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Determining Impacts of Long-Term Use of Reverse Osmosis Concentrate as Drip Irrigation Water Source on *Atriplex* species, Soil Characteristics and Microbial Communities

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14. ABSTRACT

Drought in the Western U.S. has increased interest in non-traditional irrigation sources such as brackish groundwater. RO can be used to reduce salinity but can pose disposal issues for saline concentrates. A potential safe and beneficial use of saline concentrate is irrigation of halophytic species for livestock fodder. Objectives were to determine the impacts of brackish irrigation water on soil chemical properties, soil microbial communities, and plant germination/growth. Field studies included Atriplex plantings irrigated at different salinity concentrations. Soils were analyzed for EC, ion concentration, pH, and carbon/nitrogen. Next-Generation Sequencing was conducted to assess soil microbial communities. Greenhouse studies involved planting 2 species of Atriplex in clay or sand soils irrigated with water at various salinities. Plants were measured for germination, height, IR, and SPAD. Soil EC increased with distance from the irrigation emitter. Ion concentrations, pH and carbon/nitrogen ratios varied; soil microbe communities shifted but were not statistically significantly. Germination was significant by species, soil type, and irrigation treatment. Higher salinities did not significantly affect plant height or SPAD, but significantly increased IR. Further studies are needed to develop a better understanding and management practices for safe and effective use of saline concentrate to grow halophytes for livestock fodder.

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reverse osmosis, saline concentrate, irrigation, soil chemistry, soil microbes, plant growth

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Determining Impacts of Long-Term Use of Reverse Osmosis Concentrate on *Atriplex* species, Soil Characteristics and Microbial Communities

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Determining Impacts of Long-Term Use of Reverse Osmosis Concentrate on Atriplex species, Soil Characteristics and Microbial Communities

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Acronyms and Abbreviations

Reclamation	Bureau of Reclamation
BGNDRF	Brackish Groundwater National Desalination Research Facility
EC	Electric Conductivity
IR	Infrared
OTU	Operational Taxonomic Unit
PCR	Polymerase Chain Reaction
RO	Reverse Osmosis
SAR	Sodium Adsorption Ratio
SPAD	Soil Plant Analysis Development

Measurements

°C	degree Celsius
cm	centimeter
mg/L	milligram per liter
dS/L	decisemens per liter

Contents

Executive Summary

Decreased precipitation and increased temperatures in the western region of the United States has led to an increase of pumping of groundwater to supply water for agricultural needs, much of it brackish. While reverse osmosis (RO) can be used to reduce salinity of the brackish groundwater, RO concentrate management must be addressed. Concentrate may be used to irrigate halophytic native plants such as *Atriplex canescens* and *A. lentiformis* two halophytic native plants, which can then be cultivated for livestock feed. The objectives of this study are to (1) determine the impacts of ions from brackish water irrigation on the chemical properties of the soil, (2) evaluate how the addition of saline concentrate might impact the microbial community in the soil, and (3) assess halophyte germination, growth and vigor under highly saline irrigation.

Soil samples were collected 30 cm, 60 cm and 90 cm distance from the irrigation emitters in two directions. At each distance, samples were collected from depths of 0-25 cm and 25-50 cm. Using the soil paste extract method, EC, pH, sodium, calcium, magnesium, potassium, and chloride ions were collected. Additional soil samples were collected 30 cm from the emitter at 0-20 cm and 20-40 cm depths to be split and sent out to a laboratory for Next-Generation Sequencing and analysis of carbon and nitrogen.

New Mexico Environmental Department has allowed a saline discharge permit of up to 4,200 mg/L at the Brackish Groundwater National Desalination Research Facility (BGNDRF; Bing, C. personal communication, Sept, 2021). Because saline concentrate will have a greater electrical conductivity (EC) than what is permitted to be added to the natural soil environment, a greenhouse experiment was conducted to evaluate the impacts of high levels of EC in source water on the growth and vigor *A. canescens* and *A. lentiformis* seedlings. Greenhouse treatments included 2 different soil types (sand and clay) and 2 irrigation treatments, "brackish" water at 4 dS/m or "concentrate" at 8 dS/m. Germination rates, heights, soil plant analysis development (SPAD) and infrared (IR) were recorded at 4 months after planting.

The pattern of EC did follow the expected bell shape of the wetting front; however, the ions patterns were varied. Soil microbe communities shifted slightly, but the shift was not statistically significant. A significant decrease and then increase of microbial hits (abundance of microbes in the soil) was seen in the middle of the study

Germination of plants was significant by species, soil type and water type. IR was increased when irrigated with higher saline water. Higher salinities of irrigation water did not affect plant height or SPAD, although additional studies are needed to determine if nutrient content of the plants are affected at high EC levels.

This study provides an evaluation of the impacts of highly saline irrigation water to soil chemical and physical properties, soil microbial communities, and plant growth. With continued study, a better understanding can be gained regarding the long-term impacts, safety, and benefits of using highly saline waters for irrigation of fodder species. This in turn will serve to promote better management guidelines and broader adoption of this practice, which may become imperative when facing prolonged drought conditions.

1. Introduction

1.1 Background and previous work

The western United States have experienced higher than average temperatures since 2000, with some areas seeing an average of 1° C increase from 2000 to 2018 (NOAA 2019). This increase in average temperature for the region is associated with a decrease in precipitation (NOAA 2019). Lack of surface water replenishment and increased demand has led to long lasting droughts in this region (Mekonnen and Hoekstra 2016). Cook et al. (2020) predict droughts will continue in the western United States as decreased precipitation and increased temperatures continue. Irrigation through surface water supplies have been decreasing in New Mexico, Arizona and California, causing farmers to turn to groundwater to fulfill the needs (Funk et al. 2016; Kennedy, 2021).

There are large groundwater aquifers in the southwestern United States (Reilly et al. 2008). In New Mexico, 75% of the groundwater is brackish and too saline for human consumption (Land 2016). Salinity of groundwater is also variable and brackish groundwater in the Mesilla Valley could have a salinity as high as 3.755 dS/m (Driscoll and Sherson 2016), while the Tularosa Basin, where BGNDRF is located, has salinity ranging between 4.68 dS/m to 15.63 dS/cm in brackish areas and higher in saline areas of the basin (Newton and Land 2016).

As surface water decreases and the use of brackish groundwater increases, the need for desalination also increases. If the groundwater is not treated to remove salts before irrigation, salt accumulation will occur in soils and this will affect the environment, including the physical and chemical properties of the soil (Cucci et al. 2015), the plants that are able to grow in the soil (Yang et al. 2020) and the microbial communities (Rath et al. 2019).

Soil salinity is of great concern globally, as this is a growing environmental stress. Increased soil salinity leads to a decrease in crop yields and decline in available land for agriculture (Wang and Wang 2019; Pessoa et al. 2019). Arid and semiarid regions around the world rely on brackish water for irrigation to supplement agricultural needs. However, these regions do not receive enough precipitation to ensure proper leaching of the soils to remove accumulated salts.

Use of brackish water can damage crops (Rhoades, 1984; Yang et al. 2020) and many plants are not able to compensate for additional salt (Ozturk et al. 2018; Kankarla et al. 2019), but recent studies are beginning to show some plant species can utilize brackish water irrigation sources (Yang et al. 2020; Wang and Wang 2019), and soil amendments such as gypsum may help alleviate soil salinity toxic effects (Phogat et al. 2020). Soils with an electric conductivity (EC) greater than 4 dS/m are considered to have a high ion concentration (Richards 1969). Soils are considered to be highly saline if the EC is greater than 100 dS/m, and values up to 200 dS/m have been recorded (Richards 1969). One study of two years found that while there were no macroscopic changes to soil structure when irrigated with brackish water (ECs >4 dS/m), there were microscopic changes which reduced the soil's water movement ability (Cucci et al. 2015). It has been suggested to plant halophytes in highly saline soils as a way to reduce the soil salinity levels (Quadir and Oster 2004). Addition of biomass and organic material has also been shown to help reduce salinity (Bian 2012; Sakai et al. 2012).

Long term studies are recommended to observe changes in biomass and soil characteristics (Ozturk et al. 2018; Kankarla et al. 2019).

Reverse osmosis (RO) is a common option for removing salts from source water, accounting for 85% of the operational desalination plants in the world (Eke et al. 2020). RO functions by forcing water through a membrane to collect dissolved salts and impurities (Cavalcante et al. 2019). Apart from being energy intensive, one issue with RO is the concentrate waste created in the process of desalinating (Cavalcante et al. 2019). Traditional disposal methods of the concentrate include discharge to surface waters or oceans, deep well injection, thermal evaporation, zero liquid discharge (ZLD), and irrigation of crops (Cavalcante et al. 2019). Methods that release concentrate into the environment change the ecological habitat by increasing salinity of the surface water or oceans. Deep well injection methods pose hazards to groundwater aquifers through contamination. Thermal evaporation in evaporation ponds could also potentially contaminate groundwater aquifers. Salts extracted from thermal evaporation may also need further disposal. ZLD is an expensive process, both in financial and energy terms.

An environmentally safe disposal of the concentrate is needed. Researchers are considering irrigation of halophytic plants that could then be used for feeding livestock (Flores et al., 2016; Cavalcante et al. 2019; Salem et al. 2010; Kronberg 2015). Little research has been conducted to determine the effects irrigation of saline concentrate on halophytes may have on microbial habitats for periods of time greater than a single season (Flores et al., 2016; Ozturk et al. 2018).

Few studies have looked at the rhizosphere microbial habitat of *Atriplex* species. Halophytic plants, such as *Atriplex canescens* and *Atriplex lentiformis* have phytoremediation properties (Hasanuzzaman et al. 2014) and are useful to ranchers as a fodder supply (Salem et al. 2010; Kronberg 2015). Kronberg (2015) suggests a diet with 30% *A. canescens* during late summer, fall and winter grazing would be sufficient to sustain late-lactating and non-lactating cattle while improving sustainability of grazing lands. As soil salinity increases in agricultural regions, the ability to replant grazing lands with halophytic plants which will sustain cattle becomes a priority to ensure land is appropriately managed.

The ability of *Atriplex lentiformis* and *A. canescens* to uptake soil ions to remediate the soils has not yet been studied. Sodium absorption ratio (SAR) is used to determine the sodicity of soils (Franzen et al. 2017). Sodic soil degrades the soil structure as clay particles and sodium ions fill pores, reducing water flow through the soil (Franzen et.al. 2017). Soil is described as saline if there are excess levels of soluble salts in the soil water, and is described as sodic if the SAR is 13 or greater, showing an excess of sodium ions in comparison to cations (Franzen et al. 2017). A SAR of greater than 35 will reduce nutrient availability and hydraulic conductivity of the soil (Babcock et al. 2009). Babcock et al. (2009) suggest soil sodicity be considered a problem needing attention when irrigating with brackish water and to alleviate accumulated ions, irrigation should be at least twice the required ET_0 of the vegetation.

Soil microbial communities typically change in response to variation in soil characteristics. It is well documented soil pH is a factor affecting the microbial community (O'Brien et al. 2019; Rath et al. 2019; Zhang et al. 2019). Microbes generally prefer a soil pH range between 6 and 8, as carbon availability, nitrogen availability, and microbe processes, such as decomposition occur readily at these levels (O'Brien et al. 2019; Rath et al. 2019; Zhang et al. 2019). Rath et al. (2019) also found changes in soil pH of one unit in either acidic or basic direction to be detrimental to soil microbe

community composition. Soil salinity is believed to be a factor that influences community diversity (O'Brien et al. 2019; Rath et al. 2019; Zhang et al. 2019). As salinity increases in soil, a decrease in soil microbial diversity has been recorded (Zhang et al. 2019).

The response of bacteria to soil salinity is not yet fully understood. Diversity shifts as a result of increased salinity in soils were reported in bacterial communities (Rath et al. 2019). Vayourakis et al. (2015) indicated an increase in archaeal diversity with increased salinity, while Rath et al. (2019) did not observe this shift.

1.2 Study Objectives

The objectives of this study were to develop a better understanding of the impacts of irrigation with reverse osmosis concentrate on soil physical and chemical properties, soil microbial community dynamics, and plant growth and vigor.

1.3 Research Partners

Ms. Denise Hosler (retired, Bureau of Reclamation, Denver Technical Service Center) assisted in the first year of the study including research design and set up. The lab of Dr. Pierre Jacinthe of Indiana University-Purdue University Indianapolis examined soil samples for soil microbe biomass, percent carbon, percent nitrogen, and soil respiration. Dr. Jacque Keele of the Reclamation Environmental Research Lab in Denver, Colorado, prepared soil samples for Next Generation DNA Sequencing. Mr. Randy Shaw and staff of the Brackish Groundwater National Desalination Research Facility maintained water sources and *Atriplex* field conditions. With the help of Ms. Mariah Armijo, greenhouse experimentation was completed at the New Mexico State University Agricultural Experiment Station.

2. Materials and Methods

2.1 Site Description

The field study was conducted at Reclamation's Brackish Groundwater National Desalination Research Facility (BGNDRF) in Alamogordo, New Mexico (32°52' N, 105°58' W, elevation 1322 m). The area receives on average 33.6 cm of annual precipitation. The yearly average high temperature is 23.89°C and the annual average low is 8.33°C. (NOAA 2019) The region is classified as a semi-arid desert (USGS 2001). Figure 1 shows weather data over the study period.

Two half-acre agriculture plots at BGNDRF were identified for this study. Both plots drained from the northeast to the southwest, although low points within each field allowed ponding to exist. Soil texture was identified from 16 stratified random selected soil samples in April 2017. The strata included South plot, 0-25 cm depth; South plot, 25-50cm depth; North plot, 0-25 cm; and North plot, 25-50 cm depth. Within each group, four samples were randomly selected. Soil was air dried

and passed through a 2 mm sieve. Then, using the hydrometer method (Gee and Bauder 1986), soil particle size and texture were analyzed for sand, silt and clay content.

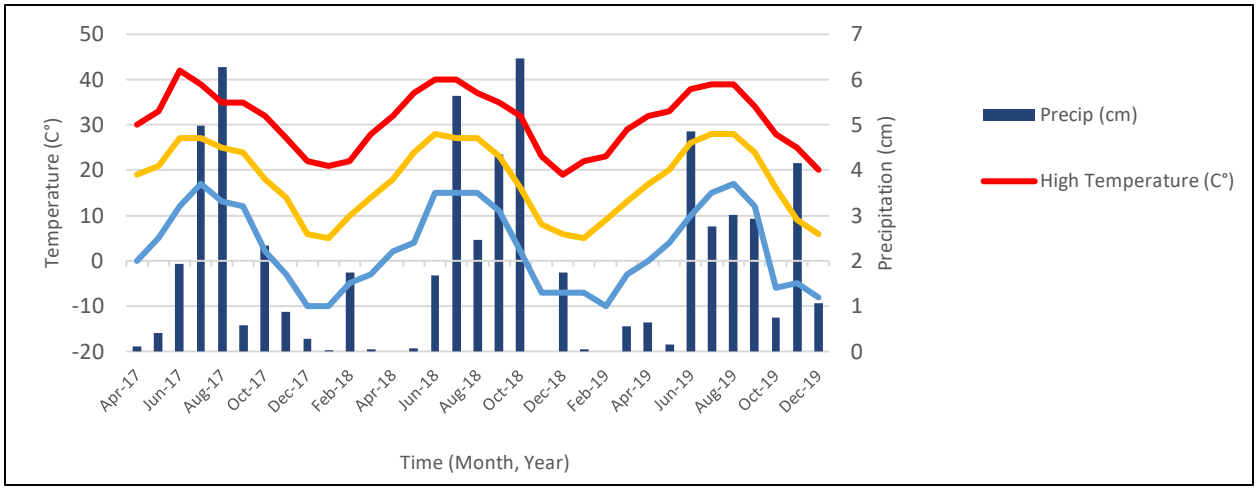

Figure 1. Weather data through the study months showing high monthly temperatures, low monthly temperatures, average monthly temperatures and precipitation.

Agriculture plots were planted between April, 2014 and October, 2015 with *Atriplex canescens* and *A. lentiformis* at a rate of 1 plant per 2 meters in each single row. Each row was spaced approximately 3 meters apart. Drip irrigation line was placed on the west side of the rows of plants. Emitters were placed at 0.3m intervals. The plots were arranged in 6 irrigation zones each. Each zone contained two rows of plants of a single species, oriented in a north to south fashion. Each zone was randomly assigned with the plant species and an irrigation rate. The plots were surrounded with 45 cm mesh fencing to prevent rabbits from entering and damaging young plants. When the study began, plants were approximately 1 m tall.

In February, 2017, all zones were leached with water from well 1 (TDS = 1,240 mg/L, BGNDRF Water Analysis, 2019). Leaching was conducted through the drip system three times per week, 120 minutes for each leaching event. This continued for 6 weeks. In April, 2017, following leaching, soil samples were collected to establish baseline data.

2.2 Irrigation

Irrigation treatments were established using the Blaney-Criddle equation (Blaney and Criddle 1962):

$$ET_0 = p(0.46T_{mean} + 8) \quad (1)$$

Where p is the percentage of sun for latitude of 35°N and T_{mean} was the average monthly temperature in degrees Fahrenheit. The resulting ET_0 was converted from inches to millimeters (Fig 2). We calculated an initial evapotranspiration (ET_0) to be 1,335 mm/year for 2017. At this ET_0, 1,753 gallons of water would need to be applied to each plot weekly. It was then determined that because in a natural setting with water availability decreasing, irrigation treatments were set at 60% and 80% of the use of water established, meaning we would need 1,402 gallons/week and 1,052 gallons/week respectively. Irrigation events occurred three times per week with a mixture of Well 2

and Well 3 at BGNDRF to create a water solution of approximately 4,200 mg/L TDS (BGDNRF Water Analysis, 2019). Irrigation events lasted for 88 minutes to represent the 60% ET_0 and 120 minutes to represent the 80% ET_0. Irrigation events were not adjusted for a changing monthly ET_0. Irrigation was suspended one week prior to soil collections and plant trimmings. Plants were trimmed to a set height of 95 cm and width of 1 m in February prior to each growing season (2017, 2018, and 2019).

2.2.1 Blaney-Criddle and Hargreaves comparison

We compared our Blaney Criddle calculations to the Hargreaves equation (Hargreaves 1985):

$$ET_0=\sum 0.0022\ R_a\ (TC+17.8)\ TR^{0.5} \quad (2)$$

Where the ET_0 is the initial evapotranspiration for the month, R_a is the solar radiation, TC is the average temperature in Celsius and TR is the temperature range in Celsius. We used this calculation to compare to the Blaney Criddle to determine if calculations were similar (Fig. 2).

2.3 Soil Chemistry

2.3.1 Soil Sample Collection

In each field, three plants were randomly selected. Selected test plants were robust in leaf coverage, not located on the edges of the field, and observed not to have variation in soil topography (dips or hills). Soil samples were collected at three distances: 30 cm, 60 cm, and 90 cm from the drip irrigation emitter in both east and west directions (Fig. 3). At each distance/direction, samples were collected with a 3 in x 24 in auger drill bit at two depths; 0-25 cm and 25-50 cm. Samples were placed in a 1-gallon Ziploc bag to be divided. Each sample was approximately 500 g. Soil sample collection was repeated in November of 2017 and 2019 as described above.

2.3.2 Chemical Analysis

To test for electric conductivity, we removed any large organic material from 100 g of soil and placed it into a cup. Deionized water was mixed into the soil, creating a paste, at a ratio of 2:1 deionized water to soil by weight. The soil paste was covered with plastic wrap and allowed to rest for 2 hours. After the soil paste rested, the pH of the soil was measured and the soil paste was then transferred to a Buchner funnel with a 100 mm diameter, grade 1 filter. Vacuum suction was applied to collect the effluent in a 50 ml tube. Electric conductivity (EC) measurements were collected from the effluent (Gavlak et al. 1994). If the EC was greater than 15 dS/m, the effluent was diluted 10% to obtain a more accurate reading. This testing was conducted in Apr. 2017, Nov. 2017 and Dec. 2019.

Magnesium, calcium, sodium, potassium, nitrate and chloride ion content were measured from the soil paste effluent using inductively coupled plasma (ICP) ion analysis (U.S. Energy Protection Agency Staff 1982). Sodium Absorption Ratio (SAR) was calculated according to the equation given by Robbins (1983):

$$SAR=\frac{[Na^+]}{\sqrt{\frac{(Ca^{2+})(Mg^{2+})}{2}}} \quad (3)$$

where Na^+ is the concentration of sodium ions, Ca^{2+} is the concentration of calcium ions and Mg^{2+} is the concentration of magnesium ions. Soil chemistry analysis was conducted at the New Mexico State University soils lab for the baseline data. Approximately 100 g of soil from each sample was sent to AgSource Laboratories (Lincoln, NE) for soil chemistry analysis of post season soils of 2017 and 2019.

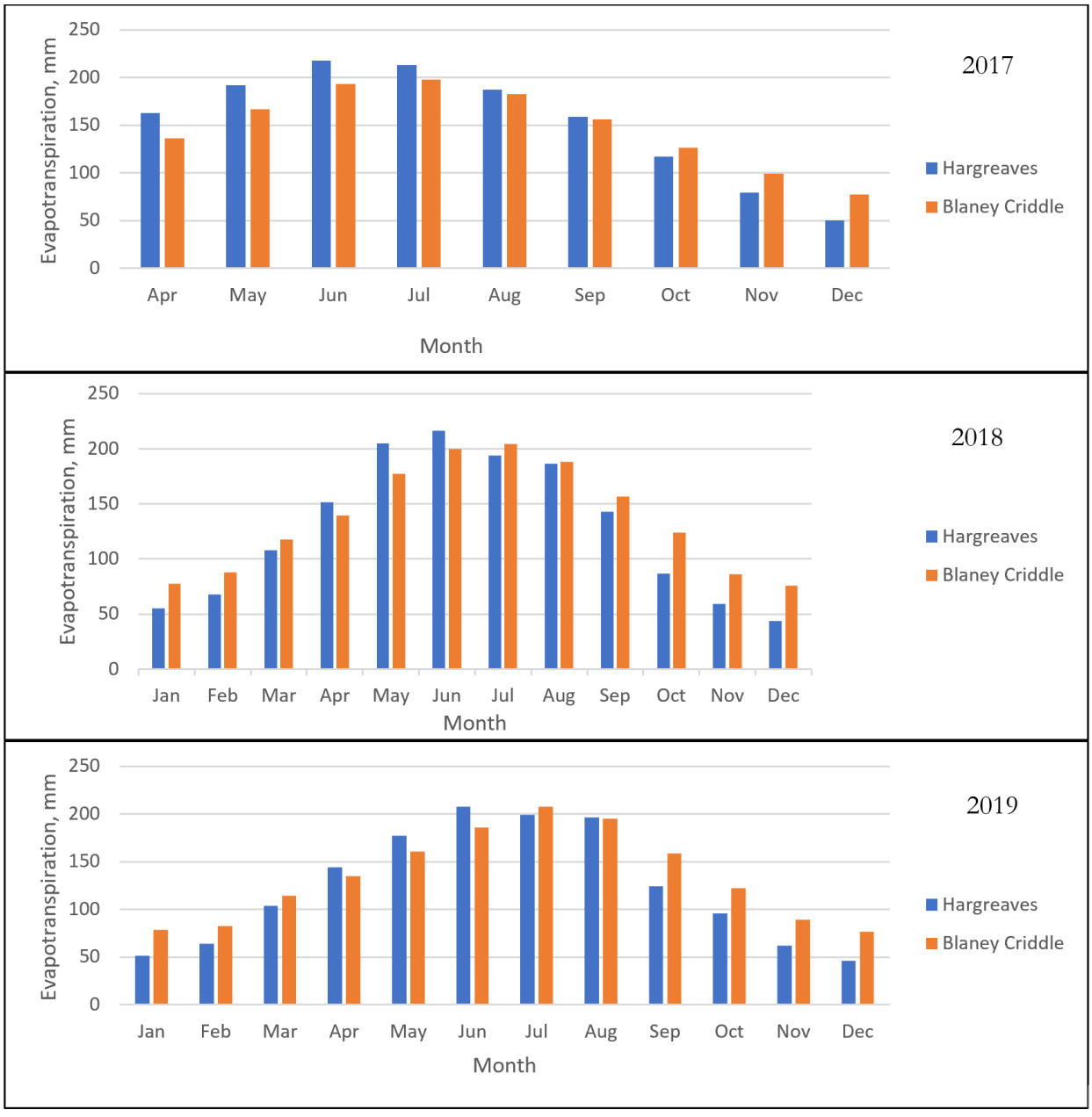

Figure 2. Evapotranspiration comparison between Hargreaves method (1985) and Blaney Criddle (1962) for Alamogordo, New Mexico during the three years of the study. Yearly evapotranspiration totals shown in millimeters.

Figure 3. Soil sample collection was conducted on either side of the plant at 30 cm, 60 cm and 90 cm from the irrigation line. Irrigation line placement moved during collection and returned to the base of the plant. Atriplex agriculture plot, Brackish Groundwater.

2.4 Soil Microbe Community

2.4.1 Soil Collection

Four soil samples were collected from each plot, two from each irrigation treatment at 30 cm from the drip line and at depths of 0-20 cm and 20-40 cm. One set of control soil samples collected outside of the irrigation area at the same depths. The soil samples were collected using a 3 in x 24 in auger drill bit from each plot, as well. Sterile techniques were observed to avoid contamination by washing the auger and soil collecting tools with bleach wipes between samples. Approximately 200 g of soil was collected for each sample in 50 ml test tubes. After irrigation was started, soil samples were collected in November, 2017 and at the end of the last growing season in December, 2019. Soil samples were shipped with ice to preserve microbial communities to designated labs in Denver, Colorado, and Indianapolis, Indiana.

2.4.2 Next-Generation Sequencing

Half of the soil samples (100 g each sample) were sent to the Bureau of Reclamation Ecological Research Lab in Denver, Colorado for DNA extraction. Samples were dried and sieved before DNA extraction was performed using a DNeasy PowerSoil Kit, with procedure followed as described in manufacturers manual (Quiagen, Hilden, Germany). Samples were then sent to RTL Genomics

(Lubbock, Texas) for Next-Generation sequencing. PCR amplification was accomplished using the 16S rRNA gene, utilizing the V4 region as the primer. PCR involved initial denaturation at 95°C for 2 minutes, followed by 30 cycles of denaturation of 95°C for 20 s, annealing at 55°C for 15 s, elongation at 72°C for 5 minutes and a final elongation at 72°C for 10 minutes. Paired-end sequencing of the PCR amplicons involved the MiSeq 250=bp paired sequencing system (Illumina) according to the manufacturer's instructions. Sequences less than 100bp were removed to reduce noise. USEARCH (Edgar, 2010) was used to continue de-noising the data by removing clusters of less than 2 members. Chimera checking was performed on OTU selections and removed if marked as "Chimeric." All sequences were trimmed to the same length. OTU clustering was performed using UPARSE and original reads were mapped to the OTUs. Using the selected OTUs, a phylogenetic tree in Newick format was constructed. Taxonomic identification was conducted using USEARCH global search algorithm

2.4.3 Soil Carbon and Nitrogen testing

Soil samples were placed on ice and shipped overnight to the Center of Earth and Environmental Sciences at Indiana University Purdue University Indianapolis. Field-moist soil samples were extracted with 0.5 M KCl (1:2 soil to solution ratio), and the extract analyzed for NH_4^+ (nitroprusside method) and NO_3^- (hydrazine reduction method) concentration using a photometric nutrient analyzer (Aquakem).

A portion of each soil sample was air-dried, sieved (2 mm) and used for determination of pH and soil C. Soil pH was measured with a pH-meter using a soil suspension (soil-to-water ratio of 1:2). An aliquot of each dried soil sample was crushed, and the fraction that passed through a 250 μm sieve was used for determination of C and N concentration by dry combustion (960 °C) using a Vario-TOC analyzer (Elementar Americas, Mount Laurel, NJ). Given the alkaline pH of most of the soil samples, samples were treated with mild acid (0.2 g of soil, 0.5 mL of 1 M HCl) to remove carbonates prior to C & N analysis.

2.5 Greenhouse Atriplex testing

2.5.1 Experimental design

Experimental soil was collected from two locations and designated sand or clay. The sand was collected from Dona Ana County at approximately 32.2387 latitude, -106.9146 longitude. The clay was collected from Fabien Garcia Research Facility of New Mexico State University in Las Cruces, New Mexico (32.2797 lat, -106.7726 lon). Soils were spread on tarps and air dried for 5-7 days, mixing to ensure uniform moisture content inside the greenhouse. Plant pots were thirty-two PVC pipes approximately 30 cm in diameter cut to 35 cm in length, with a cap attached at one end with 6 drilled holes for drainage. At the bottom of each pot, one layer of cheese cloth was applied, then gravel to a height of approximately 10 cm, and a second layer of cheese cloth. The pots containing the rocks and cheese cloth were weighed. Experimental soil of either sand or clay was then added until approximately 10 cm from the top edge of the pot. The filled pot was then weighed and bulk density of the soil was calculated. Pots were leached with fresh tap water until effluent was less than 1 dS/m. Each pot was planted with seeds of either *Atriplex lentiformis* or *A. canescens,* with 16 pots of each. Pots were watered with brine, at either 4 dS/m or 8 dS/m. This gave an experimental group to be either sand or clay, either *A. lentiformis* or *A. canescens*, and either brine at 4 dS/m or 8 dS/m.

Because field studies have been conducted using water sources of less than 4 dS/m, we compared the growth of the plants to a brackish source and a concentrate source of water.

Seed germination was measured 14 days after planting, by counting emergence of seedlings. Plant height was measured at 4 months after planting, as well as IR and SPAD of the leaves for each of the experimental groups. IR was obtained using an infrared meter. An initial IR reading was obtained, with a second reading 48 hours following. An increased IR indicates plant growth and viability. SPAD was measured using a SPAD meter. Increased SPAD indicates chlorophyll availability for photosynthesis. Measurements were recorded from midlevel leaves which were not shaded.

2.6 Statistical Analysis

Statistical analysis was conducted using R-4.0.2 (Ihaka and Gentleman, 2020). Differences for each distance from the drip emitter and soil depth compared to time and irrigation rate were determined using analysis of variance (ANOVA) tests. Differences in height, germination rate, IR and SPAD were calculated in comparison to water salinity, species and soil type also using ANOVA tests. Significance was determined at an alpha of 0.05, using a Tukey Test for between group analyses. Primary component analysis (PCA) and Pearson correlation analysis were also conducted to determine relationships between various factors in the study. Ion concentrations were log transformed for PCA and Pearson correlation analysis to ensure consistency in data interpretation. Comparison of microbial communities were analyzed using a permutational multivariate analysis of variance. Alpha diversity was calculated using R.

3. Results

3.1 Physical Analysis

Soils collected within each plot were classified using USDA textural classification (Soil Survey Division Staff 1993). The soil sample texture ranged from sandy clay loam to clay loam in the 0-25 cm depth and from sandy loam to clay from 25-50 cm depth (Table 1).

3.2 Soil Chemistry

3.2.1 Electric Conductivity (EC)

Electric conductivity showed significance in location (ANOVA p-value 1.10E-10), time (ANOVA p-value 1.46E-3), and irrigation treatments (ANOVA p-value 1.44E-2). Depth was not significant (ANOVA p-value 7.87E-1). The soil samples 30 cm from the emitters showed a significantly lower EC than samples from the 60 cm and 90 cm distances (Tukey Test p-values 1.08E-05 and 1.00E-07, respectively). The end of year 3 EC was significantly higher than baseline and the end of year 1 (Tukey Test p-values 4.0E-3 and 7.0E-3, respectively), showing an accumulation of salts.

Accumulation was significantly greater in the 60% irrigation treatment in comparison to the 80% irrigation treatment at 60 and 90 cm from the emitter. A significant interaction between location and irrigation was indicated (ANOVA p-value 1.09E-2) (Fig. 4). The distribution of data included more

Table 1. Soil texture data. Samples shown by distance from plant (30, 60, 90 cm) and depth (a=0-25 cm, b=25-50 cm) collected in April 2017, prior to treatments applied.

Sample	% Sand	% Clay	% Silt	Texture Class
30a replicate 1	59.3	24.0	16.7	Loam
30a replicate 2	37.3	50.0	12.7	silty loam
30a replicate 3	57.3	30.3	12.4	clay loam
30b replicate 1	63.3	22.0	14.7	sandy clay loam
30b replicate 2	63.3	22.3	14.4	sandy clay loam
30b replicate 3	67.6	6.7	25.6	sandy loam
60a replicate 1	51.3	36.0	12.7	clay loam
60a replicate 2	59.3	32.3	8.4	clay loam
60a replicate 3	56.0	32.0	12.0	clay loam
60b replicate 1	33.3	48.3	18.4	silty clay
60b replicate 2	49.3	38.3	12.4	clay loam
60b replicate 3	60.0	24.0	16.0	loam
90a replicate 1	39.3	49.4	11.3	clay
90a replicate 2	53.6	30.0	16.4	clay loam
90b replicate 1	39.3	46.3	14.4	clay
90b replicate 2	47.6	38.0	13.4	clay loam

variation from the mean in the 90 cm and 60 cm west collection points when compared central collection points for both irrigation rates (Fig 4). In general, the lower irrigation rate of 60% ET_0 is more variable regardless of collection location.

Soil salinity (based on EC) increased over time between both irrigation rates and both depths (ANOVA, p-value 3.53E-3) in the soil samples 30 cm from the drip irrigation emitter. There was also a significant difference between irrigation rates (ANOVA, p-value 4.02E-2), with the salinity of the soil being higher at the 80% ET_0 irrigation rate, in comparison to the 60% ET_0 irrigation rate, in the samples 30 cm from the drip irrigation emitter. This location was closely examined for EC as the same location microbial communities were observed.

3.2.2 Ion concentrations

The ions measured included calcium, magnesium, sodium, potassium, nitrogen, and chloride. The accumulation patterns of the ions varied by the end of year 3 (Fig. 5). Calcium, potassium, and magnesium ion concentrations in the soil were similar for all distances, while sodium and chloride ion concentrations were greatest at 60 cm and 90 cm distances and lowest at 30 cm distances.

3.2.2.1 Calcium

Calcium ions accumulated in significantly higher quantities in the 0-25 cm depth samples compared to the deeper 25-50 cm samples (ANOVA p-value 8.64E-06). Calcium ions also significantly

accumulated over time (ANOVA p-value 2.00E-16), with a greater quantity of ions accumulated at the end of year 3 than the baseline data. There was no statistical significance in accumulation of calcium with distance from the emitter (ANOVA p-value 3.43E-1). The greatest accumulation of calcium through the duration of the study in relation to the irrigation treatment was with the 80% ET_0 but this difference in irrigation treatments was not significant (ANOVA p-value 1.06E-1).

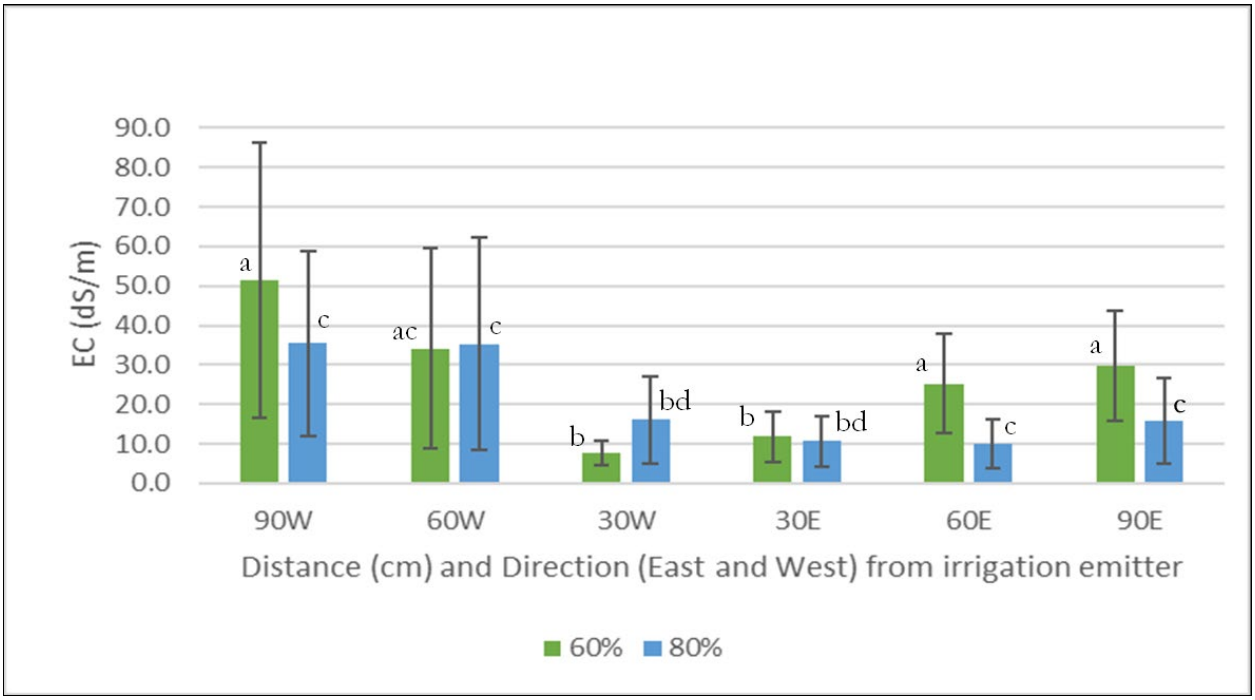

Figure 4. Electric conductivity relationship between distance from the emitter and irrigation treatment.

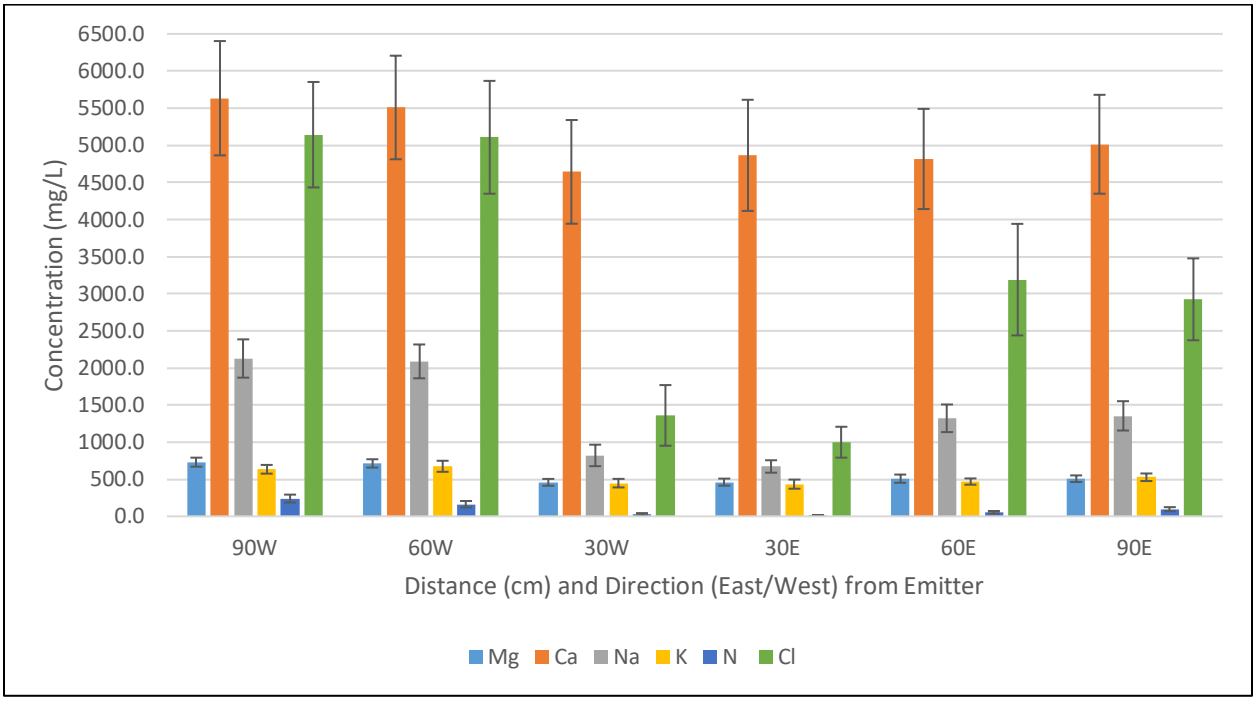

Figure 5. Ion concentrations by distance and direction from the irrigation emitter at the end of year 3.

3.2.2.2 Magnesium

Magnesium ions accumulated in the greatest concentrations 60 cm and 90 cm from the irrigation emitter (ANOVA, p-value 6.33E-4), however depth was not a significant factor (ANOVA p-value 9.65E-1). The baseline magnesium ion accumulation was significantly less than the end of growing seasons one and three (ANOVA, p-value 9.6E-12). Magnesium ions had a greater accumulation in the 60% ET_0 irrigation treatment in comparison to the 80% ET_0 irrigation treatment (ANOVA, p-value 1.6E-06), with the greatest accumulation occurring at the end of the study in the 60% ET_0. The lowest accumulation of magnesium was in the baseline, 80% ET_0 rate. The end of year 3 accumulation was significantly greater than the baseline (ANOVA, p-value 9.6E-12)

3.2.2.3 Potassium

Potassium ions accumulated at the 60 cm and 90 cm distance from the emitter at greater levels than the 30 cm distance (ANOVA p-value 1.08E-3), especially to the west of the emitter. There was a significant increase in accumulation of potassium ions over the duration of the study (ANOVA p-value 2.0E-16), which was interacting with the irrigation treatments (ANOVA 5.73E-14). Irrigation treatments alone and depth of soil samples were not significant (ANOVA p-value 4.38E-1 and 6.84E-2 respectively). When depth was related to distance from the emitter, samples of 60 cm and 90 cm had greater accumulation closer to the surface (0-25 cm) (ANOVA p-value 4.23E-2), but at the lower depth (25-50 cm), distance was not significant was not significant (ANOVA p-value 2.12E-1).

3.2.2.4 Nitrogen

The accumulation of nitrogen ions decreased during the study (ANOVA p-value 1.97E-07). The distance from the emitter had the greatest effect at the baseline sampling (ANOVA p-value 4.32E-06) and through the end of year 3 (ANOVA p-value 7.63E-10). Samples 30 cm from the emitter were less than samples at 60 cm or 90 cm, possibly due to plant uptake near the root zone. There as not a significant accumulation of nitrogen ions due to irrigation treatment (ANOVA p-value 2.63E-1) or the depth of the sample (ANOVA p-value 2.0 E-1) over the course of this study. When considered with time, there was a significantly lower concentration of nitrogen ions during the baseline sampling in association with the 60% ET_0 irrigation treatment (ANOVA p-value 1.56E-2), however the treatment had not begun and this difference cannot be attributed to the irrigation treatment affect. It is unclear why the baseline samples had lower nitrogen ion concentration at the locations of the 60% ET_0 irrigation treatment, but plant 2 of the sampling had lower initial nitrogen values as an outlier compared to the other 5 study plants.

3.2.2.5 Chlorine

Accumulation of chlorine ions was significantly higher with either of the irrigation treatments (ANOVA 7.91E-05), distance from the emitter (ANOVA p-value 1.36 E-08) but showed a significant decrease over time for all treatments (ANOVA 2.06E-09). Baseline levels of chlorine were greatest in samples farther than 30 cm from the emitter. While an ellipsoid pattern of chlorine ion concentrations did appear to be present at the end of year three, the accumulation levels of chlorine ions were significantly less than the initial samples (ANOVA p-value2.9E-3). There was a greater concentration of chlorine ions in the baseline samples at 60% ET_0 irrigation rate (p-value 9.1E-6) , which declined through the course of the study. At the end of years 1 and year 3, there was not a difference between irrigation treatments for chlorine ion accumulation (ANOVA 6.97E-1).

3.2.2.6 Sodium

Sodium ions were significantly higher at distances greater than 30 cm from the emitter across all treatments (ANOVA p-value 2.49E-08). There was a significantly greater accumulation of sodium ions with the 60% ET_0 irrigation rate (ANOVA p-value 3.72E-04), however this difference could be due to a greater concentration of sodium ions at the baseline sampling. The sodium ions of the 80% ET_0 irrigation treatment did not change in the first growing season (p-value 9.47E-1) and were significantly lower by the end of the study (p-value 1.51E-2).

3.2.3 Sodium Absorption Ratio

Sodium absorption ratios (SAR) were significantly different when comparing the irrigation treatments to the baseline to the end of year 1 and year 3 (ANOVA p-value 1.08E-03) (Fig. 6). The baseline samples found SAR near or above sodic rates of 13 at distances greater than 30 cm from the plant (ANOVA p-value 7.79E-08).

3.2.4 Soil pH

The soil of the two plots ranged from sandy loam (with 67.64% sand, 6.72% clay and 25.64% silt) to clay (with 39.28% sand, 49.44% clay and 11.28% silt). Average soil pH increased from 7.95 in the baseline samples to 8.46 at the end of year one, and decreased to 7.72 by the end of year 3. The difference in pH between end of year 1 and end of year 3 is significant (t-Test, p-value 4.02E-08).

3.2.5 PCA and Pearson Correlation Analysis

Approximately 85% of the interactions were determined by primary components one and two. The first primary component had positive associations with the ion concentration and negative associations with the plant number, irrigation treatment and distance from the emitter (Fig. 7). Component 2 was positively associated with the year collected as well as the calcium and potassium concentrations, while it was negatively associated with sodium concentration, chloride concentration and depth. The correlation matrix shows a strong positive correlation with the ions (especially between sodium and chloride) and negative correlations between the study factors and the ions.

Ion accumulation was positively correlated between the various ions and negatively correlated with the study factors (Fig. 8). The strongest positive correlation was between sodium and chloride concentrations.

3.3 Soil Microbe Community

There were slight changes in phylum composition of the microbial community over the course of this study, but these changes were not significant (Table 3). The samples had varying proportions of each phylum present (Fig. 9). Proportions of phyla changed in each sample, but there were clear differences in major phyla, such as Proteobacteria, Bacteriodes and those in the Archaea kingdom.

Over all samples collected, there were 32 categories of phylum hits, including unknown hits and unclassified. In genome sequencing, a "hit" refers to the closest sequence of DNA in the sample that matches a taxonomic group, in this case, bacterial or archaeal phyla. There were 4 phyla representing the kingdom Archaea, and 5 phyla of the kingdom of Bacteria represented in the baseline data that were not present in the end of year three samples. One phylum, Fibrobacteres, was present in the baseline samples and the end of year one samples, but not in the end of year three

samples. There were four phyla of both the kingdoms, Archaea and Bacteria, that were present at the end of the study but not found in the baseline samples. Two of these phyla (Armatimonadetes and Tenecutes) were found only in the end of year three samples. There is a decrease in total Archaea and Bacteria species from 566 to 441 from the baseline to the end of year 1, followed by an increase in total species from end of year 1 (at 441) to end of year 3 at 550 species. The number of 16S rRNA hits showed a similar pattern of decreasing from baseline to the end of year 1 and then increasing to near baseline numbers by the end of year 3 (Fig. 10). The changes in total number of species over time were significant (ANOVA, p-value 1.35E-02), with the significance being due to a decrease at the end of year 1. Irrigation also showed a significant effect on the total number of species (ANOVA, p-value 1.16E-02) due to differences between the control and both irrigation treatments, but not between the two irrigation treatments (Fig. 11). The addition of irrigation increased the total number of species (p-value 4.3E-2). Depth did not affect the total number of

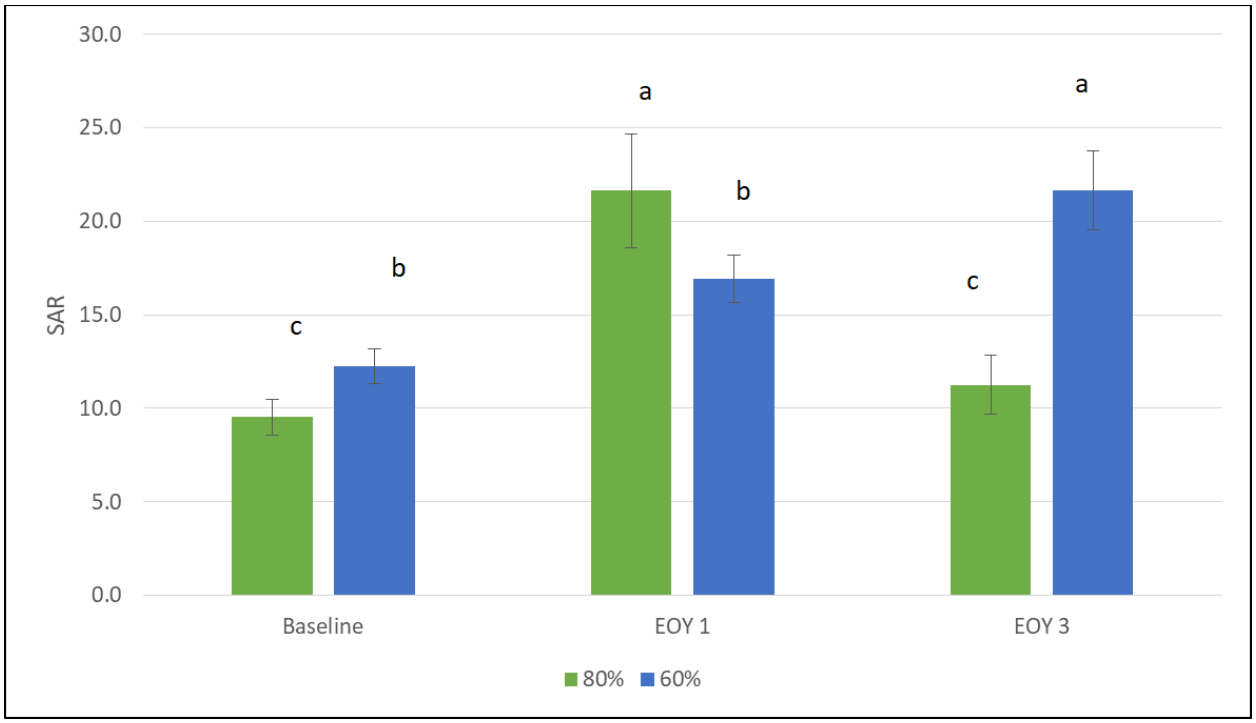

Figure 6. Irrigation treatment values for SAR over the duration of the study.

species (p-value 4.32E-1). In general, Archaeal hits increased as the total number of hits increased (Fig. 12). The control samples had significantly lower numbers of Archaea hits (ANOVA, p-value 4.9E-2) (Fig. 13).

Over all samples collected, there were 32 categories of phylum hits, including unknown hits and unclassified. In genome sequencing, a "hit" refers to the closest sequence of DNA in the sample that matches a taxonomic group, in this case, bacterial or archaeal phyla. There were 4 phyla representing the kingdom Archaea, and 5 phyla of the kingdom of Bacteria represented in the baseline data that were not present in the end of year three samples. One phylum, Fibrobacteres, was present in the baseline samples and the end of year one samples, but not in the end of year three samples. There were four phyla of both the kingdoms, Archaea and Bacteria, that were present at the end of the study but not found in the baseline samples. Two of these phyla (Armatimonadetes and Tenecutes) were found only in the end of year three samples. There is a decrease in total

Archaea and Bacteria species from 566 to 441 from the baseline to the end of year 1, followed by an increase in total species from end of year 1 (at 441) to end of year 3 at 550 species. The number of 16S rRNA hits showed a similar pattern of decreasing from baseline to the end of year 1 and then increasing to near baseline numbers by the end of year 3 (Fig. 10). The changes in total number of species over time were significant (ANOVA, p-value 1.35E-02), with the significance being due to a decrease at the end of year 1. Irrigation also showed a significant effect on the total number of species (ANOVA, p-value 1.16E-02) due to differences between the control and both irrigation treatments, but not between the two irrigation treatments (Fig. 11). The addition of irrigation increased the total number of species (p-value 4.3E-2). Depth did not affect the total number of species (p-value 4.32E-1). In general, Archaeal hits increased as the total number of hits increased (Fig. 12). The control samples had significantly lower numbers of Archaea hits (ANOVA, p-value 4.9E-2) (Fig. 13).

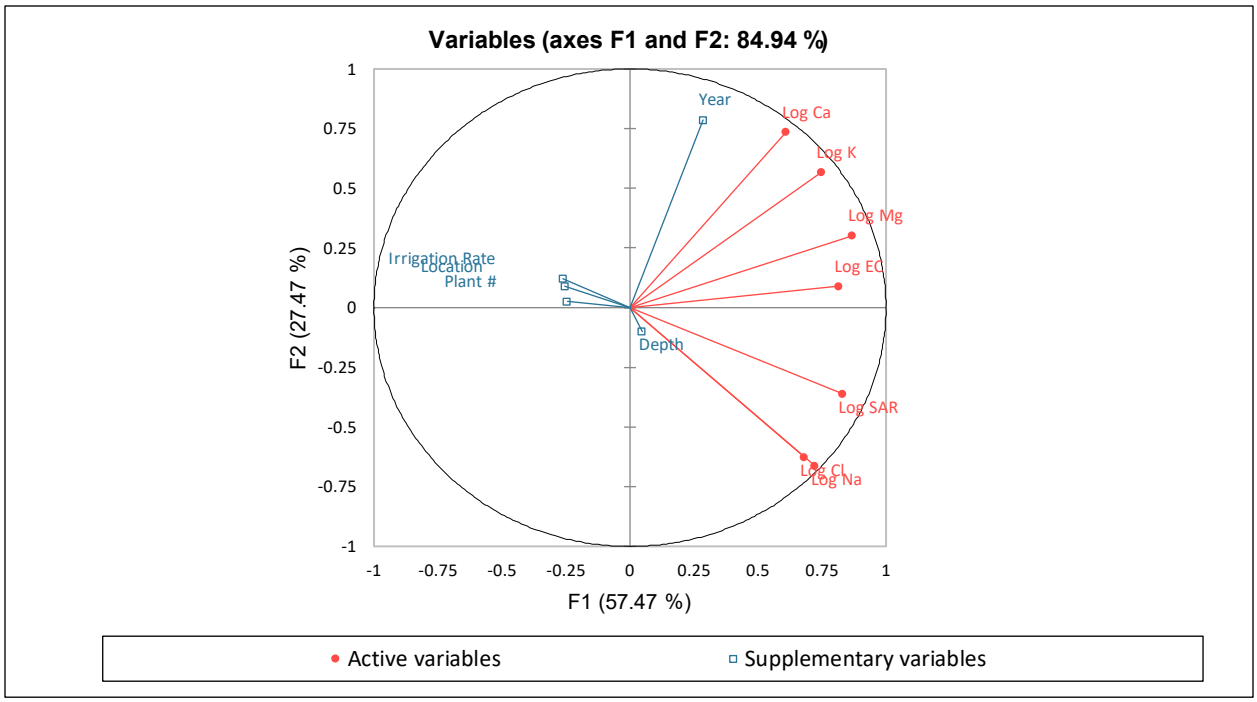

Figure 7. PCA of Factor (component) 1 and Factor (component 2).

The baseline diversity was significantly lower than the end of year 1 or end of year 3 (Permanova, p-value 9.99E-4) (Fig. 14). The lowest alpha diversity in the study was found in the baseline deep soil samples (20-40 cm) while the largest diversity was found in the 0-20 samples of the end of year 1.

Irrigation was not a significant factor in determining the diversity of the community. The greatest predictor of alpha diversity for each sample was pH (p-value 1.5E-2) and depth of sample (p-value 3.0E-2). The carbon/nitrogen ratio was significantly affected by the pH (p-value 5.2E-4) and the year of the sample (p-value 1.29E-2), but this was not a factor in determining alpha diversity (p-value 5.6E-1). Electric conductivity was not a significant determining factor for alpha diversity (p-value 4.51E-1).

3.4 Greenhouse Atriplex testing

Seedlings of *Atriplex* emerged approximately 10-14 days after planting. Germination of seedlings was significant by water type, soil type and plant type. Approximately 16% of *A. canescens* seeds germinated, while 41.25% of *A. lentiformis* seeds germinated, showing a negative correlation (Fig. 15). Germination was positively correlated with final height (Fig. 15), showing a greater height was achieved when the germination rate was greater. Salinity of irrigation treatments did not significantly affect the height of the plants, but the soil type and the species type were significant (p-value 3.47E-5 and 7.4E-3, respectfully) (Fig. 16). Infrared reflectance (IR) was significant in plants by soil type and irrigation treatment (p-values 3.67E-2 and 1.9E-4, respectively) (Fig. 17). The soil plant analysis development (SPAD) chlorophyll measurements were significantly different between species and soil types (p-values 1.81E-2 and 1.24E-2, respectively), but not significant by irrigation treatment (Fig. 18).

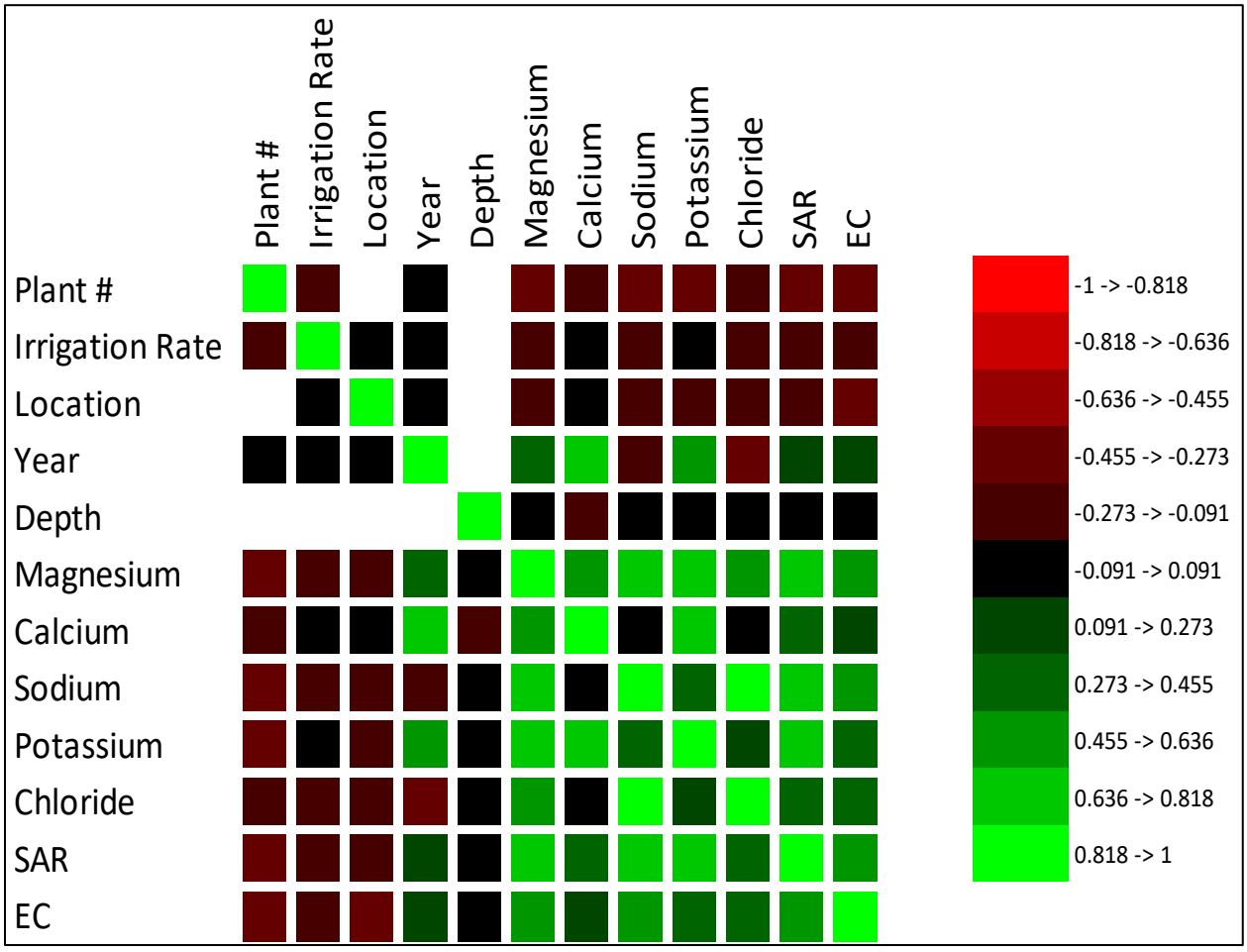

Figure 8. Pearson Correlation Matrix of soil chemistry.

Table 3. Phylum presence in baseline, end of year 1 and end of year 3 for Archaea and Bacteria kingdoms. Phyla in blue were not present in baseline samples but were present in later samples. Phyla in yellow were present in baseline samples but not found in the end of year 3 samples.

Kingdom	Phylum	Median %	Mean %	Baseline	EOY 1	EOY 3
Archaea	Crenarchaeota	1.79	1.51	No	Yes	Yes
Archaea	Euryarchaeota	0.06	0.30	Yes	Yes	Yes
Archaea	Thaumarchaeota	0.00	0.31	Yes	No	Yes
Archaea	Unclassified	2.57	2.40	Yes	Yes	Yes
Bacteria	Acidobacteria	0.60	2.23	Yes	Yes	Yes
Bacteria	Actinobacteria	28.09	24.93	Yes	Yes	Yes
Bacteria	Aquificae	0.01	0.09	Yes	Yes	Yes
Bacteria	Armatimonadetes	0.02	0.03	No	No	Yes
Bacteria	Bacteroidetes	4.83	6.56	Yes	Yes	Yes
Bacteria	Chlamydiae	0.01	0.04	Yes	No	Yes
Bacteria	Chlorobi	0.01	0.03	Yes	No	Yes
Bacteria	Chloroflexi	4.83	4.75	Yes	Yes	Yes
Bacteria	Cyanobacteria	0.37	1.00	Yes	Yes	Yes
Bacteria	Deferribacteres	0.00	0.01	Yes	No	Yes
Bacteria	Deinococcus-Thermus	0.17	1.02	No	Yes	Yes
Bacteria	Elusimicrobia	0.05	0.06	Yes	No	Yes
Bacteria	Fibrobacteres	0.00	0.15	Yes	Yes	No
Bacteria	Firmicules	1.83	2.81	Yes	Yes	Yes
Bacteria	Fusobacteria	0.00	0.02	Yes	No	Yes
Bacteria	Gemmatimondetes	1.11	1.25	Yes	Yes	Yes
Bacteria	Ignavibacteriae	0.11	0.11	Yes	No	No
Bacteria	Latescibacteria	0.21	0.21	Yes	No	No
Bacteria	Nitrospirae	0.25	0.55	Yes	Yes	Yes
Bacteria	Planctomycetes	5.71	5.35	Yes	Yes	Yes
Bacteria	Proteobacteria	25.03	26.02	Yes	Yes	Yes
Bacteria	Spirochetes	0.00	0.05	Yes	Yes	Yes
Bacteria	Tenencutes	0.00	0.01	No	No	Yes
Bacteria	Thermotogae	0.01	0.01	Yes	No	No
Bacteria	Verrucomicrobia	1.11	1.42	Yes	Yes	Yes
Bacteria	Unclassified	9.45	9.57	Yes	Yes	Yes
Bacteria	Unknown	14.80	13.74	No	Yes	No
Bacteria	Candidate NC10	0.14	0.14	Yes	No	No
No Hit	No Hit	1.84	1.98	Yes	Yes	Yes

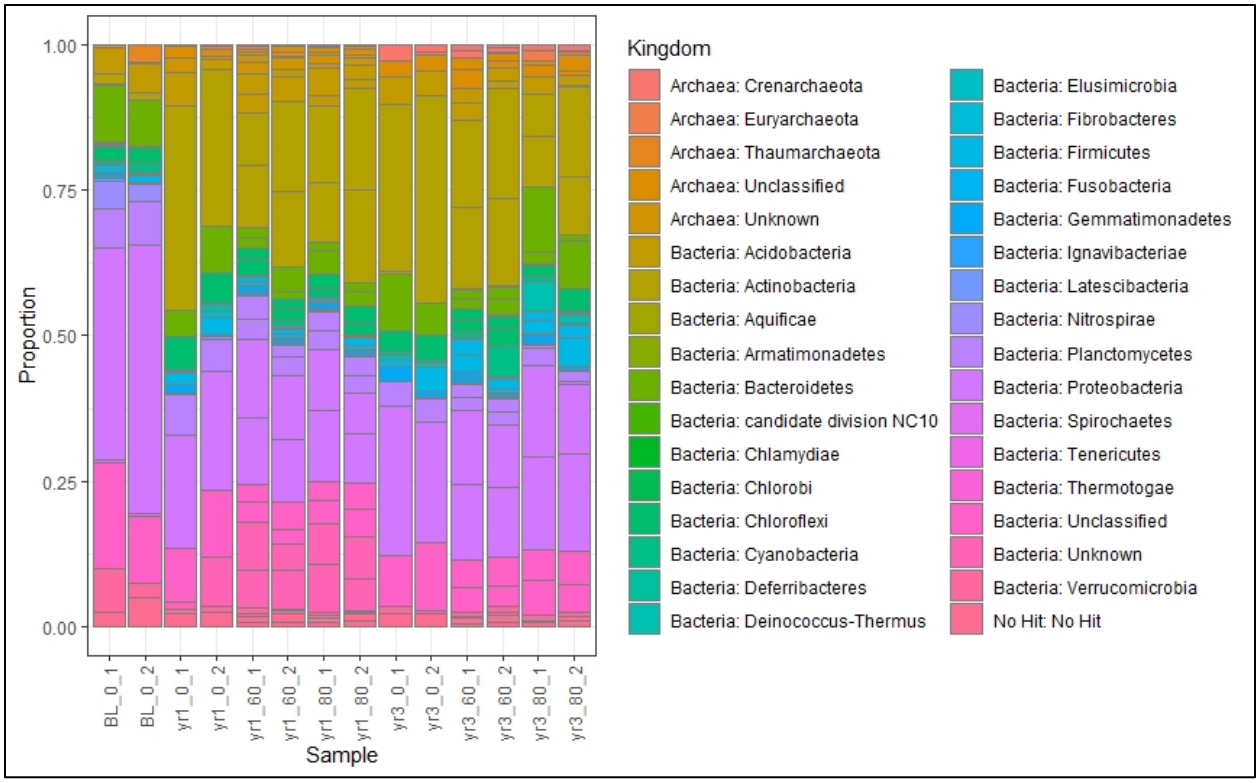

Figure 9. Bacteria and archaea species distribution from samples at various years (BL= Baseline, yr1 = end of year 1 and yr3= end of year 3), irrigation levels (0= control or no irrigation, 60= 60% ET0 and 80 = 80% ET0) and depth (1 = 0-25cm and 2= 25-50cm).

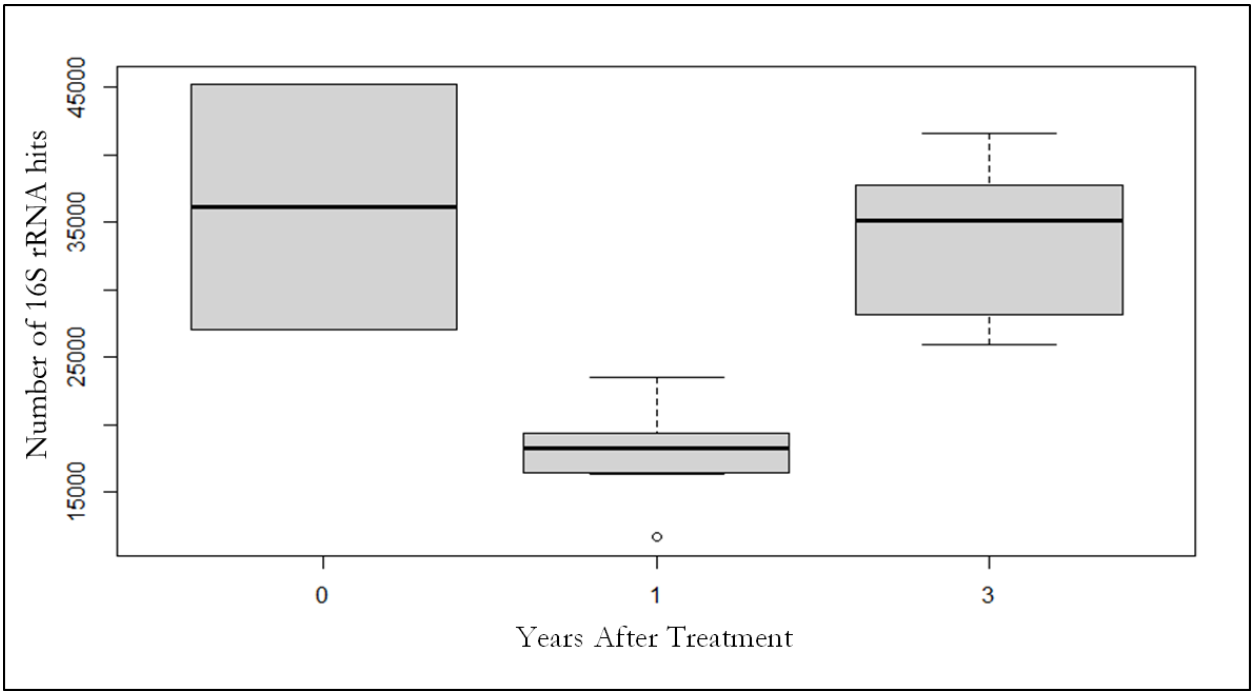

Figure 10. Number of 16S rRNA hits from soil samples collected pre-irrigation treatment (0), and 1 and 3 years after treatment (1 and 3, respectfully).

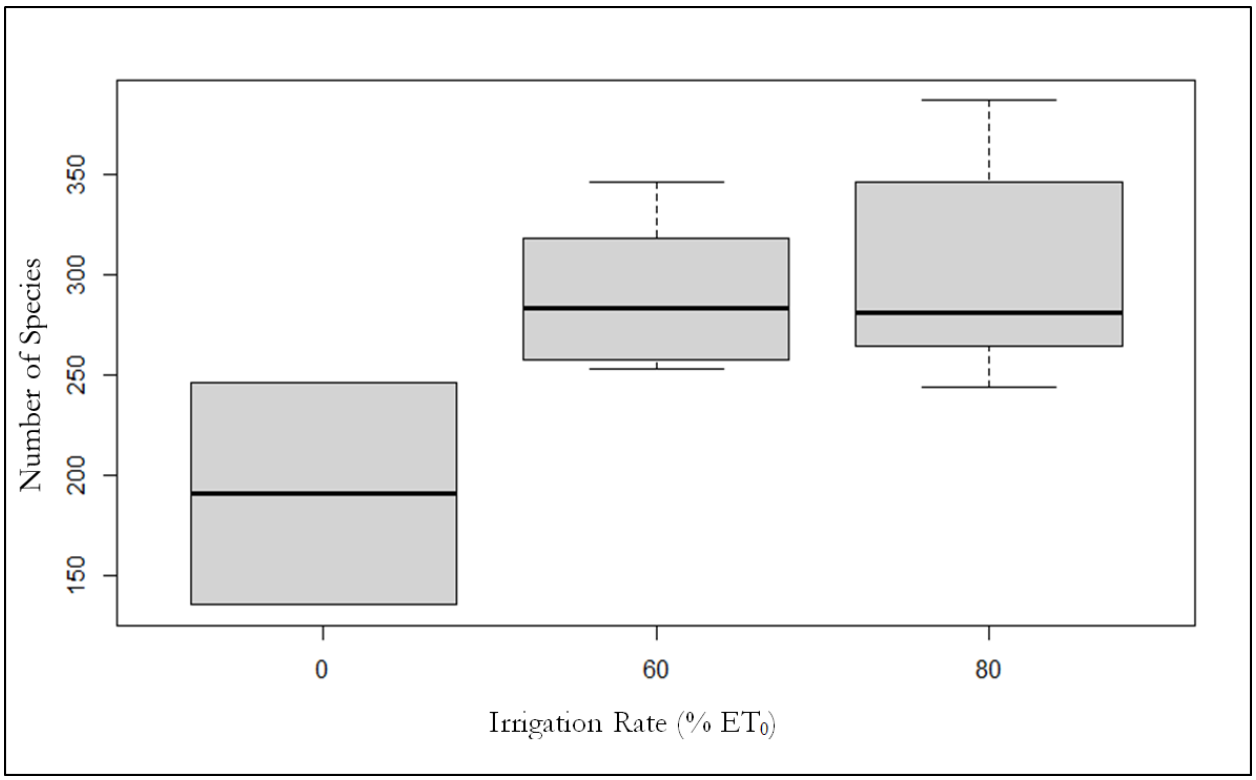

Figure 11. Number of soil microbial species from plots without irrigation (0), and at 60% and 80% irrigation treatments (60 and 80, respectfully).

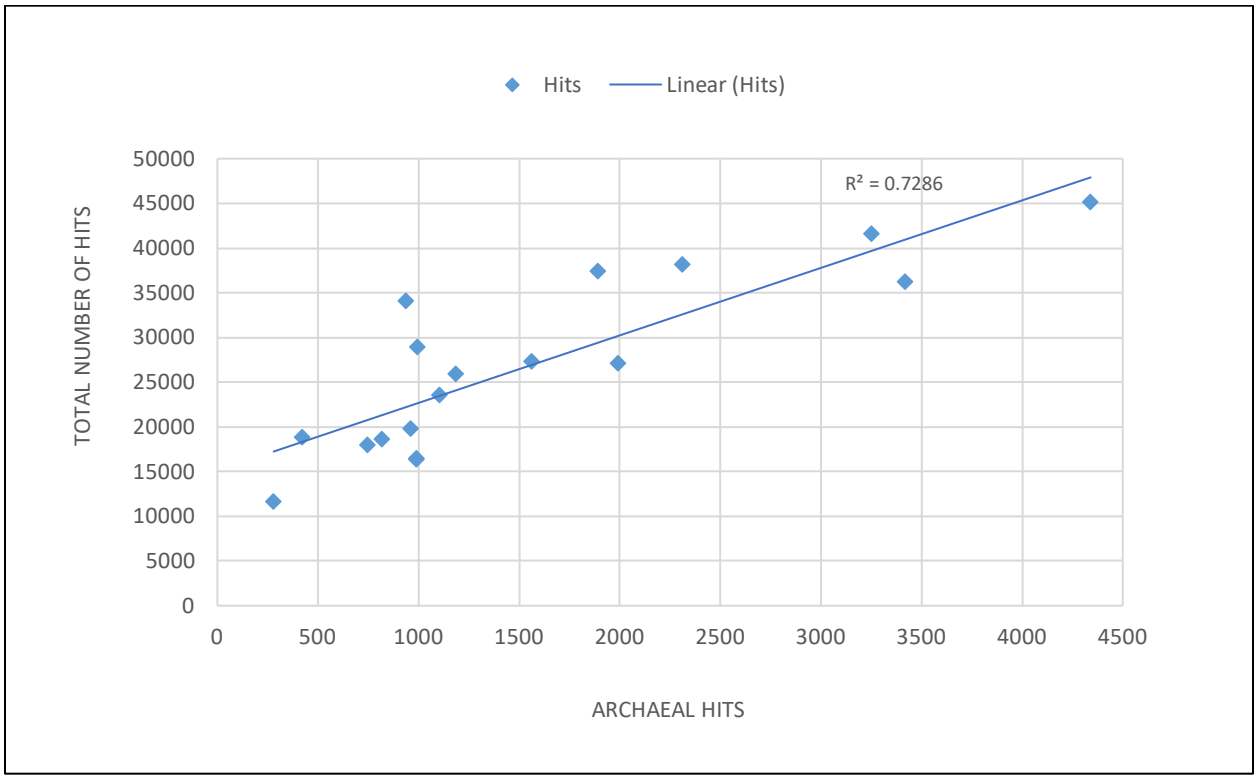

Figure 12. Total number of 16S rRNA hits versus archaeal rRNA hits.

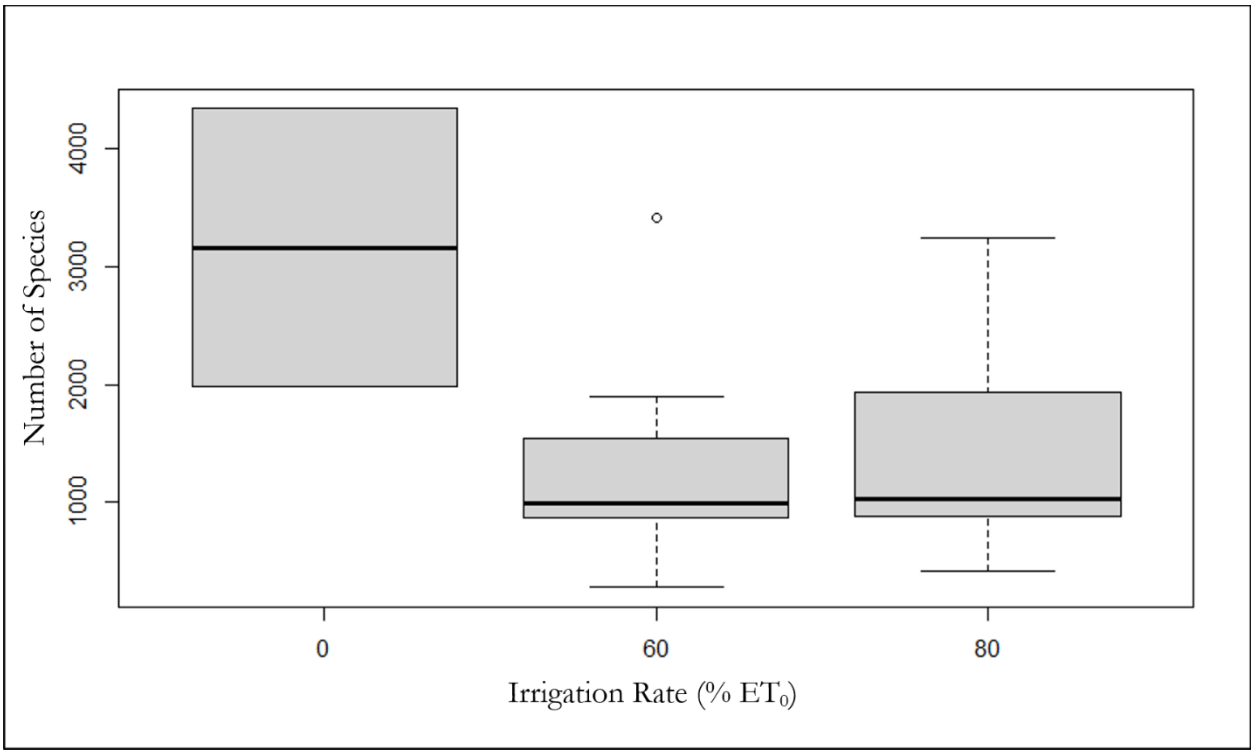

Figure 13. Number of soil microbial species from plots without irrigation (0), and at 60% irrigation treatments (60 and 80 respectfully).

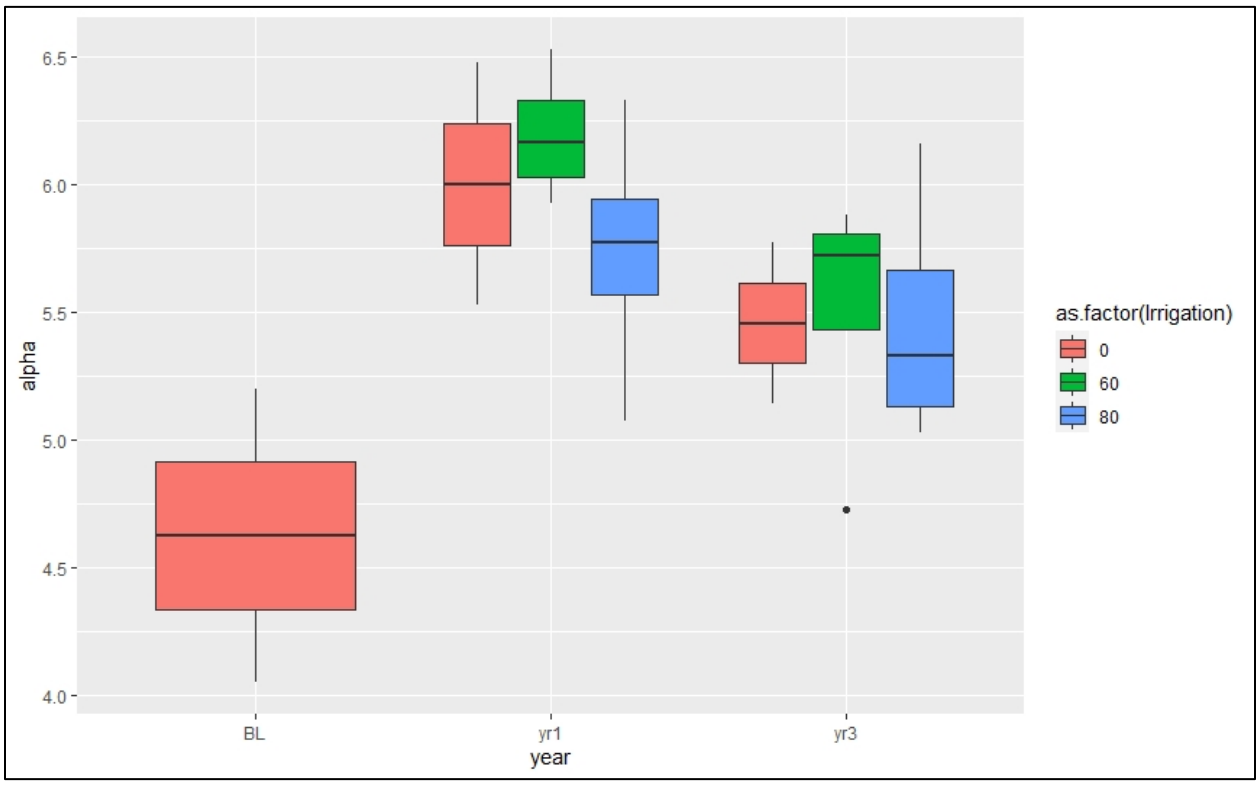

Figure 14. Alpha diversity of soil samples at different irrigation rates, with 0= control (no irrigation), 60= 60% ET_0 and 80 = 80% ET_0.

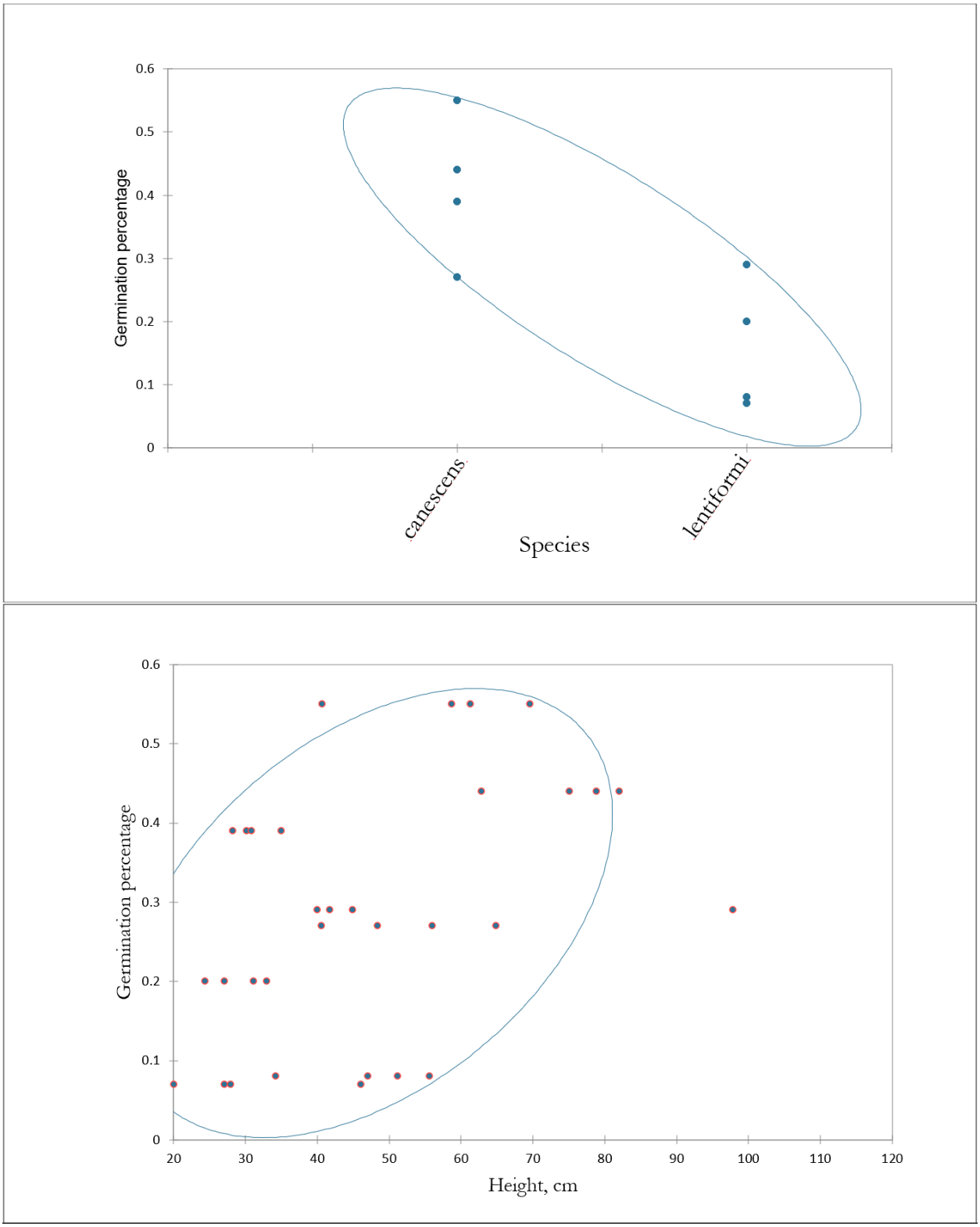

Figure 15. Pearson correlations for germination rates by species and final height.

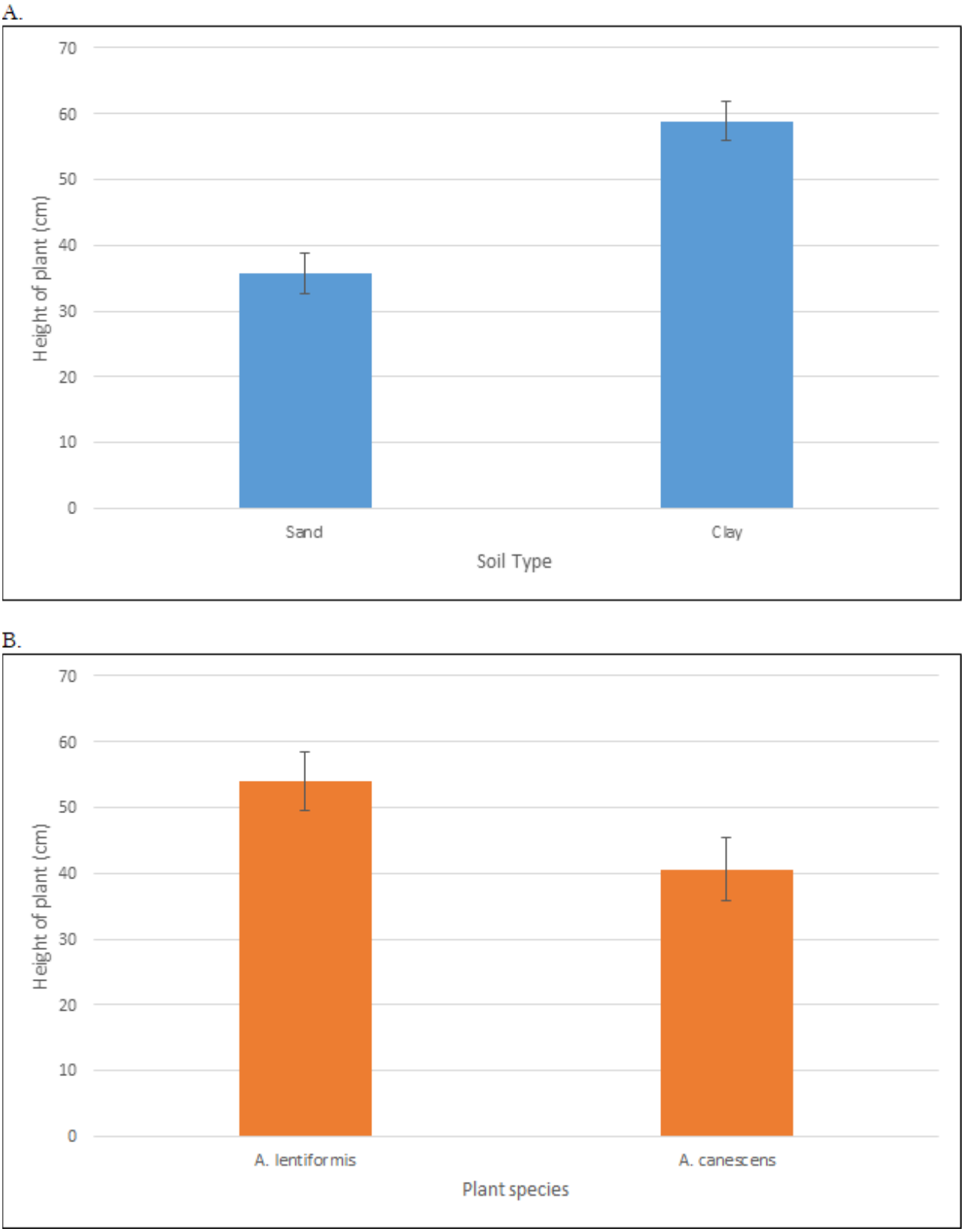

Figure 16. A) Average height of plants at the end of the growing period in sand and clay soils. B) Average height of plant at the end of the growing period of differing species.

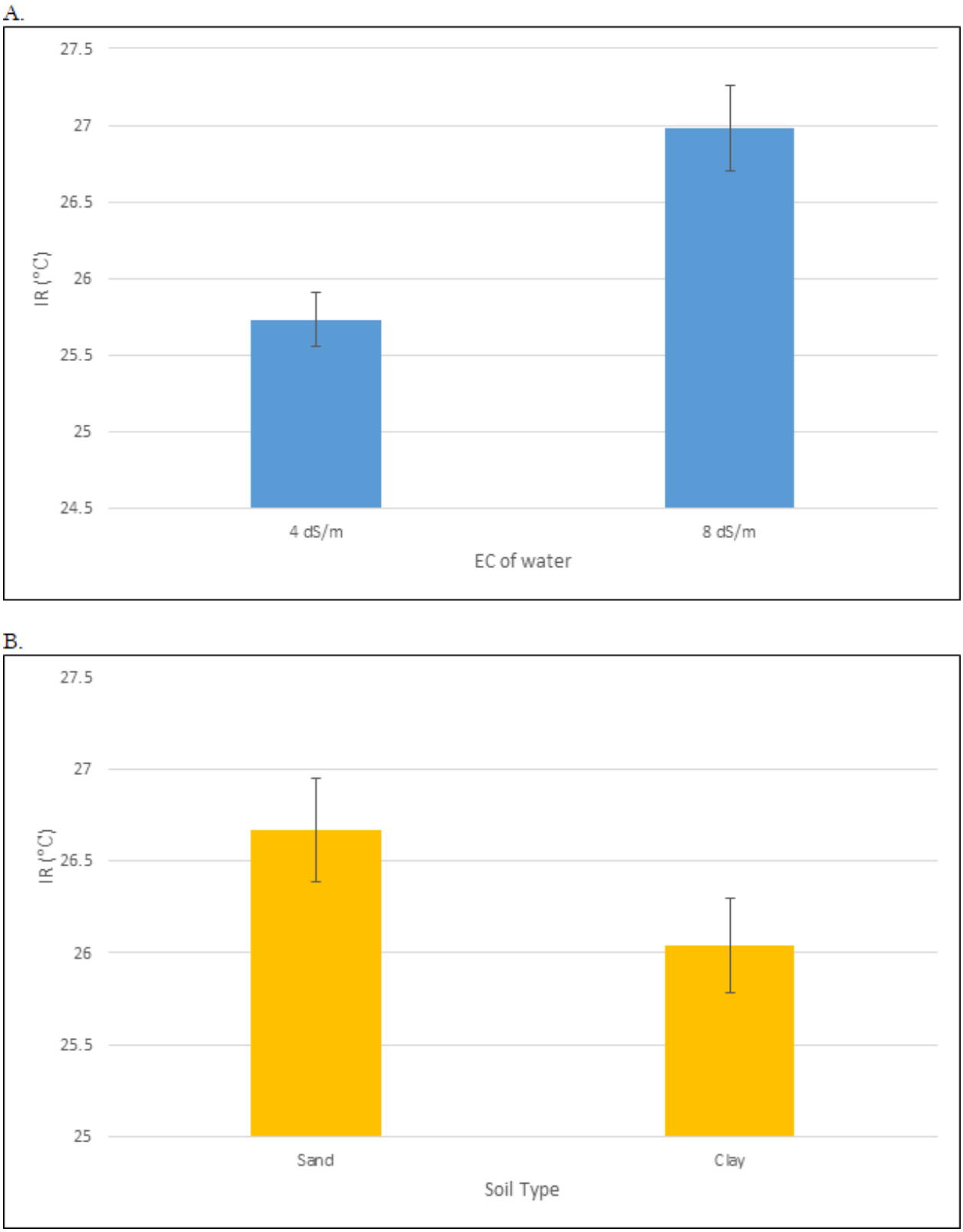

Figure 17. IR readings, A) by irrigation EC, and B) by soil type.

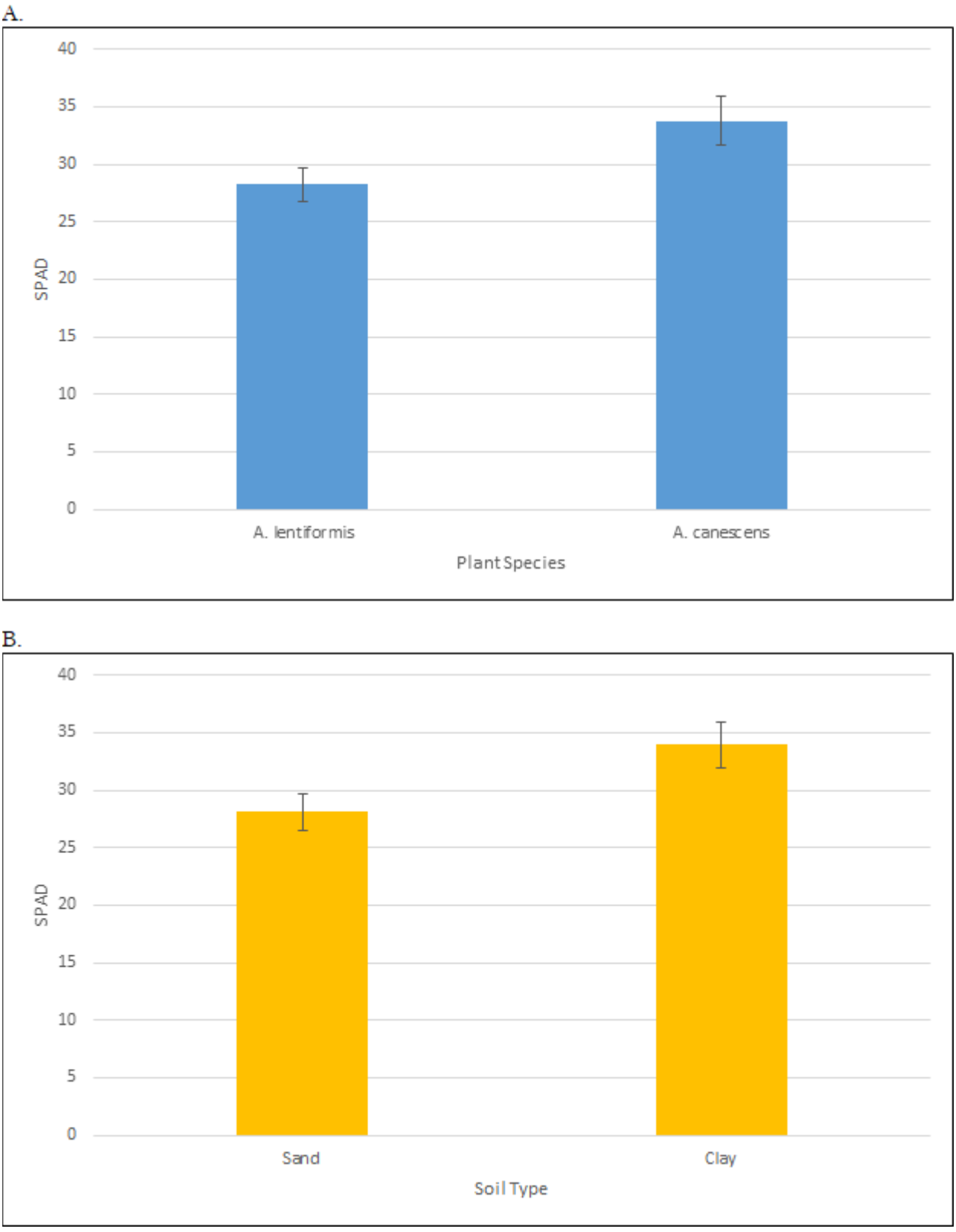

Figure 18. SPAD readings, A) by plant species, and B) by soil type.

4. Discussion

4.1 Soil Chemistry

We expected to see the ion accumulation to increase in an inverted bell shape from the drip line. To study this, we collected soil samples at three distances in two directions and two depths and tested for a selection of ions and electric conductivity. We found EC to show an increase in ions as the distance increased from the drip line. The bell curve was more clearly observed in the 60% ET_0 treatment than in the 80% ET_0 irrigation treatment (Fig. 4). Irrigation in the 80% ET_0 was applied for a longer period of time than at the 60% ET_0, which may have allowed ions to accumulate further out and down from the study area.

However, a similar pattern is not seen in all of the ions studied. Calcium concentration decreased with distance from the drip emitter. Sodium, chloride, and nitrate ion accumulation increased at the surface depth with distance. These ions may be accumulating further from 90 cm distance in a bell shape, but further testing at a greater distance would be needed. Potassium and magnesium are necessary in plant enzymatic activity, and potassium plays a large function in photosynthesis (Provin and McFarland 2014). We did not see significant changes in these ions with distance. Plant roots may have up-taken these ions at depths greater than 25 cm, resulting in a reduction in accumulation of these ions at the deeper depth.

The SAR levels exceeded the sodicity recommendations of less than 13 (Lamond and Whitney 1992; Davis, Waskom, and Bauder 2012) at 60 cm and 90 cm at both depths studied. At the end of the study, the SAR level was near sodic at 30 cm as well. The sodic soils will reduce water flow through the soil (Babcock et al. 2009; Frazen et al. 2017)

Atriplex hamilis has been documented to uptake sodium and chloride ions (Huezé 2019). If species in the same genus such as *A. canescens* and *A. lentiformis* also uptake these ions, it may explain the decrease of sodium ions observed in our study to some extent.

To reduce salt accumulation in soil, leaching is commonly used to move salts down and away from the root zone (Li and Kang 2020). Flood irrigation is the traditional method, in combination with drainage amendments, to relieve salt accumulation. Li and Kang (2020) conducted studies in areas with very shallow groundwater. Irrigation increased the elevation of the water table, which pushed salts to the surface and increased the soil salinity. Li and Ren (2020) described leaching to be 80% of the salts removed at 450 mm precipitation or freshwater irrigation in loam, sandy loam and clay loam experimental sites (as simulated in their model) in a growing season. Precipitation at the BGNDRF site averaged at approximately 185 mm (minimum of 177 mm in 2017 from April to December, peak was 205 mm in 2018 from May to October). The site did not receive enough rain to effectively leach the soil, so accumulation would be expected to occur in the root zone. The water table at the study site is approximately 25 m below the land surface (usgs.gov, 2021) (Fig 19), so a rise in water table elevation would not be expected to alter the results of this study.

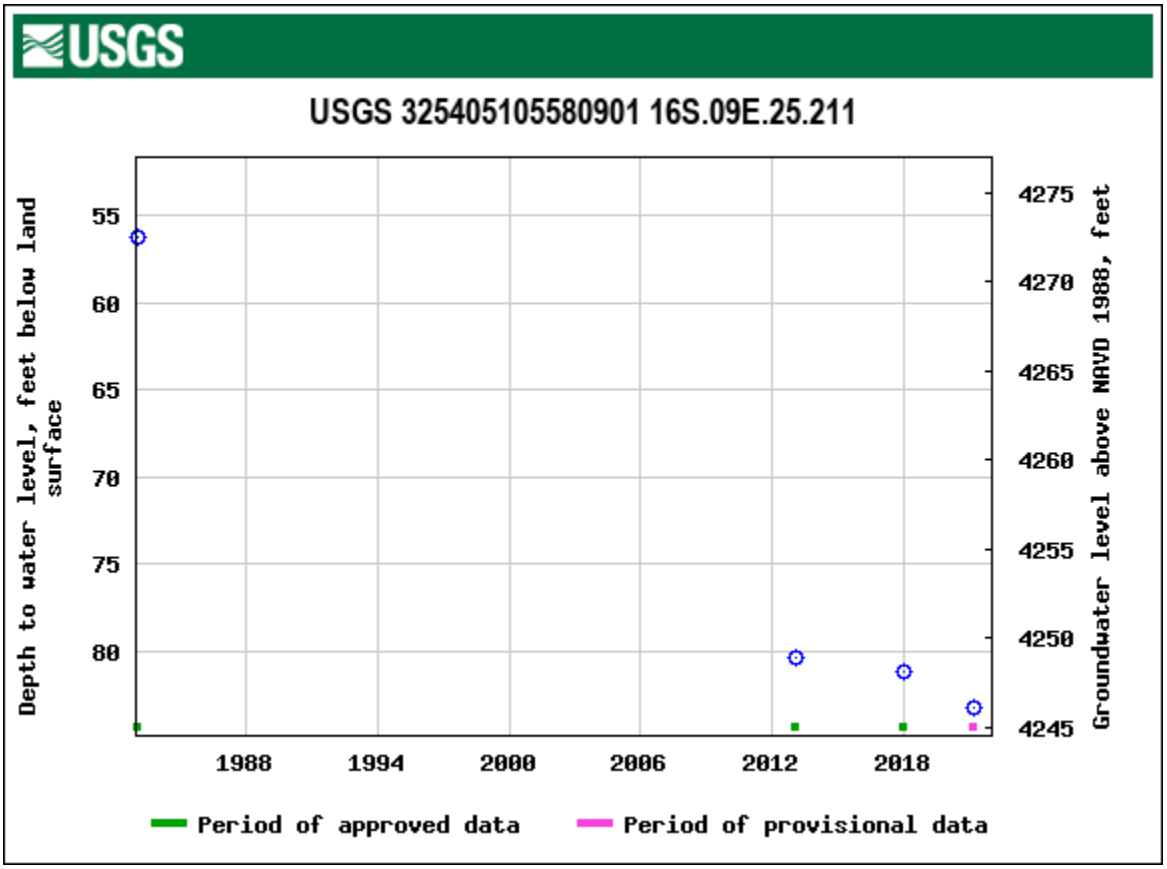

Figure 19. Depth to water table from 1983 to 2021 in Alamogordo, NM from USGS data. Obtained July 25, 2021. https://nwis.waterdata.usgs.gov/usa/nwis/gwlevels/?site_no=325405105580901

4.2 Soil Microbe Community

Over the course of the study, changes in the diversity of the microbial community were observed. We discovered 4 phyla of the kingdom Archaea in our study, as well as unclassified Archaea hits. The kingdom Archaea contain prokaryotes that typically live in extreme habitats, including those of temperature extremes, salinity extremes, pH extremes, and radiation. The phylum Crenarchaeota, was discovered only in our third-year soil samples (Table 3). Initial reports of the phylum Crenarchaeota indicated these were sulfur- and nitrate-fixing bacteria only found in extreme hot temperatures, but were later found to inhabit soil and sediments (Barns and Burggraf 1997). The member of this phylum found in this study did not contain sufficient RNA to further classify the organism to determine is genus. It was therefore not possible to determine if its occurrence was due to the increase in soil salinity or what potential environmental significance this organism may have. Carbon/nitrogen ratios are positively correlated with the presence of Archaeal species in soil environments (Li et al. 2017). The carbon/nitrogen ratio was significantly different by year and by irrigation treatment in this study, however the alpha diversity of the soil microbial community was not significantly different between samples.

Soil salinity increased with the addition of brackish water through drip irrigation. In control plots not watered with the brackish water, the diversity of the microbial community was lower than those irrigated with brackish water. This could be due to the water availability in the soil, as presence of

water is correlated with soil community composition (Li et al. 2017). However, Mukhtar et al. (2018) observed greater diversity in soil samples with greater salinity, with moderate soil salinity of approximately 5.2 dS/m showing the greatest diversity. Electric conductivity did not affect the alpha diversity significantly in this study.

The major phyla observed in this study were Actinomycetes and Proteobacteria, compromising approximately 50% of the total bacterial abundance of bacteria across all plots and irrigation treatments. Bacteroides, Chloroflexi and Planctomycetes were also found in moderate abundance (add percentages, respectively). There are 32 phyla of prokaryotes found in most soils around the world (Janssen, 2006; Sengupta et al. 2015). Generally, Proteobacteria, Acidomycetes and Actinomycetes are most common, with Bacteroidetes, Chloroflexi, Firmicules, Gemmatimondetes, Planctomycetes and Verrucomicrobia moderately common (Janssen, 2006; Sengupta et al. 2015). Armatimonadetes and Deinococcus-Thermus were found at the end of the study but not in the baseline soil samples. Deinococcus-Thermus are some of the most resistant bacteria to extreme environments (*Dienococcus-Thermus*, 2021). This phylum was found most frequently in the south plot samples closest to the surface (0-20 cm). Generally, the soil in the south plot was more sandy-loam in texture, with reduced ability to hold water in comparison to the more loamy-clay soils of the north plot. Like the Deinococcus-Thermus phylum, members of the Armatimonadetes are also thermophilic. These were only found in one soil sample close to the surface (0-20 cm). Soil temperature was not measured in this study, but the soil depth and moisture are typical for desert areas (Buxton 1924; Clark et al. 2009), allowing for thermophilic organisms to thrive in this ecological zone. Similarly, Ignavibacteriae, Latescibacteria and Thermotogae are phyla present at the beginning of the study but not at the end. It could be these organisms, which are not commonly found in soil samples and not yet well described in ecological function, could not withstand the addition of the salinity or the fluctuations in pH.

It is well documented that the pH of the soil affects the soil microbial community composition (Rousk et al. 2011; Wei et al. 2017; Tan et al. 2020). In our study, the pH of soil samples significantly increased from the baseline to the end of year one, and then decreased from the end of year one to the end of year three. While this was not a focus of our study, it is important to consider changes seen in diversity and abundance in the microbial community could potentially be attributed to the change in pH. In this study, pH was shown to be positively correlated with differences in alpha diversity. Soil sample pH remained within the optimal range of bacterial growth (Tan et al. 2020). Drastic changes of pH of near one unit or more change the ability for microbes to be active (Rath et al. 2019). While most soil microbes are generalists, the significant change in pH in soil samples in the middle of the study may have affected the microbial community.

4.3 Greenhouse *Atriplex* testing

We determined the RO concentrate significantly affected the germination rate and the IR, but did not affect the SPAD or heights of the plants. Species and soil type had a greater effect on the early growth stages of the test plants. *A. lentiformis* grew taller throughout the study in comparison with *A. canescens*. This was not a surprising result, as similar differences in species heights were observed in the BGNDRF field study; established *A. lentiformis* grew more heartily in the field (test plant circumference average approximately 10 m) than *A. canescens* (test plant circumference average approximately 7.8 m).

In attribution to salinity tolerance, Shah et al. (2017) showed leaf pigmentation and chlorophyll abundance increases with higher salinities. While there was a significant increase in plant IR readings with increased salinity, SPAD was not significantly different as salinity increased.

In an attempt to identify soil salinity based on community composition, Yilmaz et al. (2020) identified species of halophytes that can be indicators of soil salinity. While *Atriplex canescens* and *A. lentiformis* were not found in the regions of their study, other species of *Atriplex* were identified to be present in moderately saline environments (Yilmaz et al. 2020). Given that *Atriplex* species are known to clean soil environments of metallic contaminants (de Souza et al. 2014), we can consider additional uses of *A. canescens* and *A. lentiformis* for remediating soils that may have been contaminated with salts. Biomass of *A. canescens* has been found to increase as electric conductivity of soil increases, while nutrient and protein levels were not significantly changed (Mellado et al. 2018).

5. Conclusion

Currently used reverse osmosis concentrate disposal methods have the potential to disturb the environment or have a large cost associated with repurposing the extracted salts. Using reverse osmosis concentrate as a drip irrigation source for Atriplex canescens and A. lentiformis appears to be a viable option for disposal. These species of plants did not exhibit decreased growth with the increased salt concentration of the irrigation water. The alpha diversity of the soil community was not significantly changed over the three years of this study under either of the saline concentrate irrigation treatments. The metabolism of the soil community should be further researched to ensure important soil processes are not disturbed by the ion accumulation. Higher salinities of irrigation water did not affect plant height or SPAD, although additional studies are needed to determine if nutrient content of the plants are affected at high EC levels. If long-term effects of high-saline irrigation water on soil chemistry and microbial communities are proven to be negligible, growers will have the opportunity to produce Atriplex spp. for livestock fodder. while disposing of RO concentrate without significant effects to the environment

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