A COMPARATIVE STUDY ON THE EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI ON *TRIPSACUM DACTYLOIDES, ZEA DIPLOPERENNIS* AND *ZEA MAYS*

by

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DEDICATION

I dedicate this thesis to my family spanning Texas, Canada and Georgia. I specifically dedicate this to my grandfather, Ross Gingera. Even though he is no longer with us, I am forever grateful for his example of hard work, wisdom and guidance provided in a few but powerful words shared between us. I would not be the person I am today without the continued and immense support of my family.

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LIST OF ABBREVIATIONS

Abbreviation	Description
ADP	adenosine diphosphate
AMF	arbuscular mycorrhizal fungi
ANOVA	analysis of variance
ATP	adenosine triphosphate
cm	centimeter
DAE	days after emergence
DNA	deoxyribonucleic acid
DP	direct pathway
ft	feet
g	gram
HCl	hydrochloric acid
in	inch
Kg/ha	kilograms per hectare
Kpa	kilopascal
L	liter
lbs.	pounds
mg	milligram
mg/L	milligrams per liter
ml	milliliter
MP	mycorrhizal pathway
N	normal
NK	nitrogen, potassium
NPK	nitrogen, phosphorus, potassium
Pi	orthophosphate
PSB	phosphate solubilizing bacteria
RMD	relative mycorrhizal dependency
RNA	ribonucleic acid

I. INTRODUCTION

Arbuscular mycorrhizal fungi, AMF, are a subset of plant symbiotic fungi called mycorrhizae (Bazaretti et al. 2019). The term mycorrhiza is used to describe a symbiotic relationship between a fungus and a host plant's rooting system (Barman et al. 2016). Because they are obligate biotrophic organisms, AMF are entirely reliant on a host plant's nutrients to grow and replicate (Bazaretti et al. 2019). The fungi form a mutualistic symbiotic relationship with the host plant by forming arbuscules, which are highly branched structures that form within the plant cell wall but outside the plasma membrane of the root cells (Rust 2021). Arbuscules are composed of fungal hyphae ensheathed in a modified cell membrane called the peri arbuscular membrane (Wipf et al. 2019). The arbuscules serve as the site for exchanging vital plant nutrients such as phosphorus, carbon and water (Hartnett and Wilson 1999). The arbuscules are a short-lived fungal structure, while the fungal hyphae are longer living structures that can continually colonize roots as they enter the fungal domain (Rust 2021). In the mutualistic relationship, the plant will provide the AMF a source of carbon and water, and the AMF will help provide the plant phosphorus, specifically non-plant available phosphorus (Sultenfuss and Doyle 1999).

The lifecycle of AMF begins when the fungal spore germinates and forms hyphae that will grow in the direction of the intended host. The fungal spore will illicit signals that will counteract the natural immune response of the plant through physiological changes, allowing for the symbiotic relationship to begin. The external hyphae will colonize surrounding soil, so nutrients, like phosphorus, can begin to be transferred from the AMF to the host plant (Denison and Kiers 2011).

Endomycorrhiza and ectomycorrhiza are the two types of mycorrhizae that are commonly described. Ectomycorrhiza will not penetrate root cortical cells, while endomycorrhiza will penetrate root cortical cells (Barman et al. 2016). Endomycorrhiza, including arbuscular mycorrhizal fungi, evolved 450 to 500 million years ago, according to fossil records. Arbuscular mycorrhizal fungi are the most abundant endomycorrhiza with fungal presence found in more than 80% of vascular plants (Wipf et al. 2019).

Because of its dependence on a plant host to establish and grow, most modern-day AMF inoculants are established using *in vitro* methods. In the 1970's experimentation into the *in vitro* culturing of AMF began (Bazaretti et al. 2019). In 1975, Mosse and Hepper (1975) conducted the first studies on *in vitro* growing of AMF using an axenic culture. Following this, the first subculturing of *in vitro* AMF was completed by Strullu and Romand (Strullu and Romand 1986). *In vitro* culturing allows for the generation of many AMF spores in a controlled environment. Additionally, *in vitro* culturing allows for continuous subculturing, making AMF more readily available to the public (Bazaretti et al. 2019).

The addition of AMF to the root space can help fortify the plant in times of drought or pest infestation through improved nutrition and acquisition of nutrients like phosphorus (Garg and Chandel 2010, Kabir et al 1999, Wander et al. 1998). The southern Unites States experienced extreme drought in 2011 that had adverse effects on economically important soybean and corn crops. The drought jeopardized 52% of corn crops and 36% of soybean crops planted that year causing economic losses up to 7.6 billion, like what was seen in Texas (Lal et al. 2012). In production areas or circumstances where water resources are limited, AMF hyphae can help with water stress by increasing the root space of the plant, thus giving the plant access to water of lower Kpa, kilopascal, value that would otherwise not have the required pressure to

travel through the root system (Hallett et al. 2008). This is all accomplished because endophytes influence the increasing uptake of nutrients like phosphorus, which is responsible for cationic stomal movement in response to a changing bulk leaf water status (Garg and Chandel 2010).

Inoculation is the process of applying ready-made AMF, in one of many forms, to a seed or the soil it will be sown into. The inoculation process will be most effective when the native population of soil fungi is low or non-existent (Denison and Kiers 2011). There are five different ways to inoculate, either seed or soil, with AMF. Inoculation by broadcasting involves a combination of seed and inoculant, in powdered form, being spread over soil. In-furrow application involves digging a small trench below the sowing depth for the seed and placing granular or pelletized AMF into the trench which will be covered with soil before the seed is sown. Seed coating consists of applying a carrier, to help with inoculum adhesion, to the seed followed by rolling the seed in inoculum. For propagated plants, root dipping can be utilized. In this process, roots are rinsed then dipped into a mixture that contains a carrier and the inoculum. Lastly, seedling inoculation can be accomplished by growing seedlings in a potting mix that contains inoculum before transplanting the plant to a field (Adholeya et al. 2005). Once inoculated, plants will receive phosphorus uptake regulation via AMF (Sultenfuss and Doyle 1999).

Phosphorus is a plant essential macronutrient that is needed in large quantities by plants, but it is often a limiting soil nutrient for plant growth (Elser et al. 2007). Phosphorus is an essential component of nucleic acids, which make up the DNA, deoxyribonucleic acid, and RNA, ribonucleic acid, of the plant; the genetic material contained in DNA and RNA is responsible for the passage of traits to future generations. During the light phase of photosynthesis, phosphate, in the form of ATP, adenosine triphosphate, is needed to create

carbohydrates and release oxygen. A lack of proper phosphorus levels will manifest itself in the plant through decreased leaf expansion, decreased leaf surface area, decreased root growth and decreased forage quality (Sultenfuss and Doyle 1999, Toussaint et al. 2007). Phosphorus uptake can occur in one of two pathways: direct pathway, DP, or mycorrhizal pathway, MP (Smith et al. 2011).

In the DP, orthophosphate, Pi, a negatively charged phosphate ion, is present because plants and fungi uptake phosphorus in the form of this negatively charged ion. Orthophosphate will be absorbed from the plant's rhizosphere through plant Pi transporters that are in the root hairs near the root's surface. Because uptake is significantly faster than replenishment, Pi within the rhizosphere will be rapidly depleted. The MP pathway can help reduce Pi depletion. In the MP pathway, Pi will be derived from polyP hydrolysis, which is the reaction of water with a phosphate containing compound. Pi moves from the fungal cytoplasm to the peri arbuscular space through a method which is currently unknown. Localized phosphate transporters within the peri arbuscular membrane will allow for the transport of Pi into the plant cell. This movement of Pi is powered by the hydrogen ion gradient that is created when the plant transforms ATP to ADP, adenosine diphosphate, (Smith et al. 2011, Wipf et al. 2019). While AMF are helpful in the active transport of Pi across the plant cell wall, the far reach of fungal hyphae can help the plant gain access to nutrients that are physically out of the reach of the root system because hyphae can expand the reach of roots by up to 700 times (Wipf et al. 2019).

The transfer and transport of phosphorus by the arbuscular mycorrhizal hyphae creates positive feedback between the host plant, the arbuscular mycorrhizal fungi and other soil bacteria that help with making organic phosphorus soluble. Host plants will capture light rays from the sun and convert them into sugars through photosynthesis. Carbohydrates/sugars that are formed

during photosynthesis will be given to arbuscular mycorrhizal fungi in exchange for the fungi providing phosphorus from sources the plant cannot tap into on its own. The hyphae of arbuscular mycorrhizal fungi will transport phosphate solubilizing bacteria, PSB, phosphate solubilizing bacteria, to patches of organic phosphorus. The bacteria are dependent on the nutrition that comes from fungal hyphae exudates to move through the phosphorus patches in the soil. In this way, arbuscular mycorrhizal fungi have control over the interaction with PSB that will further increase the amount of phosphorus that can be taken from the soil and transported to the plant. The exudates from the fungal hyphae will further increase the growth rate for PSB, which will only serve to increase the amount of organic phosphorus in the soil that can be mineralized (Jiang et al. 2021).

While the combination of mycorrhizal fungi and phosphorus in a field setting can enhance the effectiveness of the native mycorrhizal populations, a careful balance must be maintained. The inclusion of mycorrhizal fungi may require a lesser amount of fertilizer, when compared to recommended fertilizer rates for the soil type (Ortas 2012). However, phosphorus uptake in host plants is mostly seen in early crop development stages and will decline over time (Gaur and Adholeya 2002). Applied arbuscular mycorrhizal fungi will be most effective in soils where phosphorus levels are low because a heightened level of phosphorus can suppress mycorrhizal activity, so in soils where phosphorus is already present it is important to not over apply external phosphorus (Ortas 2012).

Production of *Zea mays* exceeds 366 million tons produced annually, more than any other grain, making it one of the most important cereal grains (U.S. Grains Council 2022). *Zea diploperennis* is a previously unknown wild relative of maize that was first discovered in the Valley of San Miguel in Mexico in the late 1970's (Eubanks 1995, Nault and Findley

1981). *Tripsacum dactyloides*, eastern gamagrass, is the parent plant of *Zea mays*. *Tripsacum dactyloides* is a perennial sod forming plant that thrives in warm climates and is known for its high production rate (Benz et al. 1989). A major goal of genetic crossing among the three species is to create a perennial corn crop.

The hypotheses of this study are that higher measured phosphorus levels will be present within the leaf tissue of inoculated plants and that similarities will be seen between the species within the same treatment group.

II. LITERATURE REVIEW

Thorough experimentation has been conducted colonizing plant species such as corn, soybean, cotton, and rice and their association with AMF. The vertical distribution of mycorrhizal fungi was assessed in a field experiment where conventional tillage and no-till practices were assessed, under a planting of *Zea mays*. Measurements were taken at depths between 0-5 cm, centimeter, 5-10 cm, 10-15 cm, 15-20 cm, and 20-25 cm, in both conventional and no-till plots. In both conventional and no-till plots, the top 15 cm of the soil contained 87% of active hyphae in the conventional plot and 90% of the total active hyphae in the no-till plot. After this depth the concentration of fungal hyphae and spores diminished remarkably in both tillage systems. The density of total AMF and the metabolic activity of AMF only differed significantly between conventional and no-till systems in the top 5 cm of the soil, with conventional till having a lower soil hyphae and spore density by 40 and 50% respectively. Root colonization was not affected by the choice of tillage practice. Total hyphal density and spore density were found to be strongly correlated, with a p value below 0.001. Overall, most total hyphae density and active hyphae density was present within the top 15 cm of the soil, regardless of tillage practice (Kabir et al., 1998). In a conventional management system, the use of tillage will break up the top portion of the soil, leaving it susceptible to erosion and making it difficult for the fungal community to grow and thrive (Hussain et al. 1998). In addition, no till soils were found to have microbial biomass that was three times more concentrated than a conventionally tilled field (Mathew et al. 2012).

Differing varieties of AMF have also been evaluated for their ability to colonize roots of varying rice varieties. Two varieties of AMF that have been compared are *Funneliformis mosseae* and *Rhizophagus irregularis*. Root colonization, in a greenhouse setting, was evaluated

on 12 varieties of rice. *F. mosseae* showed stimulated plant growth in all twelve varieties, whereas *R. irregularis* only showed growth results in 7 of the 12 varieties. However, root colonization was more effective in varieties inoculated with *R. irregularis* overall. Across all varieties of rice, inoculated plants showed higher leaf levels of Pi when compared to non-inoculated plants. Across the experiment, significant yield increases were observed in inoculated plants when compared to non-inoculated plants (Campo et al., 2020).

In Brazil, Cely et al. (2016) conducted a study on the effects of mycorrhizal fungi on soybean and cotton crops in a field setting. Four treatments were used in the soybean portion of the experiment consisting of a control, AMF application, AMF application with 200 kg/ha, kilograms per hectare, NPK, nitrogen, phosphorus, potassium, fertilizer application, and AMF application with half of the fertilizer dosage applied. This was conducted in a completely randomized block design setup that had five replicates and was repeated over two seasons. For the cotton experiment, the same four treatments as the soybean experiment were used and contained five replicates in a completely randomized block design. The roots of 10 plants from each plot were randomly sampled at 30 and 80 days after emergence, DAE, days after emergence, in the soybean experiment to evaluate the mycorrhizal colonization. The soybean grains were harvested at 120 DAE, and the cotton yield was estimated at 190 DAE. Measurements that were taken include root colonization, height, plant biomass, potassium content, nitrogen content, and relative mycorrhizal dependency, RMD, relative mycorrhizal dependency. In the soybean experiment, the best grain yield was observed in the treatment groups that applied AMF and fertilizer and AMF and half fertilizer. There was a significant correlation between inoculation and yield and potassium and nitrogen tissue contents, with p values of 0.01 and 0.03, respectively, falling well below the threshold of 0.05. In the cotton

experiment, there was no significant correlation between the height of the plant and inoculation status. The strongest results were seen in the treatment group where only AMF was applied and AMF application with half fertilizer. Like with the soybean experiment, nutrient content, specifically N and P, had a significantly high correlation to cotton yield. Overall, inoculation helped plants to uptake phosphorus from the fertilizer in addition to increasing plant growth and yield (Cely et al., 2016).

Bazaretti et al. (2019) conducted a study, in Brazil, evaluating mycorrhizal fungi effects on soybean and corn in both field and greenhouse experimental settings. In the greenhouse portion, six treatments were applied including the use of one of three carriers, peat, vermiculite or rock phosphate, along with inoculation, or non-inoculation, with AMF. Each treatment had fifteen replicates in a randomized block design. After five days, germination was estimated and the number of AMF propagules, where applicable, were counted. For the soybean and corn field experiments, a randomized block design consisting of seven treatments and five replicates was enacted. The treatments included one of the above listed carriers along with AMF, or not, and phosphorus application, or not. Fertilizer doses were applied in a concentration of 200 kg/ha^{-1} of NPK fertilizer (0-20-20) for treatments where phosphorus was present and in doses of 200 kg/ha^{-1} NPK fertilizer (0-0-20) where phosphorus was not present. In the soybean experiment, cultivation occurred over four months, and the corn cultivation occurred over five months. Five plants of each corn and soybean were collected from each plot and analyzed for AMF root colonization. An additional five plants of corn and soybean were collected and analyzed for shoot dry weight. In addition, grain yield was estimated, phosphorus and nitrogen leaf contents were evaluated, and the number of AMF propagules were counted (Bazaretti et al., 2019).

In the greenhouse soybean experiment the carrier treatments without AMF inoculation showed an increased germination rate when compared to the control (no carrier and no AMF). Peat, rock phosphate, and vermiculite all showed increased germination rates at 86%, 88%, and 90% respectively. There was no difference in germination between control and AMF + peat (AMFPt). However, a decrease in germination was observed in the AMF + rock phosphate (AMFRP) and AMF + vermiculite (AMFV) at 8% and 20% respectively. In the greenhouse corn experiment, the control and peat groups had 100% germination. The rock phosphate and vermiculite showed a decrease in germination of 4% and 2%. The AMFPt treatment showed a 4% decrease in germination, while the AMFRP and AMFV showed decreases of 20% and 26% (Bazaretti et al., 2019).

In the soybean field experiment, seeds treated with AMFV + phosphorus showed a higher AMF colonization when compared to the other treatments. Compared to AMFPt without phosphorus, AMFV without phosphorus showed an increased dry shoot weight. The AMFV without phosphorus treatment group demonstrated the largest yield across the treatment groups. The leaf phosphorus content was increased in the AMFRP + phosphorus group, but it was decreased in the AMFRP without phosphorus. Leaf nitrogen content was increased in AMFRP + phosphorus and without phosphorus when compared with the other treatment groups. In the corn field experiment, AMF colonization was lowest in the AMFV + phosphorus and AMFV without phosphorus treatment groups. Shoot dry weight was highest with treatments of AMFPt + phosphorus, and it was lowest with treatment group AMFV without phosphorus. Compared to other treatment groups, phosphorus leaf content was increased with treatment AMFV without phosphorus. Lastly, nitrogen leaf content was higher in treatment group AMFRP + phosphorus (Bazaretti et al., 2019)

Pinos et al. (2019) conducted a study on the effects of combining humic substances and mycorrhizal fungi in Brazil. This experiment was conducted in a greenhouse setting, where the crops were grown in pots. The soil was sun dried for 30 days to reduce the native AMF population via heating. Three types of humic substances, organisol (HS-Org), andisol (HS-And), and Vc (HS-Vc), were used in combination with inoculation, or non-inoculation, with AMF in corn crops. In addition, there was one control treatment where no humic substances or AMF were applied. Fifty DAE the plant height was measured, along with stem diameter, shoot dry mass, root dry mass, and mycorrhizal colonization. Upon data analysis, inoculation with AMF in combination with HS-Vc significantly increased shoot dry mass, root dry mass and total dry mass by 25, 41 and 31% respectively, compared to HS-Vc without AMF inoculation. The treatment group that had both HS-Vc and AMF demonstrated a significant increase in carbohydrate content, with an increase of 81% compared to the treatment group with HS-Vc only. Plant height showed significant stimulation with the humic substance application, but not in combination with AMF application. When analyzing nutrient content, the combination of HS-Vc and AMF showed a leaf nitrogen content that increased by 14%, when compared to the control treatment. Lastly, the phosphorus content in the treatments that had both HS-Org and AMF increased significantly by up to 12%, compared to the control group (Pinos et al. 2019).

Cotton, which is grown globally, is an arbuscular mycorrhizal dependent crop, with a specific affinity for the *R. irregularis* species. The relationship between cotton and arbuscular mycorrhizal fungi is important to maintaining the high volume of production that is needed to satisfy global demand. In soils where phosphorus was readily available to the intended host cotton plant, there has been shown to be a decrease in mycorrhizal colonization and activity because the plant can gain access to phosphorus without having to exchange carbohydrates and

sugars with the mycorrhizal fungi. When the soil is low in phosphorus, there is a heightened level of colonization and activity between the host plant and fungi. This will increase the phosphorus concentrations in the roots, stems and leaves of the inoculated cotton plant. Overall yield, in a two-year field study, in inoculated plants also shows a significant increase of about 28% when compared to non-inoculated cotton plants. The relationship between arbuscular mycorrhizal fungi and the cotton plant will increase the photosynthetic rate in addition to increasing yield via boll number per plant and quality of the fiber. The quality of the cotton fiber can be demonstrated through the elongation percentage, which is typically between 3 and 7%. Inoculated plants in this study had an elongation percentage that was 0.2% higher than non-inoculated plants (Gao et al. 2020). In addition to having a positive impact on crops that are grown for consumer goods, like textiles, arbuscular mycorrhizal fungi association is also important in crops that are grown for consumption and medicinal purposes.

In crops like sweet basil, which are used for medicinal purposes as well as consumption, balancing the correct application of phosphorus with arbuscular mycorrhizal fungi inoculation can increase the shoot antioxidant content, in the form of rosmarinic and caffeic acids. Mycorrhizal fungi inoculation has been combined with increasing levels of phosphorus application in sweet basil to demonstrate the impacts on increasing phytochemical content in shoots. After a seven-week growing period, mycorrhizal species *G. intraradices* significantly increased colonization in inoculated sweet basil plants, but this species had lower phytochemical content in roots and shoots than the other two species being investigated. Additionally, *G. intraradices* had a significantly higher colonization rate of 76% compared to *G. mosseae* and *G.* caledonium which only had 39% and 15% colonization, respectively. Plant colonized by *G. mosseae* had the highest shoot caffeic acid concentration at 15 mg, milligrams, of caffeic acid

per gram of dried shoot weight. This result was not seen in the examination of rosmarinic acid, as there was no significant difference found among the three species in root or shoot concentrations. Ultimately this outcome could be attributed to the increased phosphorus application suppressing the fungal capabilities and the plant's need for the relationship with the fungus, but it demonstrates that the ability to colonize at a higher rate does not necessarily correlate to the ability to increase certain phytochemicals (Ortas 2012). The outcome of this study proved that there are two ways to increase the phytochemical content of shoots in sweet basil, by increasing phosphorus application when not inoculating with arbuscular mycorrhizal fungi or to inoculated with fungi while decreasing phosphorus inputs (Toussaint et al. 2007).

III. MATERIALS AND METHODS

The first trial of the experiment was conducted in Fentress, Texas, during the spring of 2022 (April to mid-June). The experiment's second trial was conducted in New Braunfels, Texas, about 15 miles from the location of trial 1, during the fall of 2022 (late October to mid-December). The climate of both regions is subtropical, with an average rainfall of approximately 9 in, inches annually and an average humidity of 66%. The average spring temperature of Fentress is approximately 24.4°C, and the average fall temperature of New Braunfels is approximately 16.7°C. Clay topsoil was purchased from a local landscape supplier. To rid the soil of native microorganisms, soil pasteurization was conducted. Pasteurization was achieved by placing the soil in aluminum pans and heating the soil medium to 100°C for 1 hour in a drying oven (Penn State Extension 2007).

A commercially available inoculum, Myco Bliss, was selected for the experiment. The inoculum consisted of a mixture of *Rhizophagus irregularis*, *Rhizophagus aggregatus*, *Rhizophagus proliferum*, *Rhizophagus clarus*, and *Claroideoglomus etunicatum* in a ratio of 200 propagules per gram of inoculum. For trial 1, NPK fertilizer was applied to the required treatment groups in the following amounts: 0.13 g, grams, of nitrogen, 0.10 g of phosphorus, and 0.10 g of potassium (Bazaretti et al. 2019). For the groups that required rock phosphate, the 0.10 g of phosphorus was replaced with 0.10 g of rock phosphate. The arbuscular mycorrhizal fungi inoculum was applied at a rate of 2.0 g per pot, according to the recommended application process on the product. For trial 2, the NPK fertilizer rate was doubled, so 0.26 g of nitrogen, 0.20 g of phosphorus, and 0.20 g of potassium were applied to each required treatment group. The phosphorus was replaced with 0.20 g of rock phosphate in the required treatment groups. The ratio of arbuscular mycorrhizal fungi remained at 2.0 g per pot.

This experiment was conducted as a potted experiment to reduce the introduction of non-pasteurized soil. The pots used were six in. in diameter and six in. in depth, holding an average of 0.75 L, liters. at an average weight of 2.875 lbs., pounds, (Bell and Koeppe 1971, Nass and Zuber 1971). Three plant species were used in this experiment: *Zea mays* (A), *Zea diploperennis* (B), and *Tripsacum dactyloides* (C). A total of 8 different treatments were applied to 15 replicates per species, totaling 360 plants. Planting depths varied for each of the three selected species. The planting depths required for *Zea mays*, *Zea diploperennis*, and *Tripsacum dactyloides* are 5 cm, 5 cm, and one in., respectively. For treatments that required AMF inoculation, the in-furrow method of seed inoculation was used. A small trench was dug just below the appropriate planting depth for each species, and the granular inoculum was placed in the trench. The inoculum was then covered by a thin layer of soil; one seed per pot was placed and covered with soil (Adholeya et al. 2015). Fertilizer was applied to each pot at the prescribed doses.

Treatment 1 was pasteurized soil with no AMF application and no NPK fertilizer to be used as a control. Treatment 2 was pasteurized soil with the application of AMF and no NPK. Treatment 3 was sterilized soil with AMF application and NPK fertilizer at the previously specified doses. Treatment 4 was pasteurized soil with NPK fertilizer and no AMF application. Treatment 5 was NK, nitrogen, potassium, fertilizer at the previously specified doses and AMF application. Treatment 6 was pasteurized soil with NK fertilizer and no AMF application. Treatment 7 was pasteurized soil with AMF application, NK fertilizer, and rock phosphate. Treatment 8 was pasteurized soil with NK fertilizer, rock phosphate, and no AMF application (Table 1). The treatment groups remained the same for both trials, with the only change being a doubling of the applied fertilizer amounts.

Table 1: Treatments

Treatment	AMF	Nitrogen	Phosphorus	Potassium	Rock Phosphate
1					
2	X				
3	X	X	X	X	
4		X	X	X	
5	X	X		X	
6		X		X	
7	X	X		X	X
8		X		X	X

Once planted, pots were arranged in a systematically randomized plot that measured 10 ft, feet, by 9 ft (Table 2). The systematically randomized plot design was created using a random number generator to select the first plant placed; this was done to eliminate bias regarding placement and amount of sunlight received by the plant, as two of the four sides were against a building or fence that would impede sunlight duration.

Table 2: Systematically randomized plot design

1c	1b	1a	8c	8b	8a	7c	7b	7a	6c	6b	6a	5c	5b	5a	4c	4b	4a
7c	7b	7a	6c	6b	6a	5c	5b	5a	4c	4b	4a	3c	3b	3a	2c	2b	2a
5c	5b	5a	4c	4b	4a	3c	3b	3a	2c	2b	2a	1c	1b	1a	8c	8b	8a
3c	3b	3a	2c	2b	2a	1c	1b	1a	8c	8b	8a	7c	7b	7a	6c	6b	6a
1c	1b	1a	8c	8b	8a	7c	7b	7a	6c	6b	6a	5c	5b	5a	4c	4b	4a
7c	7b	7a	6c	6b	6a	5c	5b	5a	4c	4b	4a	3c	3b	3a	2c	2b	2a
5c	5b	5a	4c	4b	4a	3c	3b	3a	2c	2b	2a	1c	1b	1a	8c	8b	8a
3c	3b	3a	2c	2b	2a	1c	1b	1a	8c	8b	8a	7c	7b	7a	6c	6b	6a
1c	1b	1a	8c	8b	8a	7c	7b	7a	6c	6b	6a	5c	5b	5a	4c	4b	4a
7c	7b	7a	6c	6b	6a	5c	5b	5a	4c	4b	4a	3c	3b	3a	2c	2b	2a
5c	5b	5a	4c	4b	4a	3c	3b	3a	2c	2b	2a	1c	1b	1a	8c	8b	8a
3c	3b	3a	2c	2b	2a	1c	1b	1a	8c	8b	8a	7c	7b	7a	6c	6b	6a
1c	1b	1a	8c	8b	8a	7c	7b	7a	6c	6b	6a	5c	5b	5a	4c	4b	4a
7c	7b	7a	6c	6b	6a	5c	5b	5a	4c	4b	4a	3c	3b	3a	2c	2b	2a
5c	5b	5a	4c	4b	4a	3c	3b	3a	2c	2b	2a	1c	1b	1a	8c	8b	8a
3c	3b	3a	2c	2b	2a	1c	1b	1a	8c	8b	8a	7c	7b	7a	6c	6b	6a
1c	1b	1a	8c	8b	8a	7c	7b	7a	6c	6b	6a	5c	5b	5a	4c	4b	4a
7c	7b	7a	6c	6b	6a	5c	5b	5a	4c	4b	4a	3c	3b	3a	2c	2b	2a
5c	5b	5a	4c	4b	4a	3c	3b	3a	2c	2b	2a	1c	1b	1a	8c	8b	8a
3c	3b	3a	3c	2b	2a	1c	1b	1a	8c	8b	8a	7c	7b	7a	6c	6b	6a

The plants were watered daily unless a rain event eliminated the need for watering. Once each species reached 50% germination, a 50-day countdown to harvest began. Each species was harvested 50 DAE (Pinos et al. 2019). Upon harvest, plants were placed into labeled brown bags and dried at 100°C for 24 hours; the shoots were then cut away from the roots to be used for analysis.

The plant tissue analysis began by dry ashing the shoots; 1.0 g of plant material was placed into a crucible in the ashing oven at 500°C for 3 hours. The sample was then removed from the oven and allowed to cool before being wet with a few drops of distilled water. Three ml, milliliters, of 6N, normal, HCl, hydrochloric acid, was added to the crucible. The crucible was heated on a hot plate until the sample was nearly dry. Five ml of 1N HCl was added to the crucible, and the sample was stirred. The sample was then transferred to a 50 ml beaker and

filled to the 50 ml marker with distilled water (Ellis et al., Haynes 1980).

Once ashing was complete, the Murphy-Riley method was used to determine phosphorus content within the plant material using spectrophotometry. An ammonium molybdate reagent was prepared by dissolving 25.0 g in 500 ml of distilled water. An antimony potassium tartrate reagent was prepared by dissolving 1.0 g in 100 ml of distilled water. A diluted sulfuric acid reagent was prepared by adding 50 ml of concentrated sulfuric acid to 950 ml of distilled water.

Five ml of the ammonium molybdate reagent was added to the beaker containing the sample and stirred. Five ml of the antimony potassium tartrate reagent was added to the beaker and stirred. One ml of sulfuric acid reagent was added to the beaker and stirred. Once all reagents were added to the sample, the sample was allowed to sit for 30 minutes, allowing for color development. The Palintest spectrophotometry machine was used to analyze the sample. The machine was blanked by placing a 10 ml test tube of distilled water into the testing chamber. Once blanked, a 10 ml test tube of the sample was loaded into the testing chamber, and the machine output a reading for phosphorus in the sample in units of mg/L, milligrams per liter, (Cho and Nielson 2017). The collected values were analyzed using ANOVA, analysis of variance, in Excel (SARE 2017).

IV. RESULTS

Comparisons by trial, treatment and species were made upon conclusion of the 50 DAE period and the ending of both trials. Treatment 1, no AMF or NPK, showed that average phosphorus absorbance for all three species was higher during trial 2 than trial 1 (Figure 1). Through ANOVA testing, there was no significant difference in average absorbance values between trial 1 and trial 2 ($p = 0.0715$). Combining both trials, there was also no significant difference in average absorbance values between species ($p = 0.6946$) (Figure 2). *Tripsacum dactyloides* showed the highest overall average absorbance.

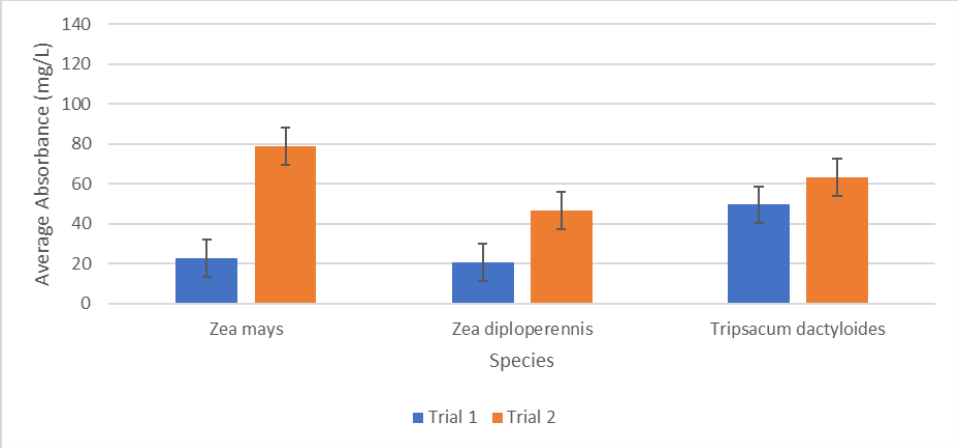

Figure 1: Treatment 1 average phosphorus absorbances by trial and species. The average absorbance of phosphorus (mg/L) separated by species and trial is shown for treatment 1(no AMF inoculation or NPK application). Trial 1 data is shown by the left bar above the species label, and trial 2 data is shown by the right bar above the species label. Across all species, trial two showed higher absorbance values, although that finding is not significant ($p > 0.05$).

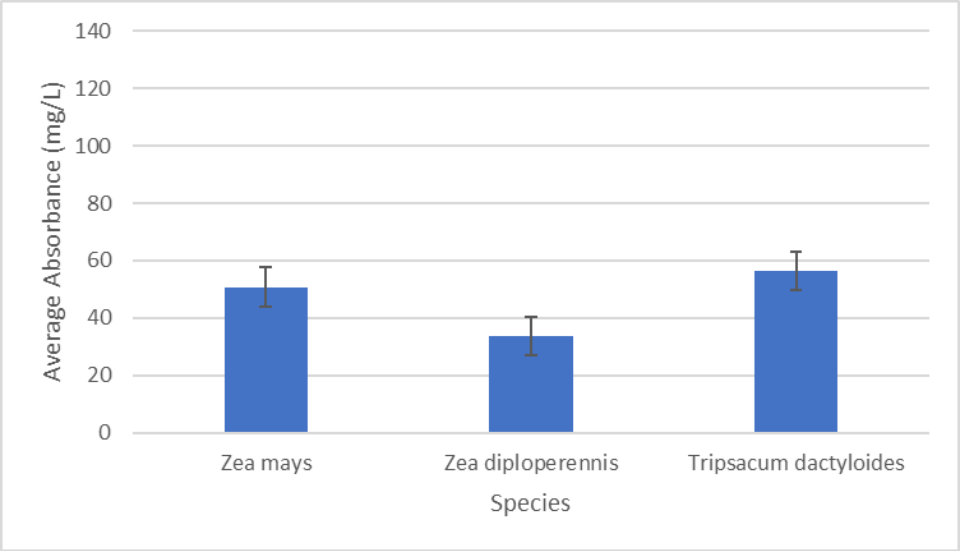

Figure 2: Treatment 1 average phosphorus absorbances across both trials, by species. The pooled average absorbance of phosphorus (mg/L) of trials 1 and 2 for treatment 1 (no AMF inoculation or NPK application). The average absorbances are separated by species. There was no significant difference among average absorbances for the species ($p > 0.05$).

Treatment 2, AMF inoculation with no NPK, showed a non-significantly ($p = 0.0779$) higher average phosphorus absorbance value in trial 2 versus trial 1 across all species, although the average absorbance in trial 2 was only higher than trial 1 in *Zea diploperennis* by approximately 4 mg/L (Figure 3). Across both trials, there was no significant difference in average absorbance between species ($p = 0.7623$) (Figure 4). *Zea mays* showed the highest overall average absorbance.

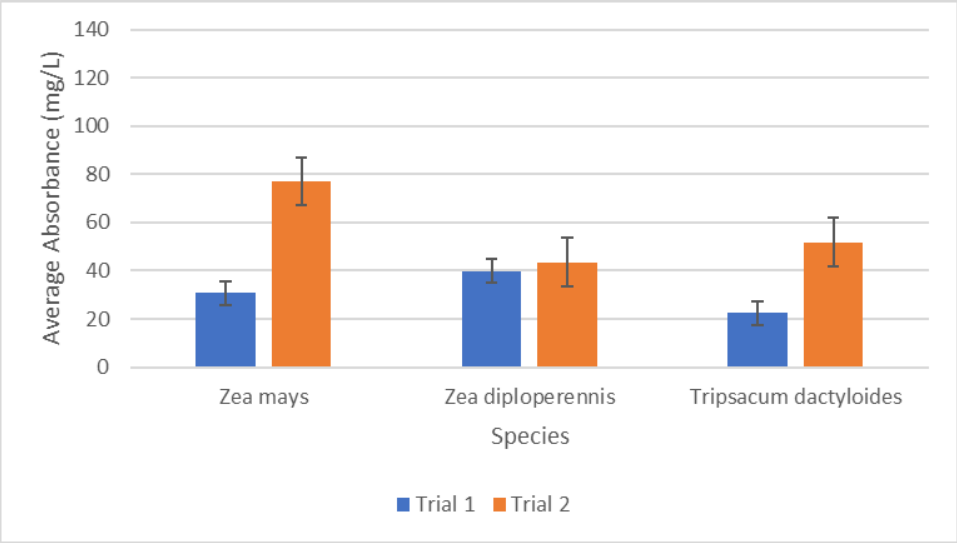

Figure 3: Treatment 2 average phosphorus absorbances by trial and species. The average absorbance of phosphorus (mg/L) separated by species and trial is shown for treatment 2 (AMF inoculation and no NPK application). Trial 1 data is shown by the left bar above the species label, and trial 2 data is shown by the right bar above the species label. Across all species, trial two showed higher absorbance values, although that finding is not significant ($p > 0.05$).

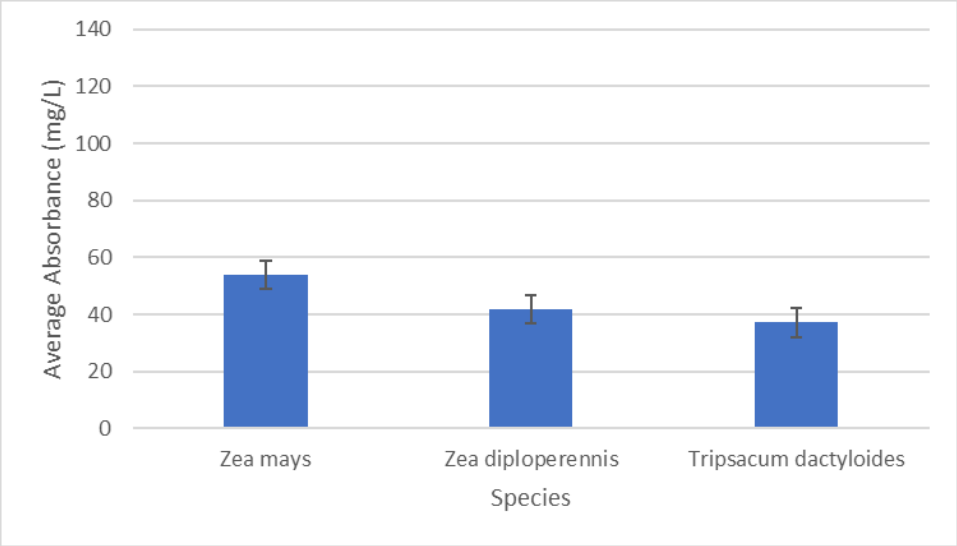

Figure 4: Treatment 2 average phosphorus absorbances across both trials, by species. The pooled average absorbance of phosphorus (mg/L) of trials 1 and 2 for treatment 2 (AMF inoculation and no NPK application). The average absorbances are separated by species. No significant difference among the average absorbances was found ($p > 0.05$).

Treatment 3, AMF inoculation with NPK application, showed a higher average absorbance in trial 2 for *Zea mays* and *Zea diploperennis*, but average absorbance was higher in trial 1 for *Tripsacum dactyloides*. There was no significant difference in absorbances between

trials for any species (p = 0.8131) (Figure 5). Across both trials, there was no significant difference in the average absorbance between trials (p = 0.4597) (Figure 6). *Tripsacum dactyloides* showed the highest overall average absorbance.

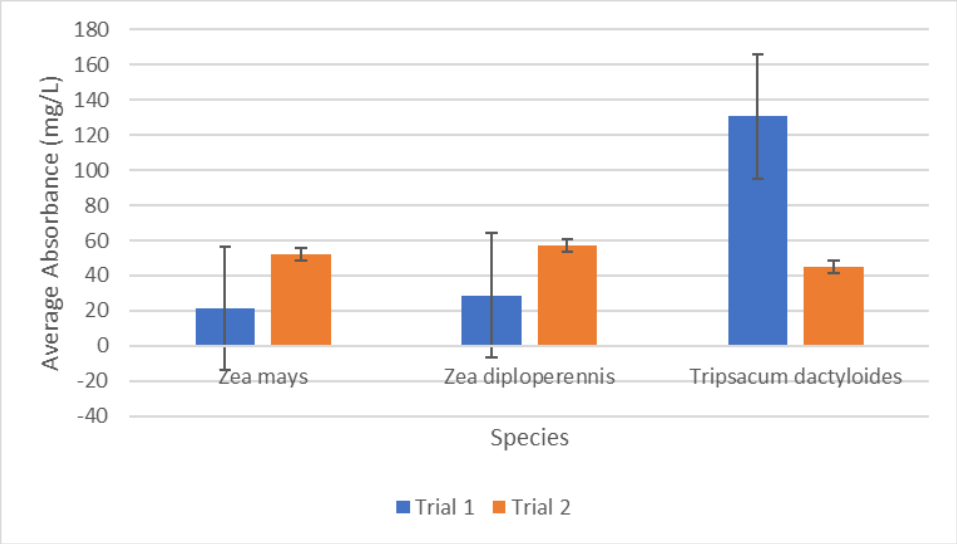

Figure 5: Treatment 3 average phosphorus absorbances by trial and species. The average absorbance of phosphorus (mg/L) separated by species and trial is shown for treatment 3 (AMF inoculation and NPK application). Trial 1 data is shown by the left bar above the species label, and trial 2 data is shown by the right bar above the species label. Variation between the trials is not significant (p > 0.05).

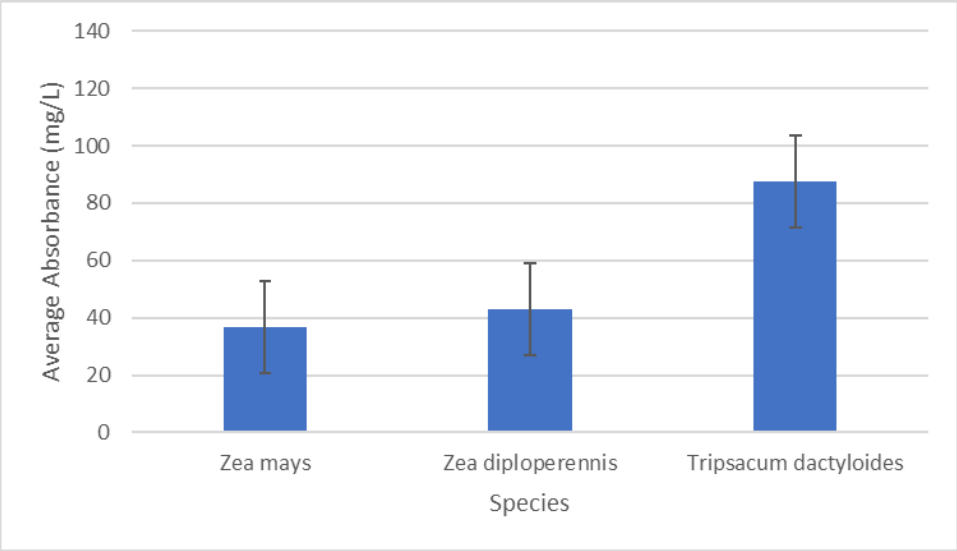

Figure 6: Treatment 3 average phosphorus absorbances across both trials, by species. The pooled average absorbance of phosphorus (mg/L) of trials 1 and 2 for treatment 3 (AMF inoculation and NPK application). The average absorbances are separated by species. No significant difference in average absorbances was observed ($p > 0.05$).

Treatment 4, no AMF with NPK application, produced higher average absorbances in trial 2 for all species. Trial 2 did not show a significant difference in average absorbances ($p = 0.1229$) (Figure 7). Overall, Zea mays had the highest average absorbance, but there was no significant difference in average absorbances between any species ($p = 0.4700$) (Figure 8).

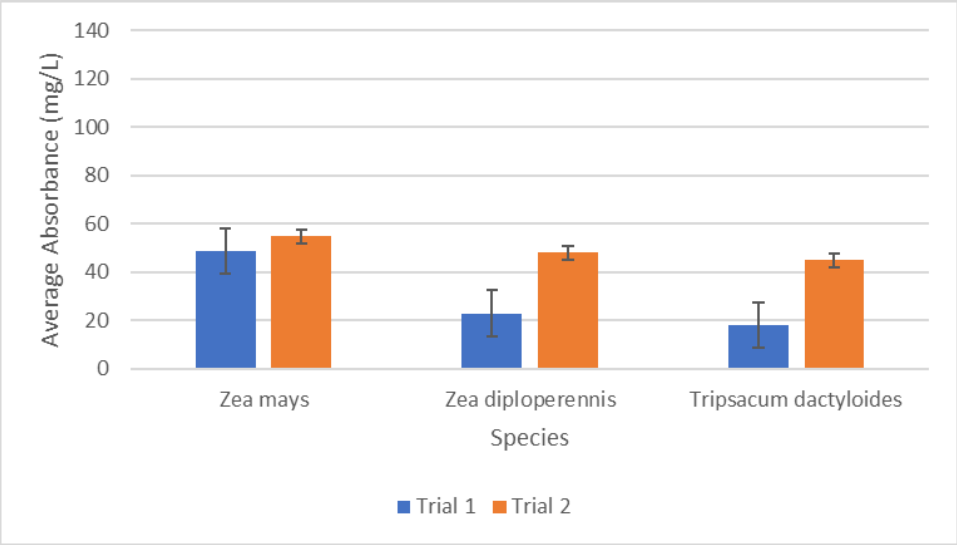

Figure 7: Treatment 4 average phosphorus absorbances by trial and species. The average absorbance of phosphorus (mg/L) separated by species and trial is shown for treatment 4 (no AMF inoculation with NPK application). Trial 1 data is shown by the left bar above the species label, and trial 2 data is shown by the right bar above the species label. Across all species, trial two showed higher absorbance values, although that finding is not significant ($p > 0.05$).

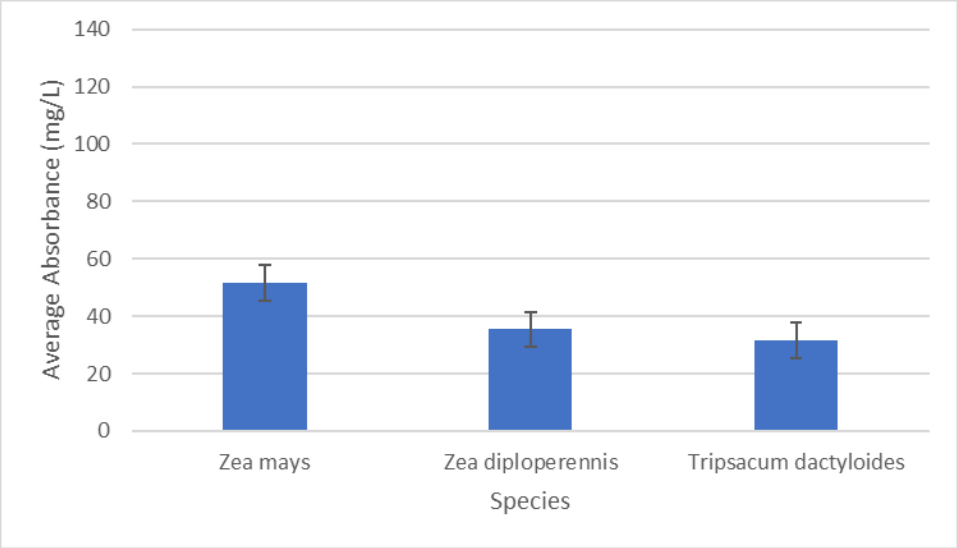

Figure 8: Treatment 4 average phosphorus absorbances across both trials, by species. The pooled average absorbance of phosphorus (mg/L) of trials 1 and 2 for treatment 4 (no AMF inoculation with NPK application). The average absorbances are separated by species. No significant difference in average absorbances was observed ($p > 0.05$).

In treatment group 5, AMF inoculation with NK application, *Zea mays* showed a higher average absorbance in trial 1, whereas *Zea diploperennis* and *Tripsacum dactyloides* had higher average absorbances in trial 2. There was no significant difference in average absorbances between trials ($p = 0.5303$) (Figure 9). Overall, in treatment group 5 *Zea mays* had the highest

average absorbance, but there was no significant difference in average absorbances between species ($p = 0.6712$) (Figure 10).

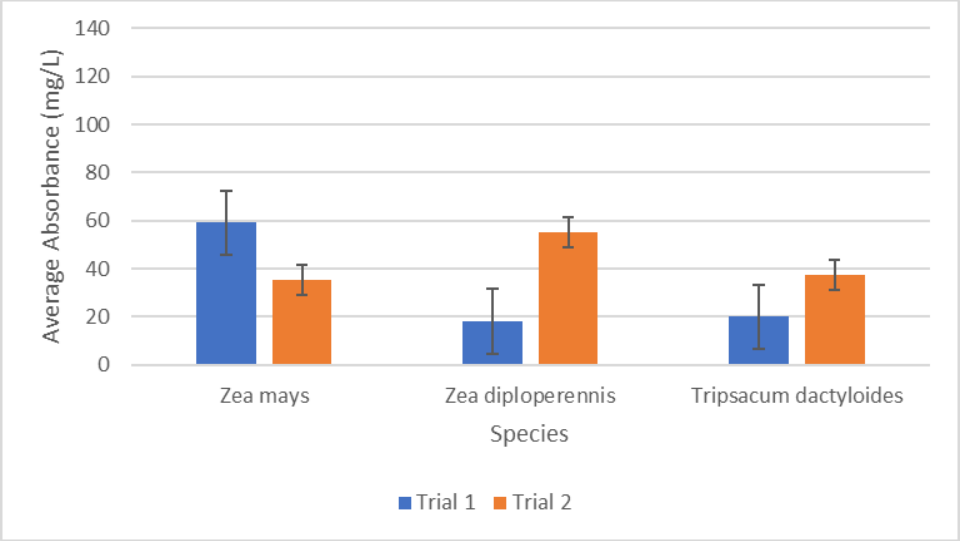

Figure 9: Treatment 5 average phosphorus absorbances by trial and species. The average absorbance of phosphorus (mg/L) separated by species and trial is shown for treatment 5 (AMF inoculation with NK application). Trial 1 data is shown by the left bar above the species label, and trial 2 data is shown by the right bar above the species label. Variation between the trials is not significant ($p > 0.05$).

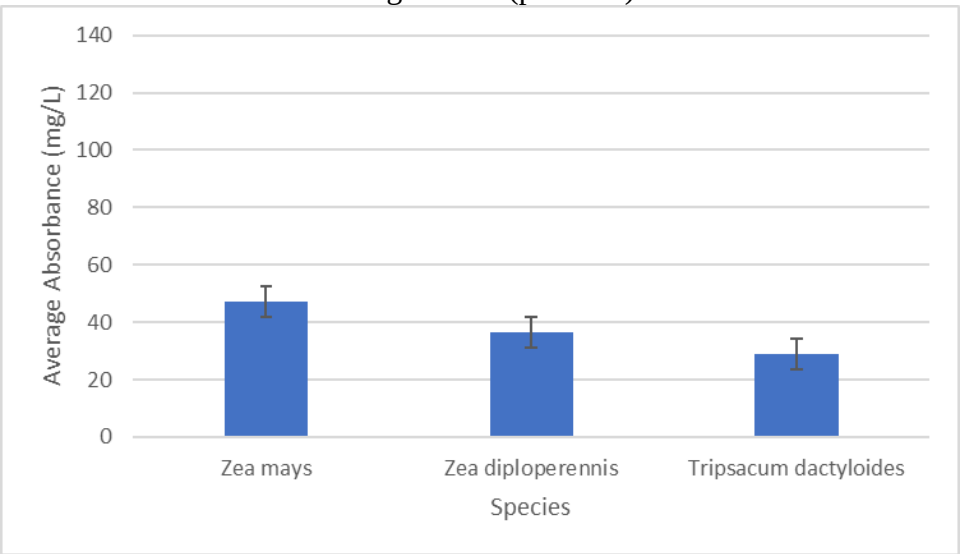

Figure 10: Treatment 5 average phosphorus absorbances across both trials, by species. The pooled average absorbance of phosphorus (mg/L) of trials 1 and 2 for treatment 5 (AMF inoculation with NK application). The average absorbances are separated by species. No significant difference in average absorbances was observed ($p > 0.05$).

Treatment 6, no AMF with NK application, produced higher average absorbance in trial 1 for *Zea mays*, but higher average absorbance in trial 2 for *Zea diploperennis*. *Tripsacum*

dactyloides did not have any surviving plants in trial 1. There was no significant variance in average absorbance between trials ($p = 0.1907$) (Figure 11). Across both trials, *Zea mays* had the highest average absorbance, although there was no significant difference in average absorbance between species ($p = 0.6995$) (Figure 12).

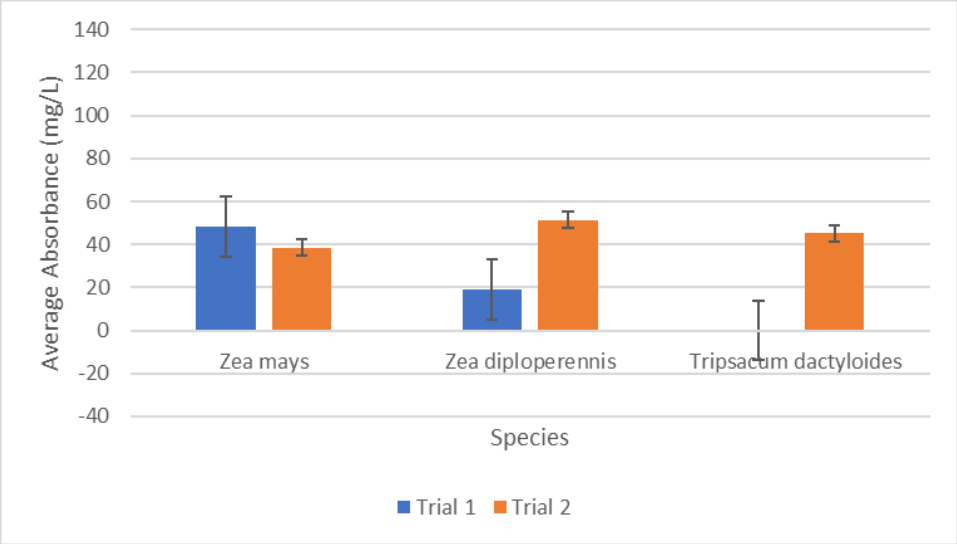

Figure 11: Treatment 6 average phosphorus absorbances by trial and species. The average absorbance of phosphorus (mg/L) separated by species and trial is shown for treatment 6 (no AMF inoculation with NK application). Trial 1 data is shown by the left bar above the species label, and trial 2 data is shown by the right bar above the species label. Data for trial 1 species C does not exist due to a lack of surviving specimens. Variation between the trials is not significant ($p > 0.05$).

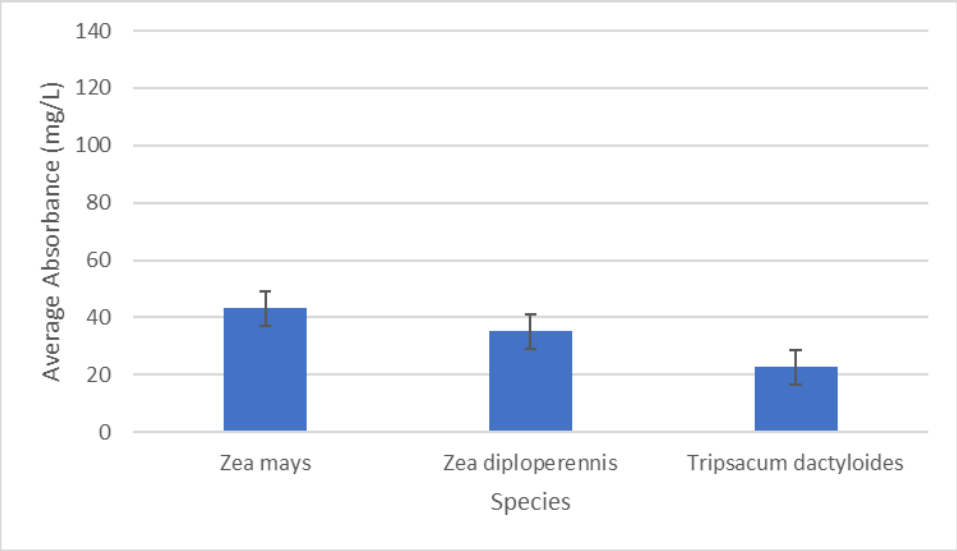

Figure 12: Treatment 6 average phosphorus absorbances across both trials, by species. The pooled average absorbance of phosphorus (mg/L) of trials 1 and 2 for treatment 6 (no AMF inoculation with NK application). The average absorbances are separated by species. No significant difference in average absorbances was observed ($p > 0.05$).

In treatment group 7, AMF inoculation with NK and rock phosphate application, *Zea mays* and *Zea diploperennis* had higher average absorbances in trial 2. *Tripsacum dactyloides* had a higher average absorbance in trial 1. There was no significant difference in average absorbances across trials ($p = 0.2246$) (Figure 13). Across both trials, there was no significant difference in average absorbances between species ($p = 0.7234$) (Figure 14). *Tripsacum dactyloides* had the highest overall average absorbance.

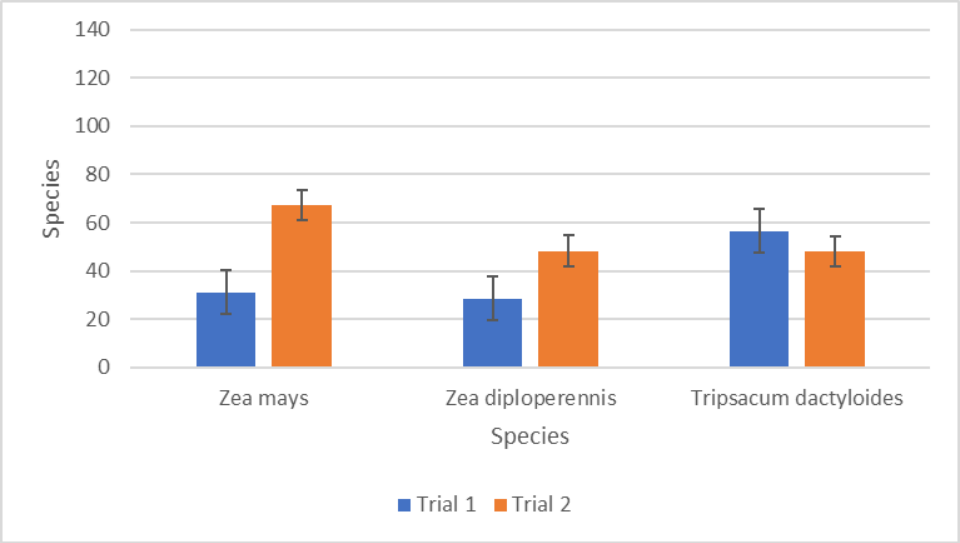

Figure 13: Treatment 7 average phosphorus absorbances by trial and species. The average absorbance of phosphorus (mg/L) separated by species and trial is shown for treatment 7 (AMF inoculation with NK+ rock phosphate application). Trial 1 data is shown by the left bar above the species label, and trial 2 data is shown by the right bar above the species label. Variation between the trials is not significant ($p > 0.05$).

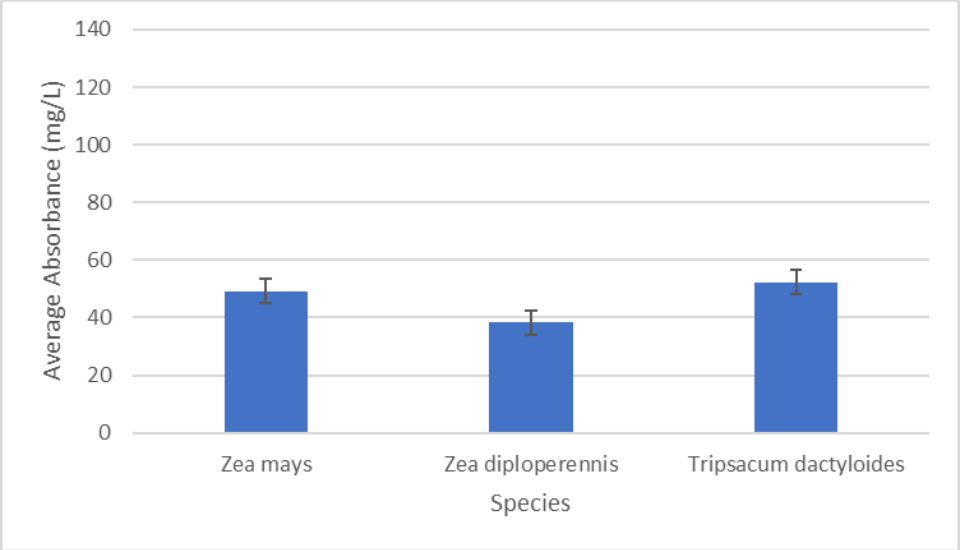

Figure 14: Treatment 7 average phosphorus absorbances across both trials, by species. The pooled average absorbance of phosphorus (mg/L) of trials 1 and 2 for treatment 7 (AMF inoculation with NK + rock phosphate application). The average absorbances are separated by species. No significant difference in average absorbances was observed ($p > 0.05$).

In treatment group 8, no AMF inoculation with NK and rock phosphate application, all species produced significantly higher average absorbances in trial 2 ($p = 0.0298$) (Figure 15). Across all trials, Zea mays had the highest average absorbance, but there was no significant difference in average absorbances between any species ($p = 0.6433$) (Figure 16).

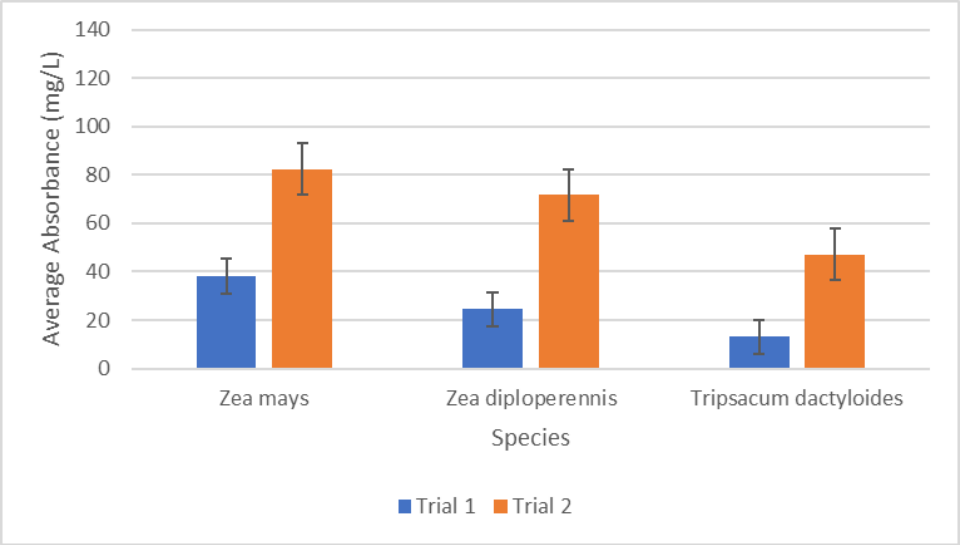

Figure 15: Treatment 8 average phosphorus absorbance readings by trial and species. The average absorbance of phosphorus (mg/L) separated by species and trial is shown for treatment 8 (no AMF inoculation with NK+ rock phosphate application). Trial 1 data is shown by the left bar above the species label, and trial 2 data is shown by the right bar above the species label. Across both trials, trial 2 showed significantly higher average absorbance ($p < 0.05$).

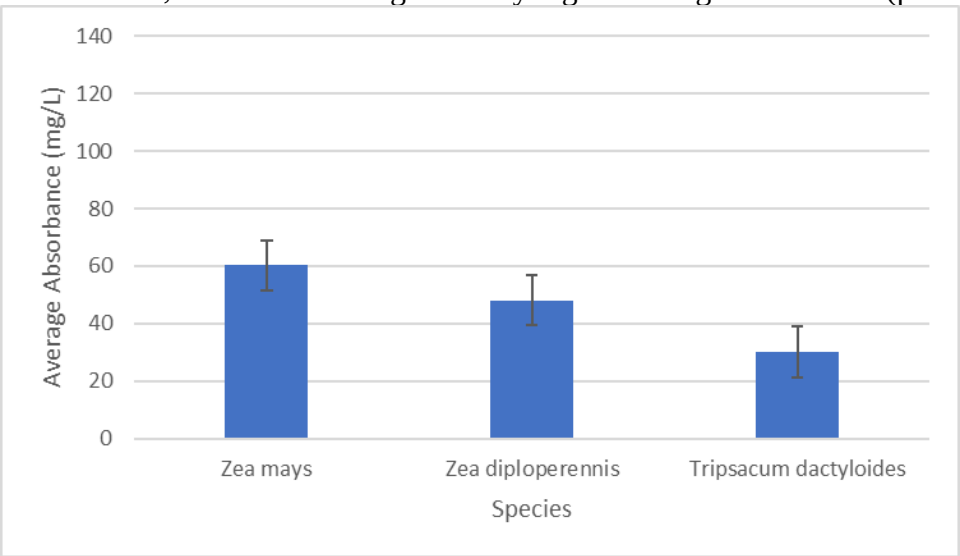

Figure 16: Treatment 8 average phosphorus absorbance readings across both trials, by species. The pooled average absorbance reading of phosphorus (mg/L) of trials 1 and 2 for treatment 8 (no AMF inoculation with NK + rock phosphate application). The average absorbances are separated by species. No significant difference in average absorbances was observed ($p > 0.05$).

Comparing trial 1 versus trial 2 data for Zea mays for all treatment groups, trial 2 produced significantly higher average absorbance readings across treatment groups ($p = 0.0119$) (Figure 17). Similarly, trial 2 produced higher average absorbance readings across all treatment groups for Zea diploperennis ($p = 0.0000$). Across all treatment groups, Zea diploperennis

showed higher average absorbances in trial 2 (Figure 18). Tripsacum dactyloides showed no significant difference in average absorbances between the two trials, but trial 1 treatment 6 failed to produce any living specimens (p = 0. 5512) (Figure 19).

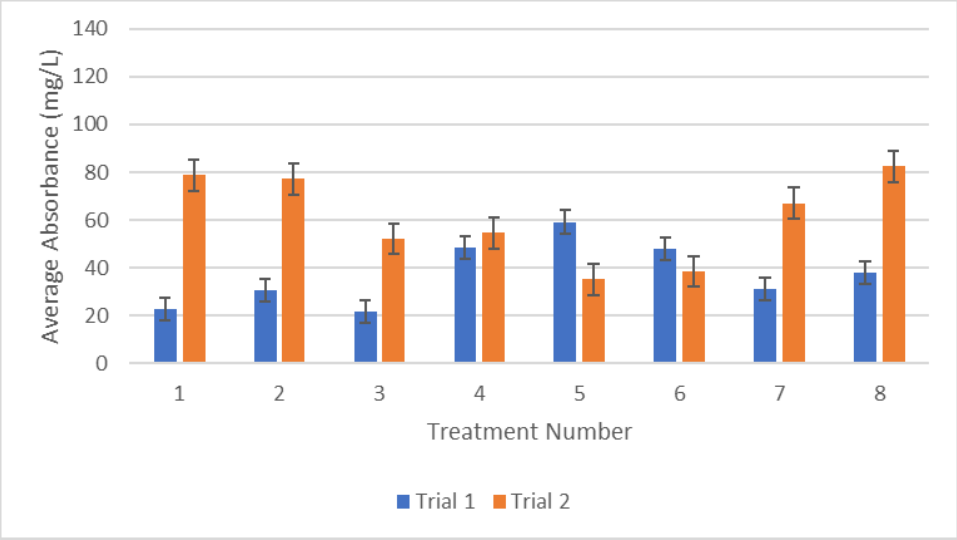

Figure 17: Average phosphorus absorbance reading for *Zea mays* in trial 1 vs. trial 2. The average absorbance reading (mg/L) of phosphorus is separated by treatment group (1-8) and trial (1 or 2). Trial 1 data is represented by the left bars, and trial 2 data is represented by the right bars. Trial 2 had a significantly higher average absorbance across all treatment groups ($p < 0.05$).

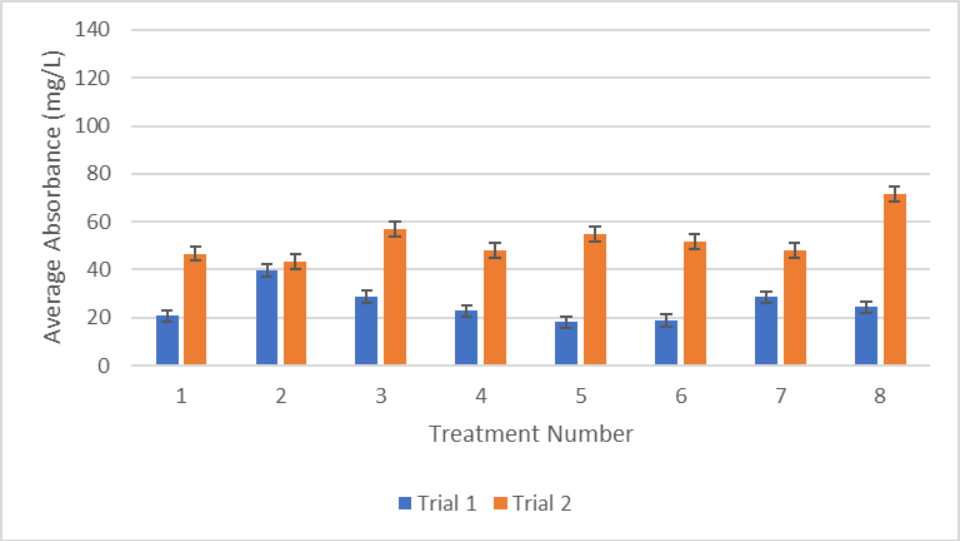

Figure 18: Average phosphorus absorbance reading for *Zea diploperennis* in trial 1 vs. trial 2. The average absorbance reading (mg/L) of phosphorus is separated by treatment group (1-8) and trial (1 or 2). Trial 1 data is represented by the left bars, and trial 2 data is represented by the right bars. Trial 2 had a significantly higher average absorbance across all treatment groups ($p < 0.05$).

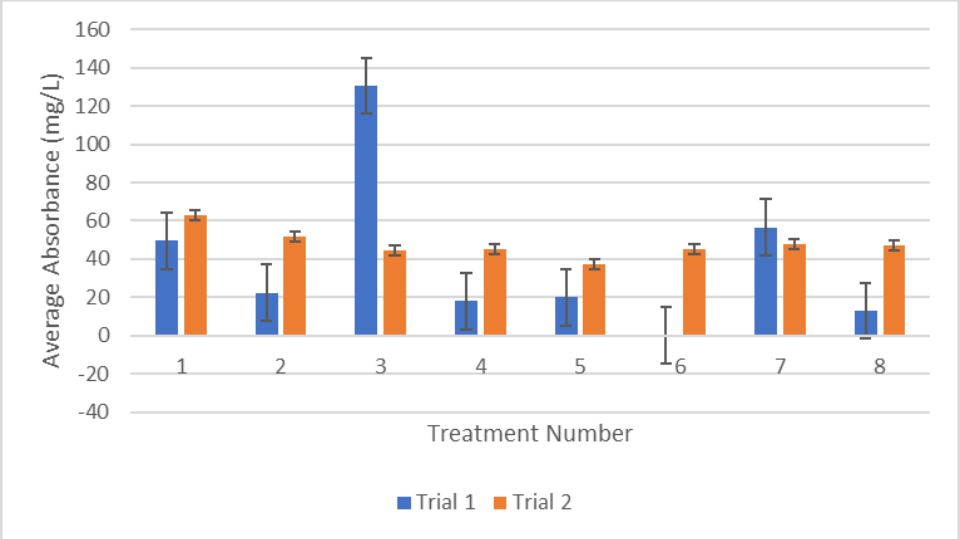

Figure 19: Average phosphorus absorbance reading for *Tripsacum dactyloides* in trial 1 vs. trial 2. The average absorbance reading (mg/L) of phosphorus is separated by treatment group (1-8) and trial (1 or 2). Trial 1 data is represented by the left bars, and trial 2 data is represented by the right bars. Due to lack of surviving specimens, there is no data for trial 1 treatment 6. There was no significant difference in average absorbance between trial 1 and trial 2 ($p > 0.05$).

One goal of this experiment was to observe the effectiveness of AMF inoculation on phosphorus absorbance values. In trial 1 on *Zea mays*, treatment groups inoculated with AMF outperformed non-inoculated groups in the control setting, no NPK, and in the treatment with only NK (Figure 20). Despite this, there was no significant difference in average absorbance

values in trial 1 between inoculated and non-inoculated treatment groups ($p = 0.7271$). In trial 2, non-inoculated *Zea mays* plants had higher average absorbance values in all treatment groups, but there was no statistically significant difference between the inoculated and non-inoculated average absorbance values ($p = 0.6942$) (Figure 21).

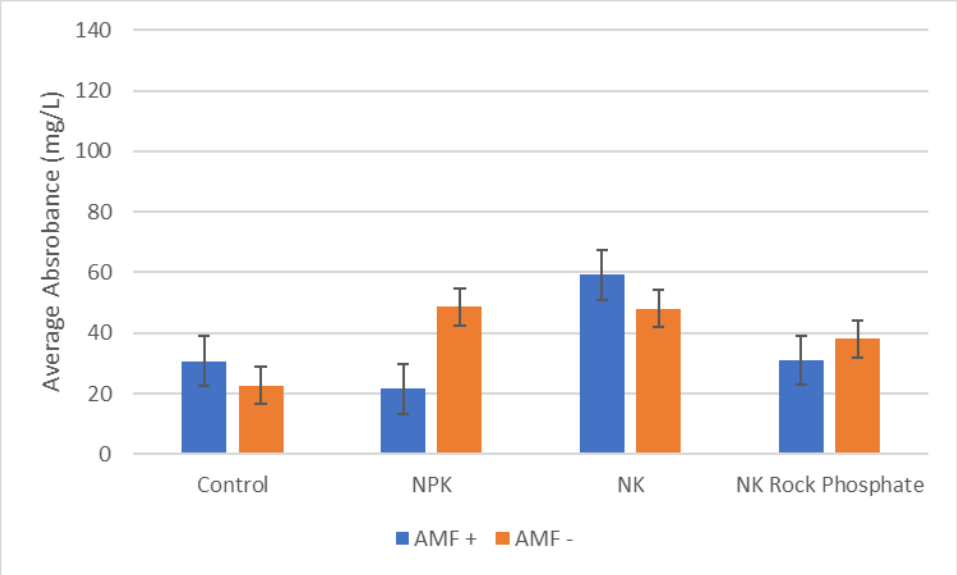

Figure 20: Trial 1 absorbance readings of phosphorus AMF inoculation versus non-inoculation in *Zea mays*. The average absorbance reading (mg/L) of phosphorus is separated based on inoculation status. Inoculated treatments are indicated by the left bars, and non-inoculated treatments are indicated by the right bars. No significant difference in average absorbance between the two groups was found ($p > 0.05$).

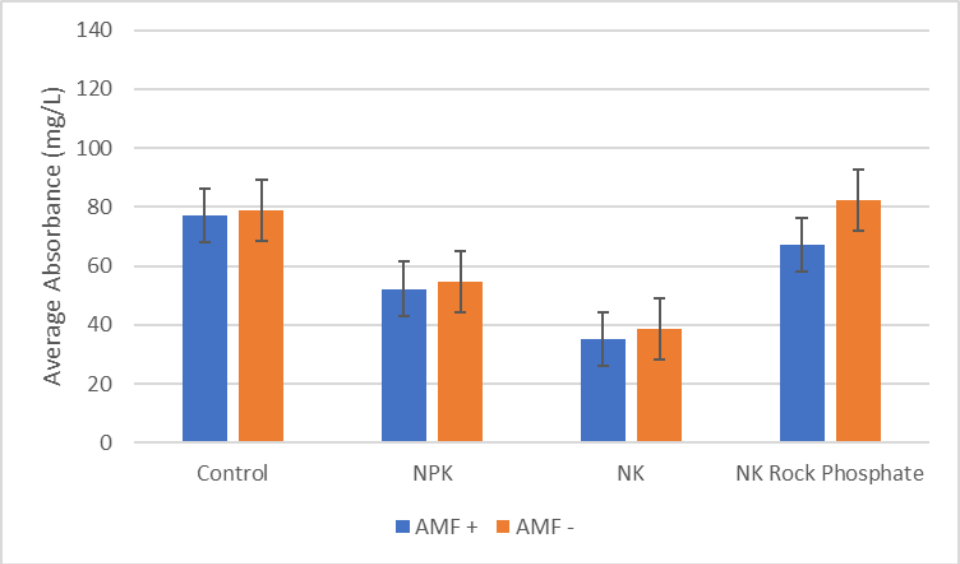

Figure 21: Trial 2 average absorbance readings of phosphorus AMF inoculation versus non-inoculation for *Zea mays*. The average absorbance readings (mg/L) of phosphorus are separated based on inoculation status. Inoculated treatments are indicated by the left bars, and non-inoculated treatments are indicated by the right bars. No significant difference in average absorbance between the two groups was found ($p > 0.05$).

During trial 1, *Zea diploperennis* had higher average absorbance values in treatment groups that were inoculated with AMF in the control group, NPK group and NK and rock phosphate group. In the treatment with only NK, the non-inoculated treatment group had higher average absorbance values by approximately 0.7 mg/L (Figure 22). In trial 1, there was no significant difference between the average absorbance values in inoculated versus non-inoculated treatment groups ($p = 0.1720$). In trial 2, inoculated groups treated with NPK and NK had higher average absorbance values than non-inoculated groups. Oppositely, non-inoculated plants performed better in the control setting and NK with rock phosphate treatment (Figure 23). There was no significant difference between the average absorbance values of inoculated and non-inoculated treatment groups ($p = 0.6119$).

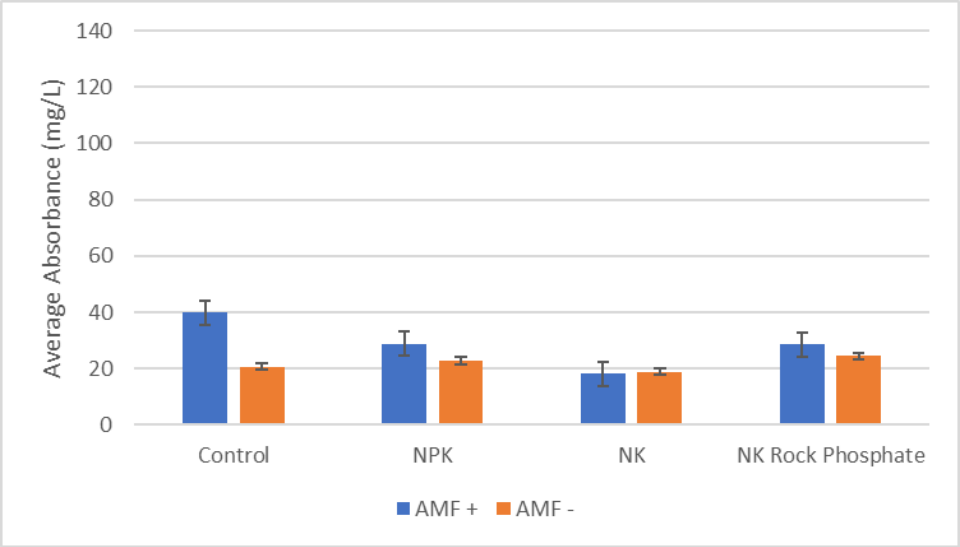

Figure 22: Trial 1 absorbance readings of phosphorus AMF inoculation versus non-inoculation in *Zea diploperennis*. The average absorbance reading (mg/L) of phosphorus is separated based on inoculation status. Inoculated treatments are indicated by the left bars, and non-inoculated treatments are indicated by the right bars. No significant difference in average absorbance between the two groups was found ($p > 0.05$).

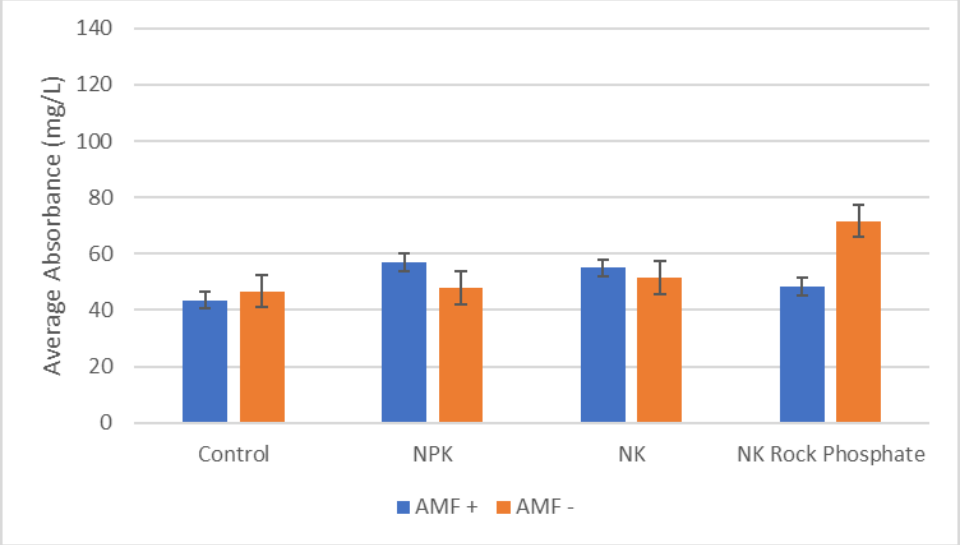

Figure 23: Trial 2 average absorbance readings of phosphorus AMF inoculation versus non-inoculation for *Zea diploperennis*. The average absorbance readings (mg/L) of phosphorus are separated based on inoculation status. Inoculated treatments are indicated by the left bars, and non-inoculated treatments are indicated by the right bars. No significant difference in average absorbance between the two groups was found ($p > 0.05$).

In trial 1, *Tripsacum dactyloides* had higher average absorbance values in inoculated treatment groups that also contained NPK, NK or NK with rock phosphate, but there were no surviving specimens in the non-inoculated with NK treatment group. In the control group, non-inoculated plants showed a higher average absorbance value (Figure 24). There was no

signficant difference between inoculated and non-inoculated treatment groups ($p = 0.2288$). In trial 2, plants inoculated with AMF had higher average absorbance values only in the NK with rock phosphate treatment group. In all other treatment groups, non-inoculated plants had higher average absorbance values (Figure 25). There was no significant difference between the average absorbance values in inoculated versus non-inoculated treatment groups ($p = 0.4172$).

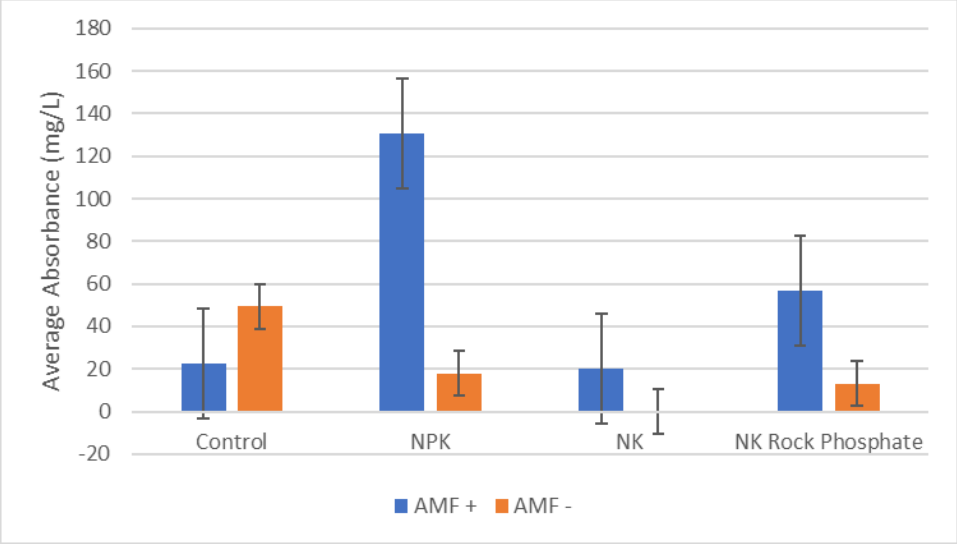

Figure 24: Trial 1 absorbance readings of phosphorus AMF inoculation versus non-inoculation in *Tripsacum dactyloides*. The average absorbance reading (mg/L) of phosphorus is separated based on inoculation status. Inoculated treatments are indicated by the left bars, and non-inoculated treatments are indicated by the right bars. No significant difference in average absorbance between the trial 1 and trial 2 was found ($p > 0.05$).

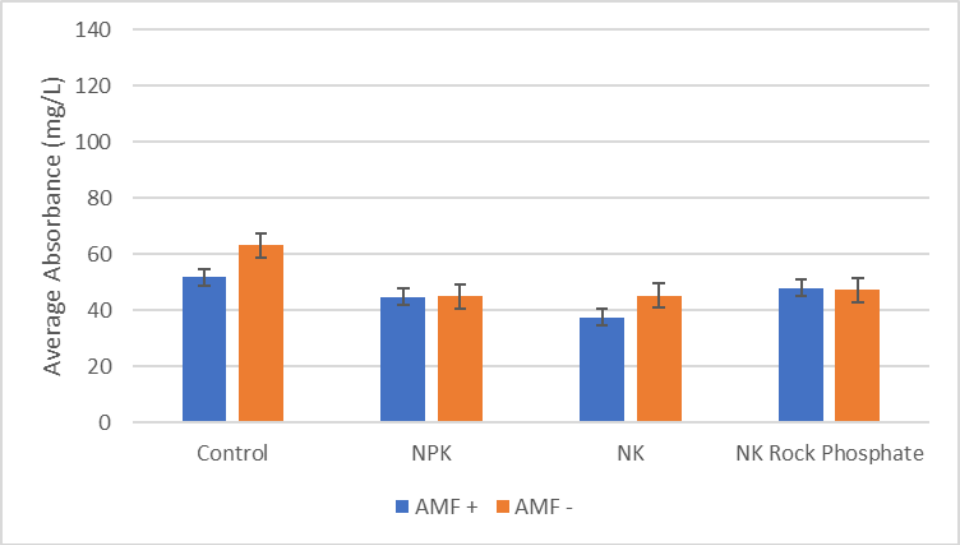

Figure 25: Trial 2 average absorbance readings of phosphorus AMF inoculation versus non-inoculation for *Tripsacum dactyloides*. The average absorbance readings (mg/L) of phosphorus are separated based on inoculation status. Inoculated treatments are indicated by the left bars, and non-inoculated treatments are indicated by the right bars. No significant difference in average absorbance between the trial 1 and trial 2 was found ($p > 0.05$).

Comparing trial 1 and trial 2, *Zea mays* plants inoculated with AMF had higher average absorbance values in trial 2 in the control group, NPK group and NK with rock phosphate group (Figure 26). There was no significant difference in average absorbance values between the two trials ($p = 0.1189$). Non-inoculated groups had higher average absorbance values during trial 2 in the control group, NPK group and NK with rock phosphate group (Figure 27). There was no significant difference in average absorbance values between the trials ($p = 0.0906$).

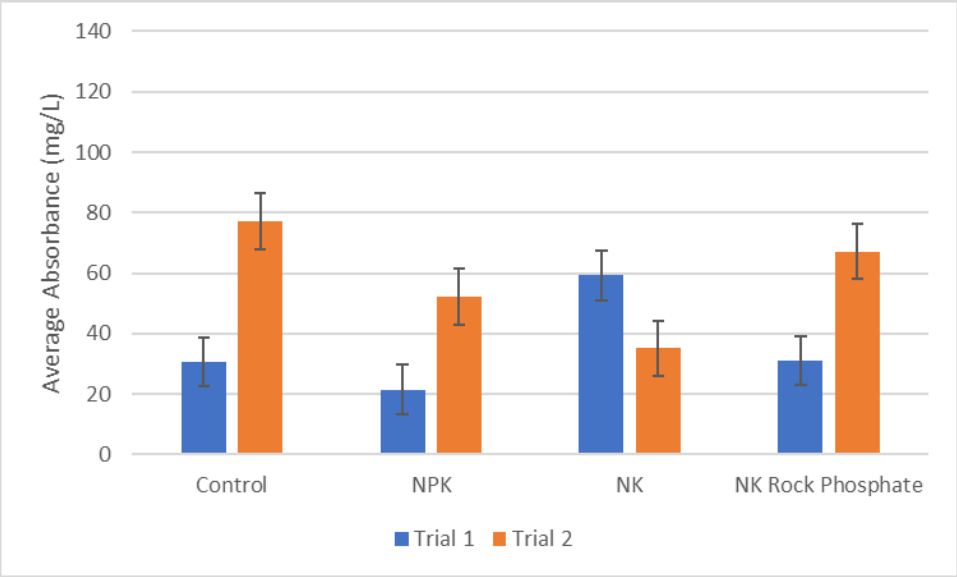

Figure 26: Trial 1 versus trial 2 average absorbance readings of phosphorus within inoculated treatment groups for *Zea mays*. The average absorbance readings (mg/L) for phosphorus are separated by trial. Data from trial 1 is represented by the left bars, and data from trial 2 is represented by the right bars. All treatment groups represented in this graph were inoculated with AMF. No significant difference in average absorbance between the two trials was found ($p > 0.05$).

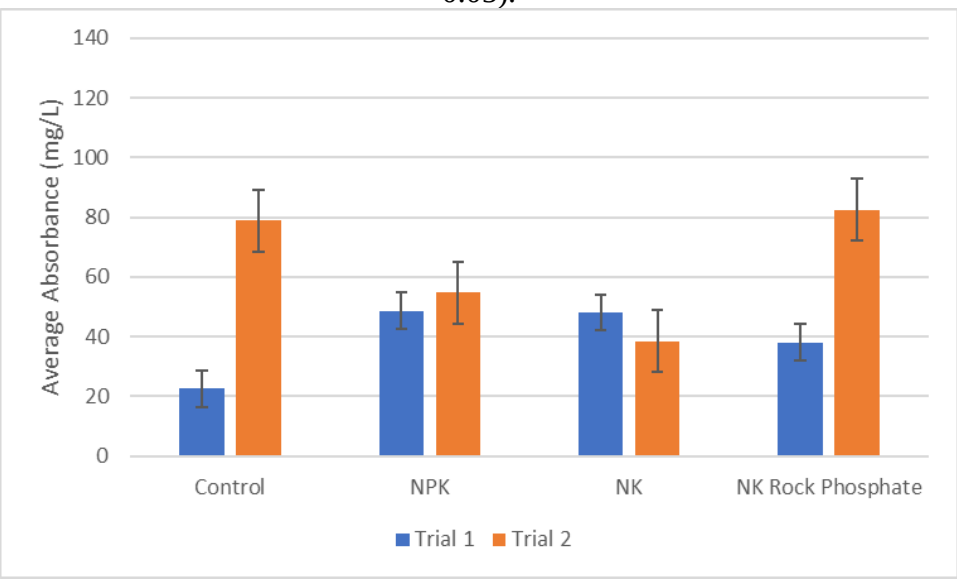

Figure 27: Trial 1 versus trial 2 average absorbance readings of phosphorus within non-inoculated treatment groups for *Zea mays*. The average absorbance readings (mg/L) for phosphorus are separated by trial. Data from trial 1 is represented by the left bars, and data from trial 2 is represented by the right bars. All treatment groups represented in this graph were not inoculated with AMF. No significant difference in average absorbance between the two trials was found ($p > 0.05$).

Zea diploperennis plants inoculated with AMF had higher average absorbance values in trial 2 across all treatment groups (Figure 28). Trial 2 inoculated plants had significantly higher

average absorbance values when compared to trial 1 inoculated plants ($p = 0.0063$). Non-inoculated plants had higher average absorbance values in trial 2 compared to trial 1 across all treatment groups (Figure 29). There was a significant difference in absorbance values between trials 1 and 2 ($p = 0.0015$).

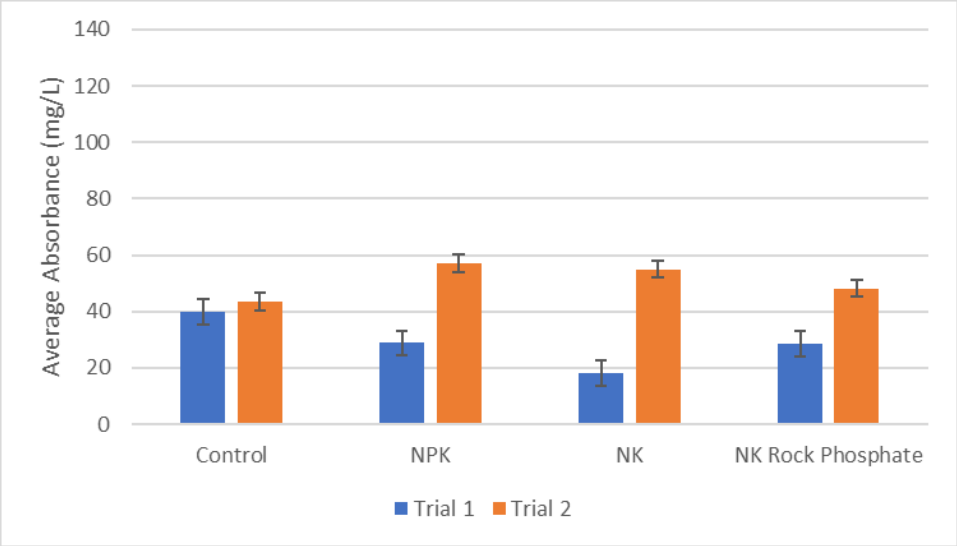

Figure 28: Trial 1 versus trial 2 average absorbance readings of phosphorus within inoculated treatment groups for *Zea diploperennis*. The average absorbance readings (mg/L) for phosphorus are separated by trial. Data from trial 1 is represented by the left bars, and data from trial 2 is represented by the right bars. All treatment groups represented in this graph were inoculated with AMF. A significant difference in average absorbance between the two trials was found ($p < 0.05$), with trial 2 having higher average absorbances.

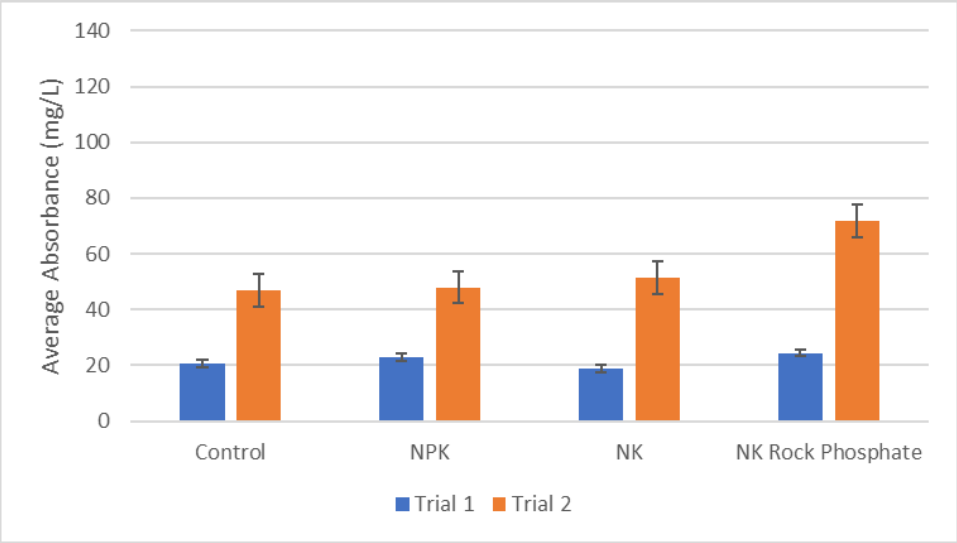

Figure 29: Trial 1 versus trial 2 average absorbance readings of phosphorus within non-inoculated treatment groups for *Zea diploperennis*. The average absorbance readings (mg/L) for phosphorus are separated by trial. Data from trial 1 is represented by the left bars, and data from trial 2 is represented by the right bars. All treatment groups represented in this graph were not inoculated with AMF. A significant difference in average absorbance between the two trials was found ($p < 0.05$), with trial 2 having higher average absorbances.

Tripsacum dactyloides plants inoculated with AMF had higher average absorbance values in trial 1 in groups with NPK and NK with rock phosphate. In trial 2, the control group and NK group had higher average absorbance values than in trial 1 (Figure 30). There was no significant difference in average absorbances between trial 1 and trial 2 in inoculated treatment groups ($p = 0.6642$). In non-inoculated groups, trial 2 had higher average absorbance values across all treatments when compared to trial 1 (Figure 31). Trial 2 non-inoculated plants had significantly higher average absorbance values compared to trial 1 ($p = 0.0384$).

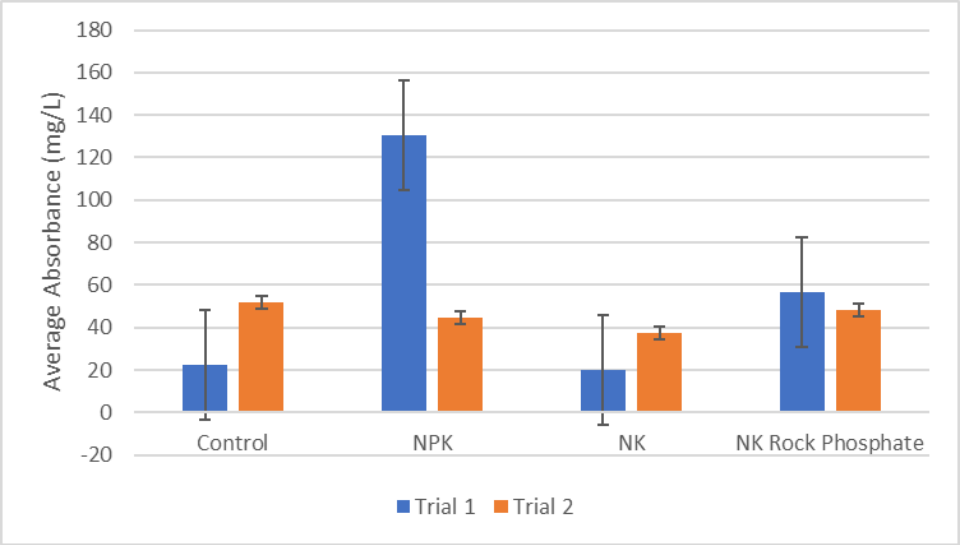

Figure 30: Trial 1 versus trial 2 average absorbance readings of phosphorus within inoculated treatment groups for *Tripsacum dactyloides*. The average absorbance readings (mg/L) for phosphorus are separated by trial. Data from trial 1 is represented by the left bars, and data from trial 2 is represented by the right bars. All treatment groups represented in this graph were inoculated with AMF. No significant difference between the absorbances from the two trials was found ($p > 0.05$).

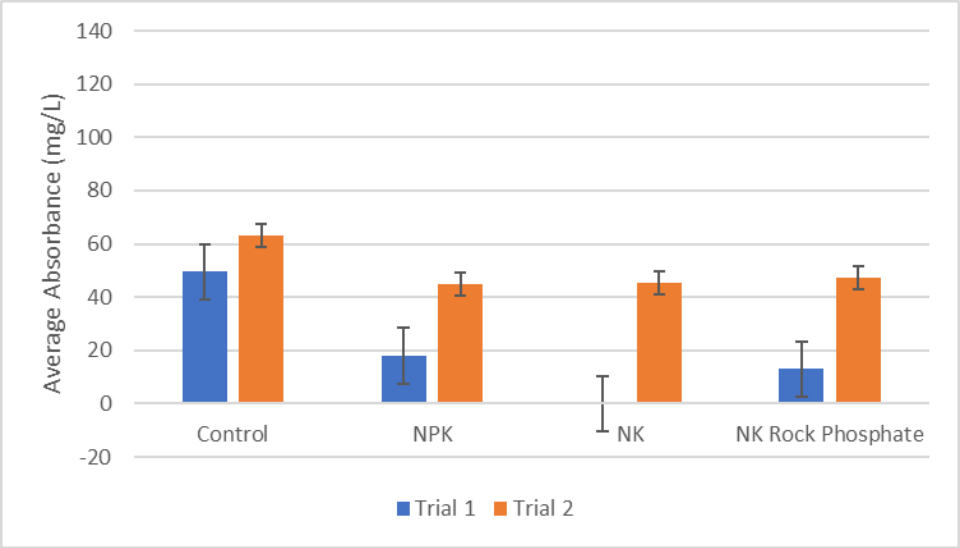

Figure 31: Trial 1 versus trial 2 average absorbance readings of phosphorus within non-inoculated treatment groups for *Tripsacum dactyloides*. The average absorbance readings (mg/L) for phosphorus are separated by trial. Data from trial 1 is represented by the left bars, and data from trial 2 is represented by the right bars. All treatment groups represented in this graph were not inoculated with AMF. A significant difference in average absorbance between the two trials was found ($p < 0.05$), with trial 2 having higher average absorbances.

A comparison of all three species over all treatment groups in trial 1was conducted. In trial 1, *Zea mays* had the highest absorbance in treatment group 5. *Zea diploperennis* had the highest absorbance in treatment group 2, and *Tripsacum dactyloides* had its highest absorbance in

treatment group 3 (Figure 32). There was no significant difference between species or treatment groups with p values of 0.5166 and 0.7722 respectively. Another comparison of all three species over all treatment groups during trial 2 was conducted. In trial 2, *Zea mays* had its highest average absorbance in treatment group 1. *Zea diploperennis* had its highest average absorbance in treatment group 8. *Tripsacum dactyloides* had its highest average absorbance in treatment group 1 (Figure 33). There was no significant difference between species or treatment groups with p values of 0.1390 and 0.2716 respectively.

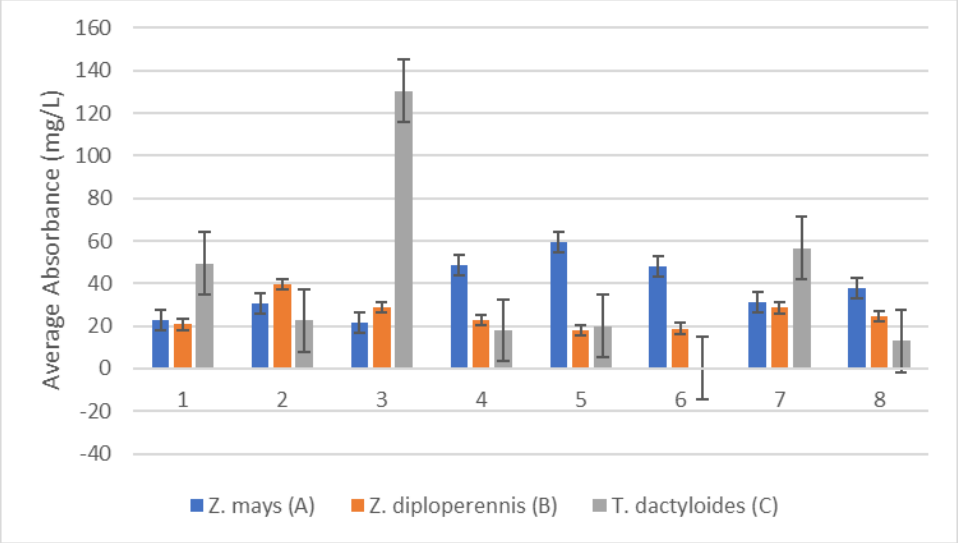

Figure 32: Trial 1 comparison of absorbances (mg/L) of all treatments across all species. The average absorbance readings for *Zea mays*, *Zea diploperennis* and *Tripsacum dactyloides* are separated by treatment number for trial 1. *Zea mays* is represented by the left bars. *Zea diploperennis* is represented by the middle bars. *Tripsacum dactyloides* is represented by the right bars. There was no significant difference between species or treatment groups ($p > 0.05$).

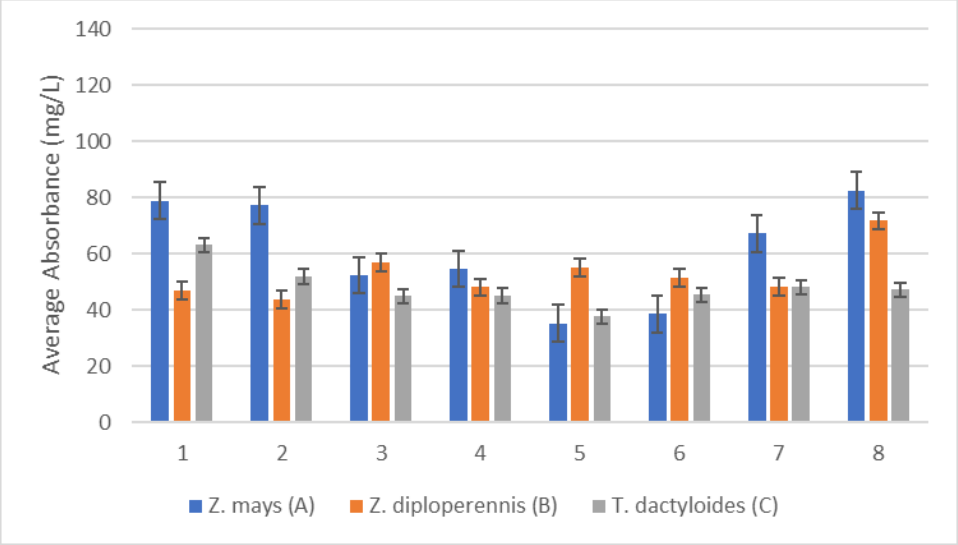

Figure 33: Trial 2 comparison of absorbances (mg/L) of all treatments across all species. The average absorbance readings for *Zea mays*, *Zea diploperennis* and *Tripsacum dactyloides* are separated by treatment number for trial 2. *Zea mays* is represented by the left bars. *Zea diploperennis* is represented by the middle bars. *Tripsacum dactyloides* is represented by the right bars. There was no significant difference between species or treatment groups ($p > 0.05$).

V. DISCUSSION/CONCLUSION

It was hypothesized that there would be significant differences in the absorbance values of inoculated plants in comparison to non-inoculated plants. In addition, it was hypothesized that similarities would be present between species of the same treatment group. The results of this study showed significant differences between the two trials as opposed to between inoculated and non-inoculated plants, which differs from previous studies conducted by Cely et al. 2016, Pinos et al. 2019 and Gao et al. 2020. Two differences existed between trial one and trial two: climate and fertilizer dosage. The fertilizer dosage used in trial one was doubled in trial two, but, similar to Cely et al. 2016, single and double dosages of fertilizer showed similar results with regards to AMF effectiveness (Cely et al. 2016). In trial one, the day and night temperatures were at their average lowest when planting was conducted, whereas in trial two day and night temperatures were at their average highest when planting was conducted. This could have impacted specifically the germination of *Tripsacum dactyloides* during trial one, as supported by a USDA study conducted in 2005. Additionally, there was a potential for empty *Tripsacum dactyloides* seed pods as evidenced by Ahring and Frank 1968.

The optimal planting temperature for *Zea mays* for the development of roots and shoots is between 20°C and 30°C (Ratajczak et al. 2023). Because *Zea diploperennis* is endangered and primarily used for research purposes, less standardized information is available about optimal planting temperature, but it is widely known to not be frost tender. In 2005, a study was carried out exploring the optimal day and night temperatures for *Tripsacum dactyloides* leaf growth and protein content. An incubator style experiment was conducted where Tripsacum dactyloides seeds were grown at three different day and night temperatures: 20°C/14°C, 27°C/22°C and 35°C/29°C. The results proved that the highest day and night temperatures (35°C and 29°C

respectively) explored had the highest leaf growth and protein content (USDA 2005). These findings align with this study in that *Tripsacum dactyloides* had a much higher germination rate in trial two, where temperatures were 10°C hotter on average at the start of the trial. Another explanation for the lack of *Tripsacum dactyloides* germination could be empty seed pods, as demonstrated by Ahring and Frank in 1968.

Ahring and Frank conducted an experiment to explore a lack of *Tripsacum dactyloides* germination due to empty seed pods. Upon visual examination by the naked eye, an empty seed pod is nearly indistinguishable from a filled seed pod. The researchers used three methods for sorting empty from filled seed pods: soaking in water, blowing with forced air and checking through hand and visual examination. Across all three sorting methods a random sample of 100 seeds were selected, and they found that anywhere from 32-48% of seed pods were empty (Ahring and Frank 1968). While this finding is notable and could have contributed a small amount to a lack of *Tripsacum dactyloides* germination in trial one, seed manufacturing processes have evolved and improved since this study was conducted. Additionally, their findings do not explain the improved germination rate in trial two. Without examining the effectiveness of inoculation, the differing temperatures present between the two trials could explain why *Tripsacum dactyloides* had a higher rate of germination in trial two compared to trial one.

Differences between inoculated and non-inoculated plants with added phosphorus have been previously reported in literature, where the addition of phosphorus does not always produce a significantly positive effect on phosphorus or nitrogen content in leaf tissue. A 2019 study conducted by Bazaretti et al. on corn and soybean produced results that demonstrated a higher level of phosphorus content within the leaf tissue of corn plants in a treatment group where

plants were inoculated with AMF but had no added phosphorus fertilizer. Oppositely, nitrogen content in corn leaf tissue increased with added phosphorus and inoculation with AMF. In soybean plants, both nitrogen and phosphorus increased with inoculation and phosphorus fertilizer application (Bazaretti et al. 2019). Because the study by Bazaretti et al. was conducted in a field setting, there could have been a phosphorus presence naturally in the soil. In soils where phosphorus is already present in sufficient quantities, AMF has been shown to not be as effective in phosphorus delivery via the MP (Bazaretti et al. 2019). The soil used in this study was purchased from a landscaping company, so the presence of phosphorus could have contributed to the limited significant differences between inoculated and non-inoculated plants. The pasteurization process used on the soil prior to planting helped decrease the naturally occurring mycorrhizal presence in the soil, but this process did not have any effect on the phosphorus content within the soil. The addition of phosphorus or nitrogen that contributes to an excessive amount of macronutrients within the soil can inhibit the effectiveness of AMF, as demonstrated in previous literature.

Lin et al. 2020 demonstrated that the addition of nitrogen and phosphorus improved overall soil nutrition, which decreased the dependence between the plant host and AMF. In trial two of this experiment, the amount of all fertilizer components was doubled. For all three species studied, trial two had several treatment groups where non-inoculated groups produced a higher phosphorus absorbance reading. Although this finding was not proven significant through ANOVA analysis, increasing the amount of nitrogen and phosphorus applied to the soil could have increased overall soil nutrition and macronutrient availability, thus reducing the need for the symbiotic relationship between AMF and the rooting system. The amount of carbon resources that can be put towards a symbiotic relationship with AMF is also reduced when the

intended host plant can attain macronutrients on its own, thus beginning metabolic processes within the plant. In addition, the addition of excess amounts of nitrogen has been proven to cause mitochondrial stress that leads to the cracking of root cell cytoplasm, which, in addition to the increased consumption of carbohydrates needed to convert ammonium into usable nitrogen forms, will decrease plant growth and development (Lin et al. 2020). A delicate balance must be maintained between inherent macronutrients and applied macronutrients to ensure maximal effectiveness of applied AMF or naturally occurring AMF.

Lower levels of phosphorus have been shown to increase AMF colonization and effectiveness, as measured through leaf tissue nutrient content. In addition, indigenous species of mycorrhizae have been shown to be more effective colonizers of in field plants (Ortas 2012) (Gao et al. 2020). Because there were some significant differences between trial one and trial two, with trial two often having significantly higher average absorbance, the threshold for excessive phosphorus may not have been reached by doubling fertilizer contents in this study, or a sufficient inherent level of phosphorus was present within the soil, which decreased the need for the symbiotic relationship between plant and AMF. A balance between macronutrients within the soil and applied macronutrients must be attained to reach maximal AMF effectiveness (Ortas 2012).

The results of this study did not demonstrate a significant difference between inoculated plants and non-inoculated plants, as was hypothesized. The species of AMF applied is dependent on the species of plant being grown, because different species of plants have a higher affinity for different species of AMF (Campo et al. 2020). In this study a mixture of several species of AMF was used, so not having a specific species of AMF for each species of plant could have contributed to the non-significant differences that inoculation made on phosphorus

absorption. However, there were no significant differences found in absorption values across species or treatments. This supports the initial hypothesis that the three species would respond similarly to each treatment due to their familial relationship. Additionally, the results of this study leave room for future research projects to further explore AMF effectiveness on the species.

Future studies could include a repetition of the study with both fertilizer levels tested during both the spring and fall seasons, to explore the effect of climate on germination rates and AMF effectiveness. With this study being in a controlled environment, potted experiment, a field study could be conducted using the same treatment groups. A study like this could also explore the importance of a balance between applied macronutrients and inherent macronutrients. If a potted experiment was repeated, the use of sterilized soil as opposed to pasteurized soil would further decrease the probability of an indigenous AMF population within the soil sample.

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