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EFFECT OF MYCORRHIZAE INOCULATION ON THE GROWTH AND SUCCESS OF THREE TAXODIUM DISTICHUM HYBRIDS IN SALINE - IMPACTED COASTAL SOILS

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By

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ABSTRACT

In 2008, Galveston Island was severely impacted by Hurricane Ike, resulting in high salt deposition in the soil and groundwater. This caused a loss of many native plant species. A study initiated to determine effective ways to promote the growth conditions of three bald cypress genotypes (*Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* crosses) in salt-affected soils. The treatments applied were mycorrhizae inoculation, fertilizer application, and a combination of mycorrhizae inoculation and fertilizer application. A total of sixty (60) trees planted in plots of three rows and divided into five randomized replication blocks of four treatments each were used. Plants were measured for total height and diameter at breast height (DBH), and tree leaf nutrient concentration over the 2021 growing season. The effect of these treatments on soil health was determined by measuring the soil microbial functional diversity and soil respiration.

None of the three treatments had a significant effect on height, diameter breast height, leaf nutrient concentration, soil microbial functional diversity, or soil respiration. This could be because of the limited spacing between the trees and the age of the trees. However, treatment with mycorrhizae alone and a combination of mycorrhizae and fertilizer showed the potential to improve the tree height and the DBH.

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1. INTRODUCTION

The salinity of soils is a worldwide concern that cuts across all continents. According to Gopalakrishnan & Kumar (2020), high salinity affects about 33% of irrigated agricultural land and 20% of overall cultivated land worldwide, this presents a significant threat to agricultural farm production, leading to adverse implications for food security, environmental health, and economic welfare. Soil salinity is one among many severe environmental issues reducing the output of crop plants and consequently affecting the economy worldwide. The reduced crop output is caused because most crop plants are sensitive to high salt concentrations present in the soil (Shrivastava and Kumar, 2015). Several salts may be introduced into soils and may or may not occur naturally (Sullia, 2004).

The word “salinity” is a term used to describe a state in which water-soluble salts (primarily sodium, potassium, magnesium, calcium, and chloride) accumulate in soils to levels where they have a detrimental effect on the growth of plants (Ebrahim, 2014). The salinity levels in the soil influence the activity of certain enzymes, photosynthesis, and turgor in the plants. Salt concentrations beyond critical levels can impair the biological and physiological functions of plants, such as germination, vegetative growth, and reproductive development. In addition, salt stress causes a decrease in plant growth and

productivity by disrupting physiological processes, particularly photosynthesis (Sudhir & Murthy, 2004).

One way to help control soil salinity is by planting trees (Nosetto et al., 2008; Horsnell et al., 2009). For example, the hydrological imbalance may be restored by replacing shallow agricultural grass species with deep-rooted trees. Planting these deep-rooted trees enables the reversal of the causal process of salinization in affected areas. (George et al., 1999; Jobbagy & Jackson, 2004; Shao et al., 2008). In Shandong Province (Yellow River Delta), one of the severely salinized regions of China, afforestation was used to control soil salinization by lowering the water table through the process of taking up water from the soil and removing it through transpiration. However, afforestation is extremely difficult in the region of the Yellow River Delta because the soil contains high salt concentrations and low levels of nutrient elements. Nutrient elements such as low soluble phosphorous (P) are limiting due to the high pH of the soil, which limits the mobilization of nutrient elements in the soil solution (Wang et al., 1993).

Biological approaches, such as inoculation of plants with mycorrhizae fungi can help to address soil salinity. Mycorrhizae are a symbiotic relationship between plant roots and fungi. Their principal function is to improve nutrient and water utilization by the host plant, providing absorption surface by a larger area than roots alone (Dighton, 2009). The fungi produce tiny threadlike structures called hyphae within or on the plant's root cells. They help plant root systems, providing increased absorption of water and nutrients,

whereas the plants supply the fungus with carbohydrates produced from photosynthesis. Mycorrhizal relationships are essential for tree species. They also help to prevent the colonization of root by plant disease-causing organisms. (Berruti et al., 2016)

Taxodium is a deciduous conifer that belongs to the family Cupressaceae, one of several genera in the family commonly referred to as cypresses. Previous literature often refers to three species: 1). *Taxodium distichum* (Bald cypress), 2). *T. ascendens* (Pond cypress), and 3). *T. mucronatum* (Montezuma cypress). However, current taxonomy places *Taxodium* as one species with three botanical varieties (Denny and Arnold, 2007).

- *Taxodium distichum (L.) Rich.var. distichum* (Bald cypress - BC)
- *Taxodium distichum var. imbricarium (Nutt.) Croom* (Pond cypress - PC)
- *Taxodium distichum var. mexicanum* (Carriere Gordon) (Montezuma cypress - MC)

In 2008, Galveston Island was severely impacted by Hurricane Ike. The hurricane resulted in high sea salt concentrations in the soil and groundwater, which affected the recovery of many plant species including trees. The loss of plant species led to many changes in the coastal environment, such as soil erosion (Williams et al., 2020). One of the most impacted plant species on Galveston Island was live oak (*Quercus virginiana*). Because this tree species is known to have moderate to a low tolerance to salinity, full recovery of the live oaks may take some time. Nearly 80% of live oaks (~55,000 live oaks) were lost due to salt-water damage on Galveston Island (Morgan, 2020).

Consequently, the island needs plant species that are more salt-tolerant than the live oaks to survive changing coastal conditions brought on by hurricanes or other impacts such as rising sea levels. A promising tree species that could replace salt intolerant species such as the live oak is bald cypress (*Taxodium distichum*). Bald cypress is a dominant overstory tree species with moderate to high levels of salt tolerance and is primarily found in the southern parts of the U.S (Lauer, 2013). These plants are tolerant to salt and are considered an essential part of the ecosystem in the Galveston area. Galveston Island is a vulnerable barrier island and is a potential habitat for the bald cypress plant.

In order to achieve superior genotypes with better qualities, further research was carried out, and crosses were made between the three major botanical varieties of *Taxodium* (Chen et al., 1987). Considerable work has been conducted in China which involved controlled crosses between BC, MC, and PC, and the subsequent selection of superior genotypes that are multiplied through cutting propagation. T302 (BC × MC) was selected in China in 1987 chiefly for growth rate and tolerance to alkaline and salt-rich coastal floodplains. T302 is registered at both the provincial and federal level and accepted for higher salt tolerance than BC and PC. Other qualities attributed to T302 included 159% faster growth than BC, good form with longer foliage retention in fall and early winter, and no knees (Chen et al., 1987). Huang et al. (2006) reported that T302 was widely adapted to a wide range of soils and climate.

Due to the geological formations on Galveston, the soil salinity levels in this region are higher than inland areas. Rainstorms, wind, and evaporation events often cause this soil salinization. With the increasing risk of salt deposition, there is a need for understanding how mycorrhizae can contribute to the salt tolerance of bald cypress.

Extensive research has been carried out on the effectiveness of biological remediation techniques to improve plant growth in saline soils. However, information describing the effectiveness of various treatments applied separately and in combination is currently lacking. Scharnagl *et al*. (2018), considered the effect of mycorrhizae on plant growth, particularly in saline soil, but not in combination with other treatments. As such, further study is required to fully comprehend and understand the ideal quantity treatments to achieve optimum yield and plant growth.

In this study, growth parameters were evaluated such as plant height, stem diameter at breast height and plant tissue nutrient levels (N, P, K, C, and Na) under the different treatments. Studying the effects of mycorrhizal inoculation and other treatments is essential to determine the best treatment required for the growth of *Taxodium distichum* in saline soils. In addition, the survival and efficient growth of *Taxodium distichum* is essential as this tree species offers a suitable replacement for the live oaks destroyed as a result of the hurricane Ike.

Research on microbial species diversity and activity is also essential to determine the soil micro-flora responsible for promoting plant growth and soil ecosystem balance to support the growth of other tree species during afforestation.

The saline soils on Galveston Island can be used to study and better understand the role mycorrhizal fungi and fertilizer application play in the survival of *Taxodium distichum,* which is essential to restoring the eco-balance of Galveston Island.

2. OBJECTIVES

The specific objectives of this study were to:

1. Examine the effect of a commercial mycorrhizae inoculation and fertilizer application on three bald cypress genotypes (crosses made between *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum*) using the following parameters.
    - Growth of trees as determined by tree height and diameter at breast height (DBH).
    - Tree leaf nutrient (N, P, K, C) and Sodium (Na) concentrations.
    - Microbial species diversity as determined by the Ecolog/EcoPlate system
    - Microbial activity as measured by a field soil respirometer.

    This study tested the following hypotheses:

    - Null Hypothesis: Mycorrhizal inoculation has no effect on plant growth and nutrient concentration in addition to no effect on soil health.
    - Alternative Hypothesis: Mycorrhizal inoculation will improve plant growth and nutrient concentration and promote soil health.

3. LITERATURE REVIEW

3.1 Soil Salinity

Soils are compound mixtures of mineral particles, water, air, organic matter, and the decaying remains of countless organisms that were once-living things. Soils have the ability to support plant life and are vital to all life on earth. However, there are various soil types, and the characteristics of these soil types affect plant growth and yield, and other life form (Yoder et al., 2022).

The characteristic of saline soils that separates them from the other soils is that they contain many soluble salts, which negatively affect the growth of plants (Dasgupta et al., 2015). In addition, the saturated soil extract of saline soils have has an electrical conductivity more than 4 dS/m at 25°C (Richards 1954).

3.1.1 Coastal Soils and Salinity

Coastal soils are usually poor for crop productivity, primarily due to soil usage, water, and climatic conditions (Gopalakrishnan and Kumar, 2020). Among the problems associated with agriculture, salinity is a significant factor responsible for reducing crop yield in coastal areas (Sudhir & Murthy, 2004). Due to the different factors that affect coastal soils, there is a need for management techniques to increase production in these areas. The coastal region represents the area between terrestrial and marine environments.

It is comprised of the shoreline ecosystems, upland watersheds that drain into coastal waters, and

the nearshore sub-littoral ecosystems influenced by land-based activities. Reduction in the productivity of this region is influenced by adverse agro-climatic conditions, salinity, acidity, waterlogging and sandy texture (Gopalakrishnan and Kumar, 2020).

Many coastal soils are saline in nature. Saline soils can be defined as soils with a conductivity of the saturation extract greater than four (4) dS m^{-1} an exchangeable sodium percentage less than 15 and a pH less than 8.5. Saline soils were formerly called white alkali soils because of a surface crust of white salts (Karen, 2005). Soil salinity is a crucial abiotic factor that reduces the success of crops.

3.1.2 Characteristics of Saline Soils

Saline soils have soluble salt concentrations that are high, which causes high osmotic pressure of the soil solution. Osmotic pressure affects plant water uptake rate growth. High soluble salt concentrations cause wilting of plants and nutrient deficiency as a salt content of more than 0.1% is injurious for plant growth.

Soil salinity tests measure the minerals and content of salts readily dissolved in water. The following mineral ions are found frequently in soil-water extract listed in order of importance: Na^+, Cl^-, Ca^{2+}, SO_4^{2-}, HCO_3^-, K^+, Mg^{2+}, and NO_3^-. Increased soil salinity, especially due to high Na^+ content has progressive and often adverse effects on soil structure, water movement, microbial and plant diversity. Soil salinity is measured

using a water-saturated soil paste extract for measurement of electrical conductivity (EC). When Na ions are in excessive concentrations, it leads to an imbalance in the ratio of monovalent cations to divalent cations. The exchangeable sodium percent (ESP) measures this imbalance, and the salt-affected soils are classified by their ESP.

Electrical conductivity (EC) of the soil saturation extract is a common measurement for the assessment of saline soils for plant growth and is expressed as dS m^{-1}. Salinity effects are negligible below two (2) dS m^{-1}. However, yields of very sensitive crops may be restricted between 2 and 4 dS m^{-1}, while yields of many crops may be restricted between 4 and 8 dS m^{-1}. On the other hand, only tolerant crops yield satisfactorily between 8 and 16 dS m^{-1}, whereas above 16 dS m^{-1}, only high-tolerant crops grow (Srivastava *et al.*, 2019).

3.1.3 Sources of Soil Salinity

There are various sources of soil salinity, with natural soil salinity occurring in hot arid and semiarid climates which receive <27 cm of annual rainfall. Soils and lands with shallow water tables can develop saline soils due to the concentration of salts and excessive water evaporation. Also, poor water quality and irrigation practices contribute to the salinization of thousands of acres of farmland each year around the world. On a global basis, salt-affected soils occupy 952.2 million ha of land. These soils make up nearly 7% of the total land area, or about 33% of the potential agricultural landmass of the world (Artiola et al., 2019).

Primary salinization is the process of salt accumulation due to natural causes, chiefly topography, mineralogy of the parent material, and water table quality. Primary mineral weathering results in the deposition of sodium and other ions implicated in saline soils. This weathering results in the release of soluble cations and anions, with the most common cations being Ca^{2+}, K^{+}, Mg^{2+}, and Na^{+}, and the most common anions being HCO^{3-}, Cl^{-}, and SO_4^{2-}.

In humid regions, cations and anions are predominantly leached from the soil system and subsequently transported via water movement to low-lying landforms or groundwater aquifers (Zinck and Metternicht, 2009). In arid, semi-arid, and sub-humid regions, such cations tend to persist in the soil-exchangeable complex or precipitate as secondary minerals after the ionic concentration in the solution attains saturation of a specific salt. The less soluble salts, gypsum ($CaSO_4\ 2H_2O$), magnesite ($MgCO_3$), and calcium carbonate ($CaCO_3$), can easily precipitate under arid and semi-arid conditions leading to a relative increase in the proportion of Na^{+} ions in the solution. Consequently, a replacement of some exchangeable Mg^{2+} and Ca^{2+} by Na^{+} on the exchange complex occurs (Bui, 2017).

This process leads to an increase in ESP values. In arid conditions, where high evaporation rates lead to the concentration of the water solution, salts with more solubility than gypsum may precipitate in the soil. These salts contain sodium carbonates (trona, nahcolite, thermonatrite), magnesium sulphates (e.g., epsomite), potassium

chloride (sylvite), magnesium chlorides (e.g., bischofite), sodium sulfates (e.g., thenardite), and the most soluble and common salt, sodium chloride (NaCl, halite).

Salts with more solubility than gypsum are called 'soluble salts' and consist of the diagnostic minerals that describe saline soils (IUSS Working Group WRB, 2015). According to The World Refence Base (WRB) international classification, soils with a high concentration of these soluble salts, either at the surface or a defined depth, are categorized as "solonchaks."

Some lithologies release relatively high amounts of cations and anions also in non-dryland regions. For example, sedimentary rocks deposited in marine environments may include evaporite strata associated with shales and marls. The weathering of evaporite strata further increases the content of dissolved salts in the circulant water. Usually, such geological formations of evaporites are relatively plastic and tend to form domes through the tectonic process. Generally, the formations of such evaporites occur in higher landscape positions, making them a potential source of salts for the surrounding land (Zinck and Metternicht, 2009).

In drylands and salt-rich groundwater bodies downslope of recharge areas, called "saline seeps," soil salinity is elevated enough to inhibit vegetation growth (Brown et al., 1982). Alluvial plains and wetlands in arid and semi-arid regions are very sensitive to primary (and secondary) salinization because they accumulate overland water flow due to their low relative elevation. Ground and surface water dynamics drive the salinization processes in these landforms. When groundwater is saline, and the water table level rises

due to river regulations or land-use change, the impact on soil surface salinity is detrimental (Jolly et al., 2008).

Coastal areas in arid and semi-arid climates face a high risk of primary soil salinization. Salts transported directly from the sea surface through marine spray or wind can be deposited on the inland soil surface. The action of wind and other weather agents is a typical way salts are deposited on oceanic islands, such as Hawaii (Whipkey et al., 2000). The aeolian salt deposition can affect lands several kilometers from the coastline. An example is the Western Australia Wheatbelt. Pannell (2001) reported considerable salt deposition (20–200 kg ha−1 year−1) by wind and rainfall at considerable distances from the coastline.

Another cause of primary salinity in coastal areas subjected to tides is saline water intrusion into rivers and groundwater. Notably, the movement of high salinity backwater from the river delta inland is responsible for this process. This backwater effect is a result of a rise in sea level (Mahmuduzzaman et al., 2014).

3.2 Plant and Microbial Communities in Relation to Soil Salinity

Soil salinity affects (directly or indirectly) both the growth and reproduction of plants owing to complex interactions between physicochemical properties of soil (salt content, poor aeration, increased crusting, reduced infiltration, water uptake reduction, and difficult root penetration) and plant morphological and physiological features (Akbarimoghaddam et al., 2011).

Salinity causes low water potential in the soil, negatively affecting plant water and nutrient uptake. Plants collect salts simultaneously with the water they use and accumulate Na^+ and Cl^- ions, resulting in toxicity to plant cells due to ion imbalances. Furthermore, enzymatic activity in cells may be disturbed. These factors trigger different responses in plants, manifested by various symptoms both at cell and organ levels (Kumar and Verma, 2018). For example, a reduction in respiration characterizes stressed plants, showing altered assimilate distribution, inhibited photosynthesis process, and lower production of new leaves.

Microbial communities, including fungi, bacteria, and nematodes, help maintain the soil health by regulating functions such as nutrient cycling, decomposition, and carbon storage. Also, microbes serve as sensitive indicators of change for soil health in response to environmental stresses, such as salinity (Egamberdieva et al., 2010). Salinity impacts aboveground biomass production and has variable effects on belowground organisms, depending on the concentration of the salts. Previous studies have shown that soils with high concentrations of chloride-based salts can damage microorganisms by disrupting the soil ecosystem processes. For example, Rietz and Haynes (2003) reported that salinity had adverse effects on the size and growth of microbial biomass, likely due to decreased rates of soil organic matter deposition and nutrient mineralization as salinity increased.

Indicators of soil biological function and health often include microbial respiration and nitrogen mineralization (Knoepp et al., 2000). Microbial respiration is

important because it is linked to decomposition, nutrient mineralization, and the global carbon cycle and is often used to measure microbial activity. Respiration rates, accompanied by decreases in soil microbial biomass, were shown to decrease in moderately saline soils suggesting that salinity has significant adverse effects on microbial activity (Muhammad et al., 2006; Wong et al., 2008).

Since microbial activity is typically more concentrated in the topsoil, salts near the soil surface may directly interfere with soil microbial processes that help control ecosystem function (Yuan et al., 2007). Not only does soil salinity directly reduce microbial activity, it also affects other microbial-related processes. Consequently, the roles these microorganisms play in various nutrient cycles, such as decomposition of organic matter and maintenance of soil fertility are affected.

3.3 Fungi as Plant Growth Promoters

Fungi are achlorophyllous, eukaryotic organisms capable of reproduction by asexual and sexual spores. Fungi are principal decomposers in the global cycle of decomposition. They can decompose anything from living and non-living matter, breaking it down to its molecular constituents. (Norton, 2012). Fungi are found in various habitats, such as in the sea, moving in the air (spores), in the soils as well as in the body of living organisms. Most of fungi can grow in multicellular form some in unicellular form (yeasts).

3.3.1 Mycorrhizae

Mycorrhizae are indigenous to soil and plant rhizospheres and are essential for sustainable agriculture. In 1885, in a study of soil microbial-plant relationships, Albert Bernard Frank introduced the Greek term ‘mycorrhiza,’ which means ‘fungus roots.’ Most terrestrial plants form symbiotic associations with soil fungi from diverse fungal taxa (Bevers et al., 2001). Mycorrhizae associations which develop around or within host plant roots are generally considered mutualistic (Kiers et al., 2010). The fungi obtain carbohydrates from the host plant while the fungi provide the plant with inorganic nutrients and water (Allen, 2006). Hyphae, thread-like extensions of the fungal body, extend beyond the depletion zone surrounding the host roots, thereby providing access to resources otherwise unavailable to the host (Smith and Read, 1997). In addition, extracellular enzymes secreted by fungi enhance the absorption of nutrients in the soil. Of the seven different types of mycorrhizae (arbuscular, arbutoid, ecto-, endo-, monotropoid, ericoid, and orchidaceous mycorrhizae), endomycorrhizae (arbuscular mycorrhizae) and ectomycorrhizae are the most abundant and widespread (Smith and Read 1997; Allen, 2006).

Endomycorrhizal fungi interact with their host by infiltrating the root cells and developing structures called arbuscules. Trees typically colonized by these endomycorrhizae include cedars (*Cedrus* spp.), cypresses (*Taxodium* spp.), junipers (*Juniperus* spp.), maples (*Acer* spp.), ashes (*Fraxinus* spp.), dogwoods (*Cornus floridus* spp.), sycamore (*Platanus* spp.), and sweetgums (*Liquidambar* spp.). On the other hand,

ectomycorrhizal fungi colonize the outside of root cells and do not form structures within the cells. Trees typically colonized by these fungi include pines (*Pinus* spp.), oaks (*Quercus* spp.), and hickories (*Carya* spp.) (Bainard *et al.,* 2011).

3.3.2 Endomycorrhizae (Arbuscular Mycorrhizae (AM))

One of the most common soil fungi is the Arbuscular mycorrhizal (AM) fungi, and nearly all plant species have associations with AM fungal species. AM can exist in all ecosystems, such as arid areas, tropical regions, sub-polar habitats, and aquatic ecosystems (Ebrahim, 2014). Aseptate, obligately symbiotic fungi form arbuscular mycorrhizae from the order glomales in the Zygomycetes. Arbuscule formation begins when a side branch of an intraradical hypha penetrates the cell wall and divides dichotomously to develop hyphae and dark septate endophytes (DSE) (Smith and Read, 2008).

In addition to arbuscules, AM may develop spores and vesicles, depending on the taxa. Vesicles are oval or round, thick-walled, multinucleated, and lipid-containing structures developed at the tip of hyphae or hyphal branches in the host cortex. They are usually formed at the end of the growing seasons and act as propagules for the next season. Asexual spores, the second type of propagule, are usually produced on extraradical hyphae. Spores contain numerous nuclei, lipid droplets, and other organelles protected by pigmented and impermeable wall layers. Spores may be dispersed by air,

water, and animals, and following germination can colonize fresh and newly formed roots of the host plants (Janos, 1980).

3.3.3 Roles of Endomycorrhizae in Promoting Plant Growth

It is estimated that over 90% of terrestrial plants harbor AM. AM are characterized by arbuscule formation; these highly conserved structures develop within the host cells without penetrating the host protoplasm. Among the biological methods to enhance plant growth in saline conditions, the function of arbuscular mycorrhizal (AM) fungi is well recognized. Crops and most native plants of semi-arid and arid areas have associations with mycorrhizae, and it has been indicated that AM fungal colonization might enhance the salt tolerance of several plants (Al-Karaki, 2000). Utilizing AM fungi as a resource to resist soil salinity has practical benefits and is considered an economical method.

The function of mycorrhizal fungi in terrestrial ecosystems is primarily in the acquisition and transportation of inorganic phosphate (P) and other nutrients from the substrate to the plants' roots, enhancing plant physiology and biomass production (Smith and Read, 2008). The benefits of harboring AM fungi, however, extend beyond nutrient uptake. AM fungi increase drought resistance (Auge, 2001), delay wilting, and elevate stomatal conductance through the uptake and transport of water to their host (Zhu et al., 2010). Increased stomatal conductance improves gas exchange via stomata, contributing

to enhanced photosynthesis in mycorrhizal plants (Dunham et al., 2003; Sheng et al., 2008).

AM also help to reduce the effects of plant pathogens and nematodes, possibly by the release of mycorrhizal metabolites that reduce nematode attraction or by the increase in cell wall thickness in the tissues at the site of infection, increasing physical barriers against pathogen invasion (Rodriguez et al., 2004; De La Pena et al., 2006) and inducing systemic resistance as in the tomato plant (Vos et al., 2012). AM have also been shown to protect from salt stress (Evelin et al., 2009) by reducing intake of Na^+ ions, inducing the expression of aquaporin (a specific protein on the plasma membrane regulating the flow of water) genes to maintain a favorable osmotic gradient, detoxifying reactive oxygen species developing from salt stress, and increasing hydraulic conductivity (Giri et al., 2007).

Similarly, increased root, shoot, and total biomass of plants colonized by DSE results from increased nutrient acquisition (Newsham, 1999; Newsham, 2010). For example, a meta-analysis of plant responses to DSE by Newsham (2010) found 19 plant species from eight families to increase average shoot P and N content by 26 and 103%, respectively, thereby increasing total, root, and shoot biomass by 138, 79, and 109% respectively, without additional inorganic nitrogen supply. In addition, DSE have been shown to reduce pathogen infection by consuming organic carbon sources that would otherwise be available as a pathogen substrate (Mandyam and Jumpponen, 2005), increasing the physical barrier to pathogens by wall thickening of exodermal cells

adjacent to hyphae in asparagus (Yu et al., 2001), and production of toxic compounds, periconisins (antibacterial) (Kim et al., 2004). Given these varied roles and contributions, it can be surmised that if DSE colonization is affected by adverse environmental factors, impacts on plant performance, plant communities, and ecosystem services may ensue.

3.3.4 Ectomycorrhizae

An ectomycorrhiza is a form of symbiosis that occurs between a fungal symbiont, or mycobiont, and the roots of plants. Ectomycorrhizae are found on the roots of around 2% of plant species, especially woody plants, including species from the birch, myrtle, dipterocarp, beech, willow, pine, and rose families. (Smith and Read., 2010; Tedersoo et al., 2010). Research focused on ectomycorrhizae are increasingly gaining importance in ecosystem management and restoration, forestry, and agriculture.

Ectomycorrhizal fungi (ECM) are widespread in their distribution but are associated with only 3% of vascular plant families (Smith and Read, 1997). These fungi belong to the phyla Ascomycota and Basidiomycota, and the ECM mutualism is thought to have been derived multiple times independently from saprophytic lineages (Hibbett et al., 2000).

Although ectomycorrhizae are not as common as AM fungi species with plant root systems, they colonize woody plants in many ecosystems (Bainard *et al.*, 2011). Ectomycorrhiza fungi positively influence host plant nutrient uptake and participate in important ecosystem processes, including carbon sequestration, nutrient cycling, and

organic matter decomposition. Most ectomycorrhizal (ECM) plants rely entirely on mycorrhizal symbionts and cannot sustain themselves in the long term without these root associations (Vlk *et al*., 2020).

Ectomycorrhizae are essential for the survival of EMF trees and successful reforestation in saline areas (Turjaman et al., 2005). In early studies, EMF plants have shown reduced Na and Cl uptake into roots compared to non-inoculated plants (Muhsin and Zwiazek, 2002; Nguyen et al., 2006), and some researchers suggested that improved edaphic stress tolerance following EMF colonization might have resulted from increased P uptake by EMF plants in P-deficient soils, leading to an increased vitality of plants (Jentschke et al., 2001)

3.3.5 Effects of Mycorrhizae Inoculation

Inoculation of mycorrhizae is a soil amelioration practice that promotes the return of indigenous mycorrhiza to soils and is especially helpful to improve shallow or nutrient-depleted soils (Newsham, 2010) A study by Al-Khaliel (2010) examined the effect of salinity stress on growth response and mycorrhizal association of peanut infected by *Glomus mosseae*. Plants with and without mycorrhizae were irrigated with one of five salinity levels (0, 0.1, 0.2, 0.3, 0.4, or 0.5M). Each treatment contained ten (10) plants grown for ten weeks. This study concluded that the salinity-stressed mycorrhizal peanuts had significantly greater fresh weight and dry biomass when compared to salinity-stressed non-mycorrhizal peanuts at the same salinization level. In

addition, the mean dry weight of salinity-stressed mycorrhizal peanuts grown at the highest salinization level (0.5M) was 87% greater than salinity-stressed non-mycorrhizal peanuts grown at the same sodium chloride (NaCl) level. Likewise, results suggested that salinity-stressed mycorrhizal peanuts had total chlorophyll and leaf nutrient concentrations higher than salinity-stressed non-mycorrhizal peanuts.

Further research has been carried out on mycorrhizal inoculation to ameliorate the effects of plants on saline soils. Ciftci *et al.* (2010) examined the effects of different arbuscular mycorrhizal fungi (AMF) species on soybean cultivars grown in high saline conditions found at four different locations in Turkey. The cultivars were treated with one of three different AMF species: *Glomusintraradices* (Gi), *Glomusmosseae* (Gm) or *Glomus fasciculatum* (Gf).

Their experiment used a 4 x 4 factorial design (four soybean genotypes, three AMF, and one control) with four arbitrary replications of ten pots each for a total of 640 pots. After six weeks of planting, the plants were harvested, and plant growth parameters such as fresh shoot weight, dry plant biomass, and nutrient content were measured. They found significant differences between the control plants and those treated with the AMF species and among soybean cultivars. In addition, plants inoculated with AMF demonstrated higher mycorrhizal root colonization than control plants, with the highest colonization by *Glomus mosseae* at 33%. Findings from this study suggested that AMF had significant positive effects on soybean cultivars, as there was a considerable

difference in nutrient content between control and inoculated plants. Thus, results suggested that AMF species helped the plants absorb nutrients.

Zhu *et al*. (2016) at the University of Copenhagen (Taastrup, Denmark) evaluated the role of arbuscular mycorrhizae in alleviating salinity stress in wheat (*Triticum aestivum* L.) grown under ambient and elevated carbon dioxide (CO_2). Earlier studies showed that a high amount of CO_2 can reduce salinity stress in plants. Salt stress inhibits plant uptake of CO_2 for photosynthesis and affects water relations and nutrient transport from the soil. During the experiment, there were two groups of wheat plants; no mycorrhizae were added to the plants in soil group one, whereas in the second group, the plants' soil was inoculated with *Rhizophagus irregularis*. Both groups were treated with three different salinity levels (S0, S1, S2), respectively, 0, 9.5, 19 dS m^{-1}, and an elevated CO_2 level.

Findings from this study suggested that NaCl stress and CO_2 elevation had significant effects. For example, arbuscular mycorrhizal root colonization decreased with NaCl stress, while CO_2 elevation increased AM fungi colonization.

In addition, a significant interactive effect between NaCl and CO_2 treatments on root colonization was discovered, as NaCl decreased root colonization to a greater degree at ambient CO_2 levels. They concluded that salt stress might reduce AM fungi's capacity to colonize by negatively impacting spore germination, growth, and spread of hyphae, thereby reducing the percentage of root colonization. Likewise, elevated CO_2 increased

AM colonization under NaCl stress, which may be due to increased root biomass resulting from higher rates of C assimilation in plants grown under elevated CO_2.

Polanco *et al.* (2008) examined the response of ectomycorrhizal American elm (*Ulmus americana*) seedlings to salinity and soil compaction. American elm root systems harbor mycorrhizal populations, including ectomycorrhizal fungi (ECM) such as *Hebeloma crustuliniforme* and *Laccaria bicolor*. It is well known that ECM improves water and nutrient intake into the plant and decreases salt intake from saline soils. In this study, the authors examined the effects of soil compaction on the salt tolerance of American elm (*Ulmus americana*) seedlings that were inoculated with the ECM fungi *Hebeloma crustuliniforme* and *Laccaria bicolor*.

The experiment was a 4×2×2 complete randomized factorial design with three fungal treatments (*H. crustuliniforme, L. bicolor,* and *H. crustuliniforme + L. bicolor* groups) and a control non-inoculated treatment, two soil compaction levels (compacted vs. non-compacted) and two salt levels (60 mM NaCl and 0 mM NaCl). This study revealed that all three fungal inoculations had a similar effect on American elm seedlings subjected to soil compaction and salt treatment. ECM fungi decreased plant dry weight and root hydraulic conduction in non-compacted soil. However, inoculants did not affect net photosynthesis levels. The study concluded that ECM seedlings exposed to 60 mM NaCl had comparable Na concentrations in leaves over the three-week treatment to non-ECM inoculated plants.

Wang *et al.* (2011) also tested the influence of ectomycorrhizal fungi on the absorption and balance of essential elements in *Pinus tabulaeformis* seedlings grown in saline soil. Soil salinity is one of the severe factors restricting the capacity to support the growth of plants in silviculture, horticulture, and agriculture. Ectomycorrhizae are essential for the success of reforestation in locations that are saline.

A study by Wang *et al.* (2011) tested the effects of three ectomycorrhizal species (*Boletus edulis, Xerocomus chrysenteron*, and *Gomphidius viscidus)* on phosphorus (P) uptake and sodium (Na) absorption in saline soils. *Pinus tabulaeformis* seedlings were grown in a factorial experiment to test biomass accumulation and plant concentrations of P, Na, and K. The seedlings were inoculated with three different ectomycorrhizal species (*Boletus edulis, Xerocomus chrysenteron*, and *Gomphidius viscidus*) and subjected to two P treatments (with extra P treatment and without extra P) under saline conditions. Since soils with high salt content were used for the experiment, extra salt was not added to the soil. 200 mL of P solution was applied every six days for additional P treatment, and distilled water was used on control plants.

All treatments were replicated four times for four weeks. The adaptation of *Pinus tabulaeformis* seedlings to salinity stress was significantly improved by the three EMF species. P nutrition had a significant role as EMF species helped plants to grow under saline conditions. However, additional P nutrient treatments did not affect biomass. Also, high levels of K uptake raised the ratio of K/Na, which may balance ion concentrations and osmotic pressure to improve salinity resistance.

A study by Bandou *et al.* (2006) examined whether the ectomycorrhizal fungus *Scleroderma bermudense* alleviates salt stress in seagrape *(Coccoloba uvifera L.)* seedlings. *Coccoloba uvifera* (*L.*) is an important food source and ornamental planting. This study tested how an ectomycorrhizal fungus (*Scleroderma bermudense*), naturally associated with seagrape, responds to saline stress under greenhouse conditions. Four levels of salt solution (0, 200, 350, and 500 mM) were applied to plants in pots. In order to prevent the plant from osmotic shock, the soil was salinized every three days for four weeks of treatment by increasing the saline concentration.

This study found that salt stress tolerance in seagrapes was significantly increased by *Scleroderma bermudense*. In addition, higher K concentration in the ECM plant leaves may make ECM plants more resistant to osmotic stress caused by salt exposure. The positive effect of *S. bermudense* on seagrape seedlings occurred under NaCl stress and stress-free conditions, showing that ECM's role in improving plant growth is not limited to saline stressed environments.

3.3.6 Effects of Mycorrhizae on Plant Tissue Nutrient Composition

The colonization of roots with mycorrhizal fungi may influence host plants growth and health status through better nutrient uptake, better resistance to drought and heavy metals, and higher resistance to pathogens. In addition, mycorrhizal symbiosis changes the number and spatial arrangement of microorganisms in the rhizosphere (Bennewitz 2007; Castellanos-Morales et al., 2010).

A major physiological parameter for determining plant photosynthetic capacity is chlorophyll concentration in the leaf. Chaiya et al. (2021) determined that increased salt concentrations significantly reduced leaf chlorophyll concentrations, which was likely brought about by the suppression of some enzymes that are part of the photosynthetic pathway, and also caused a reduction in the absorption of nutrients (e.g., magnesium (Mg) and nitrogen (N)) needed for the production of chlorophyll. On the other hand, Chaiya et al. (2021) also observed that mycorrhizae significantly increased leaf chlorophyll concentration. It was suggested that this was likely because of improved nutrient uptake and reduced-sodium concentrations in the plants, resulting in overall higher photosynthetic capability.

Water and salt stress can cause a buildup of amino acids. The extrinsic use of proline could play a major role in amplifying the tolerance of the plant to stress. In saline soils, some plants have increased a proline concentration, which acts as a protective osmolyte, helps to sustain an osmotic balance, stabilizes proteins and membranes, protects plants against free radical-induced damage, and maintains appropriate NADP+/ NADPH ratios. Aliasgharzadeh et al. (2001), carried out a study that showed that the proline concentration in the leaves was affected by arbuscular mycorrhizae.

The use of mycorrhiza is an alternative way to reduce the stress caused by salt uptake without causing an increase in the production of proline. Many authors have reported that proline concentrations increased in AMF plants compared to non-AMF plants, while other authors have reported greater proline accumulation in non-AMF plants

than AMF plants, for example, in *Ocimum basilicum* L. and *Arachis hypogaea* L. (Chaiya et al. 2021).

3.4 Activities of Organisms in the Soil with Mycorrhizal Inoculation

One of the critical components in unified plant protection is mycorrhizal fungi. Mycorrhizal fungi (MF) control the soil activity and the evolution of microorganisms present in the soil, most especially with regards to the biodiversity of organisms in the rhizosphere. They also, with the aid of saprotrophs, protect plants against soil pathogens (Jamiokowska et al., 2017). Colonization of roots by endomycorrhizal fungi causes changes in the quantity and quality of exudates manufactured by the roots, the distribution of carbon in different parts of the plant, and the rate of nutrient absorption by the plants (Jakobsen et al., 2003)

Microbiological elements of the soil control several functions, such as chemical reactions, humus formation, nutrient cycles, xenobiotic degradation, and soil structure development (Nannipieri et al., 2003). Different soil microorganisms are bio-control factors for phytopathogens and frequently show synergism of the protective effect on plants with mycorrhiza. Mycorrhizal fungi affect soil structure and activity by altering the constituents and volume of root exudates produced. In addition, enzyme activity in the soil has been shown to have a visible effect on the number of nutrients accessible to the organisms in the rhizosphere (He et al., 2010).

Researchers have invariably linked the physical quality and enzymatic activity of mycorrhizal fertilized soils to the influence of agrotechnical factors such as type and level of fertilization, cultivation system, and soil and climate conditions. The co-dependence of higher plants and rhizospheric microorganisms to a large extent involves the exchange of chemicals (Lamb and Dixon, 1999).

Mycorrhizal inoculation of soil causes the enzymatic activity in the rhizosphere to be notably higher than outside of the non-rhizosphere zone. Mycorrhization has a remarkable impact on catalase activity, and as a result, plants existing in a symbiotic relationship with mycorrhiza produce a larger quantity of enzymes (Skwaryło-Bednarz and Krzepilko, 2009).

A study by Frac et al. (2018) showed that stress brought on by drought caused a significant rise in catalase activity, and the use of mycorrhizal fungi is an element that can significantly lower stress that may be associated with drought. It implies that mycorrhizal fungi possess advantageous properties that affect the absorption of water and essential nutrients, improving the plant's ability structure and reducing oxidative stress (Mayer**,** 2019).

Enzyme activity evaluation during different stages of plant development is an indicator of physiological activity (stress) in the plant. Root fluids (efflux) and plant residues are the primary carbon sources for heterotrophic microorganisms. Additionally, enzyme activities determine the rhizosphere function and activate rhizosphere microorganisms and their metabolites (Barea *et al.*, 2005; Nihorimbere *et al.*, 2011).

Furthermore, mycorrhizae control qualitative and quantitative change in root exudates in the rhizosphere zone. On the other hand, mycorrhizal inoculation reduced the number of fungi colonizing the roots of pepper plants. The use of mycorrhiza positively influences the growth and activity of soil microorganisms, which is achieved by changing the constituents of the root exudates (Bücking *et al.*, 2008).

A relationship between mycorrhiza and the plant can also be aided by metabolites produced by organisms in the rhizosphere, particularly with the help of certain saprotrophic bacteria and fungi (Mycorrhizal helper bacteria) (Joseph and Sivaprasad, 2000).

3.5 *Taxodium distichum*: A Moderately Salt-Tolerant Species

A promising tree species that could replace salt intolerant species such as live oak is bald cypress (*Taxodium distichum*). Bald cypress is a dominant overstory tree species with moderate to high levels of salt tolerance and is primarily found in the southern parts of the U. S (Lauer, 2013). *Taxodium distichum* is a conifer indigenous to different Midwestern and eastern United States regions. It is found mostly in swampy southern coastal areas where it produces large colonies. It is also indigenous in the southern tip of Illinois, where it grows along streams. In cultivation, bald cypress grows well in wet and slightly damp soil. In high pH soils, this tree may become chlorotic.

A significant problem experienced by trees in the coastal ecosystem is salinity. The trees are vulnerable to rising sea levels and increased storm intensity and frequency

due to climate change, potentially altering inland water chemistry (Allen et al., 1997). For example, the incursion of saltwater threatens plant species present in freshwater and brackish wetlands because of the sensitivity of these species to salinity change (Zhou et al., 2010).

Bald cypress is considered a dominant tree species found along the southeastern coast and is a primary, foundational species in freshwater forest wetlands of this region (Allen et al., 1996). Some specific adverse physiological effects of salinity exposure in bald cypress, even for relatively brief exposures, have included metabolic changes associated with water stress (Munns 2002). However, seedlings of bald cypress have shown a physiological tolerance to saltwater stress, with relatively slight declines in growth, photosynthetic capacity, and stomatal conductance when exposed to increased salinity (Allen et al. 1996).

Zhou et al. (2010) observed that a bald cypress x Montezuma cypress hybrid, similar to the one used in this study, showed greater salt tolerance when compared to bald cypress tree alone. Furthermore, Montezuma cypress was observed to be generally more salt-tolerant but cannot withstand prolonged periods of water inundation. However, Bald cypress is better suited to surviving prolonged inundation, so the hybrid combination of bald cypress and Montezuma cypress yields a more salt-tolerant tree that can still withstand prolonged inundation.

The specific bald cypress utilized in this study was a hybrid bald (Taxodium distichum var distichum X Taxodium distichum var mexicanum or Taxodium X' T405',

'T407' and 'T502'), which is a hybrid cross of bald cypress with Montezuma cypress, a species native to Mexico.

Hybrid cypress was used in the study because it has shown greater tolerance to salt-affected soils than native Taxodium distichum and has the potential to serve as an ornamental tree on Galveston Island. However, the degree of Na^+ tolerance of bald cypress is still a debate among scientists. Allen et al. (1996) reported bald cypress as being moderately salt-tolerant while Conner and Inabinette (2005) had a different opinion, reporting bald cypress as sensitive to Na^+, but they did recognize, however, that bald cypress with greater Na^+, tolerances do occur in regions such as Louisiana. The tolerance of bald cypress to Na^+ varies among individual populations depending on environmental adaptations to Na^+ exposure.

4. MATERIALS AND METHODS

4.1 Description of Study Site

This study was located on Galveston Island, Texas, on the Moody Gardens property adjacent to Scholes International Airport (Appendix A). The approximate coordinate of the location is 29.275462 N, -94.858925 W. The study site has an annual average temperature ranging from 18 ºC to 25 ºC and average annual precipitation of approximately 130 cm (NOAA, 2021).

According to Soil Survey Staff (2021), the soils of the study site are composed of Mustang fine sand (Typic Psammaquents) and Madre fine sand (Sodic Psammaquents). Mustang fine sands have a characteristic of zero to one percent slope, a fine sand texture, occurrences of frequent flooding and ponding, and a classification salinity of non-saline to very slightly saline. The drainage class is poorly drained, the maximum sodium adsorption ratio (SAR) is 13, with a depth to the water table ranging from 0 to 15cm, and the Saturated Hydraulic Conductivity (Ksat) is 8.73 μm $sec^{-1.}$ The Madre soils are similar to the Mustang soils in both slope and frequency of ponding but are only occasionally flooded. The salinity varies from slightly saline to moderately saline, and the texture is fine sand. The soils are poorly drained, the maximum SAR is 40, the depth to the water table ranges from 0 to 15cm, and the Saturated Hydraulic Conductivity (Ksat) is 17.980 μm sec^{-1}(Harris, 2019).

4.2 Preliminary Study

The Stephen F. Austin State University (SFASU) Biology and Environmental Science programs have examined the impact of commercially available mycorrhizal fungi inoculants on the growth of candidate plant (bald cypress) species since 2016. The Moody Gardens experimental site is a collaboration between researchers at SFASU and Moody Gardens. In 2016, three different genotypes of bald cypress were planted to observe the effect of mycorrhizal inoculation on the growth of these trees in saline-affected soils. Consequently, in 2017, this study was initiated to determine the effect of inoculation of three Cypress genotypes with a commercial endomycorrhizae/ectomycorrhizae inoculant on plant growth and development.

4.3 Experimental Design

The effect of a commercial mycorrhizal inoculant was evaluated on three unique genotypes of bald cypress by examining the trees growth and development under elevated saline conditions. These genotypes resulted from crosses between *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum.* Plants were treated with 1) no additives of fertilizer or mycorrhizae (CONT), 2) commercial mycorrhizae alone (MYCO) 3) fertilizer alone (FERT) 4) commercial mycorrhizae and fertilizer together (MYCO/FERT) (Table 1).

A total of sixty (60) trees were planted in plots of three rows with twenty trees belonging to each genotype. Each plot measured three by four meters (m). The plots for

each row were divided into five randomized replication blocks of four treatments each. Treatments for each block were randomly assigned to make a randomized block design as shown in Table 2, while Tables 3-5 show the distribution of treatments of each tree row (genotype 405, genotype 407, and genotype 502).

Table 1. Treatments applied to *Taxodium distichum* var d*istichum* and *Taxodium distichum* var *mexicanum* crosses.
Keys: (+) indicates application of treatment; (–) indicates no treatment was applied.

Treatment	Mycorrhizae	Fertilizer
1 - Control	–	–
2 - Mycorrhizae	+	–
3 - Fertilizer	–	+
4 - Mycorrhizae + Fertilizer	+	+

Table 2. Layout of treatments for each genotype at the study site located in the Moody Gardens Property in Galveston, Texas. Each column represents one genotype of *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* crosses. **Keys**: Blue: Mycorrhizae; Yellow: Fertilizer, Green: Fertilizer and Mycorrhizae combined, White: No treatment (Control).

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Tax 502		Tax 407		Tax 405
101	Replication 1	101	Replication 1	101
102		102		102
103		103		103
104		104		104
201	Replication 2	201	Replication 2	201
202		202		202
203		203		203
204		204		204
301	Replication 3	301	Replication 3	301
302		302		302
303		303		303
304		304		304
401	Replication 4	401	Replication 4	401
402		402		402
403		403		403
404		404		404
501	Replication 5	501	Replication 5	501
502		502		502
503		503		503
504		504		504

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Table 3. Genotype 405 treatment assignment. Five randomized replication blocks of four treatments were used. Treatments for each block were randomly assigned to make a randomized block design.

Genotype 405 Treatment Assignment					
Treatment Methods	**Replicant 1**	**Replicant 2**	**Replicant 3**	**Replicant 4**	**Replicant 5**
1-Control	102	202	303	404	504
2-Mycorrhizae	101	201	302	402	501
3-Fertilizer	103	204	304	403	503
4-Mycorrhizae + Fertilizer	104	203	301	401	502

Table 4. Genotype 407 treatment assignment. Five randomized replication blocks of four treatments were used. Treatments for each block were randomly assigned to make a randomized block design.

Genotype 407 Treatment Assignment					
Treatment Methods	**Replicant 1**	**Replicant 2**	**Replicant 3**	**Replicant 4**	**Replicant 5**
1-Control	101	201	301	404	502
2-Mycorrhizae	102	204	304	401	503
3-Fertilizer	103	203	302	403	504
4-Mycorrhizae + Fertilizer	104	202	303	402	501

Table 5. Genotype 502 treatment assignment. Five randomized replication blocks of four treatments were used. Treatments for each block were randomly assigned to make a randomized block design.

Genotype 502 Treatment Assignment					
Treatment Methods	**Replicant 1**	**Replicant 2**	**Replicant 3**	**Replicant 4**	**Replicant 5**
1-Control	103	202	301	403	501
2-Mycorrhizae	101	204	302	402	503
3-Fertilizer	102	201	303	401	502
4-Mycorrhizae + Fertilizer	104	203	304	404	504

4.4 Mycorrhizae Treatment Application

In this study, three genotypes of bald cypress were inoculated with a mycorrhizal consortium of species obtained commercially from Plant Success Organics (Table 6). The study started in January 2021 and extended until November 2021 (an equivalence of two growing seasons). At the beginning of this study, three equidistant points were marked around the tree trunks; each was excavated with a 50 cm long stainless-steel soil core sampler to a depth of 20 cm to form a well around the plants' root zones (Figure 1). Next, the mycorrhizal inoculum containing nine endomycorrhizae and nine ectomycorrhizae species was added to the holes. After inoculation, the removed soil was placed back into the holes. Each inoculated tree received 250 ml of the aqueous mycorrhizal solution, delivering 22,100,000 propagules of the mycorrhizae consortium.

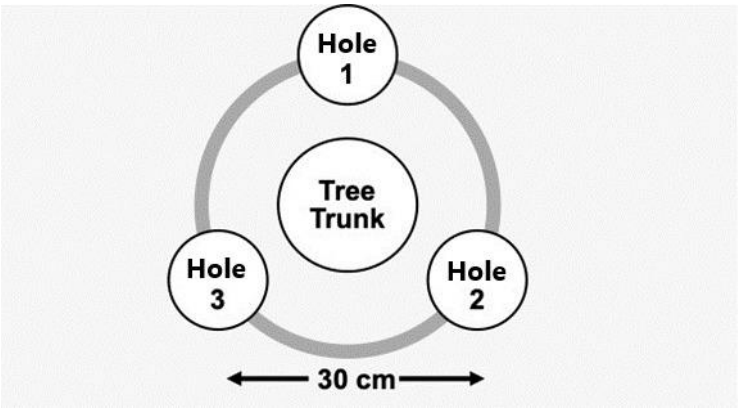


Figure 1. Design of mycorrhizae inoculation wells around the tree trunk. The space between each hole is 30 cm for each treatment tree at the study site located in the Moody Gardens Property in Galveston, Texas.

Table 6. Eighteen different mycorrhizae consortium species in commercial inoculant used in mycorrhizae treatment and a combination of mycorrhizae and fertilizer treatment.

Endomycorrhizae species	**Ectomycorrhizae species**
Glomus aggregatum	*Pisolithus tinctorius*
Glomus intraradices	*Rhizopogon luteolus*
Glomus mosseae	*Rhizopogon fulvigleba*
Glomus etunicatum	*Rhizopogon villosulus*
Glomus monosporum	*Rhizopogon amylopogon*
Glomus deserticola	*Scleroderma citrinum*
Glomus clarum	*Suillus granulatus*
Paraglomus brasilianum	*Laccaria laccata*
Gigaspora margarita	*Laccaria bicolor*

4.5 The Effect of Fertilizer Application and Mycorrhizae Inoculation on the Height and Diameter Growth of Bald Cypress Hybrids

The height and diameter at breast height (DBH) are essential parameters for this study as they served as a means to monitor the response of the plants to the applied treatments. Diameter at breast height (DBH) is considered a standard measurement system for measuring tree diameter. The diameter of the trees was measured in June during the growing season of 2021 using the method proposed by Huang et al. (2011). A stainless-steel digital caliper was used, and the DBH was measured by standing parallel to the tree at an average height of 150 cm. Also, tree heights were measured using standard calibrated measuring sticks. Fifty-four (54) trees were measured during the 2021 growing season to obtain the DBH and height for the study.

4.6 Bald Cypress Hybrid Leaf Nutrient and Sodium Concentration Analysis

Plant tissue analysis measures essential nutrient levels and identifies which elements are present in sufficient, insufficient, or excessive amounts. Leaf sampling offers a practical way of measuring the nutritional status of plants as nutrients taken up by the plant are incorporated into their tissue. On September 6, 2021, leaves were randomly selected and collected from four replicates of each treatment. The collected leaf samples were stored in labeled paper bags and left in a forced draft drying oven set at 60° C until the moisture was evaporated and a constant weight of the samples was maintained for consecutive days. After the completion of the drying process, the leaf samples were ground and submitted to the Soil, Plant, and Water Analysis Laboratory at SFASU to determine leaf nutrient concentration. The leaf tissue was analyzed for carbon (C), nitrogen (N), phosphorus (P), potassium (K), and sodium (Na) content.

4.7 Microbial Species Diversity with EcoPlate System

Microbial species diversity is an essential measure of soil health and microbial population dynamics. The Biolog EcoPlate is considered a new assay that accelerates the classification of environmental samples by employing biological activities (Gryta et al., 2014). EcoPlates contain 31 of the most common carbon sources utilized by microorganisms: amines, amides, carbohydrates, carboxylic acid, ketonic acids, amino acids, and polymers. The EcoPlates contain water as a control, and each carbon source is replicated three times on each plate. When microorganisms utilize the carbon sources, the tetrazolium reporter dye reacts to form a noticeable purple color (Sofo and Ricciuti, 2019). The 96-well plates were used to analyze collected soil samples from each of the three bald cypress tree genotypes treated with either the control, fertilizer only, mycorrhizae only, or mycorrhizae with fertilizer.

Soil samples were collected from each research plot at the study site on July 17, 2021. After removing the top layer of dry leaves, soil samples were collected from mineral soils using an auger to the depth of 20 cm. Collected soil samples were stored in a Ziplock bag and transported to SFASU where they were stored at 4°C until they could be analyzed. Each soil sample was passed through a of 2-millimeter mesh sieve to remove debris from the soil. The soil was serially diluted with a sterile phosphate buffer saline (PBS) solution to 10^{-3}. Once diluted, 100 µL of the PBS buffer/soil mix were

added to each well of a 96-well EcoPlate. The inoculated plates were then incubated at 24 °C for 48 hr.

Carbon utilization by microorganisms in the soil was determined by spectrophotometric analysis using an Ultraviolet Spectrometer. The microplate reader was set to read absorbance at a wavelength of 590 nm. A positive well for carbon utilization was indicated by absorbance of 0.51 or greater.

Functional diversity was calculated as follows:

$$\% \text{ Functional Diversity} = 100 * \frac{\text{Number of positive (purple/pink) carbon source wells}}{\text{Total number of carbon source wells (31)}}$$

Functional diversity ranges from 0 (low diversity) to 100% (high diversity).

4.8 Soil Respiration

Soil respiration is a measure of CO_2 evolution from soil. Soil carbon dioxide (CO_2) level is an important indicator of soil health as it reflects levels of plant litter, decomposition of soil organic matter as well as the soil microbial community's metabolic activity (EGM-5 PP Systems 2018). In this study, soil CO_2 levels were measured using a model EGM-5 portable CO_2 gas analyzer. The EGM-5 provides rapid and accurate measurements of CO_2 levels in the soil. For each plot, a sample area was randomly selected approximately 50 cm from each tree trunk. This area was cleaned of leaf litter that the soil surface was exposed. Before each measurement was made the respirometer

chamber was equilibrated with the surrounding atmosphere. The chamber and the unit were placed on top of the soil and recording mode was turned on. The total change in concentration of CO_2 in the air drawn into the sampler from the soil was measured for 60 seconds to determine the amount of CO_2 evolved.

4.9 Statistical Analysis

This study aimed to determine if there were significant differences between bald cypress hybrid stem height, stem diameter at breast height, and leaf nutrient levels due to mycorrhizal inoculation. Additionally, the study compared soil microbial quantity and CO_2 level with and without mycorrhizal treatment. The Shapiro–Wilk test was used to evaluate the normality of data by examining the correlation between the observed data collected and the corresponding normal scores (Ghasemi & Zahediasl, 2012). If the p-value reported by the Shapiro-Wilk test is higher than 0.05, then the data is normally distributed.

A one-way Analysis of Variance (ANOVA) test was used to statistically examine differences among treatments if the data followed a normal distribution with a significance level of 0.05. If the data did not follow a normal distribution, the Kruskal-Wallis test was used with a significance level of 0.05. Microsoft Excel was used to perform the statistical analyses.

5. RESULTS AND DISCUSSION

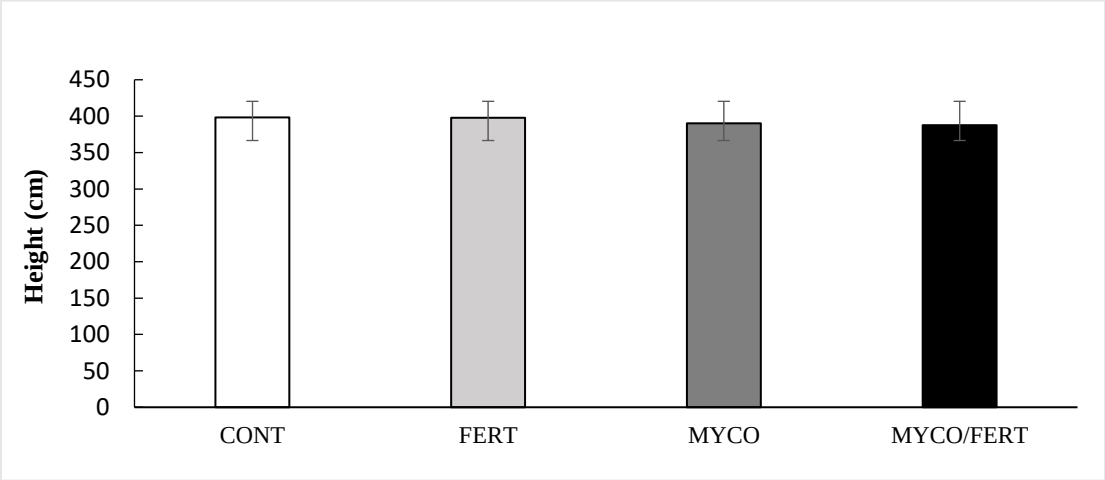
5.1 The Effect of Treatments on Tree Height and Diameter

5.1.1 Genotype 405

The control treatment had a mean tree height of 398.2 cm while trees treated with fertilizer alone (FERT) had a mean height of 397.7 cm. The trees treated with mycorrhizae (MYCO) showed a mean tree height of 390.1 cm, while a mean height of 387.6 cm was attained with the treatment involving the combination of mycorrhizae and fertilizer (MYCO/FERT) (Figure 2).

The untreated trees had a mean DBH of 82.8 mm. Treatment with fertilizer alone had a mean DBH of 94.6 mm, while mycorrhizae inoculation resulted in the mean DBH was observed of 87.3 mm. However, a combined treatment of mycorrhizae and fertilizer reached the mean DBH of 100.3 mm (Figure 2).

A



B

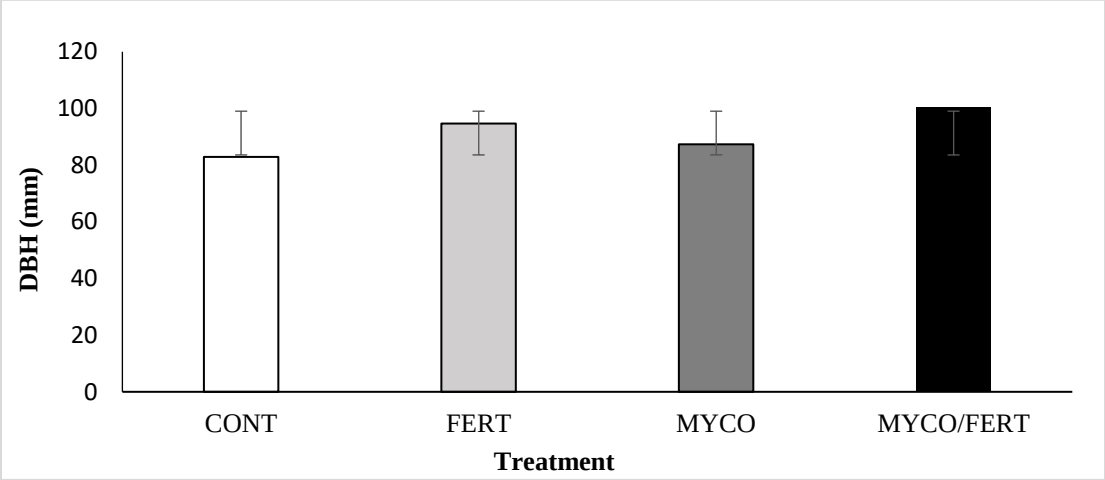


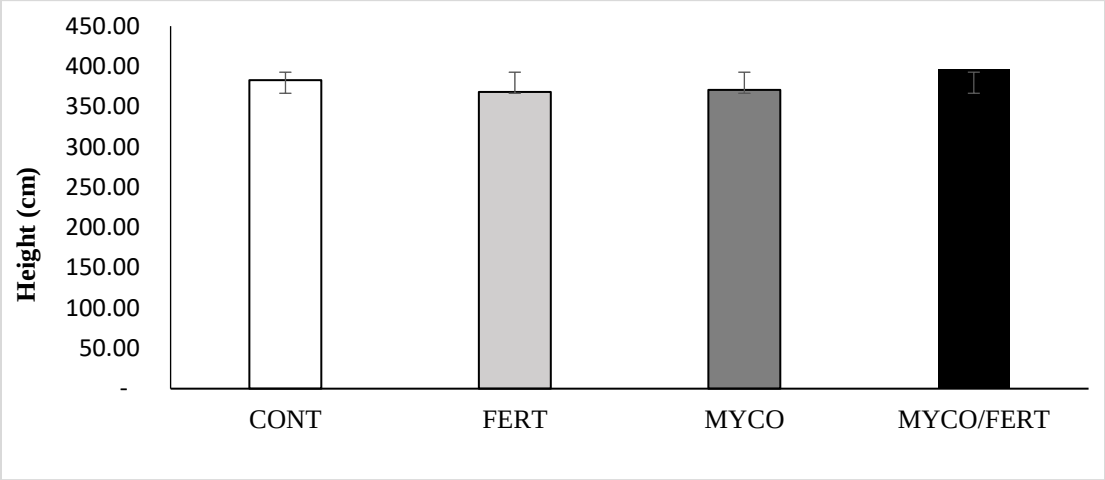
Figure 2. The effect of mycorrhizal inoculation and fertilization on tree height (A) and Diameter at Breast Height (DBH) (B), respectively, of *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* "Genotype 405" cross located in the Moody Gardens Property in Galveston, Texas.
Key: CONT: Control, FERT: Fertilizer treatment, MYCO: mycorrhizal inoculation, MYCO/FERT: mycorrhizal inoculation and fertilizer treatment.

5.1.2 Genotype 407

The control treatment had a mean tree height of 382.91 cm. Trees were treated with fertilizer alone had a mean height of 368.3 cm. Trees treated with mycorrhizae showed a mean tree height of 370.84 cm, while a mean height of 396.88 cm was attained with the treatment involving the combination of mycorrhizae and fertilizer (Figure 3).

The untreated trees had a mean DBH of 96.70 mm. Trees treated with fertilizer alone had a mean DBH of 76.49 mm, while mycorrhizae inoculation resulted in a mean DBH of 78.61 mm. However, a combined treatment of mycorrhizae and fertilizer reached the mean DBH of 111.04 mm (Figure 3).

A



B

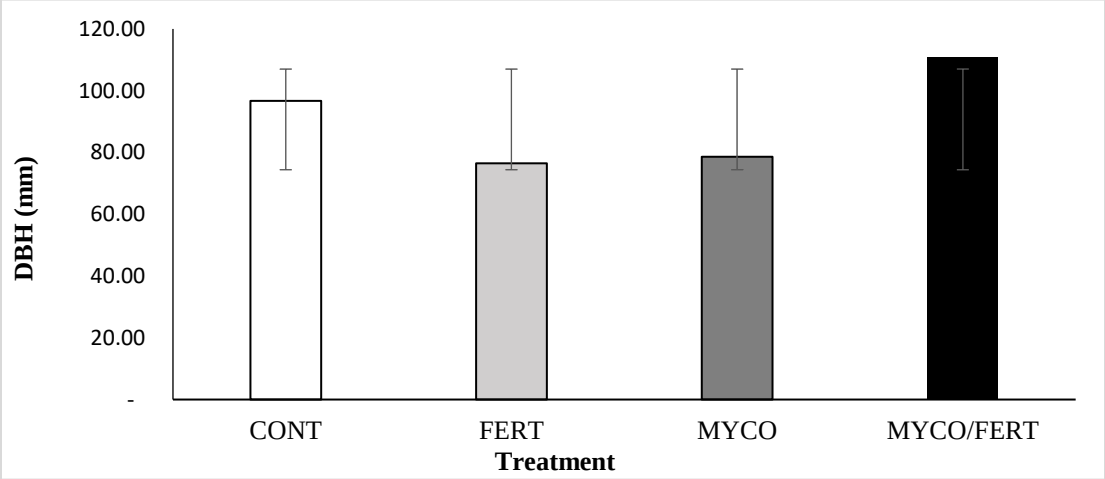


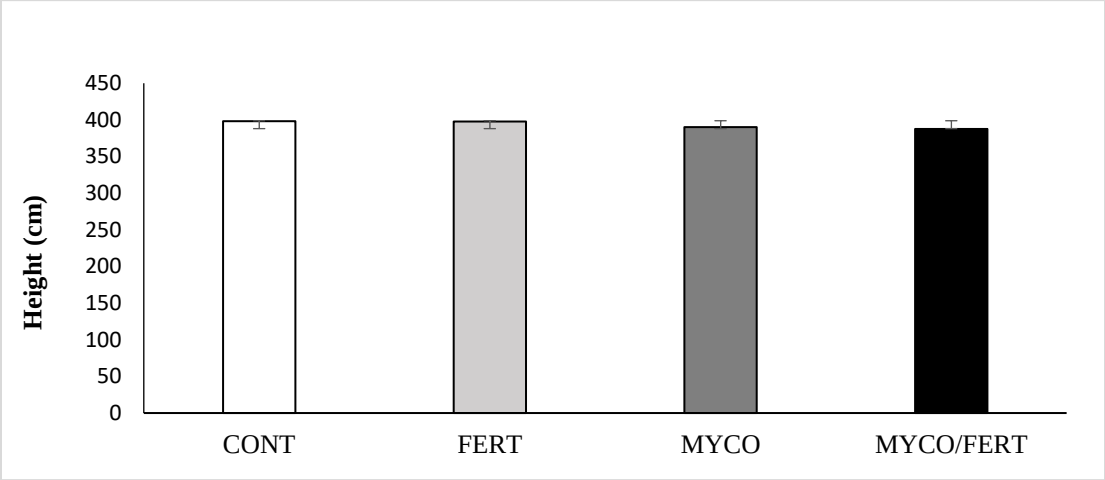
Figure 3. The effect of mycorrhizal inoculation and fertilization on tree height (A) and Diameter at Breast Height (DBH) (B), respectively, of *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* "Genotype 407" cross located in the Moody Gardens Property in Galveston, Texas.
Key: CONT: Control, FERT: Fertilizer treatment, MYCO: mycorrhizal inoculation, MYCO/FERT: mycorrhizal inoculation and fertilizer treatment.

5.1.3 Genotype 502

The control treatment had a mean tree height of 398.27 cm. Trees treated with fertilizer alone had a mean height of 397.76 cm. Trees treated with mycorrhizae showed a mean tree height of 390.15 cm, while a mean height of 387.61 cm was attained with the treatment involving the combination of mycorrhizae and fertilizer (Figure 4).

The untreated trees had a mean DBH of 90 mm. Trees treated with fertilizer alone had a mean DBH of 82.94 mm, while mycorrhizae inoculation resulted in a mean DBH of 88.75 mm. However, a combined treatment of mycorrhizae and fertilizer resulted in a mean DBH of 92.40 mm (Figure 4). Overall, for all three genotypes of *taxodium distichum* species, there was no significant difference across the treatments on DBH and height measurement.

A



B

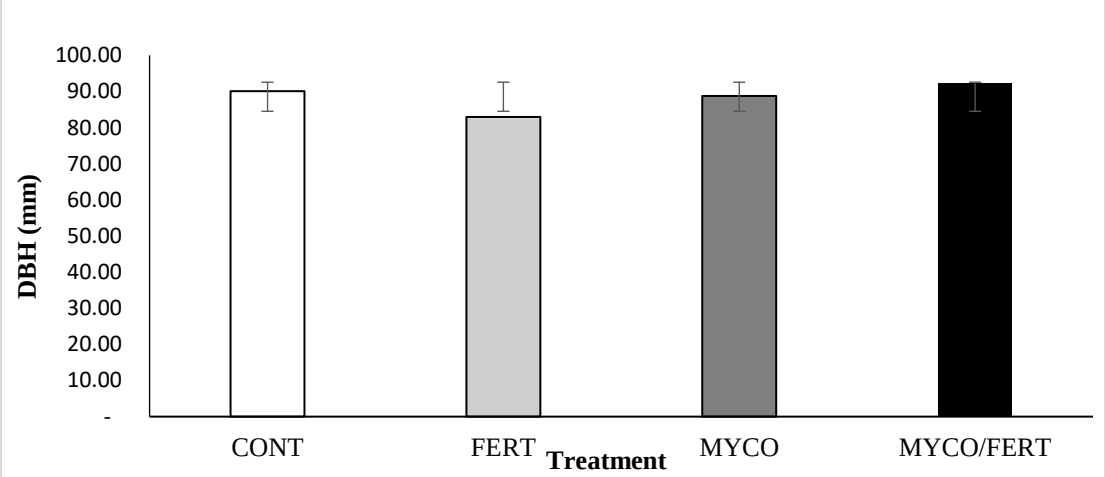


Figure 4. The effect of mycorrhizal inoculation and fertilization on tree height (A) Diameter at Breast Height (DBH) (B), respectively, of *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* "Genotype 502" cross located in the Moody Gardens Property in Galveston, Texas.
Key: CONT: Control, FERT: Fertilizer treatment, MYCO: mycorrhizal inoculation, MYCO/FERT: mycorrhizal inoculation and fertilizer treatment.

5.1.4 Statistical Analyses of the Effect of Treatment on Height and Diameter Breast Height (DBH) of Genotypes 405, 407, and 502

Tree heights of both genotype 405 and 502 species were not normally distributed. A Kruskal-Wallis test showed that the treatment means of tree height were not significantly different at a 0.05 level. For genotype 405, the Kruskal-Wallis summary statistic was $X^2 = 2.77$, $P = 0.43$ and for genotype 502 the summary statistic was $X^2 = 1.46$, $P = 0.69$.

Because tree height for genotype 407 as well as DBH measurements for genotypes 405, 407 and 502, were all normally distributed ANOVA tests were performed to determine the effect of treatment types on height and DBH. The ANOVA test showed that treatment means for these attributes were not significantly different at the 0.05 p-value (Table 7).

Table 7. Summary of ANOVA performed on the mean tree height and Diameter at Breast height (DBH) of *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* Crosses, Genotype 405; 407 and 502.
Keys: SS: sum of squares, df: degrees of freedom, MS: mean squares, F: F-statistic.

Source of Variation	*Genotype*	*Measurement*	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	405	DBH	715.23	3.00	238.41	1.20	0.35
Between Groups	407	Height	2,054.84	3.00	684.95	0.65	0.60
Between Groups	407	DBH	3,191.11	3.00	1,063.70	2.31	0.13
Between Groups	502	DBH	243.31	3.00	81.10	1.60	0.23

5.1.5 Discussion on Height and Diameter at Breast Height (DBH) of *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* Crosses Genotype 405, 407, and 502

This research did not support the hypothesis that mycorrhizal inoculation will improve plant growth and nutrient concentration and promote soil health. In contrast, these results suggest that application of mycorrhizal inoculum, fertilizer, or a combination of both on bald cypress trees did not affect plant growth, as measured by tree height and tree diameter at breast height.

One of the factors that may be responsible for no significant difference across the treatments may be due to the frequent fertilization with inorganic fertilizer which might have been washed or leached to other plots with other treatments by the action of rain. In the research carried out by Egerton-Warburton and Allen (2000); Egerton-Warburton *et al*. (2001); Adesemoye *et* al. (2008) in managed agricultural systems, it was observed that high levels of inorganic fertilizers (especially nitrogen and potassium) reduced mycorrhizal colonization of plant roots that would naturally form mycorrhizal associations when lower available nutrients are present. Consequently, the inability of plants to form mycorrhizal associations with AMF can lead to a decrease in plant canopy biomass and productivity (Koide, 1985).

Research by others has shown a symbiotic relationship between mycorrhizae and other varieties of plants. For example, it has been shown that colonization by

mycorrhizae has improved root lengths, surface area, and plant biomass for plant species such as *Trigonella foenum-graceum* and *Ehphedra aphylla* (Klinsukon et al., 2021; Kong et al., 2020; Shi-Chu et al., 2019). The results of this study, however, indicate that the bald cypress genotype tested did not significantly benefit from the application of commercial mycorrhizal inoculation. There can be several reasons for this observation, beginning with the potentially unexamined presence of native mycorrhizae in the soil where the experiments were carried out. Rittenberry et al., (2017), reported the presence of indigenous mycorrhizae in soils on Galveston Island. This could be the result of the need for plant survival, causing both plant and mycorrhizae to form an alliance in the form of symbiosis in order to withstand and survive the harsh environmental condition. On the other hand, any additional inoculation of the soil with mycorrhizae would therefore be redundant, generating no measurable difference in plant parameters.

One other potentially significant reason for the results of this study could be the commercial source of mycorrhizae. Hart et al. (2018), showed that growth and acclimatization of commercial mycorrhizae inoculants can be less vigorous than that of native species which can be due to the infrequent application or a mismatch with the soil environment. Furthermore, findings from this study may corroborate that of Chandrasekaran et al. (2014), who observed that not all AMF improve plant growth in saline soils equally well. As such, the commercial mycorrhizae used in this study may not be best suited to improve plant growth especially in salt affected soils.

Although consideration was made for the distance between the cypress trees, the one meter chosen may not have been enough to separate the trees and prevent them from influencing each other. It is not clear, however, that more distance between the trees would have improved the effects of the mycorrhizal inoculants, given the potential mismatch with the soil environment. In addition, short distances between the trees could cause treatments to migrate between control and treated trees in the field; rain or wind can also cause treatment spread between treated and control trees.

Previous studies have highlighted the lack of regulatory standards for commercially grown fungal inoculants, potentially impacting best manufacturing practices and the quality of the fungal inoculants (Hart et al., 2018). It is plausible to suggest, based on the findings of Hart *et al*. (2018) that sourcing of mycorrhizal inoculants is a crucial determinant of their applicability in improving the performance of plants. As a result, mitigating higher salinity levels in the soil for agricultural purposes might require a thorough examination of already native fungal flora. This would allow for a better selection of fungal species that would be more likely to ameliorate the soil conditions.

5.2 The Effect of Treatments on Leaf Nutrient Analysis

5.2.1 Genotype 405

The effect of each treatment on the leaf nutrient composition of genotype 405 was compared to that of the control (Table 8). The combined treatment of mycorrhizae and

fertilizer had the highest nitrogen concentration in the leaf (1350 mg/kg) while the control plant had the least nitrogen concentration (1210 mg/kg). The highest concentration of phosphorus was observed in the treatment with fertilizer (2268 mg/kg), while the least concentration was observed in the control (2082 mg/kg). Sodium concentration was highest in the treatment with mycorrhizae (3269 mg/kg) and least in the combined treatment of mycorrhizae and fertilizer (2165 mg/kg). For carbon, the highest concentration was observed in the control (4246 mg/kg) while the least was observed in the treatment with mycorrhizae (4117 mg/kg). Similarly, the concentration of potassium were found highest in the control (6190 mg/kg) and least in the treatment with mycorrhizae (5935 mg/kg). Overall, the highest concentration of nutrient was recorded for potassium with readings above 6000 mg/kg.

5.2.2 Genotype 407

The effect of each treatment on the leaf nutrient concentration was compared to that of the control for genotype 407 (Table 9). Treatment with mycorrhizae alone had the highest nitrogen concentration in the leaf (1263 mg/kg) while the control had the least nitrogen concentration (1171 mg/kg). The highest concentration of phosphorus was observed in the treatment with mycorrhizae alone (1860 mg/kg), while the least concentration was observed in the combined treatment of mycorrhizae and fertilizer (1688 mg/kg). Sodium concentration was highest in the control (4169 mg/kg) and least in the combined treatment of mycorrhizae and fertilizer (2735 mg/kg). For carbon, the

highest concentration was observed in the combined treatment of mycorrhizae and fertilizer (4294 mg/kg) while the least was observed in the control (4221 mg/kg). The concentration of potassium was found highest in the treatment with mycorrhizae alone (7500 mg/kg) and least in the combined treatment of mycorrhizae and fertilizer (5728 mg/kg). Overall, the highest concentration of nutrient was recorded for potassium, with readings up to 7500 mg/kg while the least was observed for nitrogen, with readings of 1200 mg/kg and lower.

5.2.3 Genotype 502

The effect of each treatment on the leaf nutrient composition of genotype 502 was compared to that of the control (Table 10). Treatment with mycorrhizae alone had the highest nitrogen concentration in the leaf (1331 mg/kg) while treatment with fertilizer alone had the least nitrogen concentration (1142 mg/kg). The highest concentration of phosphorus was observed in the control (2041 mg/kg), while the least concentration was observed in the treatment with fertilizer alone (1766 mg/kg). Sodium concentration was highest in the control (6911 mg/kg) while the least concentration was observed in the treatment with mycorrhizae alone (4588 mg/kg). For carbon, the highest concentration was observed in the treatment with mycorrhizae alone (4242 mg/kg) while the least was observed in the treatment with fertilizer (4104 mg/kg). Similarly, concentrations of potassium were found highest in the treatment with mycorrhizae alone (7332 mg/kg) and least in the treatment with fertilizer alone (5903 mg/kg). Overall, the highest

concentration of nutrient was recorded for potassium with readings above 7300 mg/kg, and the least was observed for nitrogen.

5.2.4 Statistical Analyses of the Effect of Treatment on Leaf Nutrient Concentration of Genotypes 405, 407, and 502

An ANOVA test showed that the treatments did not significantly affect the concentration of nutrients (N, P, K, Na, and C) in the leaves of genotype 405, genotype 407, and genotype 502 (Table 11, 12, 13) Similarly, no significant differences were observed on the effect of treatments on the concentration of nutrients in the leaves across the three taxodium genotypes when compared with the control treatment.

Table 8. Effect of treatments on leaf nutrient concentration (mg/kg) on *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* crosses "genotype 405" located in the Moody Gardens Property in Galveston, Texas.
Key: CONT: Control, FERT: Fertilizer treatment, MYCO: mycorrhizal inoculation, MYCO/FERT: mycorrhizal inoculation and fertilizer treatment.

Treatments	*Nitrogen (N)*	*Phosphorus (P)*	*Potassium (K)*	*Sodium (Na)*	*Carbon (C)*
CONT	1,210	2,082	6,190	2,338	4,246
FERT	1,338	2,268	6,178	3,201	4,210
MYCO	1,244	2,162	5,935	3,269	4,117
MYCO/FERT	1,350	2,205	6,158	2,165	4,184

Table 9. Effect of Treatments on leaf nutrient concentration (mg/kg) on *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* crosses "genotype 407**"**
Key: CONT: Control, FERT: Fertilizer treatment, MYCO: mycorrhizal inoculation, MYCO/FERT: mycorrhizal inoculation and fertilizer treatment.

Treatments	*Nitrogen (N)*	*Phosphorus (P)*	*Potassium (K)*	*Sodium (Na)*	*Carbon (C)*
CONT	1,171	1,753	6,143	4,169	4,221
FERT	1,210	1,816	6,335	3,571	4,258
MYCO	1,263	1,860	7,500	2,858	4,244
MYCO/FERT	1,195	1,688	5,728	2,735	4,294

Table 10. Effect of Treatments on leaf nutrient concentration (mg/kg) on *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* crosses "genotype 502" located in the Moody Gardens Property in Galveston, Texas.
Key: CONT: Control, FERT: Fertilizer treatment, MYCO: mycorrhizal inoculation, MYCO/FERT: mycorrhizal inoculation and fertilizer treatment.

Treatments	*Nitrogen (N)*	*Phosphorus (P)*	*Potassium (K)*	*Sodium (Na)*	*Carbon (C)*
CONT	1,291	2,041	6,164	6,911	4,190
FERT	1,142	1,766	5,903	5,932	4,104
MYCO	1,331	1,934	7,332	4,588	4,242
MYCO/FERT	1,258	2,017	6,388	6,367	4,166

Table 11. ANOVA for the mean Nutrient Analysis of *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* Crosses, genotype 405.
Keys: SS: sum of squares, df: degrees of freedom, MS: mean squares, F: F-statistic.

Source of Variation	*Genotype*	*Nutrient*	*SS*	*Df*	*MS*	*F*	*P-value*
Between Groups	405	Nitrogen	3,490,032.06	3	1,163,344.02	4.02	0.06
Between Groups	405	Phosphorus	435,690.15	3	145,230.05	2.49	0.13
Between Groups	405	Potassium	1,620,138.38	3	540,046.13	3.21	0.08
Between Groups	405	Sodium	2,953,405.58	3	984,468.53	1.08	0.41
Between Groups	405	Carbon	267,926,427.42	3	89,308,809.14	1.23	0.36

Table 12. ANOVA for the mean Nutrient Analysis of *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* Crosses, genotype 407.
Keys: SS: sum of squares, df: degrees of freedom, MS: mean squares, F: F-statistic

Source of Variation	*Genotype*	*Nutrient*	*SS*	*Df*	*MS*	*F*	*P-value*
Between Groups	407	Nitrogen	1,527,973.42	3	509,324.47	0.39	0.77
Between Groups	407	Phosphorus	726,489.34	3	242,163.11	7.88	0.06
Between Groups	407	Potassium	2,896,207.17	3	965,402.39	0.51	0.68
Between Groups	407	Sodium	3,708,570.92	3	1,236,190.31	0.75	0.55
Between Groups	407	Carbon	82,346,521.23	3	27,448,840.41	0.46	0.72

Table 13. ANOVA for the mean Nutrient Analysis of *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* Crosses, genotype 502
Keys: SS: sum of squares, df: degrees of freedom, MS: mean squares, F: F-statistic.

Source of Variation	*Genotype*	*Nutrient*	*SS*	*Df*	*MS*	*F*	*P-value*
Between Groups	502	Nitrogen	6,029,535.75	3	2,009,845.25	3.52	0.07
Between Groups	502	Phosphorus	103,446.81	3	34,482.27	0.98	0.45
Between Groups	502	Potassium	995,464.87	3	331,821.62	1.01	0.44
Between Groups	502	Sodium	8,856,696.92	3	2,952,232.31	1.75	0.23
Between Groups	502	Carbon	33,614,176.40	3	11,204,725.47	0.08	0.97

5.2.5 Discussion on Nutrient Analysis of *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* Crosses, Genotype 407

Previous studies have shown that in addition to carbon dioxide and water essential for primary production through photosynthesis, plants require several inorganic mineral nutrients that must be taken up from the soil solution into roots and that partition appropriately within the plant (Stein et al., 2017). ANOVA of the results obtained from measuring the concentration of nutrients in the leaves of *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* crosses, genotypes 405, 407 and 502, showed that there was no statistically significant difference across the various treatments.

Although there was no significant difference between the treatments and control, there were some apparent trends. A result that could be attributed to salinity of the soil, which causes the uptake of sodium and potassium ions that are usually present in large amounts in salinized soils. The result agrees with Zhou et al. (2010) who observed sodium concentration in the leaves of *Taxodium* sp. to increase with increasing salt concentration in the soil. Also, a report by Pezeshki et al. (1988) suggested significant increase in Na, K, Ca, and Mg concentration of leaf tissue in *Taxodium distichum* when subjected to saltwater flooding relative to freshwater flooding.

Furthermore, the high concentration of carbon in three *Taxodium* genotypes suggests the capacity of conversion of atmospheric carbon dioxide load to organic carbon stored in plants and its ability to reduce carbon load from the atmosphere. Similarly,

Rodriguez et al. (2015) reported a higher concentration of carbon as compared to nitrogen in the leaf tissue of different plant species in Northern Mexico.

The low concentration of phosphorus observed across the three taxa may be attributed to the salinity of the soil. Wang et al. (1993) suggested that nutrient elements such as low soluble phosphorous (P) are limiting due to the high pH of the soil, which limits the mobilization of nutrient elements in the soil solution.

5.3 Treatment Effect on Microbial Species Diversity with EcoPlate System

5.3.1 Genotype 405

The effect of the different treatments (mycorrhizae, fertilizer, and a combination of both mycorrhizae and fertilizer) on the diversity and community of microorganisms colonizing the soil-root interface was compared. Average functional diversity of the microbial community within the soil ranged from 0.44 for plots treated with mycorrhizae alone to 0.50 for plots treated with both mycorrhizae and fertilizer together (Figure 5). This suggests that there is a diverse population of microbes utilizing various carbon sources but that not all carbon sources were utilized. This also suggests that the combined treatment of mycorrhizae and fertilizer did influence functional diversity positively.

5.3.2 Genotype 407

Average functional diversity of the microbial community within the soil ranged from 0.48 for plots with a combined treatment of both mycorrhizae and fertilizer to 0.54 for plots with no treatment (control) as shown in Figure 6. This suggests that there is a diverse population of microbes utilizing various carbon sources but that not all carbon sources are utilized, and that treatment did not have significant effect on the soil microbial community of genotype 407.

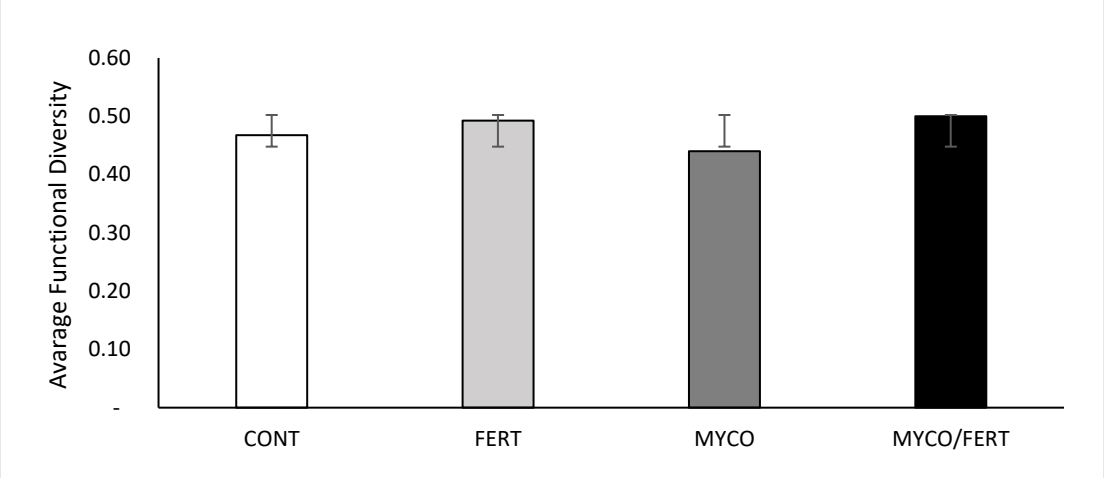


Figure 5. Effect of Treatments on the Functional Diversity of Microbial Community in Salt-affected Soil of *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* crosses "genotype 405" located in the Moody Gardens Property in Galveston, Texas.
Key: CONT: Control, FERT: Fertilizer treatment, MYCO: mycorrhizal inoculation, MYCO/FERT: mycorrhizal inoculation and fertilizer treatment.

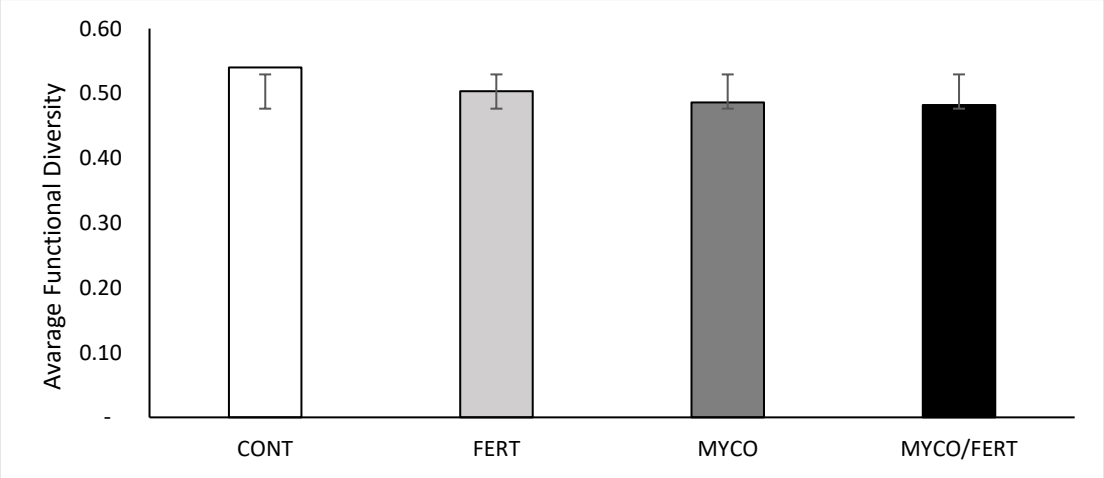


Figure 6. Effect of Treatments on the Functional Diversity of Microbial Community in Salt-affected Soil of *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* crosses "genotype 407" located in the Moody Gardens Property in Galveston, Texas.
Key: CONT: Control, FERT: Fertilizer treatment, MYCO: mycorrhizal inoculation, MYCO/FERT: mycorrhizal inoculation and fertilizer treatment.

5.3.3 Genotype 502

Average functional diversity of the microbial community ranged from 0.49 for plots treated with mycorrhizae to 0.52 for plot with no treatment (control) as shown in Figure 7.

This suggest that there is a diverse population of microbes utilizing various carbon sources but that not all carbon sources were utilized, and that treatment had no significant effect on the soil microbial community of genotype 502.

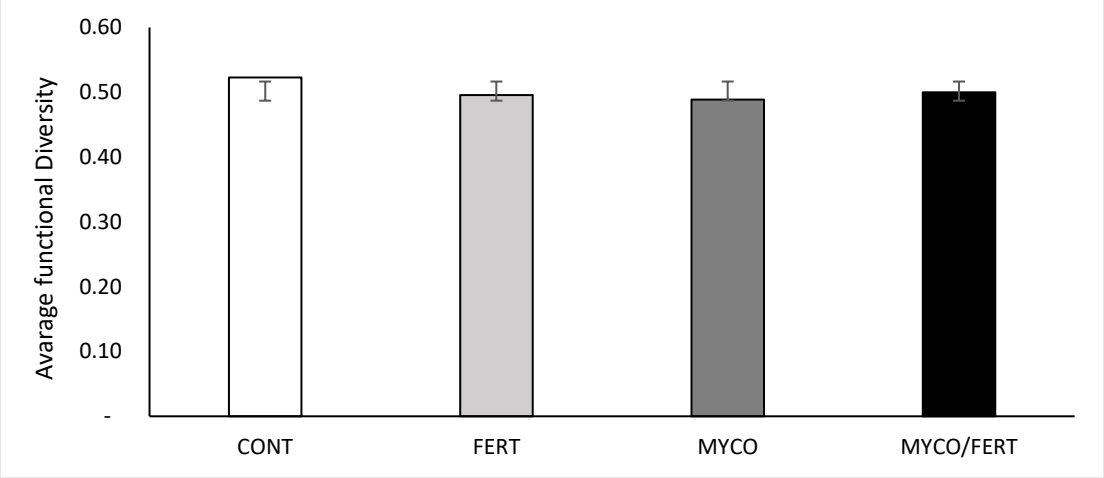


Figure 7. Effect of Treatments on the Functional Diversity of Microbial Community in Salt-affected Soil of *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* crosses "genotype 502" located in the Moody Gardens Property in Galveston, Texas.
Key: CONT: Control, FERT: Fertilizer treatment, MYCO: mycorrhizal inoculation, MYCO/FERT: mycorrhizal inoculation and fertilizer treatment.

5.3.4 Statistical Analyses of the Effect of Treatment on Microbial Community of *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* Crosses

An ANOVA analysis in each of the treatments suggested that there was no significant difference between the functional diversity observed for each treatment applied to genotype 405 P-value = 0.57, Genotype 407 P-value = 0.7 genotype 502 P-value = 0.75.

Table 14. ANOVA for the Functional Diversity of Microbial Community of *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* Crosses, genotype 405; 407 and 502.
Keys: SS: sum of squares, df: degrees of freedom, MS: mean squares, F: F-statistic.

Source of Variation	*Genotype*	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	405	0.01	3.00	0.00	0.71	0.57
Between Groups	407	0.01	3.00	0.00	0.48	0.70
Between Groups	502	0.00	3.00	0.00	0.40	0.75

5.3.5 Discussion on the Effect of Treatment on Microbial Community of *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* Crosses Genotype 405, 407, and 502

My findings did not find support for treatments increasing functional microbial community. In contrast, these results suggest that that mycorrhizal inoculation, fertilizer application and a combination of both treatments on bald cypress trees did not affect functional diversity of the microbial community as measured by microbial EcoPlate analysis.

Previous studies have shown that repeated fertilization may result in shifts in the functionality and quality of soils by directly or indirectly altering the soil's chemical, physical, and biological properties, leading to changes in fertility and the level of nutrients available (Allison & Martiny 2008; Luo et al., 2015). The results of this study align with those from Luo et al. (2015) whom also found no significant difference in the functional diversity of the microbial community after fertilizer application in their study. However, functional diversity of on the control were observed to be higher for both genotype 407 and 502 when compared with fertilizer suggesting that treatment with fertilizer could have impacted the microbial community. Cinnadurai et al. (2013) reported that long-term fertilizer experiments affect the function, community structure, and population of soil microorganisms. This agrees with the result of this study, in which fertilizer had been constantly applied even prior to the commencement of this research.

Similarly, a possible reason for no significant difference between the control and treatment (fertilizer alone) could be the choice of fertilizer.

In a study carried out in China by Luo et al. (2015) on the influence of long-term fertilization on soil microbial biomass, dehydrogenase activity, and bacterial and fungal community structure it was revealed that organic fertilizer treatments, especially organic manure treatments, significantly increased bacterial and fungal diversity when compared with inorganic fertilizer. As such, the application of organic manure in the place of inorganic fertilizer may have brought about an increase in the functional diversity of the microbial population at the soil-root interphase.

Bacterial community composition appears to be influenced and governed by saline environmental factors (Hollister et al. (2010, Zhao et al. 2018). While we did not measure correlation between soil salinity and functional soil diversity, it is evident that the soils in the study site have high concentrations of saline and this could have affected the diversity of soil microorganisms, and as such the addition of treatments may not significantly alter the diversity of soil microorganism.

Furthermore, across the three *Taxodium* genotypes (405, 407, and 502), the treatments did not show significant difference between the control and treated soil. This may be due to the activities (antagonistic) of the indigenous or autochthonous mycorrhizae present in the soil before the introduction of foreign mycorrhizae. The inoculation of these new species may have caused an antagonistic response between the

indigenous microbiota and the foreign mycorrhizae. This may have brought about a decline or no positive effect in the total functional diversity of the rhizospheric microbiota. Changey et al. (2019) found that AMF inoculation caused a decrease both bacterial and archaeal abundance and diversity. Their results also showed that AMF caused a decrease in different selection pressure on soil microbial communities according to the plant species with which they were associated.

Studies by Verbruggen et al. (2013) and Cely et al. (2016) showed that the success of AMF inoculation in agricultural soils is dependent on a number of factors such as species compatibility, habitat niche availability for AMF, and competition with native fungi. As such, compatibility is a crucial point for AMF inoculation, where some isolates could be termed as host "specialists," while others "generalists" (Öpik and Moora, 2012).

5.4 Effect of Treatments on Soil Respiration

5.4.1 Genotype 405

Treatment with a combination of mycorrhizae and fertilizer had the highest rate of CO_2 evolution at 56.75 ppm (Figure 8). For the treatment with mycorrhizae alone the CO_2 l evolution was 48.50 ppm. The mean of CO_2 evolution in the control soils was 45.75 ppm, while the lowest measurement was observed in the treatment with fertilizer alone (40.0 ppm).

5.4.2 Genotype 407

The highest average CO_2 evolution was observed in the combined treatment of mycorrhizae and fertilizer at 59.50 ppm (Figure 9). Treatment with mycorrhizae alone had the second highest reading of 51.25 ppm, closely followed by readings from the control at 51.0 ppm, while the treatment with fertilizer alone had the lowest level of CO_2 at 43.50 ppm.

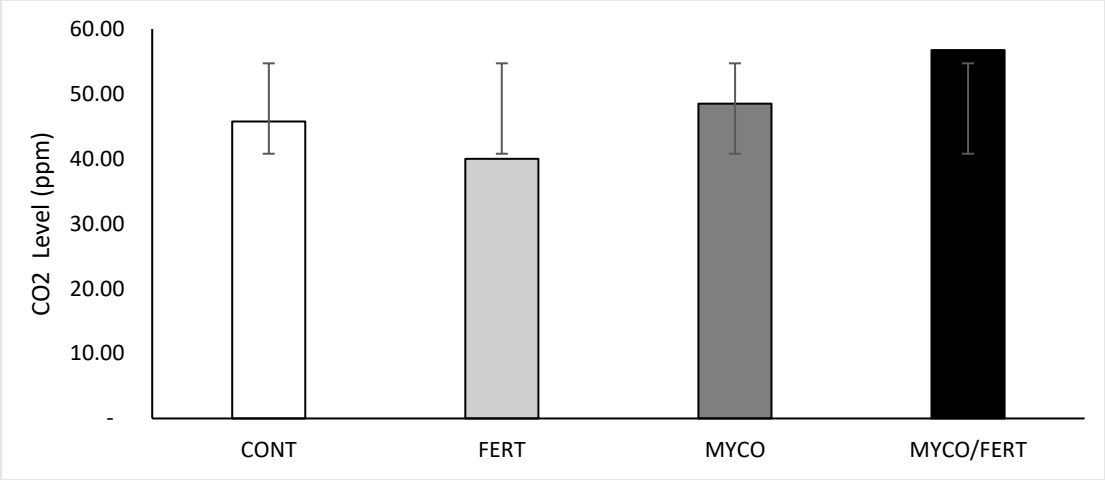


Figure 8. Effect of treatments on soil respiration in salt-affected soil of *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* crosses "genotype 405" located in the Moody Gardens Property in Galveston, Texas.
Key: CONT: Control, FERT: Fertilizer treatment, MYCO: mycorrhizal inoculation, MYCO/FERT: mycorrhizal inoculation and fertilizer treatment.

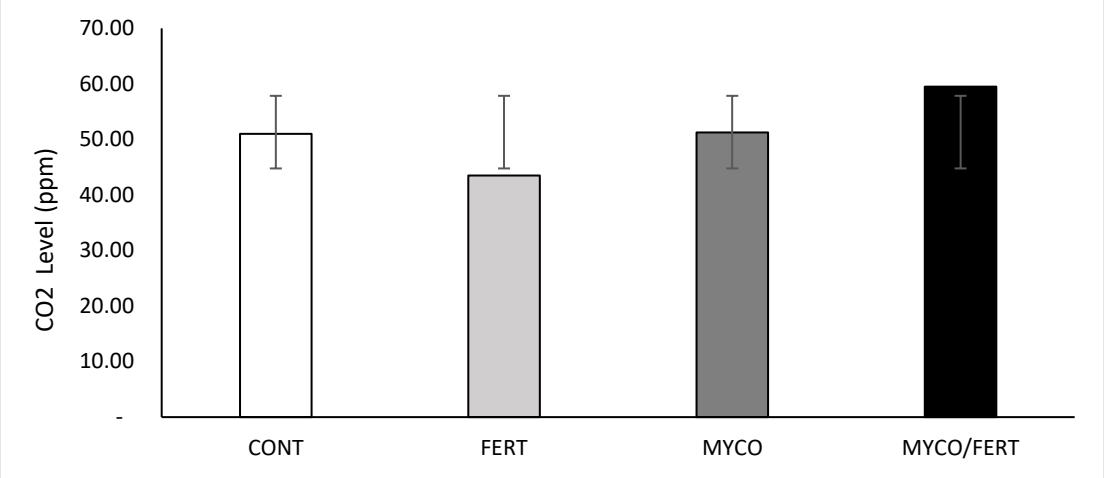


Figure 9. Effect of treatments on soil respiration in salt-affected soil of *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* crosses "genotype 407" located in the Moody Gardens Property in Galveston, Texas.
Key: CONT: Control, FERT: Fertilizer treatment, MYCO: mycorrhizal inoculation, MYCO/FERT: mycorrhizal inoculation and fertilizer treatment.

5.4.3 Genotype 502

The control treatment had the highest level of CO_2 of 44.50 ppm (Figure 10). A CO_2 level of 42.25 ppm was observed in the treatment with mycorrhizae alone. The level of CO_2 from the treatment with a combination of mycorrhizae and fertilizer was 39.25 ppm, while the least measurement was observed in the treatment with fertilizer alone, 37.50 ppm.

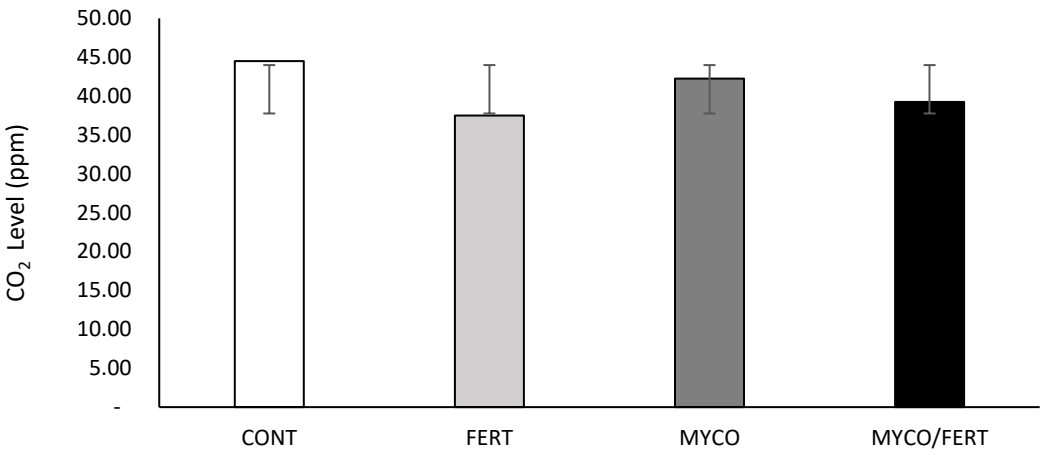


Figure 10. Effect of treatments on soil respiration in salt-affected soil of *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* crosses "genotype 502" located in the Moody Gardens Property in Galveston, Texas.
Key: CONT: Control, FERT: Fertilizer treatment, MYCO: mycorrhizal inoculation, MYCO/FERT: mycorrhizal inoculation and fertilizer treatment.

5.4.4 Statistical Analyses of the Effect of Treatment on Soil Respiration in *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* Genotypes

The effect of treatment on soil respiration as measured by CO_2 levels in the soil was assessed statistically using ANOVA at the 0.05 significance level (Table 15). There were no statistical differences in treatments of genotype 405 (P=0.36), genotype 407 (P=0.53), and genotype 502 (P=0.85)

Table 15. ANOVA Soil Respiration in *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* Crosses, genotypes 405; 407 and 502.
Keys: SS: sum of squares, df: degrees of freedom, MS: mean squares, F: F-statistic.

Source of Variation	*Genotype*	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	405	582.50	3.00	194.17	1.17	0.36
Between Groups	407	512.69	3.00	170.90	0.78	0.53
Between Groups	502	116.25	3.00	38.75	0.27	0.85

5.4.5 Discussion on the Effect of Soil Respiration in *Taxodium distichum* var *distichum* and *Taxodium distichum* var *mexicanum* Crosses Genotypes 405, 407, and 502

Soil respiration in plots supporting the three *Taxodium* genotypes (405, 407, and 502) as not significantly affected by the treatments applied. Respiration rates with treatment involving a combination of mycorrhizae and fertilizer were generally higher than that of the control and other treatments.

Tomè *et* al. (2016) and Lang *et al*. (2020), have highlighted the potentials of mycorrhizal fungi to influence soil respiration. Wang and Wang, (2018) reported higher rate of soil respiration in plots with arbuscular mycorrhizae, which could result from numerous organisms in the root (Wang and Wang, 2018). This is consistent with the result of this study in which mycorrhizae had a trended higher rate of CO_2 emission.

Lee and Jose (2003) and Phillips and Fahey (2007) reported that decrease in the rate of soil respiration could be attributed to the addition of nitrogenous fertilizer, which led to the reduction in microbial biomass and fine root production. With the higher levels of CO_2 emissions in the mycorrhizae treated soils, and it the lower levels CO_2 emissions

in the soils with fertilizer, this could be attributed to the reduction in microbial biomasses in the soil. Also, lower rate of CO_2 emission in treatment with fertilizer compared with the control could be due to the decreased allocation of carbon to roots in response to increased nutrient availability (Phillips and Fahey, 2007). This agrees with the findings of this study in which treatment with fertilizer only, had the lowest level of CO_2 emission.

However, the reduction in the soil CO_2 levels after treatment with fertilizer could be compensated for by the increased rate of organic matter decomposition with the change of carbon and nitrogen ratio in forest floor and mineral soil layers (Kim, 2008).

6. CONCLUSIONS

In summary, the treatments applied (Mycorrhizae inoculation, fertilizer application and a combination of both mycorrhizae inoculation and fertilizer application) had no significant effect on the parameters (height and DBH, soil microbial community, leaf nutrient composition, and soil respiration) considered for this study.

Even though no significant differences among the treatments were found, the treatment with a combination of mycorrhizae and fertilizer seemed to fare slightly better in terms of tree height and DBH measurements amongst the different treatments. This suggests that this treatment when applied in with the controlled quantities needed to achieve optimal yield has the potential to improve tree growth and nutrition in salt-affected soils. Additionally, it may influence soil respiration and functional diversity indirectly by affecting soil chemistry, microbial life, and physical structure.

The addition of inorganic fertilizer did not bring about a corresponding significant increase in the parameters observed. a healthy alternative fertilizer, such as organic fertilizer, could be considered. Extensive or inappropriate use of chemical fertilizers (CFs) is a major cause of nutrient imbalance in soil, leading to high losses (Krupnik et al., 2004). Because of the serious concerns associated within the continuous addition of inorganic fertilizer, adoption of organic fertilizer, which is considered more

environmentally friendly alternative should be encouraged for future research in salt-affected soils.

The high phosphorus content of the leaves indicates high phosphate availability in the soil, which can be attributed to the continuous addition of inorganic NPK fertilizer. Since high phosphate availability in the soil is known to inhibit the establishment and/or persistence of mycorrhizae, direct, non-symbiotic uptake of phosphorus by the root system is encouraged. This leads to the inhibition of mycorrhizal activity to affect plant growth. As a result of this, soils with low phosphorus content from other sites can be excavated, mixed with the salt-affected soil to reduce the available phosphorus and to enhance the optimum activity of mycorrhizae.

The presence of native fungi that have formed mycorrhizal relationships in the salt-affected soil may have brought about an antagonistic relationship with the newly inoculated species. Therefore, future research is needed to thoroughly investigate the compatibility of the autochthonous fungi with allochthonous fungi before the onset of the project. This would help to determine the suitability and compatibility of the newly inoculated species to complement or augment the activities of the indigenous species to promoting plant growth. Also, the indigenous mycorrhizae which are native residents should be investigated for future studies to know the best suited species to achieve optimum plant growth in salt-affected soils.

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APPENDIX A: STUDY SITE MAP

Map of the study site location showing distribution of preliminary sampling points mapped at the site located near Scholes International Airport to Offat's Bayou and Moody Gardens in Galveston, Texas.

VITA

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