fungal density (Kumar et al., 2008), suggesting that the AMF present in my experimental soils may not have developed to high enough densities to significantly affect soil enzymatic activities. Additionally, because the AMF inoculum did not affect phosphatase activity, the extracellular inoculum component did not seem to act as a form of nutrient supplement. If the inoculum had acted as a nutrient enrichment to the soils, we would expect the AMF positive treatments to shift either towards less phosphatase activity because of greater available phosphate, or increased in phosphatase due to increased microbial flourishing (Olander & Vitousek, 2000). Soil type did significantly impact phosphatase activities, with LSP 146 being significantly lower than PS and LSP 25R below detectable levels. This can be accounted for by the presence of heavy metals in LSP soils, which suppress the activity of soil microbial communities and phosphatase levels (Kandeler et al., 1996). Phosphatase levels below detectable levels in LSP 25R further indicate that this soil is inhospitable to not only plant growth, but also microbial development.

Conclusions

The results from my thesis find that plant growth is affected by soil type and is facilitated by the presence of AMF across soil types used. These results do not support my hypothesis that AMF facilitates plant growth to a greater degree in heavy metal soils, but my data do show greater AMF colonization of plant roots in contaminated soils. Root:shoot ratio was also seen to be significantly higher in LSP 146 soils. Additionally, plant shoot mass was found to be impacted by an interaction between soil and AMF factors, showing that AMF does impact plant growth differently depending on soil composition. Phosphatase was only seen to be impacted by soil type. Further study implementing sequencing and LSP field community results may shed light on the activities of AMF in urban brownfield soils. Findings from studying AMF in brownfield soils are applicable to phytoremediation goals. The results of this thesis show the importance in soil composition and fungal community in above ground primary production in contaminated and non-contaminated soils.

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Figures

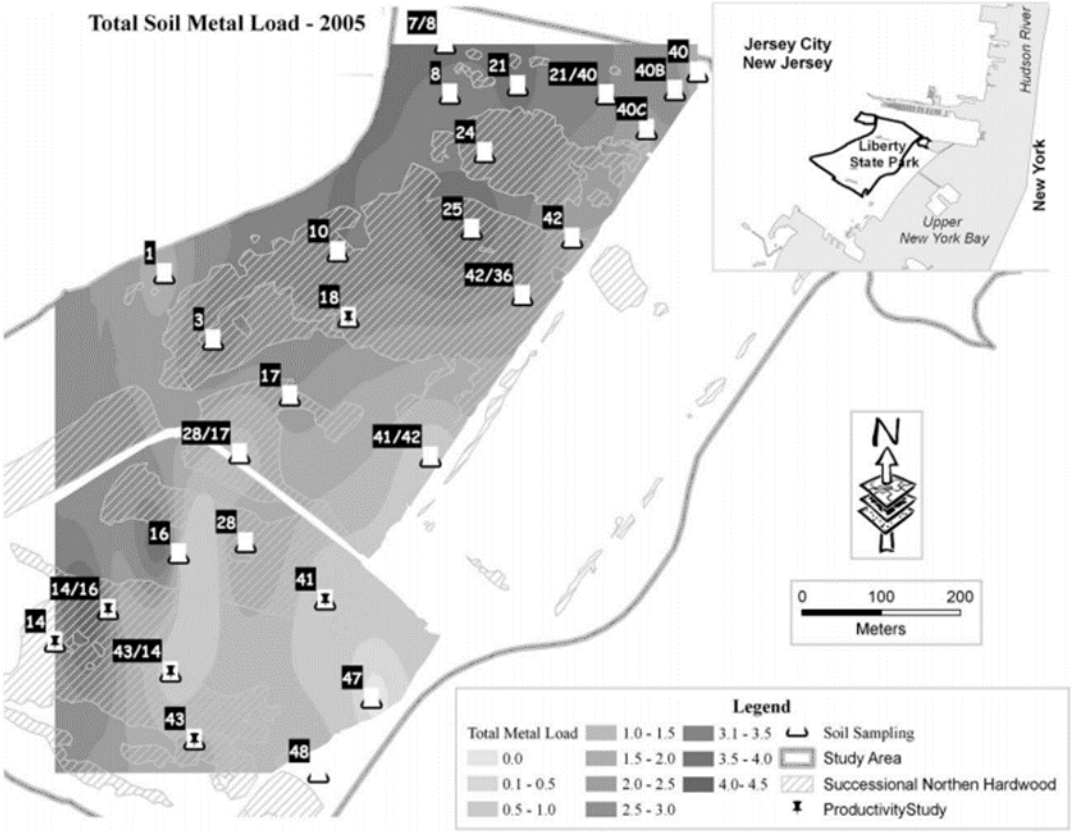


Figure 1.

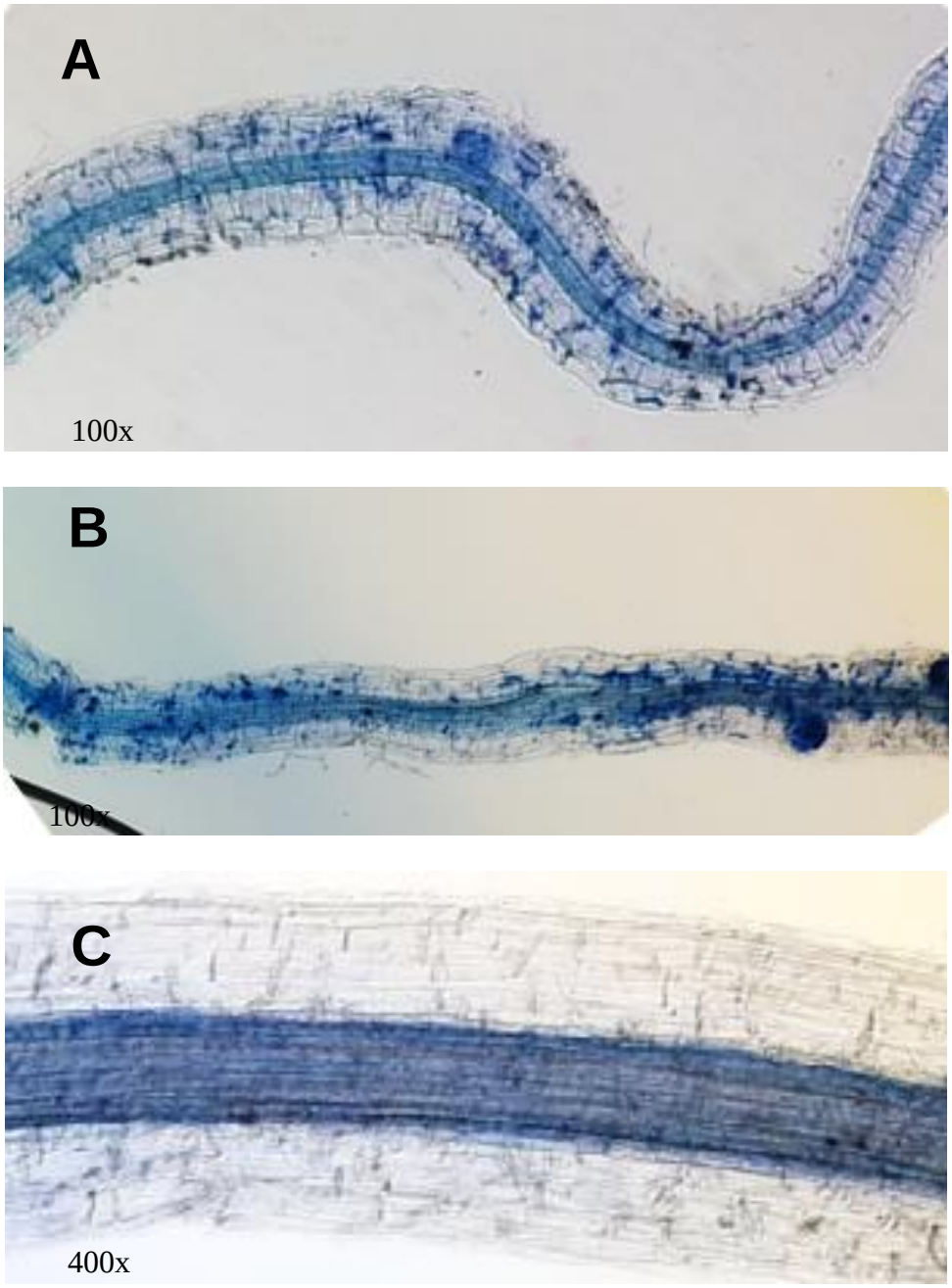
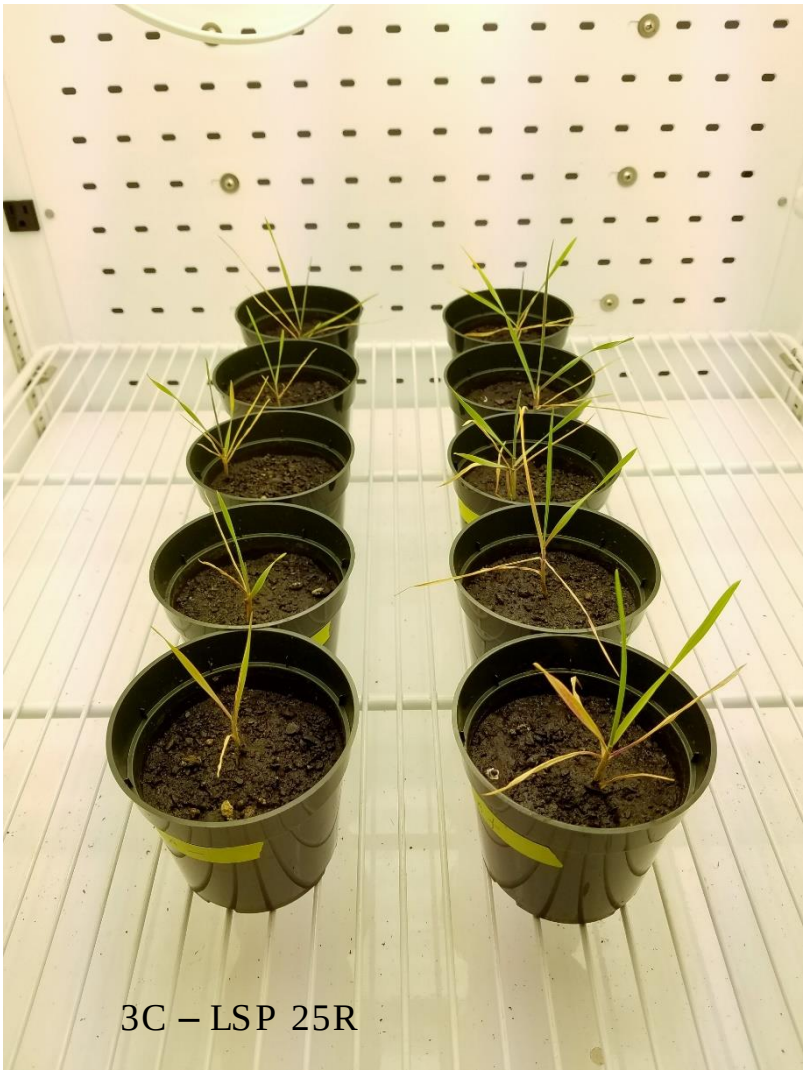


Figure 2.

Figure 3.


3B – LSP 146



3C – LSP 25R

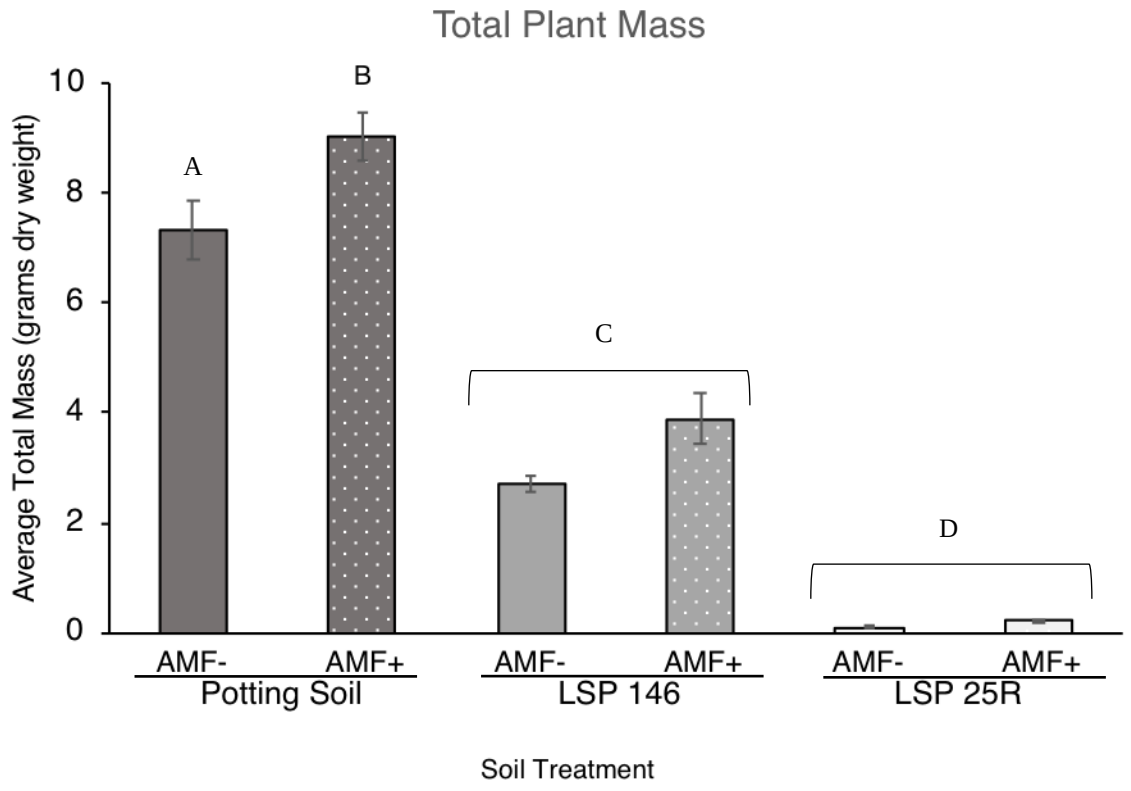
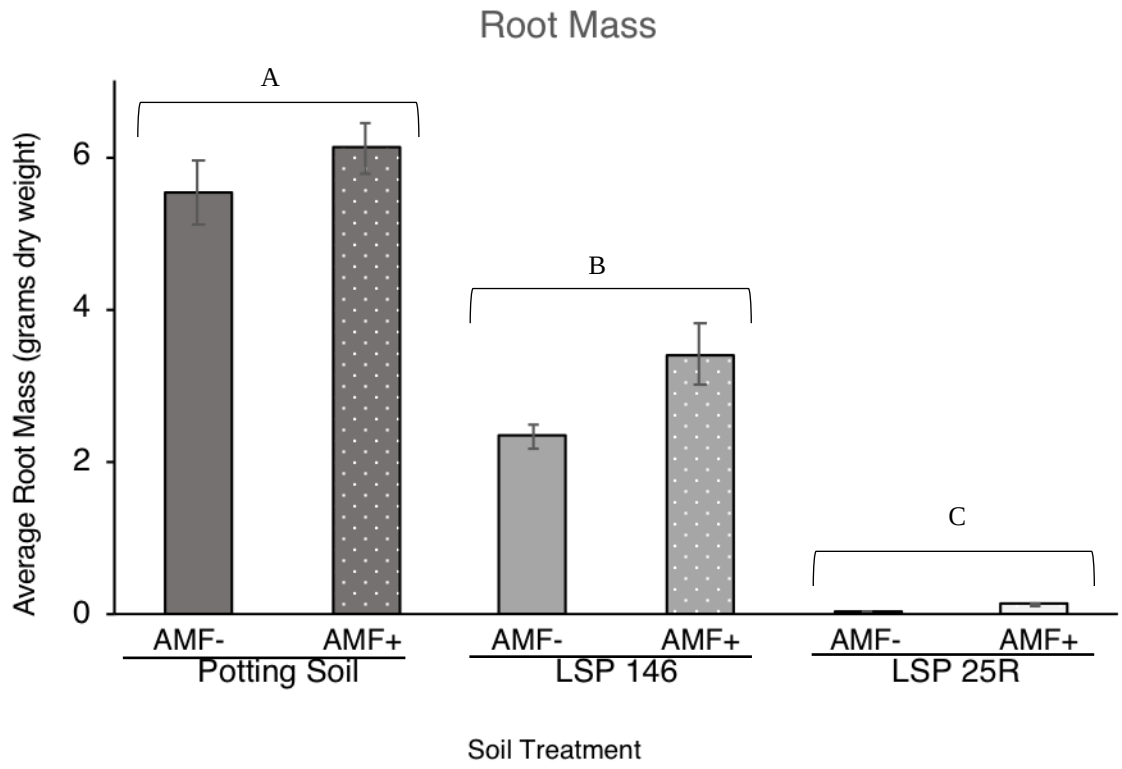


Figure 4.



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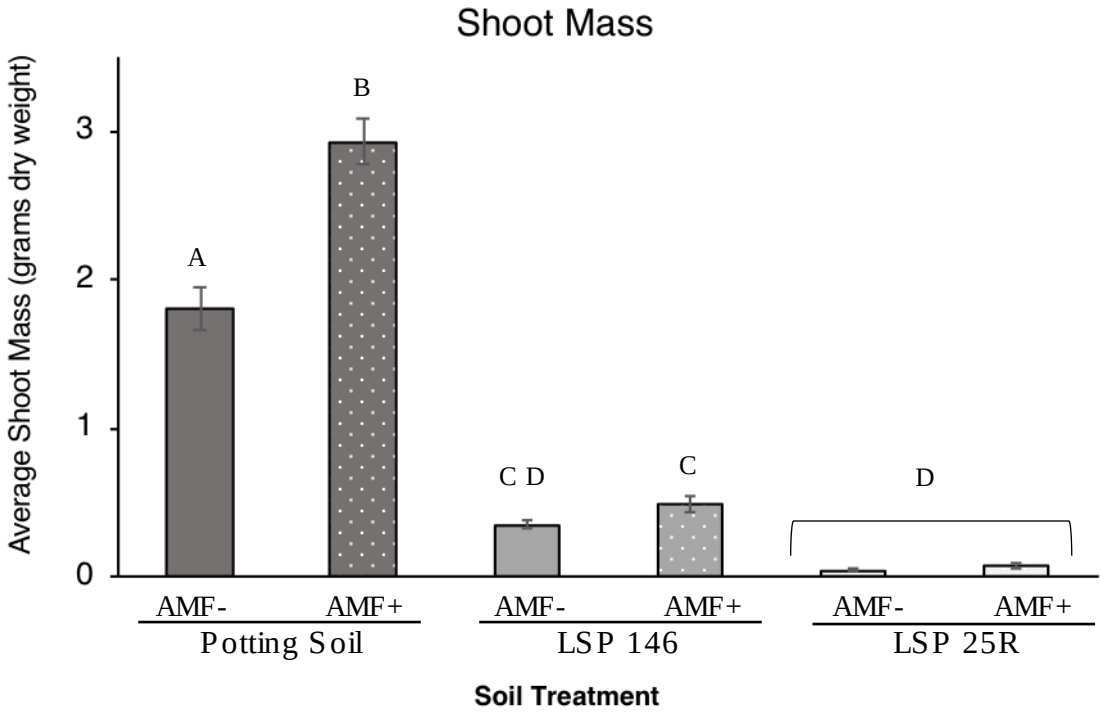
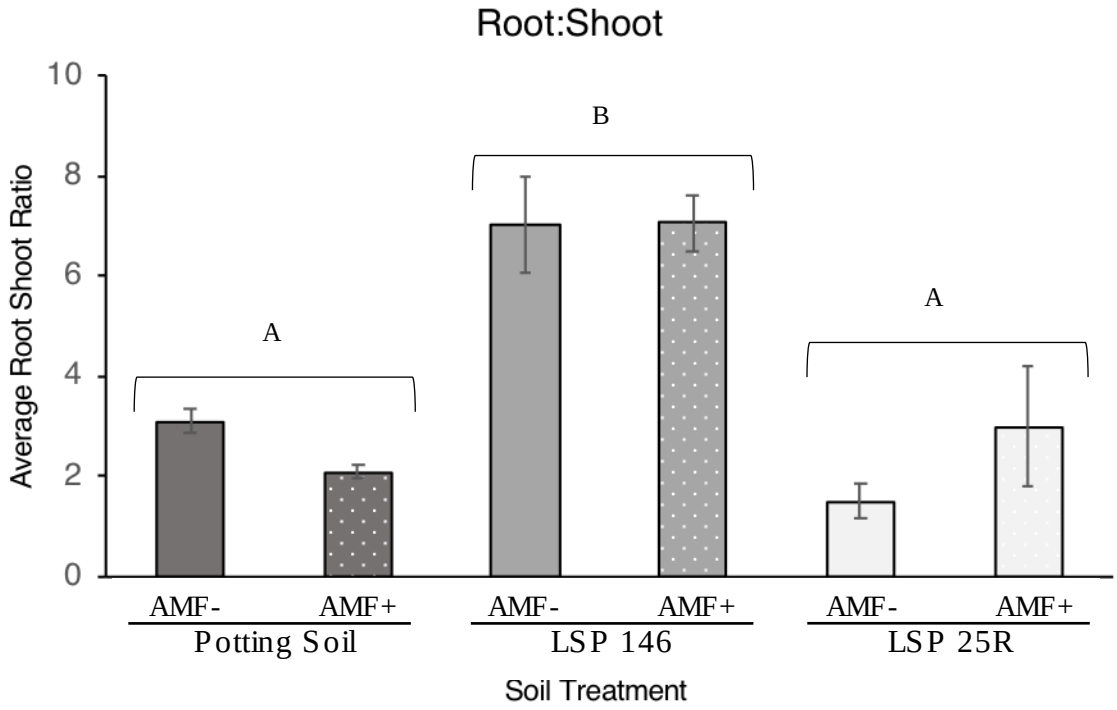


Figure 6.



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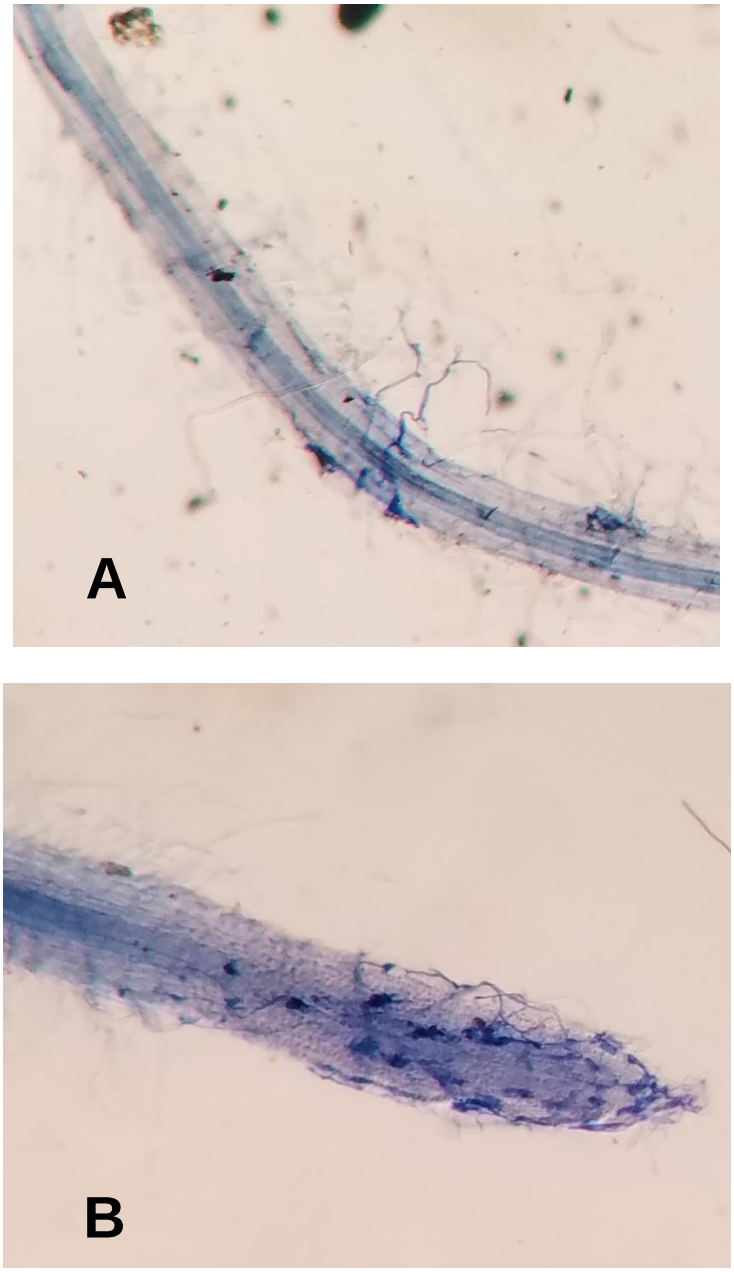

Figure 8.

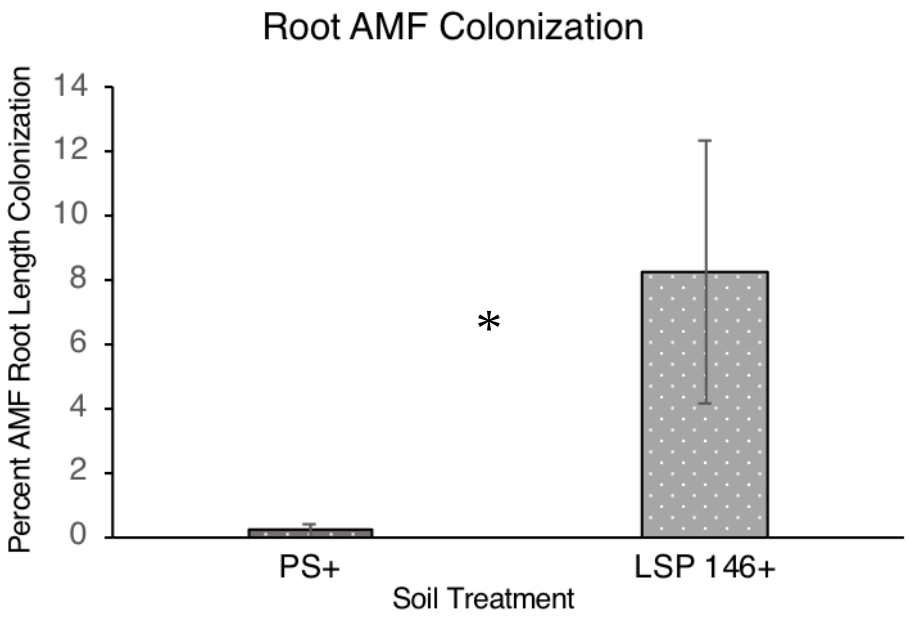

Figure 9.

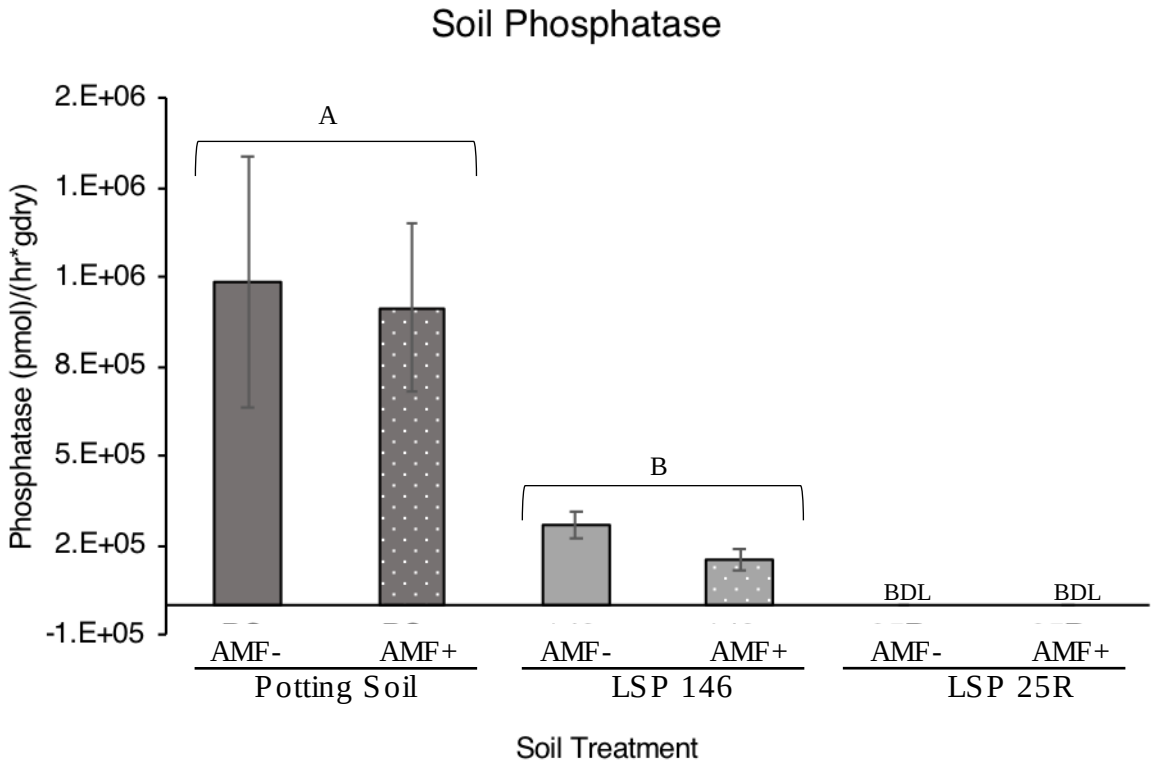

Figure 10.

Tables

Table 1.

Treatment Name	Soil Type	AMF Inoculum (+/-)	Replicates (post germination n=)
PS-	Commercial Potting Soil	-	6
PS+	Commercial Potting Soil	+	5
LSP 146-	LSP Site 146	-	6
LSP 146+	LSP Site 146	+	5
LSP 25R-	LSP Site 25R	-	6
LSP 25+	LSP Site 25R	+	6

Table 2.

Treatment	Soil mass per pot (grams)
PS AMF-	102.14 ± 1.41
PS AMF+	114.92 ± 1.10
146 AMF-	179.43 ± 1.57
146 AMF+	177.29 ± 0.81
146 AMF+	377.7 ± 2.12
25R AMF +	377.69 ± 1.57

Table 3.

Response Measured		DF	F_{34}	p-value
Average Total Plant Mass				
	Soil	2	313	<.0001
	AMF	1	14.4	.0007
	Soil*AMF	2	3.16	.0582
Average Root Mass				
	Soil	2	226	<.0001
	AMF	1	6.76	.0147
	Soil*AMF	2	1.64	.2116
Average Shoot Mass				
	Soil	2	404	<.0001
	AMF	1	36.5	<.0001
	Soil*AMF	2	23.7	<.0001
Average Root:Shoot Ratio				
	Soil	2	27.5	<.0001
	AMF	1	0.08	.7850
	Soil*AMF	2	1.57	.2256
Average Phosphatase				
	Soil	1	8.68	.0090
	AMF	1	0.12	.7287
	Soil*AMF	1	.0028	.9586