IMPACTS OF IRRIGATION WATER QUALITY ON TURFGRASS GROWTH, SOIL CHEMISTRY, AND FUNGAL COMMUNITY DIVERSITY IN TEXAS

A Thesis

by

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ABSTRACT

Sources of water for irrigation use on Texas urban soils have varied in recent years due to the decline in water supplies, urbanization, and frequent and persistent drought conditions. Municipalities are turning to other sources of water for irrigation such as wastewater treatment effluent, harvested rainwater, gray water and saline water or produced water, and others. Wastewater treatment plant effluent has become more common to use in city parks and commercial landscaping. The objectives of this study were to evaluate the relationship between irrigation water chemistry and soil chemistry, turfgrass growth and performance, and soil microbial activity. Treatments included irrigation with saline water, wastewater treatment plant effluent, municipal tap water, reverse osmosis water, and gray water. Overall, soils irrigated with saline had the highest electrical conductivity (EC), sodium, pH, sulfur, and calcium concentrations and sodium absorption ratio (SAR). Irrigation treatment did not have a significant effect on soil microbial activity and had variable effects on turfgrass growth and performance. Principal coordinate analysis and non-metric multidimensional scaling indicated no dissimilarities in fungal community composition in irrigation treatments. Relationships between soil depth, time and irrigation treatment and their effects on soil chemistry were also examined, as well as the effects of water treatment on soil fungal community composition using DNA analysis. Depth and irrigation treatment had an impact on sodium and copper. TDN, DON, salinity, and copper concentrations were significantly higher in December than in November. While relatively short-term in nature, these findings support the use of alternative sources of water for municipal and commercial turfgrass irrigation in areas that are facing water demand and supply issues.

DEDICATION

This thesis is dedicated to my friends and family. From countless discussions to listening to me banter, read aloud, and help in celebrating my tiny achievements, I would not be the scientist I am today without them. Also, a special dedication to my first adviser Dr. Ellen France, who opened my eyes to the science and sacred wonder of water.

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CHAPTER I

INTRODUCTION

1.1 Problem

Climate change, global socio-demographic and environmental changes, freshwater scarcity and freshwater demand, and unexpected impacts of extreme events are all issues that mankind is currently facing. Over the last few decades there has been an increase in demand for freshwater while there has been a decrease in available sources. With the current global water demand estimated to be 4,600 km^3 and projected to increase to 5,500 to 6,000 km^3 (20-30%) per year by 2050, pressure on freshwater sources will continue to increase (Burek et al., 2016). Along with increasing global water demand, from 2017 to 2050 the global population is projected to increase from 7.7 billion to between 9.4 and 10.2 billion with the two major contributors to be Africa at 1.3 billion and Asia at 0.75 billion (United Nations, Department of Economic and Social Affairs, 2017). Author James Ridgeway states that the ability of the world's water supply "to support human, plant and animal life is greatly in peril" (Ridgeway, 2004).

With a shortage of water and high demand, water quality also plays an important role in the availability of freshwater sources. Global increases in urbanization and industrialization are some of the many contributors precipitating the deterioration in available water sources due to the addition of unwanted sediment and chemicals (Holgate et al., 2011). Agricultural and nonagricultural pesticides (Konstantinou et al., 2006) and fertilizer runoff, trace elements in industrial waste (Nriagu and Pacyna, 1988), and heavy metal deposits caused by contaminated impervious surface runoff in urban areas (Zhao et al., 2010) are some of the major contaminants that reduce surface water quality and eventually, groundwater systems as well. In 2002, the

United States Geological Survey (USGS) reported that 22 different antibiotics, as well as other pharmaceuticals, fire retardants, and steroids, were found in 48 percent of 139 steams in 30 different states. Other countries as well as the United States are also facing deterioration in water quality. Bangladesh is currently grappling with the "largest mass poisoning" caused by inorganic arsenic seeping into groundwater resources (Smith et al., 2000). Currently, approximately half of Europe's rivers and lakes are polluted. For example, Germany and the Netherlands are suffering high levels of chemical pollutants and nitrates caused by runoff from urbanization and animal farming (European Environment Agency, 2016).

Water supply has been unable to keep pace with the rate of population increase in the last century as a result of uneven distribution, wastefulness, and poor management. Currently, 1.2 billion people or one-fifth of the world's population live in regions affected by water scarcity while other areas are on the verge of experiencing water scarcity (Watkins, 2006). To be considered an area experiencing water scarcity, the population has to experience annual water supplies dropping below 1,000 m^3 per person. Below 500 m^3 is considered "absolute scarcity" (United Nations, 2014). The major issue countries are facing is the ability to grow enough food while also having to maintain environmental and urban water needs. Growing populations increase the demand for food and with that the increase in amount of necessary water to grow that food. In reality, individuals only require around 2-5 liters of water for drinking and 20 to 400 liters of water for everyday household use. However, the consumption of water is much greater. Currently, individuals use between 2,000 and 5,000 liters of water per person per day (L/p/d), depending on what kind of food they eat and how productive the agriculture was that produced that food. The average annual amounts of water required for food consumption per person is

1,000 cubic meters, not including other every day uses such as drinking, bathing, washing household items, and recreation (Molden et al., 2007).

The two main sources that freshwater water is derived from are surface water and groundwater. Surface water resources, including rivers, lakes, and reservoirs, rely on rainfall and snowfall while groundwater resources, such as confined and unconfined aquifers, rely on recharge from surface water as it moves through the soil to the aquifer. Of the 110,000 cubic kilometers of rainfall that the Earth receives each year, about 40,000 cubic kilometers contributes to rivers and groundwater with the rest in the soil being lost to evaporation (Molden et al., 2007).

Consumers are using municipally treated public drinking water for horticulture and agricultural irrigation and for industrial purposes such as manufacturing and thermoelectricity causing a depletion in these freshwater sources in some areas (Toze, 2005; Flörke et al., 2013). Thermoelectric power and agricultural irrigation were the two largest users of water in 2010. With irrigation being the primary cause of groundwater depletion (United Nations Water, 2018). Withdrawals for thermoelectric power were 161 billion gallons per day (Bgal/d) and withdrawals for irrigation were 115 Bgal/d, while public-supply withdrawals were only 42.0 Bgal/d (Maupin et al., 2014). While agricultural irrigation relies heavily on rainfall and the timing of it, during times of drought or a period of decreased of rainfall then water will be extracted for irrigation to make up for this decline. Presently, consumers use 60% of the United States groundwater for agricultural and urban irrigation (Scanlon et al., 2012). Landscape irrigation for residential purposes ranges from 40% to 70% across the United States with the majority of withdrawals from groundwater and the balance from surface water (Hilaire et al., 2008). Freshwater surface and ground water withdrawals account for 61% of all the uses, excluding thermoelectric power, which also use saline surface water withdrawals (Maupin et al., 2014). Western states, except for

Kansas, Nebraska, Oklahoma, Texas and South Dakota, primarily use surface water for irrigation owing to the fact that the average annual precipitation is insufficient to support crops (Haupin et al., 2014). From 2005 to 2010, surface and groundwater withdrawals have seen an overall decrease, possibly caused by the decline in water quality caused by eutrophication and over-withdrawing of aquifers (United Nations, 2011). The extractions from both surface and groundwater surfaces have ultimately begun to reach their limits. The United States isn't the only country facing a decline in water sources. Multiple river basins throughout the world have been "closed" because the water is gone, and the only water left is for the local ecosystems. For example, the Colorado River in the United States, the Indus River in southern Asia, the Yellow River in China, the Jordan River in the Middle East, and the Murray Darling River in Australia all have significantly lower flows compared to their historical levels.

As citizens rely on water for any form of irrigation, many growers believe it is important for those water sources to be readily available for inexpensive extraction and not require extreme filtration. Drip irrigation technology has recently been developed and can reduce water consumptions on farms by as much as 60%; however, these systems are expensive to install, costing as much as $3,000 per acre (Chu, 2017). Due to the increasing demand both on surface and groundwater, society is beginning to turn to other sources of water for irrigation in hopes of alleviating the pressure that currently exists.

1.2 Freshwater Issues Facing Texas

Texas is currently one of the major US states that must to turn to other sources of water to be used for urban irrigation as a result of the high population densities in major urban cities such as San Antonio, Austin, Houston, and the Dallas/Fort Worth metroplex. The increase in population growth along with the high intensity droughts of Texas has placed emphasis on new techniques and technologies for water conservation. The Texas Water Development board predicts, according to the 2017 Texas State Water Plan, that from 2020 to 2070, Texas' population will grow from 29.5 million to 51 million, a 73 percent increase, and Texas water supplies in acre-feet per year will shrink from 15.2 million to 13.6 million, a 17 percent decrease (Texas State Water Plan, 2017). The current projected cost to implement the water management strategy projects recommended by the Texas Water Development Board is $63 billion in the short term if implemented immediately. It will be expensive initially, but these projects will mitigate the increased demand on water and the cost if not implemented will be $73 billion to $151 billion by 2070 (Texas State Water Plan, 2017).

Currently, there is not enough water to sustain the growing needs of the Texas population. There are cities that have resorted to using recycled wastewater, gray water, and other unlikely water sources for irrigation and potable water requirements. Residents of Dallas, Houston, and San Antonio currently use 213, 134 and 149 gallons of water per day per person, respectively (Henry, 2011). In order to alleviate some of these water scarcity issues, certain cities are introducing the recycling of toilet water, more commonly known as "toilet to tap" or black water. El Paso is presently experiencing river drought with the Rio Grande and the aquifers that are not being replenished quickly enough to satisfy its population demand. Therefore, the El Paso Water Utility is recycling its wastewater with multiple types of filtration and disinfectant treatments in order to make it safe for drinking and irrigation (El Paso Water Utility, 2007).

Efficient water use, reduction of pollutants, proper management of water sources, and good land use decisions together could help alleviate some of the current demand on water sources. A better understanding of the innovative recycling of water sources that countries and states are adopting could also prove beneficial and help minimize the impacts of urbanization and industrialization.

1.3 Treated Wastewater Treatment Plant Effluent

Worldwide water shortages are forcing countries to turn to different sources of water for agricultural irrigation. Treated municipal wastewater is readily available to be used and reused when natural resources are being depleted or are too limited. It is a source that remains constant and the reuse of the water can have environmental benefits. Raw municipal wastewater typically contains organic and inorganic material or dissolved minerals, toxic chemicals, and pathogens such as bacteria, viruses, and helminths. The chemical makeup of wastewater depends on the socioeconomic characteristics of that community and the amount of industrial and commercial properties (Hanjra et al., 2012), and therefore, can vary in chemical and biological composition. The discharge of the effluent into rivers, lakes, and reservoirs can severely degrade the surrounding environments. The high concentrations of inorganic and organic nutrients in the discharged effluent, for example nitrogen and phosphate, can be applied as a fertilizer and reduce the dependence on manufactured fertilizers (McCarty et al., 2011).

Due to the presence of possibly harmful organisms, there is a public concern and lack of public acceptance for using treated effluent to irrigate not only for agriculture, but also for municipal homes. Strict monitoring regulations have been implemented on the reuse of treated wastewater effluent to ensure the safety of humans and animals eating the agricultural crops to children outside playing on urban lawns. Cirelli et al. (2011) conducted a study in Eastern Sicily on the long-term effects of treated crops intended for human consumption. Tertiary-treated municipal wastewater was applied to tomato and eggplant crops using surface and subsurface drip irrigation. They reported elevated *E. coli* in the irrigation water, which was significantly above the 50 CFU 100 mL^{-1} Italian mandatory limits, but neither *Salmonella* nor helminth eggs were detected. Only two eggplant samples contained 10^2 CFU/100 g of fecal coliforms and fecal streptococci. Tomato samples in contact with the soil or plastic mulch were contaminated by the high presence of microbes in those substrates. The tomato and eggplant crops not only successfully grew on the irrigated plots but their yields were also significantly higher than the plots irrigated with a freshwater source. Overall, they found that the microbiological quality of the two crops was generally maintained even though *E. coli* in the treated wastewater was over the regulatory limits.

Along with health safety, soil and plant quality are also a concern when using treated wastewater for irrigation. There are currently over 1000 effluent irrigation systems that are operating in the U.S. (Feigin et al., 2012). One study performed in Arizona over a 3.2 year period found that secondary-treated sewage effluent (STSE) irrigation caused a significant increase in soil sodium and EC compared to the potable water, as a result of higher concentrations in the effluent; however, the SAR and EC values were not high enough to damage the bermudagrass (Hayes et al., 1990). Another study performed in Colorado also saw an 89 to 95% increase in sodium, a 28 to 50% increase in boron, and an 89 to 117% increase in phosphorus, but did not see values high enough to cause soil deterioration (Qian and Mecham, 2005). In the Region of Murcia, Spain, Pedrero et al. (2010) found that treated wastewater is an adequate alternative water resource for citrus tree irrigation.

Heavy metals and trace organics are also a public concern for using treated effluent as a source of water when the effluent is from an industrial or from a less treated source. Typically, heavy metals are removed during the primary stage of treatment while endocrine disrupting chemicals (EDC) and pharmaceutically-active compounds (PhAC) are removed during the secondary stage of treatment. While the concentrations in the effluents are low and the required ingestion needs to be high and over a long period of time, there is still public concern. One study performed by Angelova et al. (2004) found that flax and cotton took up heavy metals when grown in heavily contaminated soils, but the concentrations detected in the leaves and seeds were only small in comparison to the concentrations present in the soil. EDCs include estradiol compounds, and while effluent concentrations are not usually high enough to have endocrine capabilities in the human body, have been known to have significant impacts on other animals in the local ecosystem including problems relating to the size and development of male gonads in juvenile male alligators to the complete feminization of male amphibians, reptiles, and fish (Toze, 2005; Hayes et al., 2011). The major concern for PhACs, even more so than being taken up by crops, is causing antibiotic resistance in soil and water microorganisms.

Even with the different concerns of the public, most of these can be managed through proper treatments and application of the treated effluent. Metals and trace chemicals, organic and inorganic nutrients, and microorganisms can usually be removed from the wastewater during the different treatment stages of a water treatment plant. The exception being salts and other cations and anions that require reverse osmosis membrane filtrations, which is expensive and economically difficult to implement solely for irrigation purposes. However, even these contaminants can be removed with proper leaching techniques that are dependent on the soil profile. Treated wastewater effluent has the potential to reduce the use of other water sources and will last as long as the proper treatment and effective management practices are in place.

1.4 Gray Water

Gray water is defined as wastewater generated from residential sources such as baths, showers, dishwashers, hand and kitchen sinks and washing machines with the exclusion of inputs from toilet flushing (blackwater) (Jefferson et al., 2004). Because gray water is readily available and accessible it has an untapped potential for being an alternative water resource for irrigation, especially in arid or semi-arid conditions where fresh water is scarce. Gray water accounts for 50-80% of the total water use in domestic residences (Al-Hamaiedeh & Bino, 2010). The amount of gray water produced is directly related to the amount of water readily available to the household. Discharges can range from 180-300 L/p/d to 30-80 L/p/d to 9-50 L/p/d depending on if there is more than one tap inside the house, a tap outside of the house, or the house is 250 m or more from a standpipe (Rodda et al., 2011). Similar to treated wastewater effluent, there is public concern with using raw gray water as an alternative to fresh or ground water sources.

Gray water composition varies significantly from residence to residence and depends on how it is stored, the type of occupants in the household, its source and if it is raw or treated. When gray water is being used for applications, such as irrigation or vehicle washing, certain water quality requirements based on organic compounds such as those contained within total phosphorous and total nitrogen; solids, and microbial content must be met (Jefferson et al., 2004). Homeowners that irrigate ornamental and food plants with untreated gray water have concerns about the health risks and the potential for gastrointestinal disease transmission by gray water (Rose et al., 1991; Busgang et al., 2018). One study performed in Arizona by Casanova et al. (2001) measured and compared chemical and microbial quality from a single-family home with two adults and the same home years earlier when two adults and one child lived there. They found that there was no difference in total coliform levels between each household but an increase of fecal coliforms along with turbidity and biological oxygen demand in the household with two adults and one child. They also found that the overall chemical, microbial and physical qualities of the untreated gray water fell between the ranges of raw and treated wastewater and secondary effluent. Because of the high presence of enteric pathogens in gray water, gastrointestinal health is the biggest issue facing the implementation of gray water recycling programs.

Similar to treated wastewater effluent, there are public concerns on how gray water will affect the soil and plant quality. A study performed in the Israeli Negev desert by Gross et al. (2005) found elevated levels of boron in gray water treated plots resulting in plant toxicity. Due to gray water containing food matter, grease, and surfactants, irrigation may create hydrophobic soils that prevent soil infiltration and increase surface runoff and erosion (Leas et al., 2014). Another study used raw gray water on three different soil types (sand, loam, and loess) and saw a significant number of surfactants, coliform bacteria, and oil and grease in the soil compared to the treated gray and freshwater (Travis et al., 2010). Al-Hamaiedeh & Bino (2010) conducted a study in Jordan and compared treated and raw gray water and their effects on the properties of soil and irrigated plants. Their study saw an increase in salinity, SAR, and organic material in the soil over time; however, the chemical properties of the irrigated olive trees and vegetable crops were not adversely affected, but the biological quality of some of the vegetable crops were affected. Holgate et al. (2011) examined the impacts of gray water, municipal tap water, and harvested rainwater on soil under the grass species perennial ryegrass (*Lolium perenne* [L]). They found dissolved organic C losses to be two-to-four times greater in the soil irrigated with gray water and municipal tap water relative to the soils irrigated with only to the harvested rainwater. They attributed these losses to the temperatures in the greenhouse plus the addition of nitrogen and phosphorous from the gray water inputs resulting in increased litter decomposition and increased production of DOC in the gray water treatments (2011).

When gray water is recycled residentially, it has several potential economic incentives that include but are not limited to: reducing the amount of income allocated to purchasing water for irrigation, decreasing the demand for chemical fertilizers, increasing the overall quantity of water available for irrigation, and increasing the potential for higher biomass yields in crops (Leas et al., 2014). On a global scale, adopting gray water recycling practices may lower groundwater extraction rates and freshwater demands (Leas et al., 2014). Koussis et al. (2010) even found that treated gray water can reduce the pressure caused by saltwater intrusion in coastal aquifers. The treated gray water will create a larger supply of reusable brackish groundwater, which is more energy- and cost-efficient to desalinate than pure seawater.

The quality of the gray water and its intended use should be considered when creating the reuse guidelines to reduce both environmental and health risks. Already, there are various recycling treatments that are currently being considered for treating raw gray water such as storage, sedimentation, filtration, biological treatment and disinfection (Rose et al., 1991). With the implementation of proper management and safe recycling programs, gray water has the potential to be reused and to alleviate the growing pressure on freshwater sources to be used for irrigation.

1.5 Saline Water

Saline water sources or water sources that have previously been deemed too saline for use for irrigation are now being considered as an alternative solution to increase water available for irrigation. Saline water has relatively high concentrations of dissolved salts. However, recycled water does not always inherently contain high concentrations of salts. In coastal areas, the water to be used for irrigation may already be salty or the pipes carrying the groundwater to the water treatment facility may travel through areas of salt water (Parsons et al., 2010). The salinity of the water source is measured as electrical conductivity, which varies depending on the source, location, treatment, and time of year (Niu & Cabrera, 2010).

One major concern when irrigating using a water source with elevated salt levels is plant damage in response to salinity stress. Salt tolerance is defined as the plant's inherent ability to withstand the effects of high salts in the root zone or on is leaves without significant adverse effects such as growth or yield reduction (Grieve et al., 2008). There are some plants known as halophytes that can balance the osmotic and ion changes by absorbing the salt ions and sequestering them in the vacuoles of the cells. Chavarria et al. (2019) performed a two-year study over using subirrigation water containing Instant Ocean on 10 commonly used cultivars representing warm season turfgrass species. They found that the majority of cultivars, with the exception of 'Celebration' bermuda grass and seashore paspalum, fell below acceptable turf quality at EC of 15 dS m^1. Tifway, an intermediate tolerant turfgrass, had increased shoot biomass in year one but reduced biomass in year two at the 15 dS m^{-1}. A study performed by Dudeck et al. (1983) on eight bermudagrass cultivars exhibited only a 22% reduction in top growth at 9.9 dS m^{-1} while root growth increased to 270% above the plants irrigated with no salt treatment.

1.6 Sodic Water

Water that contains higher sodium (Na^+) concentration relative to concentrations of calcium (Ca^{2+}) and magnesium (Mg^{2+}) is considered to be sodic water. Soils can become sodic if there is not proper drainage throughout. Depending on the soil profile, there can be an increase in pH, a reduction in hydraulic conductivity, permeability, or both through the phenomena of swelling dispersion. In turn, this causes a decrease in readily available nutrients and water for plants (Rodda et al., 2010). Qadir and Oster (2004) found that an accumulation of salts and Na^+

in soils could be remediated by the addition of calcium to replace the excess Na^+ from the cation exchange sites through a managed amount of leaching. However, excess amounts of exchangeable sodium in soils can cause stunted growth and arrested cell development in plants. With the proper management practices and use of plants that are more salt tolerant, water sources that are saline or sodic may be used as an alternative source of water for irrigation.

CHAPTER II

IMPACTS OF IRRIGATION WATER QUALITY ON SOIL CHEMISTRY, TURFGRASS GROWTH, AND FUNGAL COMMUNITY DIVERSITY

2.1 Introduction

Municipalities must turn to alternative sources of water for irrigation including saline, recycled water, and gray water, as a result of the increasing depletion of available water sources (Qadir and Oster, 2003; Qian and Mecham, 2005; Rodda et al., 2011). One strategy to help with the use of alternative water sources is the choice of turfgrass and consideration of climate for that particular municipality. For example, in areas where groundwater tends to be more saline or sodic the selection of a salt-tolerant turfgrass may prove to be more appropriate for the landscape. Another strategy would be the appropriate soil or fertilizer type to help with the turfgrass growth. The soil is the reservoir for water and the source of most of the essential nutrients for turfgrass plants (Carrow et al., 2001).

In order to accurately meet the nutrient requirements for turfgrass it is important for managers to understand the formation of the soil. Soil formation is dependent on five major factors: climate, vegetation and other living organisms, topography or relief, and time (Jenny, 1941). Soils in warm, humid climates have a different soil profile than soils in warm, arid climates. The parent rock and microorganisms can also have a significant impact on soil formation. All of these chemical, physical, and biological factors coupled with the input water source play an important role in how managers culture their turfgrass landscape.

2.1.1 Impacts of Essential Nutrients and Metals in Water Sources on Soils and Turfgrass

The primary nutrients that managers focus on are N, P, and K due to the large amounts required by each type of turfgrass. Previous researchers have sought to determine the importance of essential nutrients for turfgrass quality. Synder and Cisar (2000) performed a study examining the effects of K:N fertilization ratios on a ‘Tifgreen’ bermudagrass (*Cynodon dactylon* (L.) Pers. x C. *transvaalensis* Burtt Davy) over a 3-year period in south Florida. They found that there were significant deficiencies of K in the absence of K fertilization and increasing beyond a 0.5 to 1 ratio had no significant effect on the growth or visual quality of the bermudagrass. They also observed that N fertilization had a significant influence on visual quality ratings during the duration of the study. Phosphorus is also important because it plays a number of roles because of its ability to form high energy pyrophosphate bonds in adenosine diphosphate (ADP), adenosine triphosphate (ATP), and other phosphates. Growth of bermudagrass has been reported to decline in Florida under excessive P, but cool-season grasses can tolerate high levels (Carrow et al., 2001).

A few other essential nutrients that managers must consider are Na, Ca, Mg, S, Fe, Mg, Zn, B, and Cu. All of these nutrients are found in wastewater treatment plant effluent, gray water, and saline water and can cause problems for turfgrass managers if the nutrient levels are toxic or insufficient. Some of these issues include but are not limited to chlorosis, reduced growth or yield, loss of shoot density, distorted appearance of new leaves, and root toxicity. Managers will need to perform a full irrigation water quality analysis to determine the current conditions of the soil and to determine what measures will possibly be needed in the future such as leaching, mowing, or fertilization (Carrow et al., 2001).

2.1.2 Fungal Community Composition

Microorganisms in soils are important in the management of turfgrass and play a key role in processes such as soil structure formation, decomposition of organic matter, toxin removal, and the cycling of nutrients (Garbeva et al., 2004). They can also help in the suppression of disease and promotion of plant growth. One such study indicated a dependence of *Senna spectabilis*, part of the legume family, on the symbiotic relationship with mycorrhizal fungi (Kung'u et al., 2008). Furthermore, because of mycorrhizal fungi's ability to increase nutrient uptake and increase plant growth in water-stressed plants, the vesicular arbuscular mycorrhizal fungi (AMF) inoculation increased total shoot height by 100%, as well as root collar diameter increased by 25%, and leaf number increased by 84%.

This symbiotic relationship is beneficial in not only legumes but also other plant and grass species as well. Bermudagrass (*Cynodon* spp.) and St. Augustine grass (*Stenotaphrum secundatum* (Walt.) Kuntze 'Raleigh') are two of the more commonly used types of turfgrass for sports facilities and municipal lawns respectively in Central Texas. Due to Texas being highly prone to droughts, mycorrhizal fungi can be crucial to plants and soil function (TWRI, 2011). Wu et al. (2011) examined the effects of AMF versus non-mycorrhizal plant growth above ground, post cutting of bermudagrass (*Cynodon dactylon L.)*. They not only found aboveground biomass to be significantly higher in AMF plants but also found increased chlorophyll contents, possibly indicating that AMF facilitates greater rates of photosynthesis. While there has been significant research on the relationship between mycorrhizal fungi and plants and mycorrhizal fungi and soil, the research is limited surrounding how actual water chemistry directly affects mycorrhizal fungi as well as other fungal species in the soil.

2.2 Objectives and Hypotheses

Objectives

The major objective of this study was to investigate the different sources of water for irrigation and their effects on turfgrass growth and physical properties, soil chemistry, and fungal community diversity within turfgrass soils. The five different irrigation sources were 1) saline, 2) wastewater treatment plant effluent, 3) sodic municipal tap water, 4) reverse osmosis water, and 5) gray water.

Hypotheses

H_{O1} There will be no significant differences in chemistry of soil receiving the wastewater treatment plant effluent, gray water, saline water, and municipal tap water treatments compared to the control treatment.

H_1 There will be significant differences in soil nutrient concentrations in the wastewater treatment plant effluent, gray water, saline water, and municipal tap water treatments compared to the control treatment due to an input of ions and nutrients.

H_{O2} There will be no significant difference in soil nutrient concentrations at different soil depths.

H_2 There will be significant differences in soil nutrient concentrations at different depths.

H_{O3} There will be no significant interaction between depth and irrigation treatments on soil chemistries.

H_3 There will be a significant interaction effect between depth and irrigation treatments on soil chemistries.

H_{O4} There will be no significant interaction between date and irrigation treatments on soil chemistries.

H_4 There will be significant interaction effect between date and irrigation treatments on soil chemistries.

H_{O5} There will be no significant differences in turfgrass growth among irrigation treatments.

H_5 There will be significant differences in turfgrass growth among irrigation treatments.

H_{O6} There will be no significant differences in microbial activity due to irrigation treatment.

H_6 There will be significant differences in microbial activity due to irrigation treatment.

H_{O7} There will be no significant differences in fungal diversity among irrigation treatments.

H_7 There will be significant differences in fungal diversity among irrigation treatments.

2.3 Materials and Methods

2.3.1 Field Study Site

The experiment occurred during the growing season from June through November of 2016 at Texas A&M University Turfgrass Field Research Site in College Station, TX. The field research site was previously used for dairy farming until its close in 2003. The native soil at the field study site is described as a Zach series (fine, smectitic, thermic Udertic Paleustalf). The site received a cumulative annual rainfall of 1106.4 mm for the year of 2016. The area designated for the experimental plot received no previous irrigation except for precipitation events that occurred throughout the year. The average daily temperature was 20.3°C for 2016, and average temperatures ranged from 17.3°C to 28.7°C during the experimental months (Figure 1). The cumulative daily rainfall during those months were 56.4 mm, 3.6 mm, 174.8 mm, 50.6 mm, 60.2 mm, and 97.5 mm, respectively. These data were collected from on-site weather stations (Campbell Scientific, Logan, UT) at the Turfgrass Research Field Laboratory in College Station, TX.

2.3.2 Experimental Design

The experiment was conducted on newly established Tifway (*Cynodon dactylon* × *C. transvaalensis Burtt Davy)* hybrid bermudagrass. To prepare for the sod installation, active ingredient (Ranger Pro Herbicide, Monsanto Company, St. Louis, Missouri) was applied at a rate of 0.7 mL/m^2 using a carrier volume of 37.4 mL/m^2 to kill all vegetation (EPA Reg #524-517). The sod was installed in 41.6 by 35.6 cm pieces. After the planting of the Tifway plot in mid May 2016, the plot was established naturally with the precipitation events (Figure 2) that occurred during the month of May which was 305.3 mm cumulative precipitation (Texas A&M AgriLife Extension). The area of the plot was 30.5 m by 1.8 m. with a total area of 55.8 m^2

(Figure 3). The study design was a randomized complete block design. There were four replicates per treatment with a total of 5 treatments. The study area was randomly segmented into 20 smaller 0.71 m^2 (0.84 m x 0.84 m) plots with a barrier 0.61 m between each individual plot and a 0.30 m barrier between the plots and the surrounding native environment (Figures 3-5). Fertilization of the plots occurred once at the beginning of the study and again during mid-summer, using a 21-7-14 N-P-K fertilizer containing 25% sulfur coated urea at a rate of 4.9 kg N ha^{-1} (URI-PELS S.R. 21-7-14 25% Sulfur Coated Urea, American Plant Food Corporation, Galena Park, Texas, USA). There was an army worm infestation during the month of September and the insecticide Talstar (EPA Reg $279-3206) was applied at a rate of 1.1 mL/m^2 using a carrier volume of 407.5 mL/m^2.

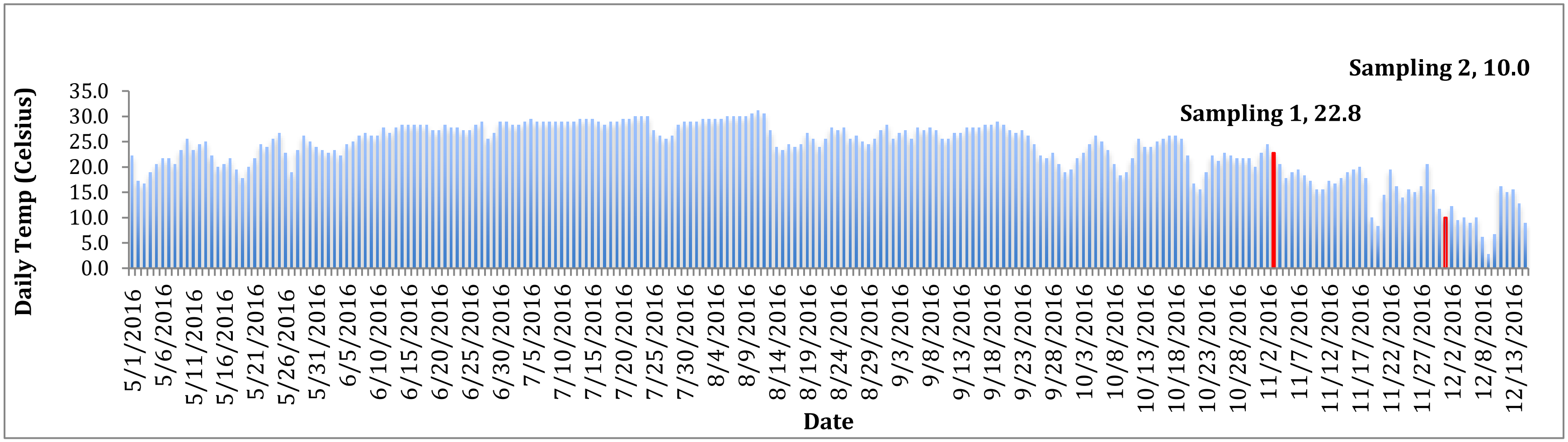

Figure 1. Daily temperature for the duration of the experiment, May - November. Values next to sampling date indicate the temperature for that sampling date.

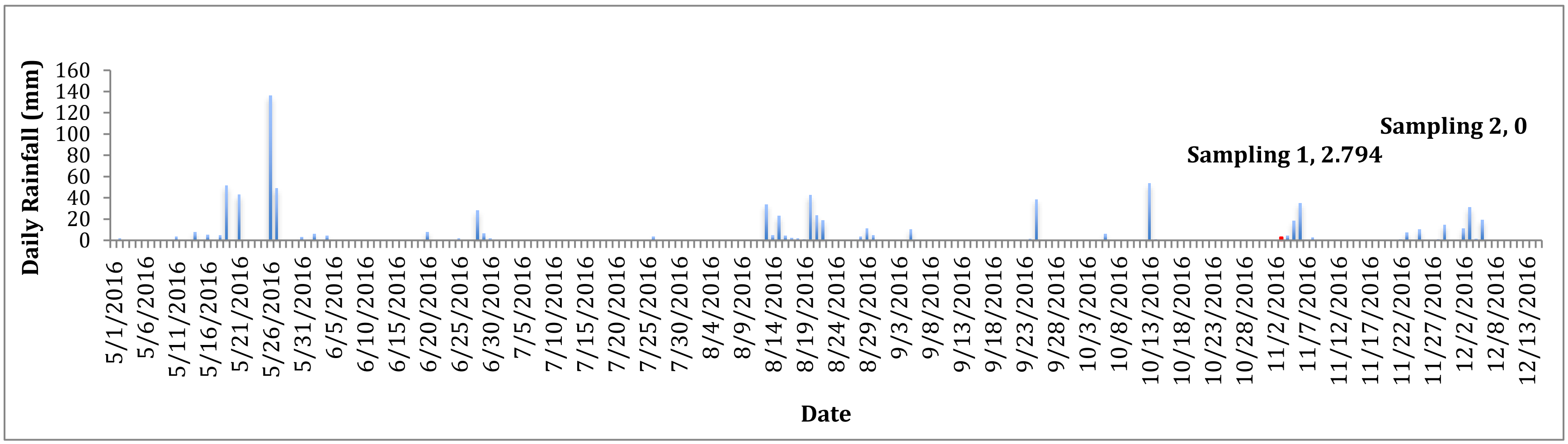

Figure 2. Daily rainfall values for the duration of the experiment, May – November**.** Values next to sampling date indicate the precipitation event for that sampling date.

Figure 3. Image of entire Tifway plot. Red paint indicates the area to be watered per treatment replicate block.

Figure 4. Image of 0.84 m x0.84 m Tifway and treatment plot within the replicate block. Red paint indicates the area to be watered per treatment replicate block.

Figure 5. Image of 0.84 m x 0.84 m Tifway plot surrounded by buffer. Red paint indicates the area to be watered per treatment replicate block.

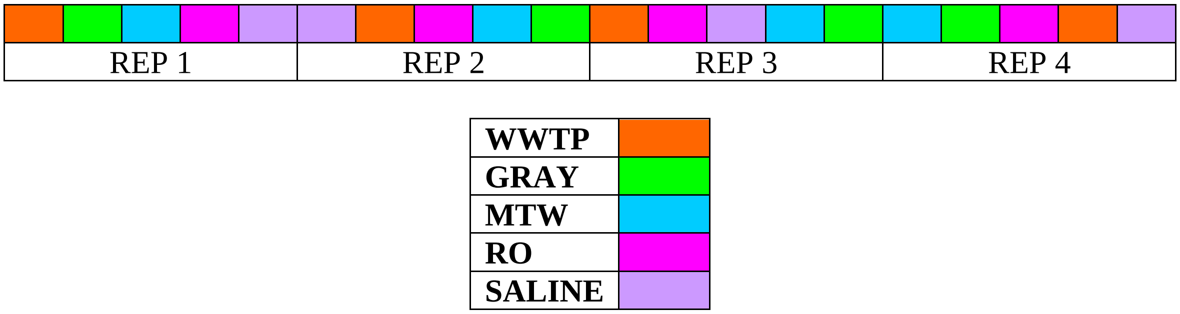

Figure 6. Diagram of the experimental plot plan treatments. WWTP = wastewater treatment plant effluent, GRAY = gray water, MTW = municipal tap water, RO = reverse osmosis water, and SALINE = saline water.

This experiment consisted of five water treatments; 1) reverse osmosis water (control), 2) sodic municipal tap water, 3) gray water, 4) saline water, and 5) wastewater treatment plant effluent (Figure 6). Irrigation of treatment plots consisted of the application of 42.7 L of the respective irrigation water twice weekly, in order to supply 2.54 cm of irrigation per week. According to the Texas Water Development Board, warm-season turfgrass such as hybrid bermudagrass requires approximately 2.54 cm of rainfall or irrigation per week during the growing season (Texas Water Development Board, n.d.). Therefore, the irrigation applications occurred twice weekly from June through November. Rainfall was not accounted for when scheduling irrigation requirements. Rain gauges located on site determine the actual amount of rainfall contributed for that week (Figure 2).

2.3.3 Irrigation Water

The gray water was prepared by using of 10 mL of Tide detergent (Tide; ingredients listed in Table 1) added to 18.93 L of MTW based on the assumption that 40 mL of detergent is used for a medium load (20 gallons or 76 L) of laundry in a front-loading washing machine. Two 18.93 L containers and one 7.57 L container was used for the collection of gray water and the saline water. Each batch was prepared within 48 hours of irrigation. In addition, a sub-sample was collected from each prepared batch, mixed, and then run for chemical analysis.

The saline water was prepared using of 69.42 g of Instant Ocean (Instant Ocean); ingredients listed in Table 1) added to 18.93 L of RO water in order to obtain an electrical conductivity (EC) of 6.51 dS m^{-1}. An irrigation water is considered saline when EC is > 4 dS m^{-1}, pH is < 8.5, exchangeable sodium percentage (%ESP) < 15 and SAR is < 13 (Davis et al., 2007). The saline water was prepared within 48 hours of irrigating and a sub-sample was taken to run for chemical analysis.

The municipal tap water from College Station, Texas that was used for the experiment is a groundwater sourced from three different aquifers: the Carrizo-Wilcox, Sparta, and Yegua and is mainly disinfected with chlorine gas. The water was obtained from a faucet located in the Heep Center Building at Texas A&M University. Reverse osmosis (RO) water, derived by pressure-driven membrane filtration, was collected from a RO faucet located in the Heep Center Building at Texas A&M University. The RO treatment was chosen as the control treatment due to it being the water treatment with no nutrients or contaminants added. Normally, MTW would have been used as the control treatment; however, the MTW in College Station has average sodium concentration of 206 ± 25 mg/L and toxicity to turfgrass can be anywhere from 70-210 mg/L with severe toxicity being above 210 mg/L (Aitkenhead-Peterson et al., 2018; Carrow et al., 2001). Sub-samples of irrigation water were taken monthly for chemical analyses.

Wastewater treatment plant effluent was collected from the Carter’s Creek wastewater plant in College Station, Texas each morning prior to irrigation. The wastewater that goes to the treatment plant travels through a primary and secondary treatment followed by UV disinfectant.

Sub-samples were taken at every collection for chemical analyses (Table 2). The chemical composition of treated wastewater may change dependent on precipitation events.

All water treatments were collected or prepared the within 48-hours of irrigation. They were collected and prepared in two, 18.9 L containers and one, 7.6 L container. As the turfgrass irrigation recommended is 2.54 cm each week, the first 1.27 cm of water was applied on a Monday and the second 1.27 cm was applied on the Thursday each week of the study (0.08534 m^3 x 1 m^3/1000 L = 85.34 L of water per treatment per week or 82.67 L irrigated twice weekly) Sub-samples were taken from every new batch of gray, saline, and wastewater effluent, and sub-samples were taken monthly for municipal tap and reverse osmosis water. All sub-samples were frozen at -20° Celsius until chemical analysis.

Table 1. Ingredients in the Tide detergent and Instant Ocean products used for the gray water and saline water treatments. Ingredients are as listed in current MSDS sheets.

Irrigation Treatment	Product	Ingredients listed on MSDS sheet
Gray	Tide detergent	Biodegradable surfactants (anionic and nonionic) and enzymes Sodium carbonate Benzenesulfonic acid, mono-C10-16-alkyl derivs. Silicic acid, aluminum sodium salt, sodium salt Carbonic acid disodium salt, compd. with hydrogen peroxide Sodium 2-(nonanoyloxy)benzenesulfonate Poly(oxy-1,2-ethanediyl), alpha-sulfo-omega-hydroxy-, C10-16-alkyl ethers
Saline	Instant Ocean	Sodium Chloride (CAS # 7647-14-5) Magnesium Chloride (CAS # 7791-18-6) Sodium Sulfate (CAS # 7757-82-6) Calcium Chloride (CAS # 10043-52-4) Potassium Chloride (CAS # 7447-40-7)

2.3.4 Chemical Analysis of the Irrigation Water Treatments and Soil Water Extracts

The irrigation water analysis tested for the following constituents: pH, EC, salinity, NO_3-N, NH_4-N, PO_4-P, dissolved organic carbon (DOC), total dissolved nitrogen (TDN), Ca, Mg, Na, K, B, S, P, Fe, Cu, and Mn. The pH and conductivity of each extract sample was recorded prior to filtration. Solutions were filtered using ashed (500 ˚C for 4 hr) Whatman GF/F filters (nominal pore size 0.7 μm). Dissolved organic nitrogen (DON) was estimated by deducting NO_3-N + NH_4-N from TDN.

Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were measured with high temperature platinum-catalyzed combustion using a Shimadzu TOC-VCSH and Shimadzu total measuring unit (TNM-1) (Shimadzu Corp, Houston, TX, USA). Dissolved organic carbon was quantified as nonpurgeable carbon using USEPA method 415.1, which entailed acidifying (2N HCl) the sample and sparging for 4 min with C-free air. Ammonium-N was analyzed using the phenate hypochlorite method with sodium nitroprusside enhancement (USEPA method 350.1), and nitrate-N was analyzed using Cd-Cu reduction (USEPA method 353.3). Orthophosphate-P was quantified using the ascorbic acid, molybdate blue method. Colorimetric methods were performed with a Westco Scientific Smartchem Discrete Analyzer (Westco Scientific Instruments Inc. Brookfield, CT, USA). Water blanks, replicate samples, National Institute of Standards and Technology (NIST) traceable standards, and check standards were run every 12^{th} sample to monitor instrument precision.

The trace metals were detected and measured using inductive coupled plasma mass spectrometry (ICP) analysis. The average and standard deviation for each chemical constituent in each of the different irrigation treatments that were used in this experiment are listed in Table 2.

Table 2. Irrigation water treatment chemistry. All values are mg/L unless otherwise indicated. The values in parenthesis in bold indicate averages and values not in bold indicate standard deviation.

Constituent		Grey	MTW	RO	Saline	Effluent
pH		**9.39**	**9.43**	**8.48**	**9.08**	**9.05**
		0.19	0.01	0.04	0.25	0.10
EC	dS/m	**1.05**	**1.05**	**0.02**	**6.51**	**1.20**
		0.05	0.01	0.00	0.26	0.04
Salinity	(S/m)	**1.01**	**0.98**	**0.02**	**5.87**	**1.17**
		0.05	0.01	0.01	0.38	0.03
SAR		**30.98**	**30.26**	**2.47**	**18.27**	**15.71**
		1.35	0.20	0.06	0.58	0.49
ESP	%	**97.73**	**97.82**	**91.85**	**78.97**	**91.84**
		0.12	0.02	0.47	0.50	0.28
NO_3-N		**0.16**	**0.18**	**0.00**	**0.01**	**16.96**
		0.10	0.07	0.00	0.01	4.24
NH_4-N		**0.01**	**0.00**	**0.01**	**0.01**	**0.24**
		0.01	0.01	0.00	0.04	0.20
PO_4-P		**0.11**	**0.19**	**0.00**	**0.01**	**3.88**
		0.01	0.02	0.00	0.01	0.44
DOC		**97.04**	**3.22**	**0.58**	**0.90**	**10.00**
		5.81	0.23	0.08	0.25	1.65
TDN		**3.31**	**0.31**	**0.00**	**0.03**	**20.74**
		0.17	0.10	0.00	0.06	1.89
DON		**3.14**	**0.12**	**0.00**	**0.03**	**3.55**
		0.19	0.17	0.00	0.06	3.01
Ca		**2.88**	**2.93**	**0.36**	**37.30**	**9.59**
		0.15	0.19	0.14	2.82	0.41
Mg		**0.58**	**0.39**	**0.02**	**110.17**	**2.08**
		0.19	0.02	0.01	7.55	0.15
Na		**246.31**	**234.41**	**6.25**	**1021.70**	**251.89**
		7.49	5.14	0.72	60.02	6.88
K		**2.21**	**1.90**	**0.19**	**35.66**	**12.62**
		0.27	0.16	0.14	2.18	0.29
B		**1.55**	**0.35**	**0.33**	**0.81**	**0.49**
		0.43	0.02	0.02	0.06	0.03
SO_4		**41.56**	**20.19**	**0.40**	**264.07**	**32.83**
		9.65	1.38	0.16	12.57	1.07
P		**0.20**	**0.20**	**0.01**	**0.02**	**3.80**
		0.02	0.01	0.01	0.00	0.42
Fe		**0.01**	**0.01**	**0.01**	**0.01**	**0.02**
		0.00	0.00	0.00	0.00	0.00
Zn		**0.01**	**0.01**	**0.01**	**0.01**	**0.05**
		0.00	0.00	0.00	0.01	0.01
Cu		**0.02**	**0.01**	**0.01**	**0.01**	**0.01**
		0.01	0.00	0.00	0.00	0.01
Mn		**0.01**	**0.01**	**0.01**	**0.01**	**0.01**
		0.00	0.00	0.00	0.00	0.00
ESP is exchangeable sodium percentage						

2.3.5 Evaluation of Turfgrass Growth and Performance

The evaluation for turfgrass growth was based on clipping biomass. Mowing occurred every 10-14 days, but the clippings were harvested once a month for clipping biomass. The clippings were not returned after mowing. Five harvesting dates included 15 July, 29 August, 28 September, 24 October, and 11 November 2016. If rainfall had recently occurred, clipping was withheld until the turfgrass was no longer saturated. The 20 individual plots were mowed at a height of 5.1 cm using a Snapper (21") 190cc Hi-Vac Push Lawn Mower. The grass clippings were collected using one-gallon paint strainers. The collected clippings were oven-dried at 65° C for 72 hr. The clippings were then weighed, and the obtained values were then divided by the number of growing days (approx. 21 to 45 days) that occurred pre-mowing to determine the growth rates.

Digital images were taken post-mowing throughout the growing season from July to November (16 June 2016, 18 July 2016, 5 October 2016, 27 October 2016, and 17 November 2016) using a Canon PowerShot SD790IS equipped to a light-box, 60.96 cm x 45.72 cm with a camera hole height of 40.64 cm. The purpose of the light box was to cancel out outside light and create uniform light within the box (Karcher and Richardson, 2005). The study analyzed the images taken for percent green cover using digital image analysis software SigmaScan (SigmaScan, SPSS, Chicago, IL) (Karcher and Richardson, 2005). The SigmaScan software is designed to create an average hue saturation and brightness level (HSB), which is a color space based upon human perception of color level for each image (Hejl, 2014). The images taken in June were taken at the beginning of the study and were not taken post-mowing.

2.3.6 Chemical Analysis of Soil Samples

To allow a full growing season to adjust to the different types of irrigation waters, soil samples were collected during the months of November and December to assess the effect of the different irrigation water on soil chemistry. For the chemical analysis at different soil depths, two core samples per plot were taken 2 December 2016 using a 5.1 cm diameter corer at 0-2.5 cm, 2.5-7.6 cm, and 7.6-12.6 cm depth increments.

The samples were allowed to air dry at 25° C then homogenized and sieved (2 mm and 0.5 mm) in preparation for chemical analysis. Approximately 3 g of soil was combined with 30 mL of DDW. Actual soil and water weights were measured and recorded. The soil/DDW solution in the centrifuge tubes were shaken at 60 rpm for two hours. The soils were then centrifuged using a S-34 fixed angle rotor at 16,000 rpm for 15 minutes at 19,974 g-force (Thermo Fisher Scientific LLC S, Asheville, NC, USA). Salinity, pH, and EC were recorded on unfiltered supernatant. The soil water extracts were then filtered with ashed (500 ◦ C for 4 hours) Whatman GF/F filters (nominal pore size 0.7 μm) to remove any floating organic material and the weight of the extract recorded. DDW was added to the extract (1:1 by weight) to ensure enough sample size. The extracts were then analyzed for NO_3-N, NH_4-N, PO_4-P, DOC, TDN, Ca, Mg, Na, K, B, S, P, Fe, Cu, and Mn.

Dissolved organic carbon and TDN were measured with high temperature platinum-catalyzed combustion using a Shimadzu TOC-VCSH and Shimadzu total measuring unit (TNM-1) (Shimadzu Corp, Houston, TX, USA). DOC was quantified as non-purgeable carbon using USEPA method 415.1, which entailed acidifying (2N HCl) the sample and sparging for 4 min with C-free air. Ammonium-N was analyzed using the phenate hypochlorite method with sodium nitroprusside enhancement (USEPA method 350.1), and nitrate-N was analyzed using Cd-Cu reduction (USEPA method 353.3). Orthophosphate-P was quantified using the ascorbic acid, molybdate blue method. Colorimetric methods were performed with a Westco Scientific Smartchem Discrete Analyzer (Westco Scientific Instruments Inc. Brookfield, CT, USA). DON was calculated as TDN–(NH_4–N + NO_3–N). Water blanks, replicate samples, NIST traceable, and check standards were run every 12th sample to monitor instrument precision.

The trace metals were detected and measured using inductive coupled plasma mass spectrometry (ICP) analysis.

Salt accumulation on the irrigated plots was monitored starting at the end of September through October with bi-weekly measurements of EC using a handheld EC meter (FieldScout EC 110 Meter, Spectrum Technologies, Inc., Aurora, IL). The readings were obtained for depths 0-2.54 cm and 0-7.62 cm unless the soil was too saturated with water due to rainfall. This was to compare the conductivity readings in the field to those obtained during the chemical analysis.

SAR was calculated using the formula: $SAR = \frac{Na}{\sqrt{\frac{(Ca)+(Mg)}{2}}}$ and %ESP was calculated using the formula: $exchangebale\ \left\{\frac{Na}{Ca+Mg+K+Na}\right\} x\ 100$.

2.3.7 Solvita CO_2 Respiration Test

All 60 of the December samples were evaluated for CO_2 respiration, as an indicator of microbial activity, using the Solvita CO_2-C Burst test (Solvita, CO_2-Burst, Mount Vernon, Maine). The samples were allowed to air dry at 25° C then homogenized and sieved (2 mm and 0.5 mm) in preparation CO_2 testing. Forty grams of the dried soil was placed into the 50 mL plastic capillary cup. The cup was then placed into a 475 mL jar and 20 mL of deionized water was added into the jar while avoiding spilling on the soil. The CO_2 detector probe was then added alongside the capillary cup with the gel probe facing the outside of the jar to be easily viewed. The lid of the jar was then sealed and allowed to incubate for 24 hr in an incubator at

20° C temperature. After the 24-hour incubation period, the probe was removed and read by the Solvita Digital Color Reader. The reader read for both color and mg CO_2/kg of soil.

2.3.8 Fungal Community Composition

At the end of October, before the turfgrass transitioned into dormancy, two soil samples were taken from each individual plot using a 2.5 cm diameter core sampler for the 0 - 12.6 cm depth and frozen at -20°C until chemical and DNA analysis. The frozen samples were homogenized and, using the same methodology from sections 2.3.4 and 2.3.6 were tested for pH, EC, salinity, NO_3-N, NH_4-N, PO_4-P, DOC, TDN, Ca, Mg, Na, K, B, S, P, Fe, Cu, and Mn.

The soil nucleic acids were extracted using the PowerSoil DNA Isolation Kit from MoBio Laboratories Inc. (Carlsbad, California), according to a slightly modified manufacturer's protocol. Instead of 0.25 g of soil sample, 0.50 g of soil sample was used. During step five of the manufacturer's protocol, the PowerBead Tube was vortexed for 20 minutes instead of 10. During step 15, 625 µL of the solution from step 14 was loaded multiple times onto a Spin Filter instead of the 675 µL. Finally, during step 20 only 50 µL of Solution C6 was added to the white filter membrane instead of the recommended 100 µL. The slight changes were made to the protocol in order to have optimum DNA yield from the DNA Isolation Kit.

Concentrations of purified DNA were determined by spectrophotometry (NanoDrop-1000, Waltham, MA). Extracted samples were shipped to the MR DNA Laboratory in Shallowater, Texas for analysis. The internal transcribed spacer 1-2 (fungi) PCR primers were used in a single-step 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA). These were the following conditions: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds, 72° C for 1 minute, and finally, 72° C for 5 minutes. Sequencing was performed on an Ion Torrent PGM following the manufacturer's guidelines. Sequence data were processed using a proprietary analysis pipeline. The sequences were depleted of barcodes and primers, then sequences <150 bp removed, sequences with ambiguous base calls, and with homopolymer runs exceeding 6 bp were also removed. Sequences were de-noised, OTUs generated and chimeras removed. Operational taxonomic units (OTUs) were defined by clustering at 3% divergence (97% similarity). Final OTUs were taxonomically classified using BLAST against a curated database derived from GreenGenes, RDPII and NCBI (www.ncbi.nlm.nih.go and http://rdp.cme.msu.edu).

2.3.9 Statistical Analyses

An initial multivariate analysis of variance using one factor with post hoc Tukey (HSD) tests was applied to the data to determine if there were any significant differences between the control treatment (RO) and the other four treatments: 1) WWTP effluent, 2) gray water, 3) municipal tap water, and 4) saline water ($p > 0.05$). An initial multivariate analysis of variance using one factor with post hoc Tukey (HSD) tests was applied to the data to determine if there were any significant differences between the three depths: 1) 0-2.54 cm, 2) 2.54-7.62 cm, and 3) 7.62-12.7 cm ($p < 0.05$).

A multivariate analysis of variance with two factors was applied to the data to determine if there was a significant effect of treatment or depth or an interaction between treatment and depth on CO_2 respiration and pH, EC, salinity, NO_3-N, NH_4-N, PO_4-P, DOC, TDN, Ca, Mg, Na, K, B, S, P, Fe, Cu, Mn, SAR, and ESP ($p < 0.05$). If there was a significant interaction, a one-way analysis of variance (ANOVA) with post hoc-Tukey (HSD) tests was performed on the nutrient for all irrigation treatments to test the hypotheses that irrigation treatment and depth would have a significant effect on soil nutrients.

If there was no depth by treatment interaction, data were pooled cross depths from both sampling months of the study; and a multivariate analysis of variance with two factors was used to determine if there was a significant effect of date, treatment, or an interaction between date and treatment on pH, EC, salinity, NO_3-N, NH_4-N, PO_4-P, DOC, TDN, Ca, Mg, Na, K, B, S, P, Fe, Cu, Mn, SAR, and ESP ($p < 0.05$). If there was a significant interaction, a one-way analysis of variance (ANOVA) with post hoc-Tukey (HSD) tests was performed on the nutrient for all irrigation treatments to test the hypotheses that irrigation treatment and date would have a significant effect on soil nutrients.

For nutrients with no significant date by treatment interaction, data were pooled cross both dates, and one-way analysis of variance (ANOVA) was performed with a post hoc Tukey (HSD) test on nutrients to determine there was an irrigation treatment main effect ($p < 0.05$).

A multivariate analysis of variance with two factors was used to determine if there was a significant effect of date, treatment, or an interaction between date and treatment on clipping biomass and percent green cover. If there was a significant interaction, a one-way analysis of variance (ANOVA) with post hoc-Tukey (HSD) tests was conducted on the clipping biomass and percent green cover for all irrigation treatments to test the hypotheses that irrigation treatment and date would have a significant effect on clipping biomass and percent green cover. A multivariate analysis of variance with two factors was used to determine if there was a significant effect of depth, treatment, or an interaction between depth and treatment on soil microbial activity.

In order to determine if fungal community composition was significantly different between samples, one-way permutational multivariate analysis of variance (PERMANOVA) was conducted on fungal species percentage data. The same matrix was used to perform one-way analysis of similarity (ANOSIM) to double check the result of the PERMANOVA and evaluate dissimilarities between treatments. While there were hundreds of species returned, only species representing greater than 1% of the community were used for comparison. The fungal community structure was estimated using Bray-Curtis distances and then visualized using principal coordinate analysis (PCoA) and non-metric multidimensional scaling analysis (nMDS) using dissimilarity matrices (Hammer et al., 2001) in PAST 4.X (University of Oslo 2020).

2.4 Results

2.4.1 Comparative Analysis of Soil Depth Chemistry

A repeated univariate linear model of analysis of variance with a post hoc Tukey test was applied to the soil chemistry and treatments to determine if there were significant differences between the control treatment and the other four treatments (Table 3). The null (H_{O-1}) hypothesis was rejected. There were no significant differences in NO_3-N, NH_4-N, PO_4-P, Ca, Mg, K, B, P, Zn, and Cu between the control treatment and saline water, gray water, wastewater treatment plant effluent, and municipal tap water treatments. The alternative hypothesis (H_1) was accepted. pH, EC, salinity, DOC, TDN, DON, Na, S, Fe, Mn, SAR, and ESP showed significant differences between the saline and control treatments ($p < 0.05$) and was verified by Tukey's Studentized Range post-hoc analysis.

Dissolved organic carbon, TDN, DON, and Fe showed significant differences between the control and gray water and WWTP effluent. Sodium, SAR, and ESP showed significant differences between the control and all four other treatments. The saline treatment had a significantly higher pH, EC, salinity, S, and Mn, than the control water treatment ($p < 0.05$). The DOC, TDN, DON and Fe concentrations in the saline, WWTP effluent, and gray water treatments were significantly higher than the MTW and control treatments ($p < 0.05$). All four treatments had significantly higher concentrations of Na, and SAR and ESP compared to the control treatments ($p < 0.05$; Table 3).

Due to these significant differences, a univariate linear model of analysis of variance was applied to all dependent variables to determine if there was a significant effect of depth (Table 4). The second null hypothesis (H_{O-2}) was rejected. The alternative hypothesis (H_2) was accepted. pH, EC, NH_4-N, Mg, P, and S showed significant differences between depth 1 and depths 2 and

3 but showed no significant differences between depth 2 and depth 3 ($p < 0.05$). There were significant differences in depth 1 and 3 for salinity, Zn, SAR, and ESP ($p < 0.05$). Orthophosphate-P, DOC, TDN, DON and Na showed significant differences between all three depths ($p < 0.05$). There were no significant differences between depth 1 and depth 2 for NO_3-N; there were no significant differences between depth 1 and depth 3 for Cu ($p < 0.05$; Table 4).

Table 3. Multiple Comparisons of soil depth chemistries for each treatment. Values in bold indicate significant difference between control and treatment at $p < 0.05$, those not bolded indicate $p > 0.05$.

Soil Nutrient	TMT	TMT	Sig	Soil Nutrient	TMT	TMT	Sig
pH	RO	WWTP	**0.822**	Na	RO	**WWTP**	**<0.001**
		MTW	**0.323**			**MTW**	**<0.001**
		SALINE	**0.006**			**SALINE**	**<0.001**
		GRAY	**0.349**			**GRAY**	**<0.001**
EC	RO	WWTP	0.926	S	RO	WWTP	0.893
		MTW	1			MTW	0.854
		SALINE	**<0.001**			**SALINE**	**<0.001**
		GRAY	0.702			GRAY	0.462
Salinity	RO	WWTP	0.941	Fe	RO	**WWTP**	**0.007**
		MTW	0.933			MTW	0.475
		SALINE	**<0.001**			**SALINE**	**0.005**
		GRAY	0.744			**GRAY**	**0.038**
DOC	RO	**WWTP**	**0.041**	Mn	RO	WWTP	0.675
		MTW	0.996			MTW	0.834
		SALINE	**<0.001**			**SALINE**	**<0.001**
		GRAY	**<0.001**			GRAY	0.991
TDN	RO	**WWTP**	**0.008**	SAR	RO	**WWTP**	**<0.001**
		MTW	0.996			**MTW**	**0.001**
		SALINE	**0.03**			**SALINE**	**<0.001**
		GRAY	**0.005**			**GRAY**	**<0.001**
DON	RO	**WWTP**	**0.024**	ESP	RO	**WWTP**	**<0.001**
		MTW	0.985			**MTW**	**<0.001**
		SALINE	**<0.001**			**SALINE**	**<0.001**
		GRAY	**0.003**			**GRAY**	**<0.001**

Table 4. Multiple Comparisons of nutrients at different depths. Values bolded indicate significant difference between control and treatment at $p < 0.05$, those not bolded indicate $p > 0.05$.

Soil Nutrient	DEPTH	DEPTH	Sig.	Soil Nutrient	DEPTH	DEPTH	Sig.	Soil Nutrient	DEPTH	DEPTH	Sig.
pH	1	2	**<0.001**	DOC	1	2	**<0.001**	P	1	2	**0.032**
		3	**<0.001**			3	**<0.001**			3	**<0.001**
	2	1	**<0.001**		2	1	**<0.001**		2	1	**0.032**
		3	0.241			3	**<0.001**			3	207
	3	1	**<0.001**		3	1	**<0.001**		3	1	**<0.001**
		2	0.241			2	**<0.001**			2	0.207
EC	1	2	**0.031**	TDN	1	2	**<0.001**	Zn	1	2	0.39
		3	**0.008**			3	**<0.001**			3	**0.035**
	2	1	**0.031**		2	1	**<0.001**		2	1	0.39
		3	0.859			3	**<0.001**			3	0.426
	3	1	**0.008**		3	1	**<0.001**		3	1	**0.035**
		2	0.859			2	**<0.001**			2	0.426
SALINITY	1	2	0.092	DON	1	2	**0.001**	Cu	1	2	**<0.001**
		3	**0.034**			3	**<0.001**			3	0.961
	2	1	0.092		2	1	**0.001**		2	1	**<0.001**
		3	0.898			3	**0.033**			3	**<0.001**
	3	1	**0.034**		3	1	**<0.001**		3	1	0.961
		2	0.898			2	**0.033**			2	**<0.001**
NO_3-N	1	2	0.71	Mg	1	2	**<0.001**	SAR	1	2	0.155
		3	**<0.001**			3	**<0.001**			3	**0.003**
	2	1	0.71		2	1	**<0.001**		2	1	0.155
		3	**<0.001**			3	0.977			3	0.254
	3	1	**<0.001**		3	1	**0.002**		3	1	**0.003**
		2	**<0.001**			2	0.977			2	0.254
NH_4-N	1	2	**<0.001**	Na	1	2	**<0.001**	ESP	1	2	0.833
		3	**<0.001**			3	**<0.001**			3	**0.014**
	2	1	**<0.001**		2	1	**<0.001**		2	1	0.833
		3	0.602			3	0.018			3	0.059
	3	1	**<0.001**		3	1	**<0.001**		3	1	**0.14**
		2	0.602			2	0.018			2	0.059
PO_4-P	1	2	**0.008**	S	1	2	**<0.001**				
		3	**<0.001**			3	**<0.001**				
	2	1	**0.008**		2	1	**<0.001**				
		3	**<0.001**			3	0.072				
	3	1	**<0.001**		3	1	**<0.001**				
		2	**<0.001**			2	0.072				

Since there were significant differences between the control and the other four treatments for EC, salinity, DOC, DON, Na, S, Fe, Mn, and SAR, a univariate analysis of variance with two factors was applied to determine if there was significant interaction between depth and the treatment on all nutrients ($p < 0.05$; Table 5). The null hypothesis ($H_{O\text{-}3}$) was rejected. There was no significant interaction between depth and pH, EC, salinity, NO_3-N, NH_4-N, PO_4-P, DOC, DON, TDN, Ca, Mg, K, B, S, P, Fe, Cn, Mn, SAR, and ESP ($p < 0.05$). The alternative hypothesis (H_3) was accepted. There was significant interaction between depth and irrigation treatment for Na and Cu. A one-way analysis of variance (ANOVA) with post hoc-Tukey (HSD) tests was conducted on the nutrient for all irrigation treatments to test the hypotheses that irrigation treatment and depth would have a significant effect on soil nutrients. (Figure 7-8).

Table 5. Effect of depth, irrigation treatment, and interaction of *depth*treatment* on soil chemistry. Values in bold indicate a significant interaction effect of depth x treatment at $p < 0.05$, those not bolded indicate $p > 0.05$

	pH	**EC**	**SALINITY**	**NO_3-N**	**NH_4-N**	**PO_4-P**	**DOC**	**DON**	**TDN**	**Na**	**Ca**
Depth	<0.001	0.006	0.029	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.548
Treatment	0.013	<0.001	<0.001	0.019	0.46	0.274	<0.001	<0.001	<0.001	<0.001	0.018
Treat*Depth	0.468	0.549	0.523	0.778	0.796	0.912	0.469	0.27	0.713	***0.002**	0.889
	Mg	**K**	**B**	**S**	**P**	**Fe**	**Zn**	**Cu**	**Mn**	**SAR**	**ESP**
Depth	<0.001	0.728	0.755	<0.001	<0.001	0.66	0.045	<0.001	0.296	0.005	0.013
Treatment	0.001	0.379	0.393	<0.001	0.794	0.002	0.733	0.013	<0.001	<0.001	<0.001
Treat*Depth	0.119	0.419	0.056	0.946	0.674	0.454	0.377	**<0.001**	0.159	0.595	0.646

2.4.1.1 Sodium

The saline treatment at depth 1 had the significantly greatest concentration of Na (489.2±46.2 μg/g). The saline water treatments at depth 2 (368.5±48.4 μg/g) and depth 3 (347.1±35.2 μg/g) were significantly greater than all other treatments except for the gray water treatment at depth 1 (308.3±32.1 μg/g).

The WWTP treatments at depth 1 (308.3±32.1 μg/g) and depth 3 (347.1±35.2 μg/g) were significantly higher than all other treatments except for the gray water treatments at depth 1 (308.3±32.1 μg/g) and depth 2 (211.1±29.7 μg/g). The WWTP treatment at depth 2 (146.5±18.6 μg/g) was not significantly different from the RO (71.1±17.0 μg/g) and municipal tap (185.6±17.0 μg/g) water treatments at depth 1, the municipal tap (153.7±28.1 μg/g) and gray (211.1±29.7 μg/g) water treatments at depth 2, and the WWTP (138.1±4.2 μg/g), the municipal tap (122.4±22.1 μg/g), and the gray (144.9±19.2 μg/g) treatments at depth 3.

The RO treatment at depth 3 (29.6±20.9 μg/g) and depth 2 (42.1±19.4 μg/g) had the significantly lowest concentrations of Na (29.6±20.9 μg/g; $p < 0.05$) compared to all the other treatments except for the RO treatments at depth 1 (71.1±17.0 μg/g). The RO treatment at depth 1 was not significantly different from the WWTP (146.5±18.6 μg/g) at depth 1, the WWTP at depth 2 (138.1±29.7 μg/g), and the municipal tap (122.4±22.1 μg/g) and gray water (144.9±19.2 μg/g) treatments at depth 3.

Municipal tap water treatments at depth 1 (185.6±17.0 μg/g) and depth 2 (153.7±28.1 μg/g) were not significantly different from the WWTP (146.5±18.6 μg/g), the municipal tap water (153.7±28.1 μg/g), and gray water (211.1±29.7 μg/g) treatments at depth 2, and the

WWTP (138.1±4.2 μg/g), the municipal tap (122.4±22.1 μg/g), and the gray water (144.9±19.2 μg/g) treatments at depth 3.

The gray water treatment at depth 1 (308.3±32.1 μg/g) was significantly higher than all other treatments except the WWTP at depth 1 (267.6±20.9 μg/g) and the saline treatments at depth 2 (368.5±28.4 μg/g) and depth 3 (347.1±35.2) μg/g. The gray water treatment at depth 2 (211.1±29.7 μg/g) was not significantly different from the gray water treatment at depth 3 (144.9±19.2 μg/g). As the soil gets deeper, there is an overall trend of decreasing Na concentrations.

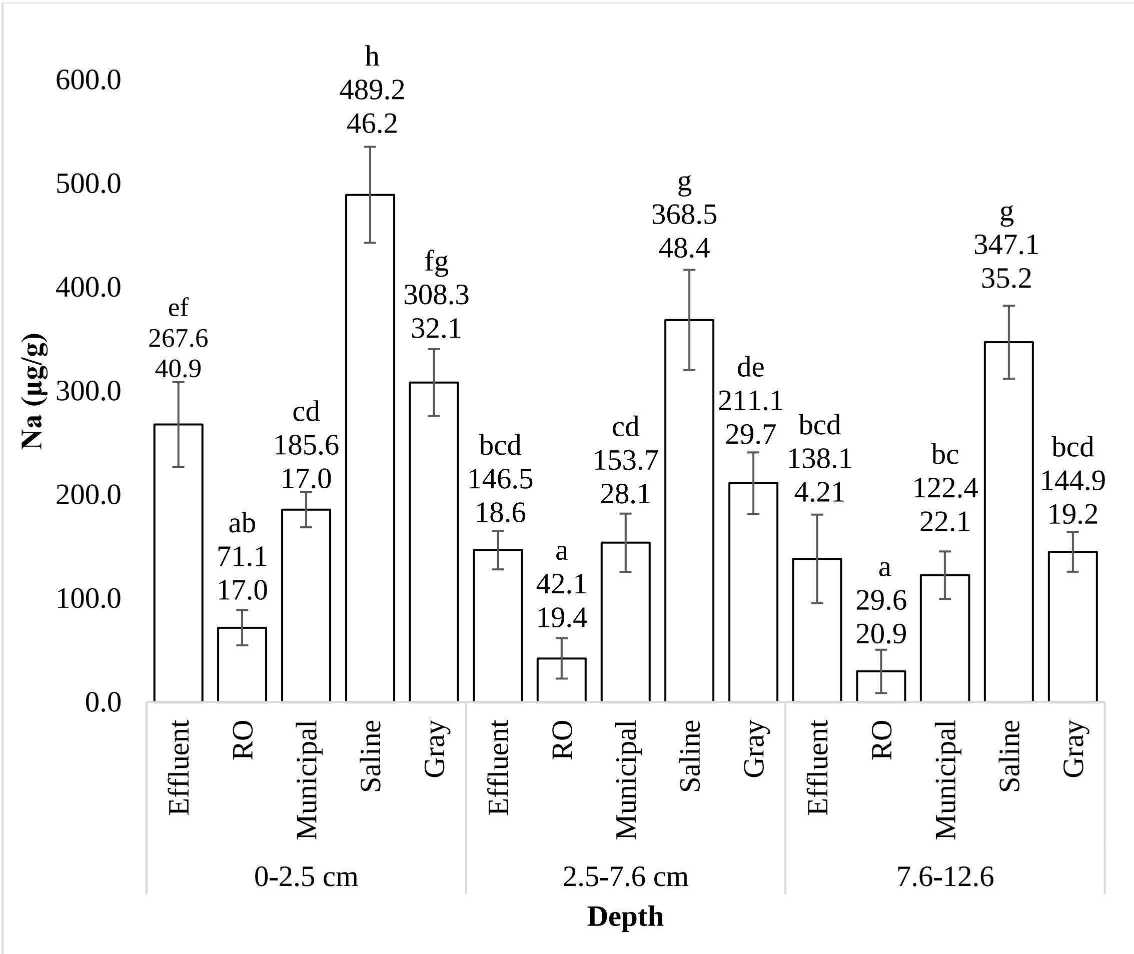

Figure 7. Average soil Na among depth and treatments. Different letters indicate significant difference ($p < 0.05$ Tukeys HSD post-hoc test). Error bars are the standard deviation of the mean. Values above each bar indicate mean and standard deviation.

2.4.1.2 Copper

The only significant difference in Cu was at depth 2. The municipal tap water treatment had the significantly highest concentration of Cu (0.58±0.21).

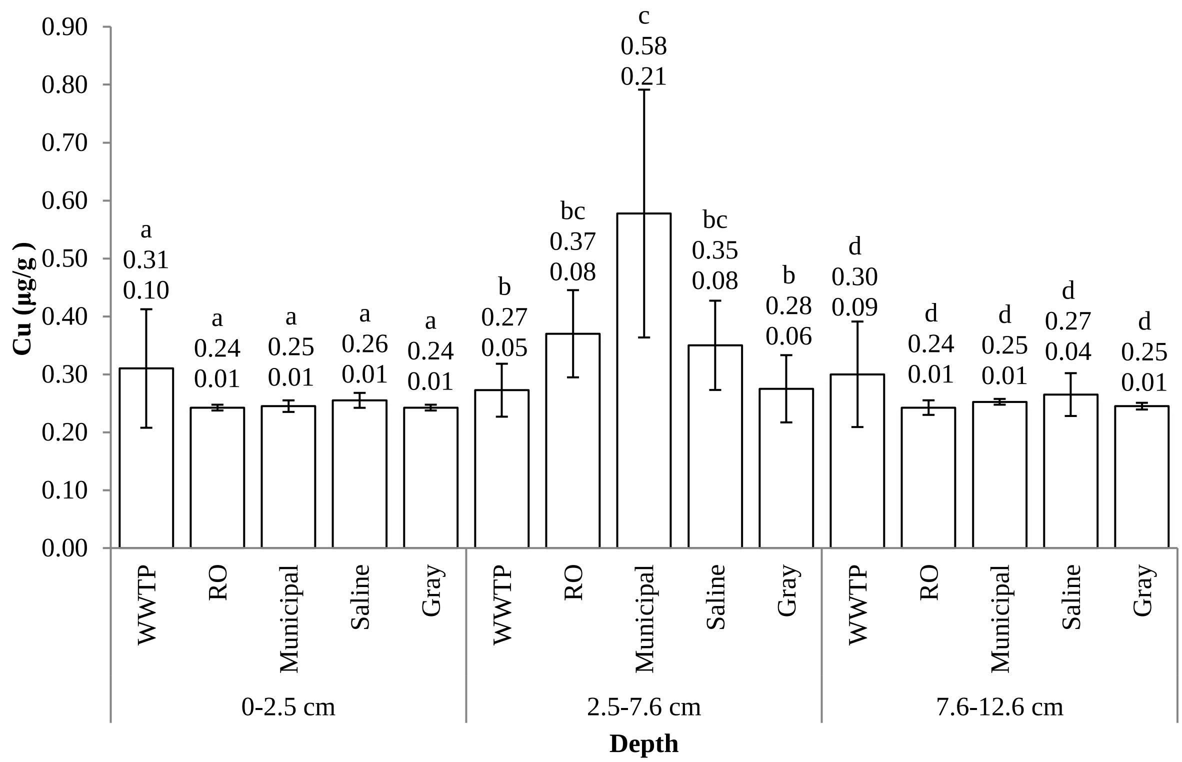

Figure 8. Average soil Cu among depth and treatments. Different letters indicate significant difference (p <0.05 Tukeys HSD post-hoc test). Error bars are the standard deviation of the mean. Values above each bar indicate mean and standard deviation.

2.4.2 Date Series of Soil Chemistries

Because of there being only a few depth by treatment interactions, the depths were pooled together for each sampling event. The soil chemistries were examined at both dates to see if there were any significant date by treatment interactions (Table 6). The null hypothesis (H_{O-4}) was rejected. There was no date by treatment interaction for pH, EC, NO_3-N, NH_4-N, PO_4-P, Ca, Na, Mg, K, B, P, Fe, Zn, S, Mn, and SAR. The hypothesis (H_4) was accepted. Salinity, DOC, TDN, DON, Cu, and ESP had significant date by treatment interactions (Figure 9-13).

Table 6. Effect of date, irrigation treatment, and interaction of *date*treatment* on soil chemistry. Values in bold indicate a significant effect of date*treatment at $p < 0.05$, those not bolded indicate $p > 0.05$.

	pH	**EC**	**SALINITY**	**NO_3-N**	**NH_4-N**	**PO_4-P**	**DOC**	**TDN**	**DON**	**Na**	**Ca**
Date	<0.001	<0.001	<0.001	<0.001	<0.001	0.072	<0.001	<0.001	<0.001	0.207	0.164
Treatment	<0.001	<0.001	<0.001	0.001	0.886	0.553	0.003	0.023	0.109	<0.001	0.047
Date*Treat	0.649	0.185	**0.016**	0.788	0.215	0.834	**0.002**	**0.006**	**0.001**	0.540	0.556
	Mg	**K**	**B**	**S**	**P**	**Fe**	**Zn**	**Cu**	**Mn**	**SAR**	**ESP**
Date	<0.001	0.439	0.051	0.019	0.928	<0.001	0.008	0.600	0.148	0.261	0.584
Treatment	0.036	0.274	0.563	<0.001	0.101	0.335	0.850	0.069	0.153	<0.001	<0.001
Date*Treat	0.640	0.562	0.735	0.356	0.217	0.933	0.612	**0.049**	0.067	0.262	**0.041**

2.4.2.1 Salinity

There were no significant differences in soil salinity for the WWTP (0.05±0.01 ppt), RO (0.03±0.01 ppt), gray (0.04±0.01 ppt), and municipal tap (0.04±0.01 ppt), water treatments in the November sampling and the WWTP (0.07±0.00 ppt), RO (0.06±0.01 ppt), gray (0.05±0.02 ppt), municipal tap (0.08±0.01ppt), water treatments for the December sampling. The WWTP (0.07±0.00), and the municipal tap (0.08±0.01 ppt), water irrigated soil had significantly higher salinity than the RO (0.03±0.01 ppt), gray (0.04±0.01 ppt), and municipal tap (0.08±0.01 ppt), irrigated soils. The saline water irrigated soils for both sampling dates had the significantly highest concentrations of salinity: November (0.12±0.02 ppt), December (0.12±0.01 ppt; Figure 9).

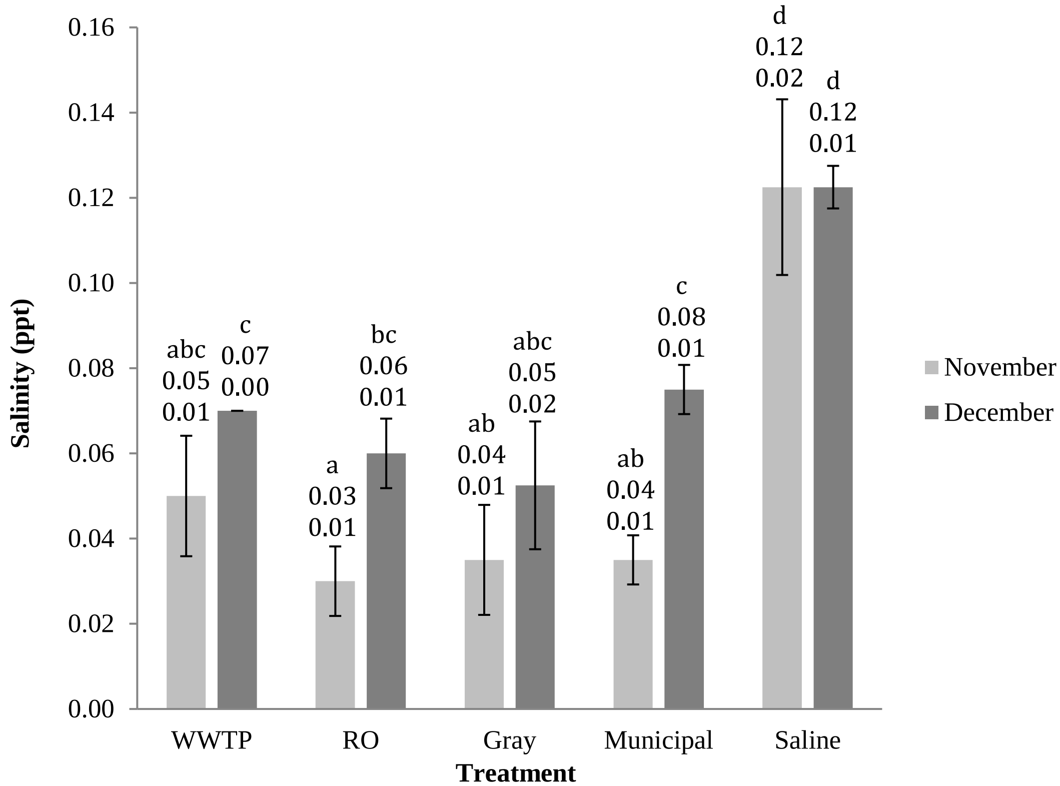

Figure 9. Average soil salinity among date and treatments. Bars with the same letter are not significantly different between months based on Tukey's HSD ($p < 0.05$). Error bars denote standard deviation of the mean. Values above bars indicate mean (top) and standard deviation (bottom).

2.4.2.2 Dissolved Organic Carbon

The DOC for the WWTP treatment at the December sampling (432.7±49.0 μg/g) was significantly greater than the WWTP (284.2±53.2 μg/g), RO (267.1±26.5 μg/g), gray (255.5±45.3 μg/g), municipal tap (266.3±53.9 μg/g), saline (256.0±46.0 μg/g) treatments at the November sampling. The DOC for the WWTP for the December sampling was not significantly different than any other treatment at the December sampling. The DOC for the WWTP (284.2±53.2 μg/g) treatment at the November sampling was not significantly different than any other treatment at the November sampling. The DOC for the WWTP (284.2±53.2 μg/g) treatment for November was significantly lower than the municipal tap (472.3±54.8 μg/g) and saline water (520.5±14.4 μg/g) treatments at the December sampling.

The DOC for the RO treatment at the December sampling (359.9±45.3 μg/g) was significantly lower than the municipal tap (472.3±54.8 μg/g), and saline water (520.5±14.4 μg/g) treatments for the December sampling and significantly greater than the gray (255.5±45.3 μg/g), and saline water (256.0±46.0 μg/g) treatments for the November sampling. The DOC for the RO treatment at the November sampling (359.9±45.3 μg/g) was not significantly different from any other treatment for the November sampling but significantly lower than the municipal tap (472.3±54.8 μg/g), and saline water (520.5±14.4 μg/g) treatments for the December sampling.

The DOC for the gray water treatment at the December sampling (369.1±9.7 μg/g) was significantly lower than the saline water (520.5±14.4 μg/g) treatment for December sampling and significantly greater than the gray (255.5±45.3 μg/g) and saline water treatments for the November sampling (256.0±46.0 μg/g). The DOC for the gray water treatment at the December sampling (369.1±9.7 μg/g) was not significantly different from both municipal tap water treatments at the November (266.3±53.9 μg/g) and the December (472.3±54.8 μg/g) samplings.

The DOC for the gray water treatment at the November sampling (255.5±45.3 μg/g) was significantly lower than all other treatments in the December sampling and was not significantly different from any treatment in the November sampling.

The DOC for the municipal tap water treatment at the December sampling (472.3±54.8 μg/g) was significantly greater than both the municipal tap (266.3±53.9 μg/g) and saline (256.0±46.0 μg/g) water treatments for the November sampling. The DOC for the municipal tap water treatment for the December sampling (472.3±54.8 μg/g) was not significantly different from the saline water treatment for the December sampling (520.5±14.4 μg/g).

The DOC for the saline water treatment at the December sampling (520.5±14.4 μg/g) was significantly greater than all other treatments at each sampling date except for the WWTP (432.7±49.0 μg/g) and municipal tap (472.3±54.8 μg/g) water treatments for the December sampling (Figure 10).

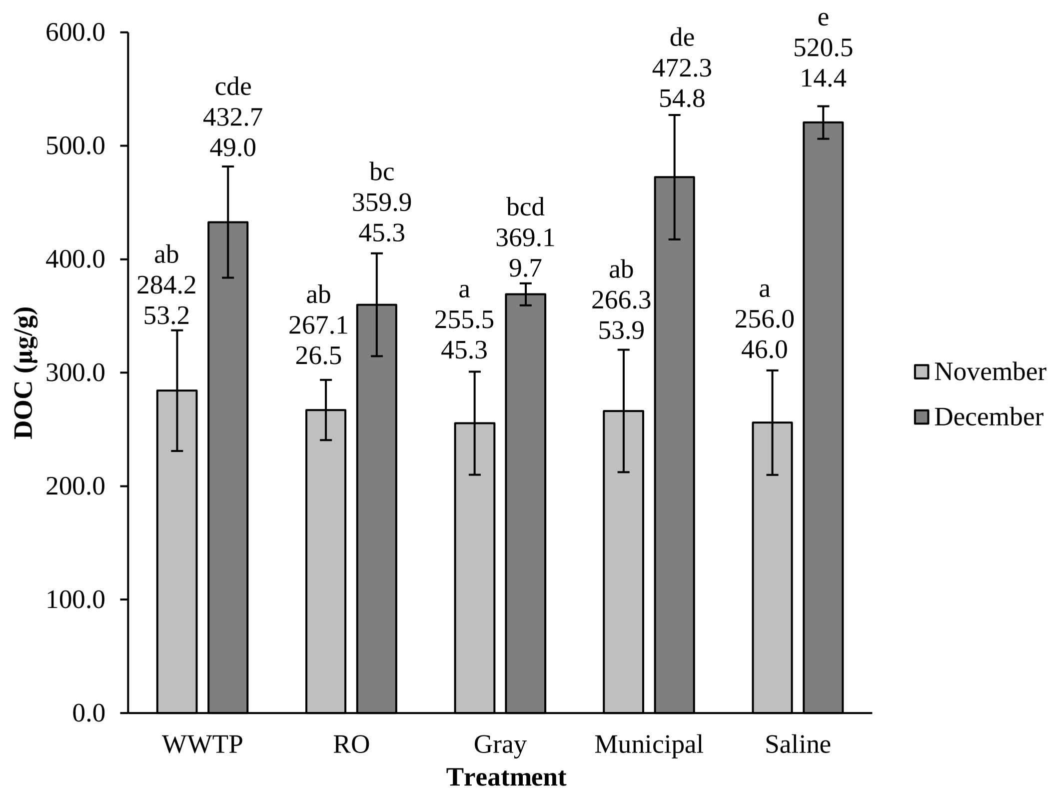

Figure 10. Average soil DOC among date and treatments. Bars with the same letter are not significantly different between months based on Tukey's HSD ($p < 0.05$). Error bars denote standard deviation of the mean. Values above bars indicate mean (top) and standard deviation (bottom).

2.4.2.3 Total Dissolved Nitrogen

The TDN concentrations for all treatments in the November sampling were not significantly different; the TDN concentrations for all treatments in the December sampling were not significantly different. The TDN in all treatments for the December sampling was significantly greater than their counterparts in the November sampling (Figure 11).

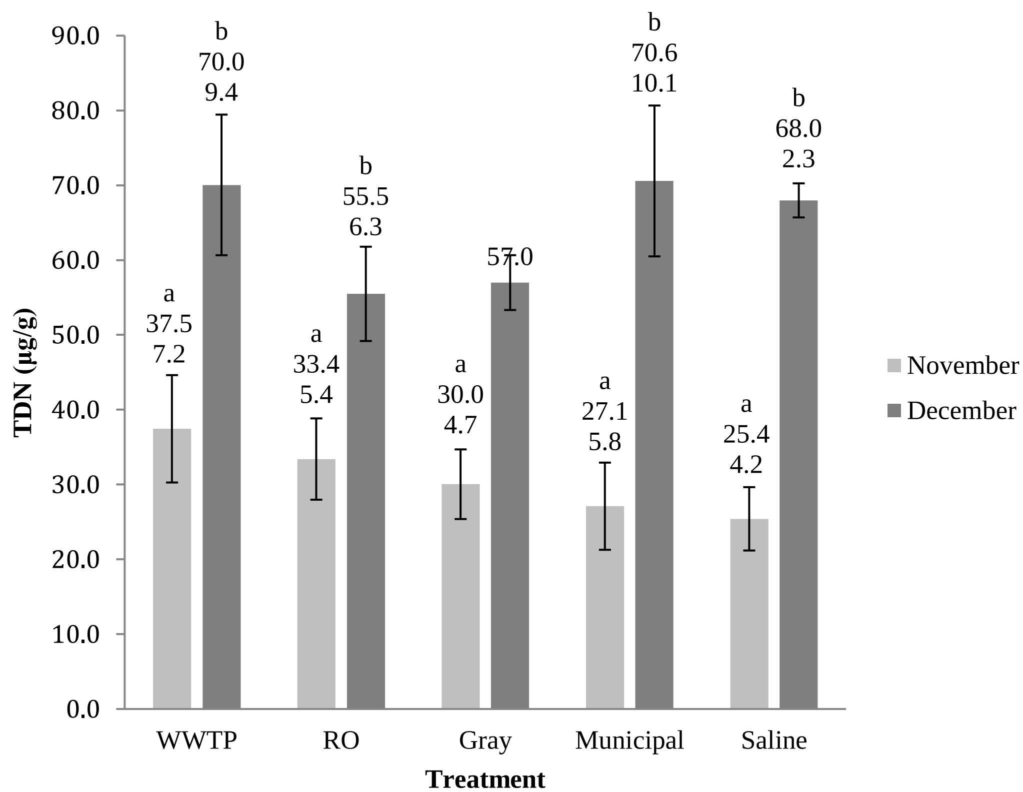

Figure 11. Average soil TDN among date and treatments. Bars with the same letter are not significantly different between months based on Tukey's HSD ($p < 0.05$). Error bars denote standard deviation of the mean. Values above bars indicate mean (top) and standard deviation (bottom).

2.4.2.4 Dissolved Organic Nitrogen

There were no significant differences in DON concentrations between any of the treatments for the November sampling. The saline treatment (50.3±4.8 μg/g) for the December sampling had the significantly greatest concentration of DON with the except of the municipal tap (47.0±5.4 μg/g) and the WWTP (44.6±5.8 μg/g) treatments for the December sampling. The saline water treatment for the November sampling (21.7±4.2 μg/g) was significantly lower than the WWTP (44.6±5.8 μg/g) , RO (34.6±5.2 μg/g), gray (36.3±2.9 μg/g), and municipal tap (47.0±5.4 μg/g) water treatments for the December sampling.

The WWTP treatment (23.2±4.5 μg/g) DON concentration for the November sampling was significantly lower than the WWTP (44.6±5.8 μg/g), gray water (36.3±2.9 μg/g), and the municipal tap (47.0±5.4 μg/g) for the December sampling. The WWTP for the December sampling (44.6±5.8 μg/g) was significantly greater than the RO (27.6±5.1 μg/g), gray (23.5±5.2 μg/g), municipal tap (22.2±2.8 μg/g), and saline (21.7±4.2 μg/g) water treatments for the November sampling. The WWTP for the December sampling was not significantly different from the RO (34.6±5.2 μg/g), gray (36.3±2.9 μg/g), and municipal tap (47.0±5.4 μg/g) for the December sampling.

The RO treatment (27.6±5.1 μg/g) DON concentration for the November sampling was significantly lower than the municipal tap (47.0±5.4 μg/g) water treatment for the December sampling. The RO treatment for the November sampling was not significantly different from the RO (34.6±5.2 μg/g) and gray water (36.3±2.9 μg/g) treatments for the December sampling. The RO treatment for the December sampling was significantly greater than the municipal tap (22.2±2.8 μg/g) and saline (21.7±4.2 μg/g) water treatments for the November sampling and significantly lower than the municipal tap (47.0±5.4 μg/g) for the December sampling.

The gray water treatment (23.5±5.2 μg/g) DON concentration for the November sampling was significantly lower than the gray water (36.3±2.9 μg/g) and municipal tap (47.0±5.4 μg/g) water treatment for the December sampling. The gray water treatment (36.3±2.9 μg/g) for the December sampling was significantly greater than the municipal tap (22.2±2.8 μg/g) and saline (21.7±4.2 μg/g) water treatments for the November sampling. The gray water treatment for the December sampling was not significantly different for the municipal tap (47.0±5.4 μg/g) for the December sampling.

The municipal tap (22.2±2.8 μg/g) water treatment for the November sampling was significantly lower than the saline water (50.3±4.8 μg/g) treatment for December sampling (Figure 12).

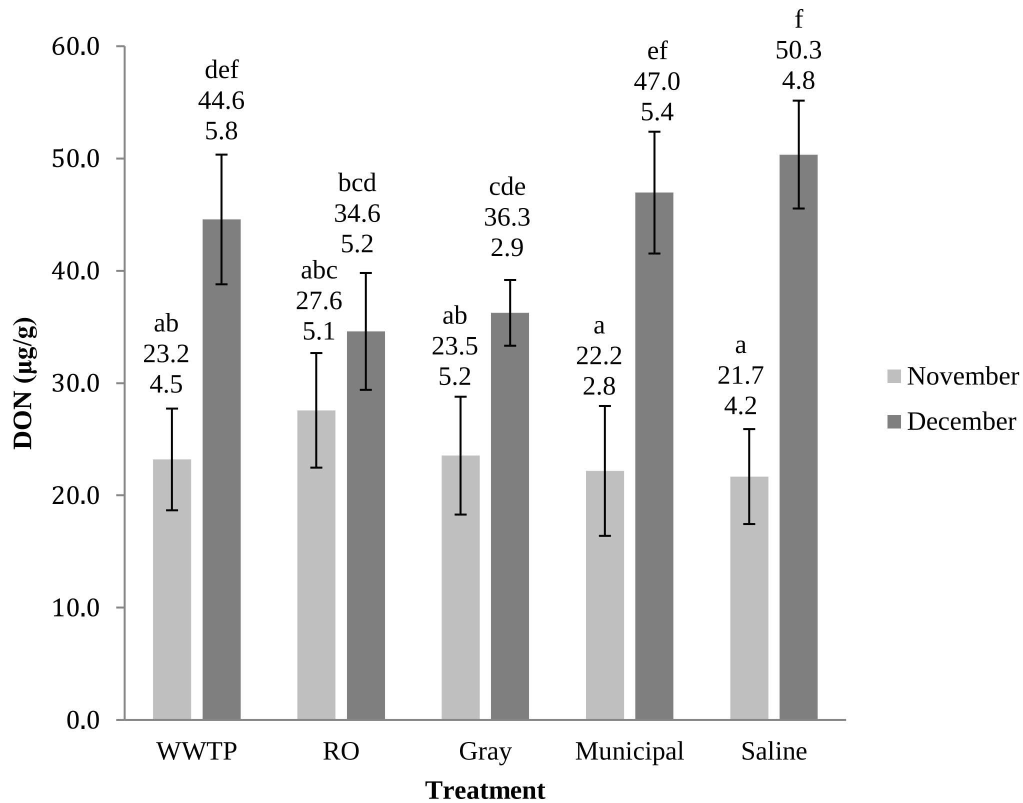

Figure 12. Average soil DON among date and treatments. Bars with the same letter are not significantly different between months based on Tukey's HSD ($p < 0.05$). Error bars denote standard deviation of the mean. Values above bars indicate mean (top) and standard deviation (bottom).

2.4.2.5 Copper

The Cu concentration for the WWTP treatment in the November sampling (0.9±0.9 μg/g) was significantly greater than the RO (0.12±0.0 μg/g), gray (0.2±0.0 μg/g), municipal tap (0.2±0.1 μg/g), and saline (0.2±0.1 μg/g) water treatments for the November sampling. The Cu concentration for the WWTP in the November sampling was not significantly different from the WWTP (0.3±0.0 μg/g), RO (0.3±0.0 μg/g), gray (0.4±0.1 μg/g), municipal tap (0.3±0.0 μg/g), and the saline (0.3±0.0 μg/g) water treatments for the December sampling. There were no significant differences in Cu concentrations between the treatments in the November samplings and their December counterparts with the exception of WWTP in the November sampling (Figure 13).

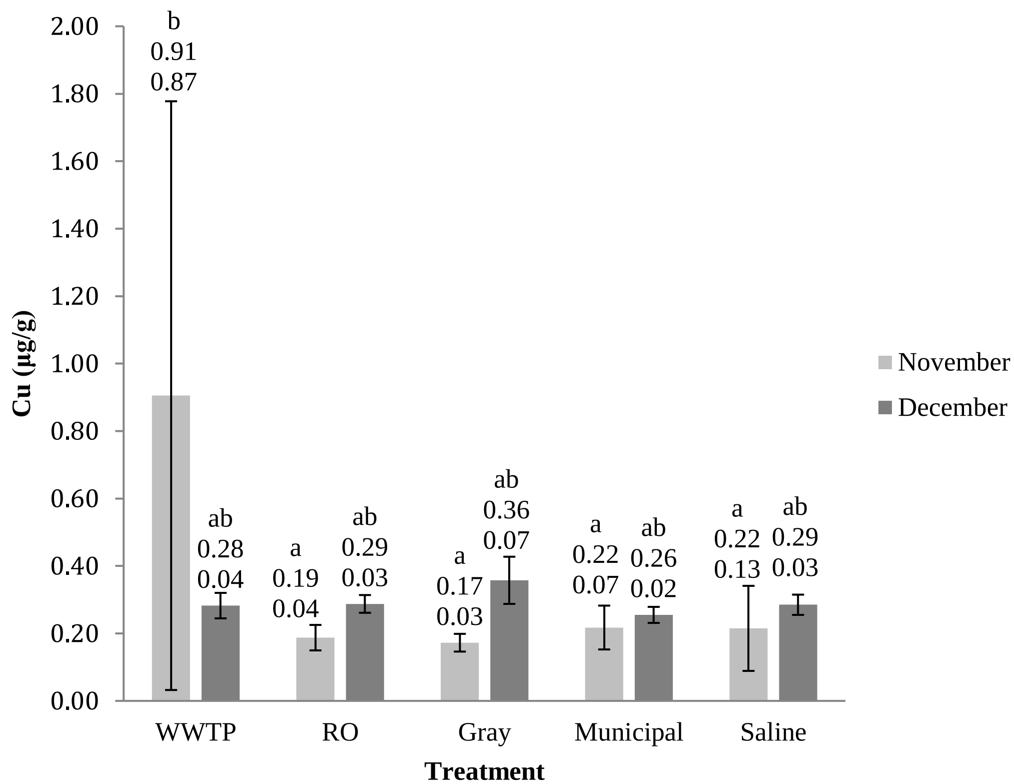

Figure 13. Average soil Cu among date and treatments. Bars with the same letter are not significantly different between months based on Tukey's HSD ($p < 0.05$). Error bars denote standard deviation of the mean. Values above bars indicate mean (top) and standard deviation (bottom).

2.4.2.6 Exchangeable sodium percentage

There were no significant differences in ESP between the WWTP (39.3±5.4 %), municipal tap, (39.2±7.0 %), and saline (52.3±13.1 %) water treatments for the November sampling and the WWTP (39.5±3.9 %), gray (37.7±30 %), municipal tap (48.6±7.3 %), and saline (43.2±7.5 %) water treatments for the December sampling. The saline water treatment ESP from the November sampling and the municipal tap water treatment ESP from the December sampling were significantly higher than the RO treatments at the November (17.1±8.1%) and December (12.2±1.9 %) samplings and the gray water (27.0±8.8 %) treatment in the November sampling. RO treatments had the significantly lowest ESP in comparison with the other treatments at each sampling date with the exception of the gray water treatment at the November sampling (Figure 14).

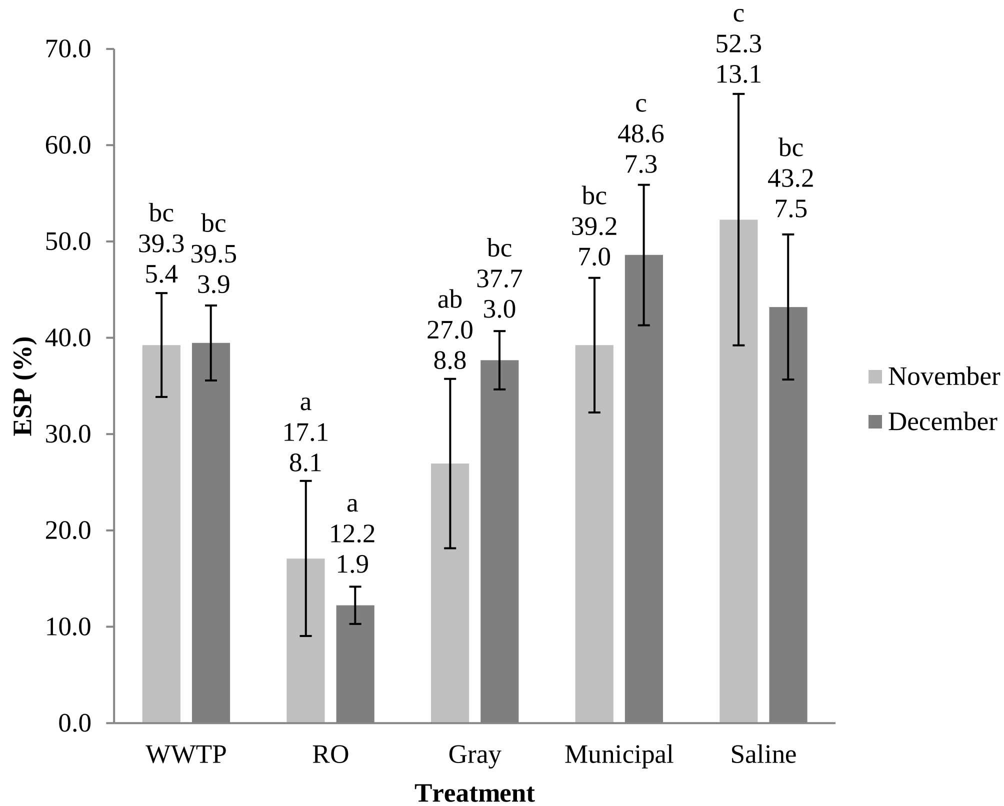

Figure 14. Average soil ESP among date and treatments. Bars with the same letter are not significantly different between months based on Tukey's HSD ($p < 0.05$). Error bars denote standard deviation of the mean. Values above bars indicate mean (top) and standard deviation (bottom).

2.4.3 Treatment Main Effect on Soil Chemistries

After examining the different interactions between depth by treatment and date by treatment, the treatment main effect on soil treatments was examined by pooling the data from the two sampling dates: November and December. Based on the univariate analysis, there was a significant treatment impact on pH, EC, NO_3-N, Na, Ca, Mg, S and SAR. One-way analysis of variance (ANOVA) was performed with a post hoc Tukey (HSD) test on nutrients to determine there was an irrigation treatment main effect. However, if an interaction was discussed for a nutrient in the previous section then it is not included in this section. ANOVA showed no significant differences in Mg or NO_3-N concentrations among the five different irrigated soils so it will not be discussed in this section.

Table 7. Main effect of treatment on soil chemistry. Values in bold indicate a significant effect of treatment at $p < 0.05$, those not bolded indicate $p > 0.05$.

	pH	**EC**	**SALINITY**	**NO_3-N**	**NH_4-N**	**PO_4-P**	**DOC**	**TDN**	**DON**	**Na**	**Ca**
Date	<0.001	<0.001	<0.001	<0.001	<0.001	0.072	<0.001	<0.001	<0.001	0.207	0.164
Treatment	**<0.001**	**<0.001**	<0.001	**0.001**	0.886	0.553	0.003	0.023	0.109	**<0.001**	**0.047**
	Mg	**K**	**B**	**S**	**P**	**Fe**	**Zn**	**Cu**	**Mn**	**SAR**	**ESP**
Date	<0.001	0.439	0.051	0.019	0.928	<0.001	0.008	0.600	0.148	0.004	0.004
Treatment	**0.036**	0.274	0.563	**<0.001**	0.101	0.335	0.850	0.069	0.153	**<0.001**	<0.001

2.4.3.1 pH

The pH levels in the WWTP (7.55±0.24), municipal tap (7.64±0.17), and gray water treatments (7.66±0.18) were not significantly different from the pH levels in the RO and the saline water treatments. The RO and saline water treatments had significantly different pH levels. The RO treatment had a significantly lower (7.43±0.25) pH level than the saline water treatment (7.79±0.19; Figure 15).

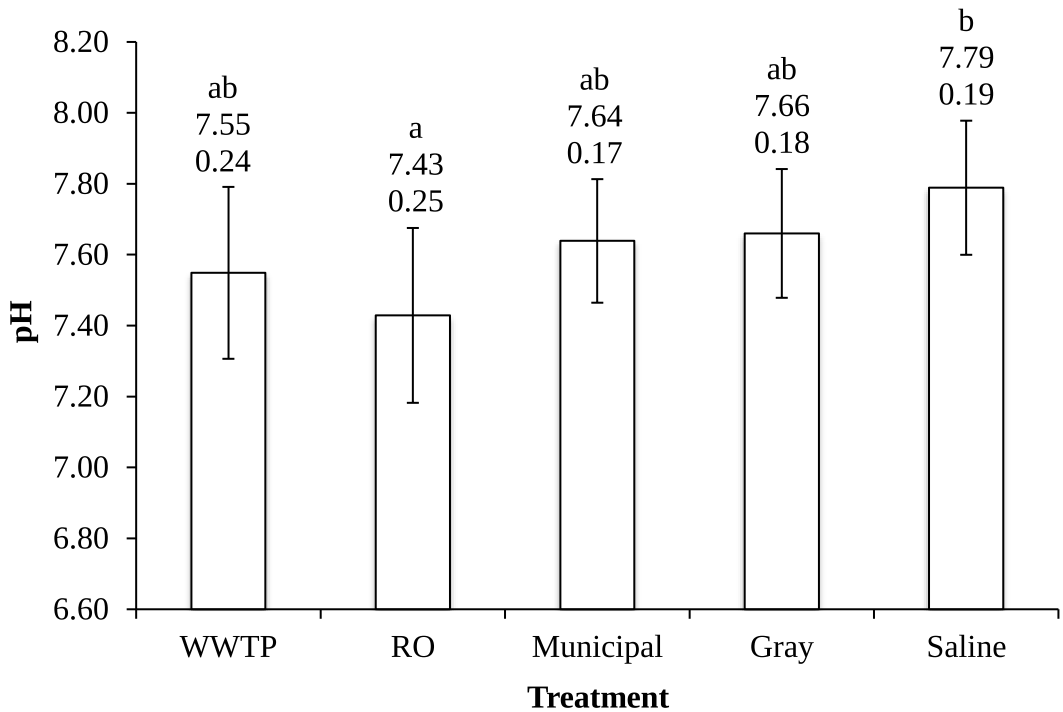

Figure 15. Average soil pH among irrigation treatments. Different letters indicate significant difference among treatments at $p < 0.05$ (Tukeys HSD post-hoc test). Error bars are the standard deviation of the mean. Values above bars indicate mean and standard deviation.

2.4.3.2 EC

Soil EC ranged from 0.09±0.02 dS m^{-1} for the RO treatment; 0.10±0.02 dS m^{-1} for the municipal tap treatment; 0.12±0.02 dS m^{-1} for the WWTP treatment; 0.12±0.03 dS m^{-1} for the gray water treatment; and 0.24±0.03 dS m^{-1} for the saline water treatment. There was no significant difference in EC between the WWTP, RO, municipal tap, and gray water treatments. Saline had the significantly greatest soil EC (Figure 16).

The field readings of EC also reflect these similar results. The saline treatment had the significantly greatest EC concentrations for both depths at the study plot. There were no significant differences between the WWTP, RO, municipal tap, and gray water treatments for both depths at the study site (Table 8).

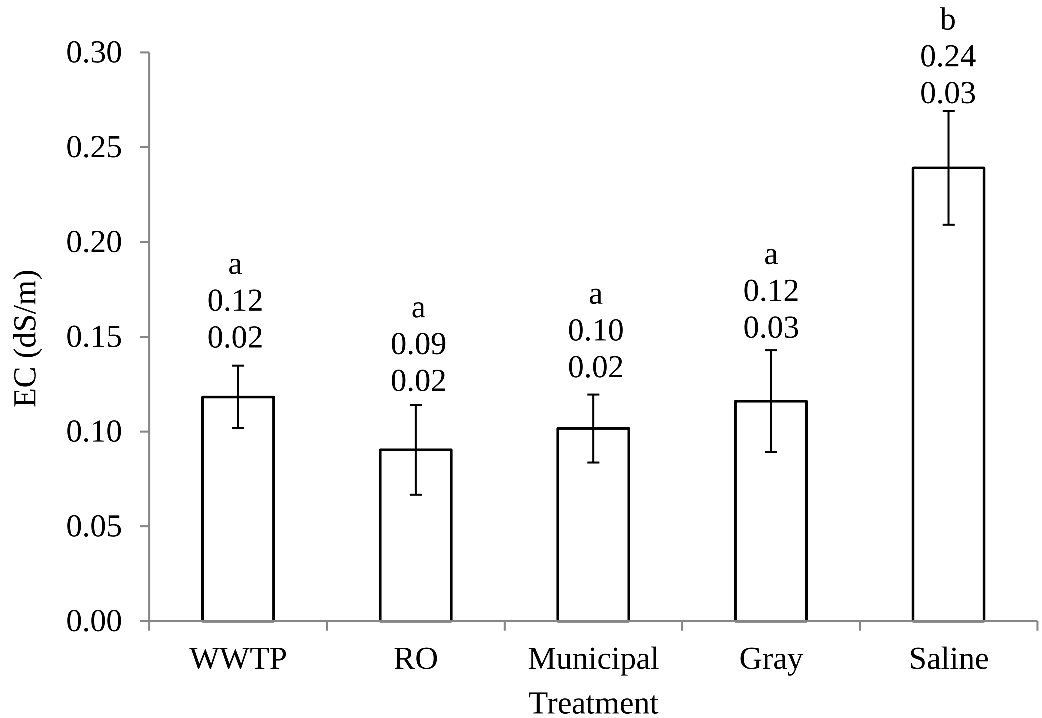

Figure 16. Average soil EC among irrigation treatments. Different letters indicate significant difference among treatments at $p < 0.05$ (Tukeys HSD post-hoc test). Error bars are the standard deviation of the mean. Values above each bar indicate mean and standard deviation.

Table 8. Average field EC at two soil depths. Different letters indicate significant difference among treatments at $p < 0.05$ (Tukeys HSD post-hoc test). Values indicate mean and standard deviation.

Treatment	Depth cm	Average Soil EC µS/cm	St. Dev
WWTP	2.54	909.2**a**	89.6
	7.62	580.7**A**	10-66
Saline	2.54	1918.4**b**	610.4
	7.62	1304.3**B**	15-36
RO	2.54	647.3**a**	60.4
	7.62	364.3**A**	7.62
Gray	2.54	910.2**a**	102.6
	7.62	538.6**A**	13-71
MTW	2.54	880.3**a**	209.0
	7.62	540.8**A**	15-56

2.4.3.3 Calcium

Soil Ca concentrations ranged from 135.59±55.63 µg/g for the gray treatment; 141.85±43.44 µg/g for the WWTP; 172.41±55.97 µg/g for the RO treatment; 172.41±187.36 µg/g for the municipal tap treatment; and 342.06±241.03 µg/g for the saline water treatment. The only significant difference in Ca concentration was between the gray water and saline water irrigated soils. The saline water irrigated soil had a significantly greater concentration of Ca than the gray water irrigated soil (Figure 17).

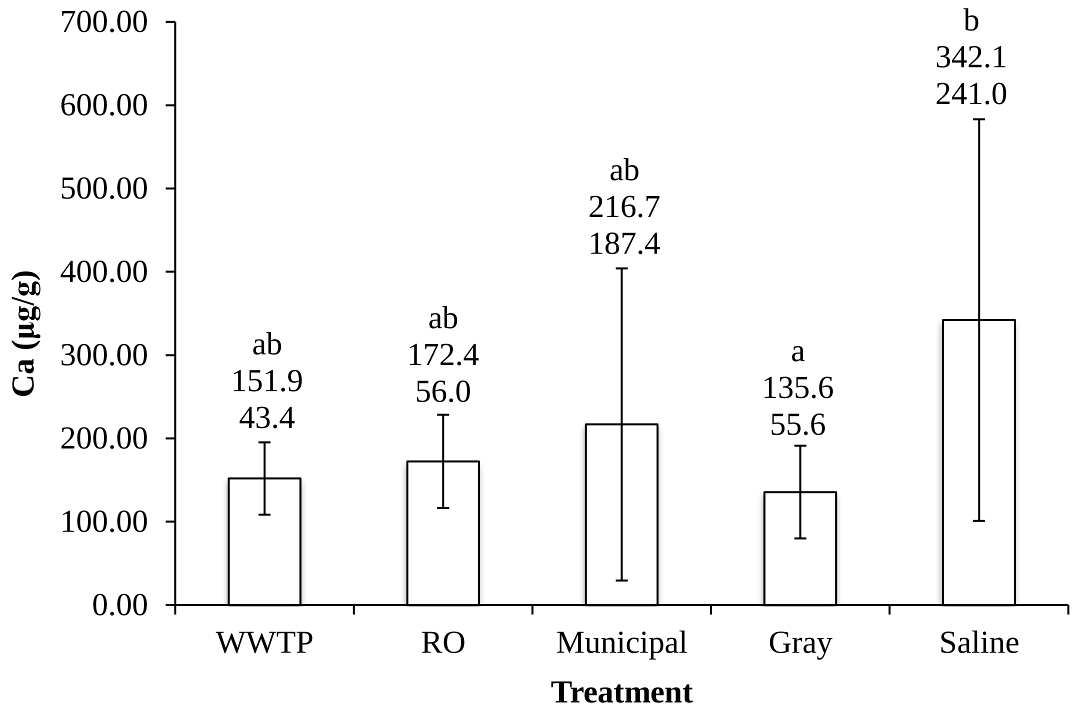

Figure 17. Average soil Ca among irrigation treatments. Different letters indicate significant difference among treatments at $p < 0.05$ (Tukeys HSD post-hoc test). Error bars are the standard deviation of the mean. Values above individual bars indicate mean and standard deviation.

2.4.3.4 Sodium

There were no significant differences in Na concentrations between soils that had been irrigated with the WWTP, municipal tap, and the gray water treatments. The saline irrigated soil had significantly higher concentration of Na, 403.48±90.75 µg/g while the RO irrigated soil had significantly lower concentration of Na, 49.17±14.45 µg/g. Soil Na ranged from 183.75±21.27 µg/g for WWTP, 49.17±14.45 µg/g for RO, 132.43±30.51 µg/g for municipal tap, 190.42±43.93 µg/g for gray water, and 403.48±90.75 µg/g for saline water (Figure 18).

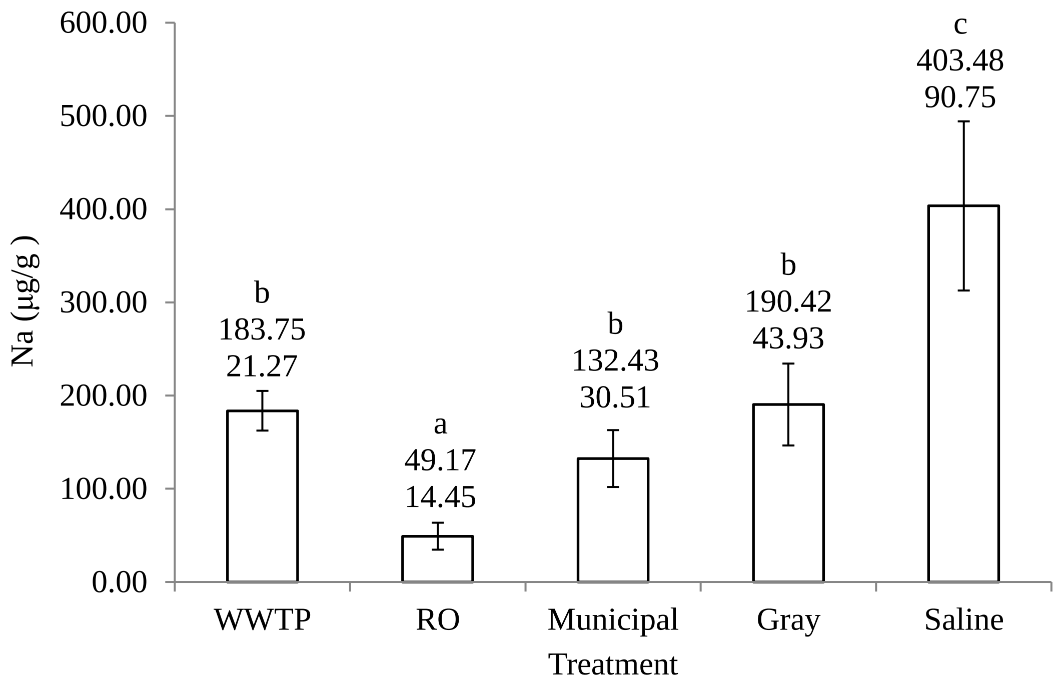

Figure 18. Average soil Na among irrigation treatments. Different letters indicate significant difference among treatments at $p < 0.05$ (Tukeys HSD post-hoc test). Error bars are the standard deviation of the mean. Values above each bar indicate mean and standard deviation.

2.4.3.5 Sulfur (S)

Soil S concentrations ranged from 16.10±5.62 for the RO treatment; 13.63±1.69 µg/g for the municipal tap treatment; 17.71±1.55 µg/g for the WWTP treatment; 18.19±3.65 for the gray water treatment; and 24.74±6.71 µg/g for the saline water treatment. There were no significant differences among the WWTP, RO, the municipal tap, and gray water treatments. The saline water treatment had the significantly greatest soil S concentration (Figure 19).

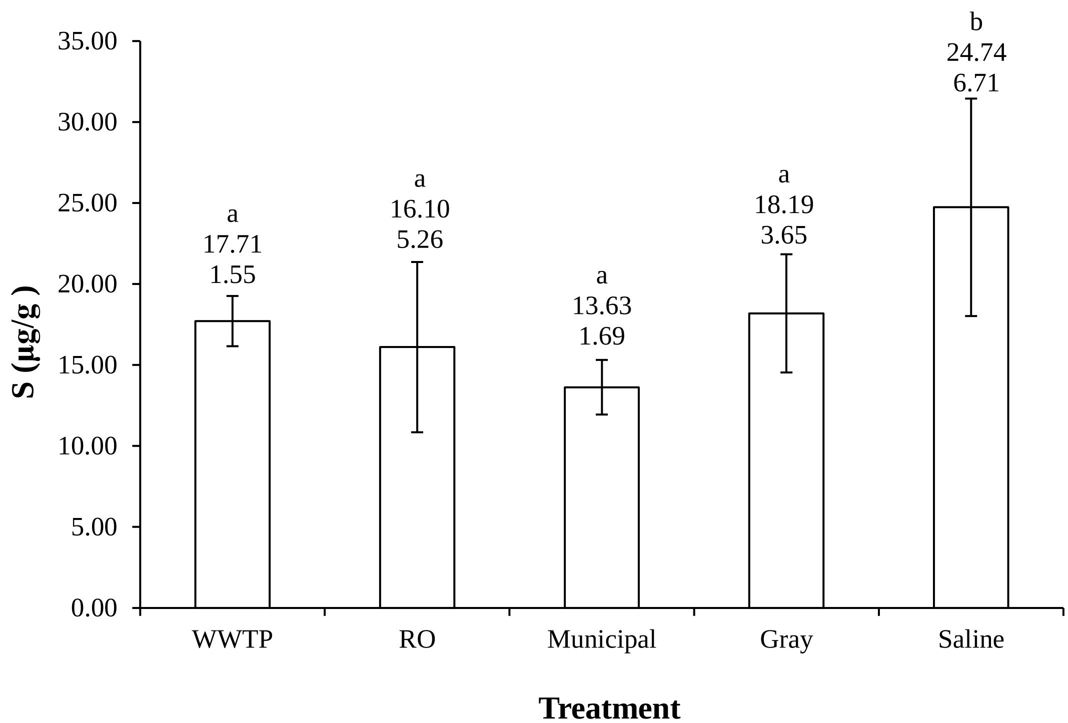

Figure 19. Average soil S among irrigation treatments. Different letters indicate significant difference among treatments at p <0.05 (Tukeys HSD post-hoc test). Error bars are the standard deviation of the mean. Values above each bar indicate mean and standard deviation.

2.4.3.6 SAR

There were no significant differences in SAR between the WWTP (1.16±0.18) and the municipal tap (0.83±0.23) and gray water (1.32±0.38) irrigated soils. Also, there were no significant differences in SAR between the RO (0.30±.12) and municipal tap water irrigated soils. RO treatment had significantly lower SAR when compared to the WWTP, gray water, and saline water irrigation treatments. Saline had a significantly higher SAR than all other irrigation treatments (2.10±0.70; $p < 0.05$; Figure 20).

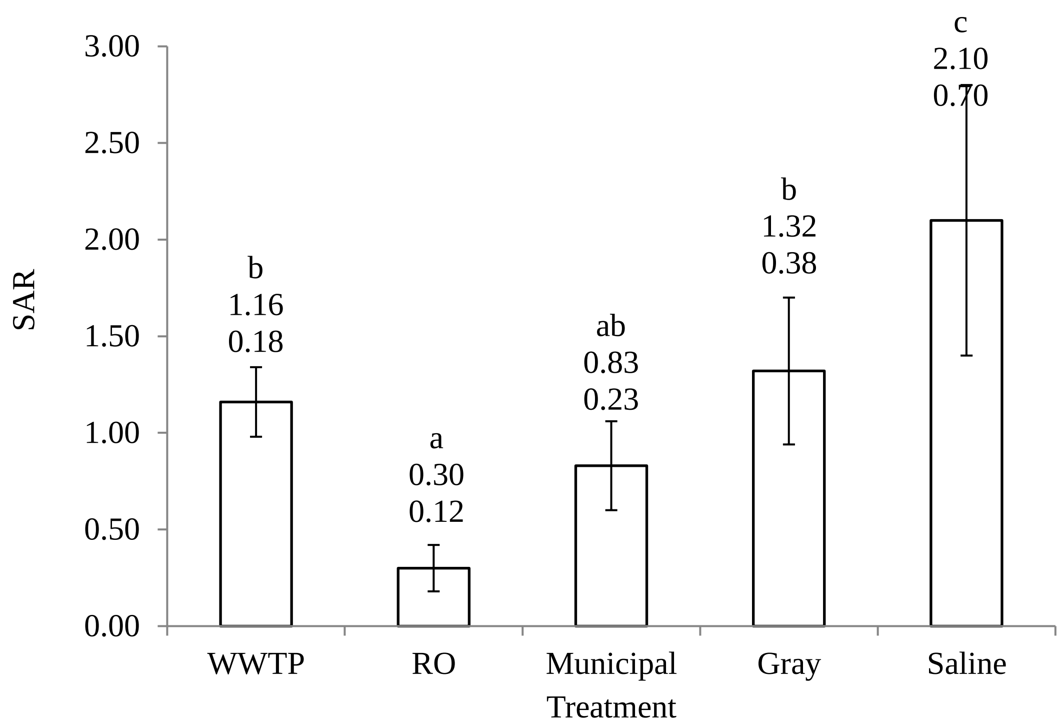

Figure 20. Average soil SAR among irrigation treatments. Different letters indicate significant difference among treatments at $p < 0.05$ (Tukeys HSD post-hoc test). Error bars are the standard deviation of the mean. Values above each bar indicate mean and standard deviation.

2.4.3.7 Time Series of Turfgrass Clippings

A multivariate analysis of variance with two factors was conducted to determine if there was a significant effect of date, treatment, or an interaction between date and treatment on clipping biomass. There was a significant date x treatment interaction on clipping biomass ($p = 0.045$). Where there was a significant date x treatment interaction, parameters were presented separately by sampling date, and an ANOVA was performed to examine if there was a significant treatment main effect at each sampling date. The null hypothesis, $H_{O\text{-}5}$, was rejected. Treatment had no significant effect on clipping biomass for sampling dates 1, 3, and 5. The hypothesis, H_5, was accepted. At sampling date 2, the clipping biomass was significantly larger for the RO treatment (2.71±0.18 g m^2 per day) relative to the saline water treatment (1.90±0.25 g m^2 per day; Figure 21). At sampling date 4, the clipping biomass was significantly larger for the WWTP treatment (3.57±0.37 g m^2 per day) relative to the gray water treatment (2.33±0.44 g m^2 per day; Figure 22).

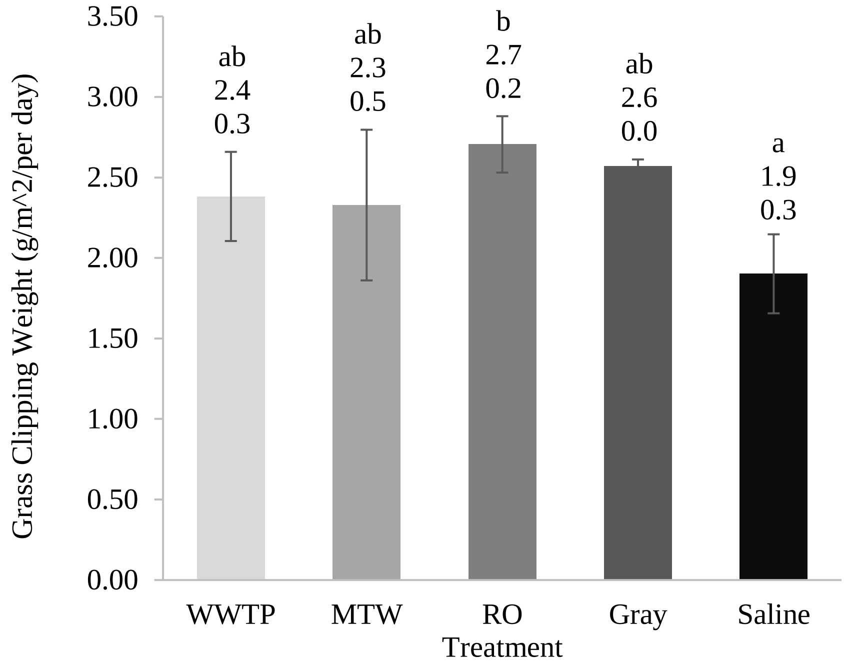

Figure 21. Analysis of variance for treatment main effect on grass clippings during sampling 2, 29 August 2016, of the study. Different letters indicate significant difference among treatments at $p < 0.05$ (Tukeys HSD post-hoc test). Error bars are the standard deviation of the mean. Values above each bar indicate mean and standard deviation.

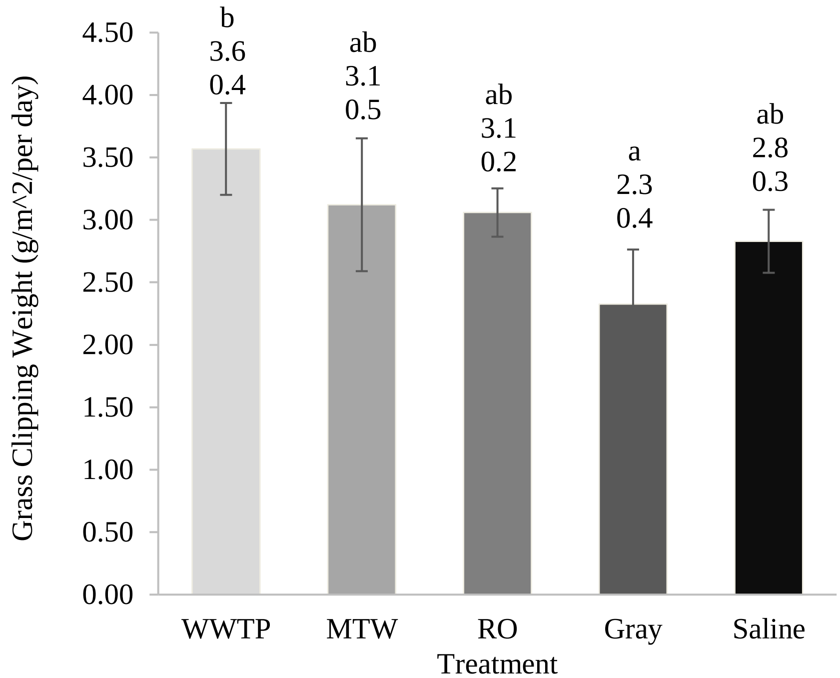

Figure 22. Analysis of variance for treatment main effect on grass clippings during sampling 4, 24 October 2016, of the study. Different letters indicate significant difference among treatments at $p < 0.05$ (Tukeys HSD post-hoc test). Error bars are the standard deviation of the mean. Values indicate mean and standard deviation.

2.4.3.8 Time Series of Percent Green Coverage

A multivariate analysis of variance with two factors was conducted to determine if there was a significant effect of date, treatment, or an interaction between date and treatment on percent green cover. There was a significant date x treatment interaction on percent green cover ($p = 0.015$). Where there was a significant date x treatment interaction parameters were presented separately by sampling date, and an ANOVA was performed to determine if there was a significant treatment main effect at each sampling date. Treatment had no significant effect on percent green cover for sampling dates 1, 2, 3, and 5. At sampling date 4, the percent of green coverage was significantly greater for the RO (59.2±5.6 %) and gray water treatments (55.2±1.2 %) relative to the WWTP treatment (37.2±8.9 %; Figure 23).

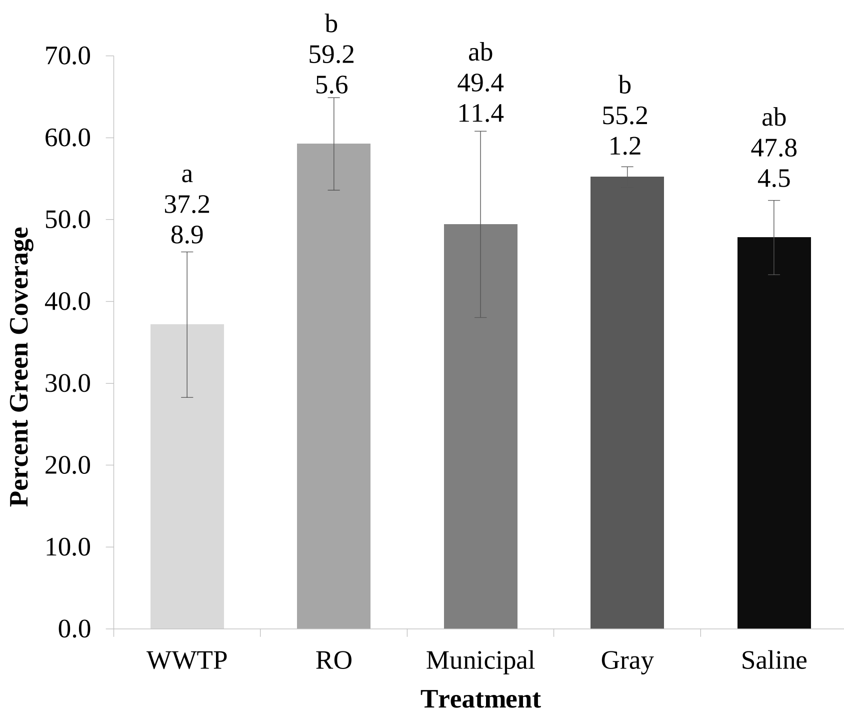

Figure 23. Analysis of variance for treatment main effect on percent green cover during sampling 4, 27 October 2016, of the study. Different letters indicate significant difference among

treatments at $p < 0.05$ (Tukeys HSD post-hoc test). Error bars are the standard deviation of the mean. Values indicate mean and standard deviation.

2.4.3.9 Solvita CO_2-C Burst test

A multivariate analysis of variance with two factors was applied to the data to determine if there was a significant effect of treatment, depth or an interaction between treatment and depth on CO_2 respiration. There was no significant depth by treatment interaction (Table 9). A one-way ANOVA was performed with a post hoc Tukey (HSD) test to determine there was an irrigation treatment main effect on microbial activity ($p < 0.05$). The null hypothesis, H_{O-6} was failed to be rejected. There were no significant differences in soil microbial activity due to irrigation treatments (Table 9). The hypothesis H_6 was rejected.

Table 9. Effect of depth, treatment, and interaction of depth*treatment on released CO_2-C. Values in bold indicate a significant effect of date*treatment at $p < 0.05$, those not bolded indicate $P > 0.05$.

	Soil CO_2
Depth	**<0.001**
Treatment	**0.044**
Depth*Treat	0.6413

2.4.3.10 Fungal Species Percentage Analysis

To determine if there were any significant differences in fungal community composition, PERMANOVA and ANOSIM tests were performed on species percentages. The fungal community structure was estimated using Bray-Curtis distances and visualized it using principal coordinate analysis (PCoA) and non-metric multidimensional scaling analysis (nMDS) using dissimilarity matrices.

Based on the ANOSIM results, there were no statistically significant differences in fungal community composition ($R = -0.027$, $p = 0.674$) in all five water treatments (Table 10). Similarly, PERMANOVA results showed no significant treatment effect on fungal community composition ($p = 0.431$; Table 11).

Table 10. Result of ANOSIM pairwise comparison of fungal species percentages associated with the water treatments at the end of October. $P < 0.05$.

	WWTP	Saline	RO	Gray	MTW
WWTP	-				
Saline	0.4426	-			
RO	0.9467	0.8227	-		
Gray	0.256	0.3706	0.5126	-	
MTW	0.339	0.5081	0.8212	0.3466	-

Table 11. Result of PERMANOVA pairwise comparison of fungal species percentages associated with the water treatments at the end of October. $P < 0.05$.

	WWTP	Saline	RO	Gray	MTW
WWTP	-				
Saline	0.7967	-			
RO	0.8268	0.7785	-		
Gray	0.2571	0.3245	0.2911	-	
MTW	0.1941	0.3222	0.4915	0.3406	-

Nonmetric multidimensional scaling (nMDS) plot was created using the Bray-Curtis distance matrices for relative abundances of species percentages in the different water treatments at the end of October. Fungal community composition showed no significant differences between the five water treatments (Figure 24). Among the water treatments, only saline showed a slightly dissimilarity compared to the other treatments. Principal coordinate analysis (PcoA) plot was created using the Euclidean distance matrices to visualize the similarities or dissimilarities of the species percentages in the different water treatments (Figure 25). The null hypothesis, H_{0-7} was failed to be rejected, there were no significant differences in fungal diversity among treatments. The hypothesis H_7 was rejected.

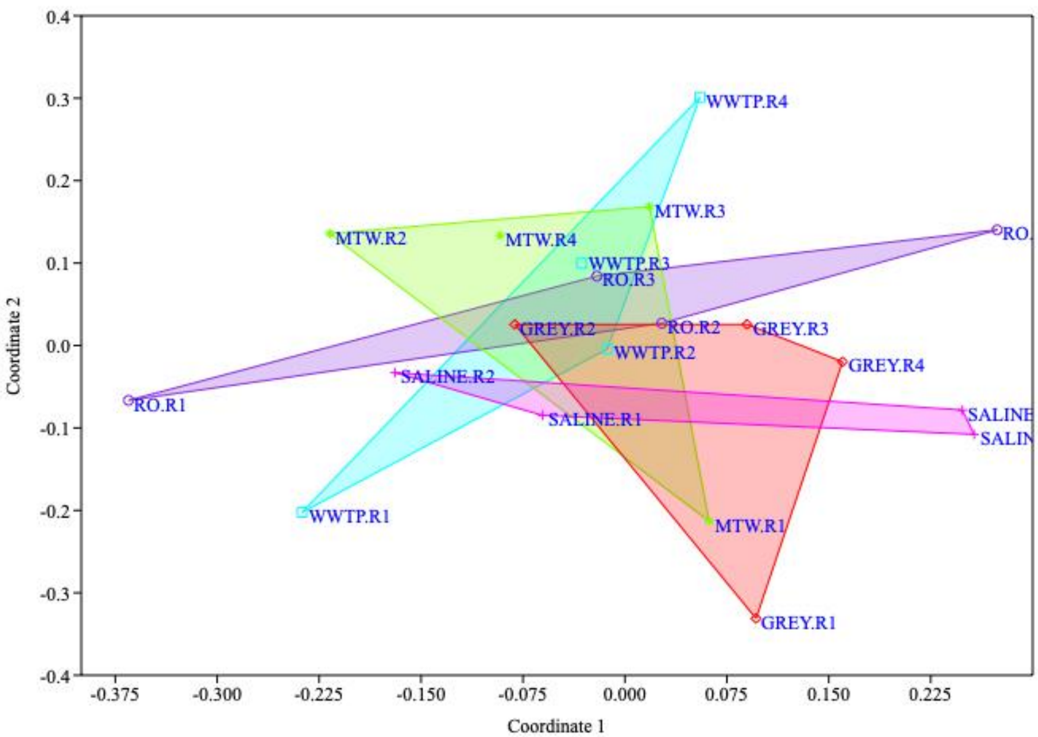

Figure 24. Nonmetric multidimensional scaling (NMDS) of species percentages in soil irrigated with WWTP, RO, municipal tap, gray, and saline water.

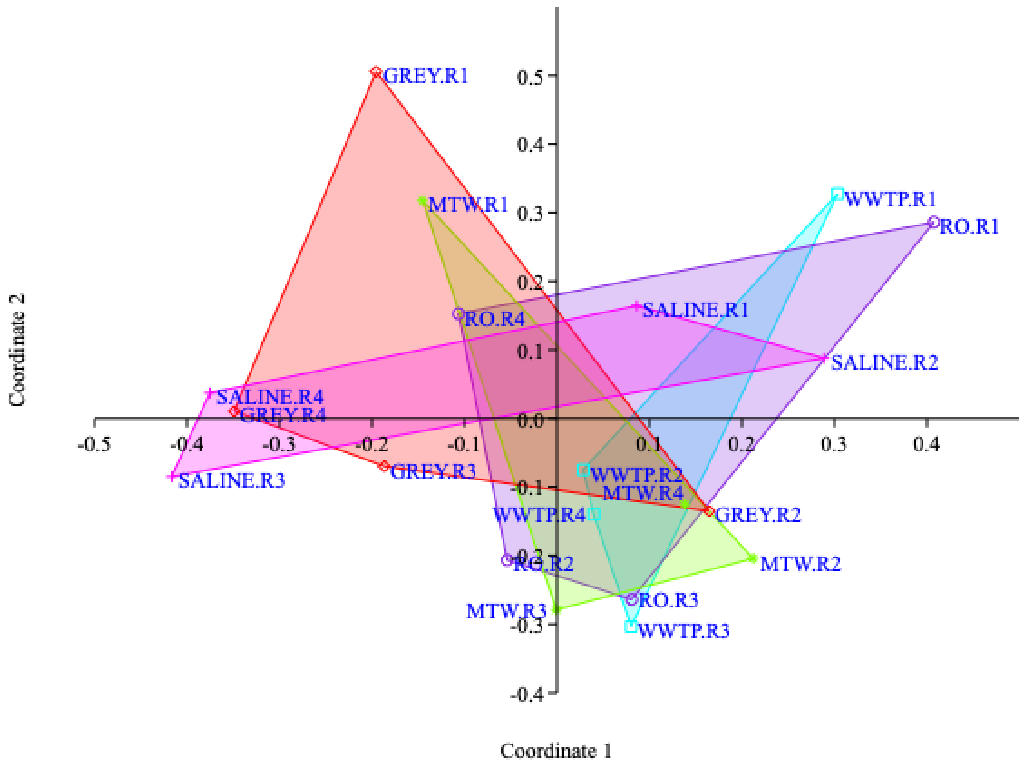

Figure 25. Principal coordinate analysis (PcoA) of species percentages in soil irrigated with WWTP, RO, municipal tap, gray, and saline water.

2.5. Discussion

Due to declining volumes of fresh and groundwater on the planet countries are examining the effect of alternative water for irrigating municipal landscapes (Watkins, 2006). This study examined the effect of alternative water sources for irrigating turfgrass on soil chemistry, turfgrass growth and soil microbial communities.

2.5.1 Alternative water sources

The intention of the study was to assess the effect of sodic, saline, treated wastewater effluent and a gray water on soil chemistry under turfgrass, turfgrass growth and soil fungi as well as CO_2 evolution from the soil. The sodic water was the municipal tap water in the city, which had a SAR of 30.3±0.2 and ESP of 98%. The gray water was the municipal tap water with the addition of tide detergent and this brought the SAR up to 31.0±1.3 with an ESP of 98%. The treated wastewater effluent is derived from the city's municipal water so expectations were that it would have a similar SAR and ESP and additional NO_3-N and PO_4-P; the SAR of this water was 15.7±0.5 and ESP was 92±0.28%. The saline water, created by using "instant ocean" had a relatively high SAR of 18.3±0.6 and ESP of 79±0.50% which, although the electrical conductivity was high put it in the realms of sodic water rather than saline water in terms of its pH, SAR and %EC (Davis et al. 2007). The RO water was not entirely pure water and had a SAR of 2.5±0.1 and ESP of 92±0.5% illustrating the difficulty of sodium removal by reverse osmosis systems. Knowledge of SAR and ESP input values helps in the understanding of the effects these alternative irrigation sources have on soils and their microbial function and activity, as well as the chemistry and effect on turfgrass quality.

2.5.2 Water extractable soil chemistry

2.5.2.1 Dissolved Organic Carbon

Soils irrigated with treated wastewater effluent typically have higher concentrations of DOC than soils irrigated with freshwater sources (Jueschke et al., 2008). Jueschke et al. (2008) investigated the use of freshwater and treated wastewater effluent over a number of years on soils underneath orchard and field crops: corn, sorghum, grapefruit, avocado and cotton soils in Israel. They found that the DOC concentrations in freshwater irrigated soil ranged from 26.40 to 22.21 μg/g (December 2002, June 2003); and the DOC concentrations in treated wastewater effluent soil ranged from 51.52 to 47.52 μg/g (December 2002, June 2003). Overall, they found that DOC increased in in soils when irrigated with treated wastewater effluent in the short term but in the long-term DOC decreased. This may be a result of high sodium content in the wastewater effluent which in the long term allowed the percent C to decline and thus the extractable DOC. The concentrations reported by Jueschke et al. (2008) were much lower than the present studies' concentrations resulting from irrigation of turfgrass with alternative water sources which ranged from 284.2 to 432.7 μg/g for treated effluent, 267.1 to 359.9 μg/g for RO, 255.5 to 369.1 for gray water, 266.3 to 472.3 for municipal tap water, and 256.0 to 520.5 μg/g for saline water. One reason for this discrepancy may be the way the soils were extracted in the two studies, which can make a large difference in DOC recovery (Carillo-Gonzalez et al. 2013). It would be expected that SAR of the Jueschke et al. (2008) municipal tap water and wastewater effluent derived from that tap water would be much lower than observed in the current as their water source was freshwater while mine was groundwater beneath marine clay. Overall, we observed higher concentrations of DOC in treated effluent irrigated soils than in the RO treated soils although concentrations were not significantly higher. We also observed higher concentrations in DOC in December versus earlier months. To tease apart the reasoning behind the difference in DOC in the present study and that of Jueschke et al. (2008) we turned to a prior study, Steele and Aitkenhead-Peterson (2013), examined the effect of different potable water irrigation chemistry across the State of Texas on water extractable DOC and DON. They reported that as the SAR of the irrigation water increased then the extractable DOC also increased. It is likely that the irrigation water used in the Jueschke et al. (2008) study had a lower SAR and that is why the current study DOC concentrations were higher.

Another study by Rosa and Debska performed on soils under agricultural use in Poland (2018) found that $CaCl_2$-extractable DOC concentrations in soils were changing throughout the year. They found that highest differences in organic carbon occurred in soils sampled in autumn (highest in November) and spring (lowest in March). However, in the second year of the study they saw an inverse dependence with higher concentrations of DOC in the spring as compared with samples taken in late autumn, indicating that DOC was migrating deeper in the soils due to irrigation. The current study consisted of only one growing season of monitoring while the Rosa and Debska (2018) study consisted of two years. There is a possibility that more variations could have seen in the treatments if the study was for multiple growing seasons. The volume of rainfall vs volume of irrigation water would make a difference

In a study performed by Karavin et al. (2016) on *Juglans regia* L. in the Middle Black Sea Region of Turkey found that as salinity in irrigation water increased so did the litter decomposition rate. However, other studies in the literature have said the opposite: Roache et al. (2006) showed leaf mass loss caused by a decomposition decrease as a result of increasing salinity. In the current study, there were high sodium concentrations in the Instant Ocean in the saline solution (520.5 μg/g) possibly causing an increase in litter decomposition which can be responsible for the high concentration of DOC in the soil irrigated with the saline solution. Steele and Aikenhead-Peterson (2012) reported that more DOC was leached from senescent vegetation when exposed to higher SAR than those with lower SAR and that saline solution did not leach DOC at all. As the saline solution in the current study had a SAR of 18.3 and ESP (79%) and high pH expectations are that it was the concentration of sodium in the solution that had the effect of increasing DOC.

The increase in DOC from October to November could be caused by an increase in organic matter. As turfgrass decomposes, nutrients increase in the soil providing energy for microbes. It has been noted that exudates released from plants are the major source of organic inputs into the rhizosphere called the rhizosphere effect (Rovira, 1965; Bertin et al., 2003; Jones et al., 2009). But, exudate from plant roots is typically very biodegradable unless an excess of nitrogen occurs (Aitkenhead-Peterson and Kalbitz 2005) which certainly may be the case in the WWTP irrigation treatment. Drake et al. (2012) found that exudates that contain C and N significantly increased microbial respiration, biomass, and the activity of exo-enzymes that decompose labile components of soil organic matter, while decreasing the activity of exo-enzymes that degraded recalcitrant soil organic matter. From this perspective, the results in the current study support the increases in DOC during dormancy is a result of the cycling of plant biomass and exudates through microorganisms and very likely caused by high SAR of the irrigation solutions.

2.5.2.2 Total dissolved nitrogen (TDN) and dissolved organic nitrogen (DON)

Studies have shown significant differences in TDN leachate among dates due to greater turfgrass assimilation of inorganic nitrogen during the warmer months versus cooler months (Wherley et al., 2009). Bermudagrass is considered a perennial warm-season grass and is most active during the late spring and summer months. There is a natural competition for nutrients between plants and microbes. Wherley et al. (2009) conducted a study to determine the NO_3-N uptake efficiency during both growth and dormancy cycles of Tifway Bermudagrass (*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt Davy). Wherley et al. (2009) found that uptake was greatest during the summer months when the turfgrass was growing rapidly, and less than 80 to 90% of NO_3-N remained in the soil after the Bermudagrass became dormant in January. The total dissolved nitrogen concentrations in the soil chemistry from different treatments in the current study were not significantly different between treatments at each sampling date. The TDN concentrations in soil chemistry from the treatments in October were significantly lower than all treatments in November. Since TDN and DON follow the same trend and there was no significant date x treatment effect for inorganic nutrients, it can be assumed that DON is causing the significant differences in TDN for December sampling.

As the turfgrass activity decreased and its tissues begin to decompose, during the cooler temperatures in November, there was increased availability of DON along with DOC in the soil. DOC and DON exists in the soil in the form of amino acids and other precursors for more complex compounds such as chlorophyll, proteins, enzymes, hormones and nucleic acids (Carrow et al., 2001; Figure 25). Since there was not a pattern in rainfall, it can be assumed that DON concentrations in both samplings was not impacted by precipitation (Figure 26). Soil temperature has a great influence on nitrogen transformations (Nedwell, 1999). Nedwell (1999) performed a study on psychrophiles, mesophiles, and thermophiles at different temperatures. He found that affinity for organic and inorganic substrates decreases consistently as temperatures drop below the optimum temperature for growth. In the current study, there were lower temperatures during November, ranging from 20.6° to 8.3° C then in October, ranging from

26.1° to 15.6° C. The temperatures are similar to the temperatures shown in Nedwell (1999) with his ranges showing greater affinity as temperatures approached 20° C. If affinity for DON by microbes along with microbes themselves in the soil were decreased by the cooler temperatures in November, then this could explain why DON was significantly higher than October DON concentrations for various treatments.

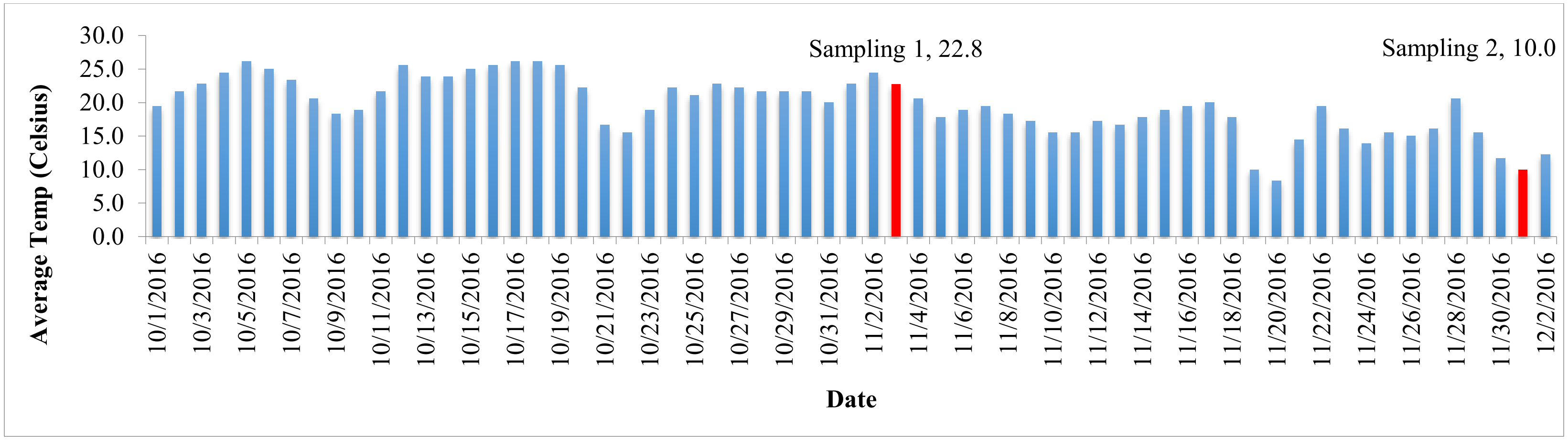

Figure 25. Daily temperature for October – November. Red bars indicate dates soil samples collected.

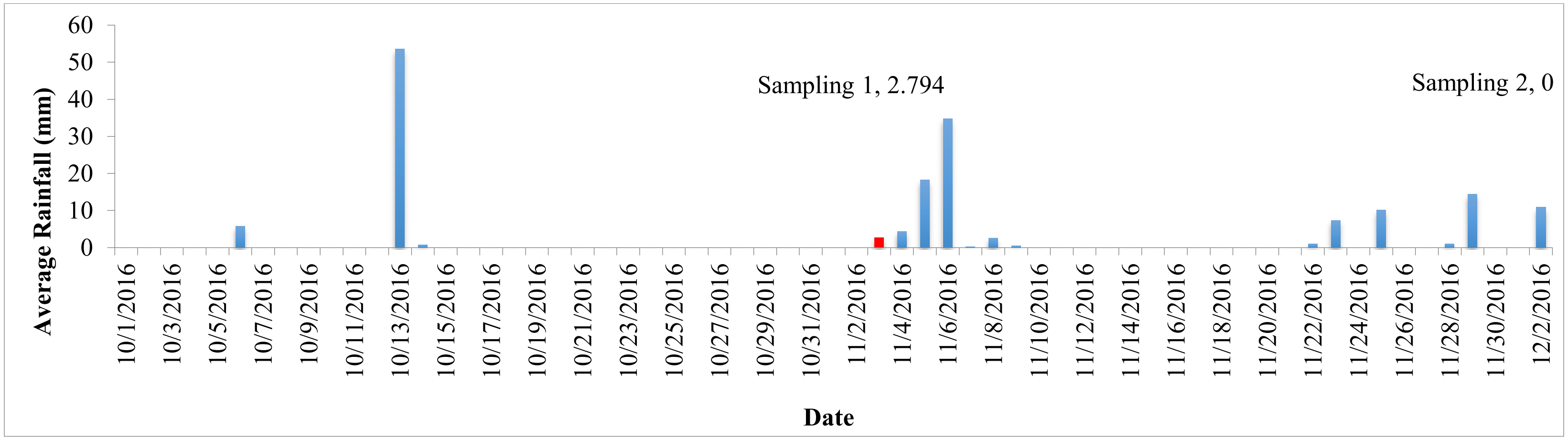

Figure 26. Daily precipitation for October – November. Red bars indicate dates soil samples collected.

2.5.2.3 pH

Soil pH can influence turfgrass and other plants by a variety of mechanism (Carrow et al., 2001). In soils where pH is greater than 7.0, basic cations such as Ca, Mg, K, and Na dominate the exchange sites and OH^- activity exceeds H^+ activity (Carrow et al., 2001). The pH in soils from the different irrigation treatments were not significantly different with the exception being the saline irrigated soils were significantly greater than the RO water irrigated soil for pH. The pH levels in the current study ranged from 7.43 for RO to 7.79 for saline treatments, which are considered to be alkaline; however, nutritional stresses and impacts on microbial populations in alkaline soils are less common compared to acid soils (Carrow et al., 2001). Hayes et al. (1990) used potable well water and treated effluent water and saw an overall significant decrease in soil pH under both irrigation sources over the study period of 16 months. There was no date x treatment interaction on pH for the current study but that could be due to the study only being for one turfgrass growing season and only two soil samplings.

2.5.2.4 Salinity and EC

Multiple studies have examined the effects using of sewage, domestic or raw, on soil hydraulic properties (Hayes *et al*, 1990; Lado, and Ben-Hur, 2009). Hayes et al. (1990) investigated the use of secondary-treated effluent on soil chemistry under turfgrass soils for 16 months in Tucson, Arizona. They found that effluent water results in increased concentrations of ions in the effluent irrigated plots versus the potable water irrigated plots. Their irrigation effluent and potable water quality EC ranges were 0.65-0.91 dS m^{-1} and 0.2-0.2 dS m^{-1}, respectively. In the present study, the saline irrigated soils, had significantly higher EC (0.24±0.03 dS m^{-1}) in the water extracts than the other irrigation treatments. These values were well below input irrigation values of 1.05±0.05 dS m^{-1} for gray water, 1.05±0.01 dS m^{-1} for municipal tap, and 6.5±0.26 dS m^{-1} for saline, and 1.20±0.04 dS m^{-1} for WWTP effluent in the current study suggesting that the excess ions in solution had either leached beyond the root zone to a deeper, unmeasured depth, run off during a rain event or were taken up by the turfgrass.

Similarly, to the previous study, Lado and Ben-Hur (2009) examined the impact of using secondary-treated effluents on hydraulic properties of semiarid and arid soils in various areas of Israel. The average EC of the effluents they used were 1.8 dS m^{-1} and 2.0 dS m^{-1}. These EC values are higher than WWTP effluent values but lower than saline treatment values in the present study. They found that the EC and salinity values were higher in the effluent-irrigated soils at both topsoil and sub soil depths, ~1.5 and >4.0 m. and resulted in salt accumulation in the soils. However, another study found that soils irrigated with both the freshwater sourced municipal tap water and the effluent saw an overall decrease in salinity over the course of the study with an initial spike in the first year. In the current study, irrigating with saline water at 6 dS m^{-1} resulted in higher soil salinity and EC due to greater input of salt ions as compared to, WWTP effluent, RO, gray water, and municipal tap water. The source of the salt ions was from Instant Ocean with the dominant four ions being chloride, sodium, magnesium and potassium (Table 1).

Chavarri et al. (2019) also used Instant Ocean in their greenhouse study using 10 commonly used cultivars representing warm-season turfgrass species. Salinity treatments were opposed on grasses for 10 weeks via subirrigation, followed by a 4-week freshwater recovery period. They saw Tifway (moderately tolerant) turfgrass quality decrease from 6.8 to 5.8 with 2.5 dS m^{-1} salinity exposure and a greater decrease in turfgrass quality from 2.5, 2.7, 0.5 with 15 dS m^{-1}, 30 dS m^{-1}, and 45 dS m^{-1}, respectively. Bermudagrass (*Cynogon dactylon*) salinity threshold is 4.3 dS m^{-1} (Carrow and Duncan, 2011). The highest EC values seen in the present study for both the water-extractable EC and the EC at field site were 0.2 dS m^{-1} and 1.9 dS m^{-1}, respectively. Based on the Carrow and Duncan (2011) salinity threshold, the salinity levels in the current study did not reach thresholds to cause any major impact on the turfgrass.

2.5.2.5 Sodium and SAR

Mancino and Pepper (1992) examined the use of secondary sewage effluent and how it affected soil quality. They found that effluent water resulted in significantly higher soil sodium levels than potable water. The sodium of the effluent and potable irrigation waters ranged from 80 to 94 mg L^{-1} and 14 to 30 mg L^{-1}, respectively in their study (Mancino and Pepper, 1992). The ranges observed by Mancino and Pepper (1992) are lower than the values sodium and SAR measured in the present study for input irrigation water. The sodium values in the present study were 6.25 mg L^{-1} for RO, 251.89 mg L^{-1} for treated effluent, 251.89 mg L^{-1} for gray water, 234.41 mg L^{-1} for municipal tap, and 1021.70 mg L^{-1} for saline, with the significantly highest concentrations of Na being in soils irrigated with treated saline water then gray, municipal tap, and WWTP effluent with RO irrigated soils being significantly lowest. In the current study, it is possible that the high concentrations of sodium in the soil irrigated by saline water is attributed to sodium chloride being the main ingredient in Instant Ocean rather than the high sodium domestic tap irrigation water. Instant Ocean was mixed with RO and not municipal tap water. The inputs of sodium for WWTP effluent, municipal tap, and gray water, while significantly higher than RO, were not significantly differently when compared to the saline solution. However, the significant difference in sodium between WWTP effluent, municipal tap, and gray compared to the RO water treatments can be attributed to the high sodium domestic tap water used in each treatment.

Several studies have investigated the effect of using either alternative water sources for irrigating turfgrass or other crops on soil chemistry (Holgate et al. 2011; Chaganti, et al., 2020) or differently sourced (ground vs freshwater) on soil chemistry beneath turfgrass (Steele and Aitkenhead-Peterson 2012). Chaganti et al. (2020) investigated the effects of using freshwater derived municipal tap water and treated municipal effluent on soil chemistry under sorghum over a two-year period in El Paso, Texas. They examined the chemical properties of the soil at two different depths: 0-15 and 15-30 cm. They observed an increase in the SAR in both types of water irrigated soils, which is attributed to the accumulation in the lower layers as a result of leaching from the surface layer. However, soils treated with gypsum saw decreases in SAR with the addition of Ca in the soil, a commonly recommended method for treating sodic soils (Davis et al. 2007) where Ca will displace Na from soil exchange sites and allow it to leach further down the soil profile. Chaganti et al. (2020) reported that soil sodicity was significantly higher in soil effluent treated plots versus the fresh water derived tap water. For the present research, the Na concentrations were significantly higher in the saline treatment compared to the other treatments. In contrast with Chaganti et al. (2020), the current study saw an overall trend of significantly decreasing concentrations of Na as the soil became deeper. This could suggest a salt accumulation in topsoil as a result of Na adsorption on exchange sites thus replacing K, Mg and Ca and insufficient rainfall to leach the excess salt to deeper soil depths (Lado and Ben-Hur, 2009). The high concentrations of sodium in the soil irrigated by saline water is more than likely attributed to sodium chloride being the main ingredient in Instant Ocean rather than the high sodium irrigation water since Instant Ocean was mixed with RO and not municipal tap water.

In Holgate (2010) and Aitkenhead-Peterson et al. (2009) it was speculated that high DOC and DON concentrations in turfgrass soil in south-central Texas were most strongly related to high sodium and bicarbonate in irrigation water. Bicarbonate was not included in the present study; however, the water extractable soil sodium concentrations ranged from 50.5 to 47.8 μg/g for RO, 183.4 to 184.1 μg/g for treated effluent, 111.0 to 153.9 μg/g for gray water, 159.4 to 221.4 μg/g for municipal tap, and 405.4 to 401.6 μg/g for saline water, with the highest concentrations being in soils irrigated with treated effluent, municipal tap, and saline water. In the current study, irrigated soils with the greatest concentrations of DOC also contained the greatest concentrations of sodium: saline, gray and WWTP irrigation treatments. The data supports the supports the correlation between sodium and DOC that both Holgate (2010) and Aitkenhead-Peterson et al. (2009) reported in their studies.

2.5.3 Secondary Nutrients

2.5.3.1 Calcium

Turfgrasses are very efficient in taking up Ca and the soil contains a number of compounds in which Ca is a constituent. There are instances, when irrigation water is high in Na such as seawater irrigation, when the addition of Ca would be useful in enhancing salt tolerance of plants (Carrow et al., 2001). Albakawneh et al. (2016) examined the effects of the use of gray water on Jordan soil EC and soil quality parameters for two years. The average irrigation inputs for Ca for their study, 170 mg L^{-1}, were higher than irrigation inputs in the current study with highest being 37.30 mg L^{-1} for saline and 9.59 mg L^{-1} for effluent. They saw an overall decrease in soil Ca after 2 years of using treated gray water. They attributed this decrease to the precipitation of calcium carbonates and the addition of carbonates from decomposition of organic material by microorganisms (Albakawneh et al. 2016). In the present study, the lowest concentration of Ca was seen in the gray water irrigated soils; however, carbonates were not analyzed so we cannot attribute the lowest Ca amounts to calcium carbonate precipitation.

2.5.3.2 Sulfur

In the current study, the saline irrigated soils had the significantly highest concentrations of S and all other treatments were not significantly different. This could possibly be attributed to sodium sulfate being a main ingredient to Instant Ocean, which was used for the saline irrigation solution. Rodda et al. (2011) found that irrigation with either the hydroponic solution or gray water resulted in significantly higher concentrations of S than tap water irrigated soils. Turfgrass deficiencies are shown through symptoms of reduced shoot growth and yellowing of the leaf tip and turfgrass grown under high N are most susceptible to S deficiency (Carrow et al., 2001).

High S levels in the soil can lead to production of H_2S, which can be phytotoxic, and the formation of FeS and MnS_2 leading to a black coloration in the turfgrass (Carrow et al., 2001).

2.5.4 Micronutrients

Some of the micronutrients required for turfgrass growth and development are Fe, Mn, Zn, and Cu. Toxicities from excessive levels of Mn and Fe are common, but toxicities for Cu and Zn are more localized to areas contaminated by heavy metals from added soil amendments, airborne deposits from nearby industries and overapplication of micronutrient containing fertilizers (Carrow et al., 2001). The data results for Cu were highly variable across depths and date. For reference, only the municipal tap water treatment at depth 2 (2.5- 7.6 cm) was significantly different from all other treatments. Only the Cu concentration in the WWTP effluent irrigated soil for the November sampling was significantly higher than the other November treatments; all other treatments at each date were not significant. The standard deviations are also high for Cu making it difficult to draw conclusions. However, it has been noted in study the Hayes et al. (1990) study that there was no significant influence of potable or effluent irrigation on soil extractable Cu concentrations.

There were no significant differences in Fe, Zn, or Mn in the present study at any depth or between sampling dates. There was also no treatment main effect on any of these nutrients. The irrigation inputs contained very low levels of all three of the nutrients; they ranged between 0.0.1-0.02 mg L^{-1} for Fe, 0.01-0.05 mg L^{-1} for Zn, and 0.01-0.01 mg L^{-1} for Mn. Mancino and Pepper (1992) and Hayes et al. (1990) both had low levels of inputs as well (<0.01 mg L^{-1}). Hayes et al. (1990) reported that both potable and treated effluent increased Fe and Mn in the soil after 1.3 years but saw a decrease in Zn and no change in Cu. Mancino and Pepper (1992) reported an increase in Fe and Mn after 3.3 years in both potable and treated effluent with Fe being higher in treated effluent irrigated soils. They reported no change in Zn.

2.5.5 Clipping Biomass

Use of wastewater to irrigate turfgrass can significantly affect biomass produced and this is likely attributed the fertilizer effect of high NO_3-N and PO_4-P concentrations found in effluent. A study performed by Castro et al. (2011) saw greater phytomass yields in wastewater irrigated plots versus the potable water plots. They also saw no significant speeds of growth between the two irrigation treatments. Another study performed by Holgate (2010) saw the highest biomass production in bath water irrigated treatments and the lowest in the unfertilized tap water treatments. From their study, the bath water treatment had the highest inputs of Na while the treatment in the current study that had the highest inputs of Na was saline. However, the saline irrigated clipping biomass for the second sampling had the lowest Na concentration. This lack of clipping biomass could possibly be due to osmotic stress caused by high salt concentrations in the saline treatment (Dean et al., 1996). Evanylo et al. (2010) found no significant differences in biomass between potable and reclaimed water on bentgrass and bermudagrass during the growing season of the turfgrasses in 2004. The highest biomass at sampling 2, 29 August 2016, was the irrigated with WWTP and the lowest was irrigated with gray water. The highest biomass at sampling 4, 24 October 2016, was irrigated with RO and the lowest was irrigated with saline water. It was difficult to make any assumption as to why we were seeing different treatment effects since the sampling dates were not back to back. However, there was an overall decrease in average clipping biomass from sampling 2 to sampling 4 with the exception of gray water for sampling 4. Out of 5 sampling dates, these two were the only ones that showed significant differences.

2.5.6 Percent Green Cover

There have been multiple studies performed using the Sigma Scan images analysis to analyze percent green cover (Richardson et al., 2001; Hejl et al., 2016; Johnson and Leinauer, 2004). Johnson and Leinauer (2004) examined warm and cool turfgrasses and their responses to different levels of irrigation water salinity and sprinklers versus drip irrigation. The three ranges of irrigation salinity were: potable water 0.6-1.2 dS m^{-1}, high saline (geothermal) 3.1-5.0 dS m^{-1}, and a 50/50 blended mix ranged from 2.0-3.0 dS m^{-1}. For saline irrigated grasses, the highest percent green cover was for Seadwarf and Seaspray (paspalum cultivars) and the lowest was Bluegrass. Another study used chlorophyll instead of percent green cover and found that Bermudagrass had higher levels of chlorophyll than paspalum cultivars under high salinity levels (Kairmi et al., 2018). Out of all five sampling dates, only sampling 4, 27 October 2016, showed significant differences in percent green cover. The RO irrigated treatment reached almost 60% green coverage and gray water irrigated treatment reached 55% green cover.

2.5.7 Soil Microbial Activity

There have been many studies performed using the Solvita CO_2-Burst test to determine soil health and nutrient release (Qin et al., 2019; Moore et al., 2019; Bateman et al., 2019). Qin et al. (2019) used the Solvita CO_2-Burst test to determine if the presence of humic substances could have a long-term influence on soil microbial activity in bell pepper rhizospheres. Humic substances are considered to be the chemical or biological decomposition products from plant are animal residuals (Qin et al., 2019). They found that although humic substances enhanced soil respiration they did significantly affect rhizosphere biomass (Qin et al. 2019). They also found that soil organic carbon did not correlate with soil respiration (Qin et al. 2019). These are similar to the results of the current study. The soils irrigated with the saline treatment had the significantly highest DOC concentrations but there were no significant differences in soil released CO_2. There was no significant treatment effect on the amount of CO_2 released from microbes in the soil. However, there was a depth effect on CO_2 released. As the soil became deeper, the less CO_2 was released. In Engelhardt et al. (2018), they found that soil depth was the main factor in shaping differences among soil bacterial and fungal communities, where plant-soil microbial coupling is tightest.

2.5.8 Fungal Community Composition

Soil microbes play key roles in ecosystems and influence a large number of important ecosystem processes, including nutrient acquisition, carbon cycling, nitrogen cycling, and soil formation (Van der Heijden et al., 2008). Mycorrhizal fungi (MF) provide resistance to disease and drought, and supply a range of limiting nutrients including N, P, Cu, Fe, and Z to the plant in exchange for carbon (Van der Heijden et al., 2008). Arbuscular mycorrhizal fungi are associated with two thirds of all land plants and are among the most abundant functional groups of soil microorganisms being present in almost any ecosystem investigated (Bender et al., 2014). The MF are present in soils as spores and hyphae in soil or as colonized roots. Arbuscular MF establish a mutual symbiosis with a turfgrass by developing a network of external hyphae that may extend the root surface area up to 40 times. This symbiotic relationship allows turfgrasses to explore a larger soil area and volume for nutrient uptake through the production of enzymes and excretions of organic substances (Visconti et al., 2020).

It has been reported that irrigation with treated wastewater could have the potential to decrease fungal diversity. Holgate et al. (2011) observed a significant decrease in fungal community 18:2 ω6c for all their irrigation treatments and a significant decrease in fungal 18:1

ω9c for their municipal tap water and gray water irrigation treatments in a greenhouse study. A study performed by del Mar Alguacil et al. (2012) investigated the effects on long-term irrigation with freshwater and urban wastewater on AMF diversity in a semiarid orange-tree orchard in southeast Spain. Their soil samples consisted of five replicates of wastewater and freshwater rhizosphere soil samples. Using DNA analysis, del Mar Alguacil et al. (2012) reported greater diversity in AMF composition in freshwater irrigated soils compared to soils irrigated with wastewater. While the current study did not examine the AMF species specifically, we found no significant differences in fungal species among different irrigation water treatments. The difference in fungal community composition results between the current study and del Mar Alguacil et al. (2012) may be the time length of soil exposure to irrigation. Their study was conducted after 43 years of irrigation while the duration of the present study was 5 months. Another study by Chen et al. (2017) also found that irrigation with aquaculture wastewater could dramatically reduce soil microbial functional diversity. Chen et al. reported that increased soil salinity, especially Cl concentration caused a decrease in diversity indices and carbon source utilization (2017). Dang et al. (2019) determined that treated wastewater increased bacterial OTUs and inhibited fungal OTUs. There were no significant differences observed in the nMDS or PCoA of the DNA data suggesting that the water sources were not selective for the different soil fungal populations

CHAPTER III

CONCLUSION

3.1 Limitations to the study

There were significant differences among soil chemistry which was a function of quality of irrigation water used. Higher concentrations of nutrients were detected in soils irrigated with saline water. Depth and date significantly affected soil chemistry. However, the data did not account for leaching due to rainfall, which could allow for variations among irrigation treatments. The study was also conducted during the rainy season in Central Texas, which allows for a high amount of leaching and a low amount of salt buildup. However, the study does not consider periods or seasons of low rainfall, allowing for salt buildup and a low amount of leaching. Furthermore, soils that contains excess salts can cause a decrease in microbial activity as a result of osmotic pressure and toxic ions (Yan et al., 2015).

The soil sampling conducted occurred at the end of the growing season; no sampling occurred either throughout the growing season or during the dormant season. This limited sampling time does not allow for the confirmation of fungal community composition and diversity during the dormant season or early growing season. However, a study conducted by Bennett et al. (2013) demonstrated that fewer AMF species were able to colonize during the colder months, indicating that fungal diversity may actually be decreased during the turfgrass dormancy season. PCoA and nMDS analysis of the DNA species data showed no dissimilarities in fungal composition between treatments.

The scope of the study is limited to the fungal community; therefore, the study will not fully represent the microbial community within the soil samples evaluated. There have been multiple studies performed that show the diversity of bacteria in soil to be very extensive and some species of bacteria also have a symbiotic relationship with fungi (Torsvik et al., 1996; Nannipieri et al., 2002; Kung'u' et al., 2008).

3.2 Summary and Recommendations

Sources of water for irrigation use in Texas urban soils have varied in recent years because of a decline in water supplies, an increase in urbanization, and frequent and persistent drought conditions. Treated wastewater effluent and recycled water sources containing low salt concentrations could be of importance in sustaining a freshwater supply. It can possibly be used as a fertilizer depending on its input nutrients. This study examined the effect of using alternative sources of water for irrigation of Tifway hybrid bermudagrass (*Cynodon dactylon x C. transvaalensis*) on soil chemistry, turfgrass growth and performance, microbial activity, and fungal community diversity.

Based on the water quality guidelines for golf course irrigation set by the Golf Course Superintendents Association of America, the water sources used in this study would be adequate for municipal and landscape irrigation. None of the results in this study exceeded the 'slight to moderate' for degree of problem. While the saline irrigation treatment had an input EC of 6.51 dS m^{-1}, the soil EC at the field research site did not exceed 1.9 dS m^{-1}, which is in the slight to moderate range for degree of problem (Harivandi, 2007). More studies are needed to more accurately determine what type of irrigation source would be best used for irrigation of bermudagrass.

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