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Soil Biogeochemistry in California Processing Tomatoes under Deficit Irrigation

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Soil Biogeochemistry in California Processing Tomatoes
under Deficit Irrigation

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Abstract

The negative effects of population growth and agricultural intensification on soil resources are further exacerbated by drought in arid regions like the California Central Valley. Deficit irrigation management techniques are used to navigate water shortages and work to maintain adequate production while limiting water inputs. This thesis examines the effect of four deficit irrigation management schedules via subsurface drip on the physicochemical properties of soil cropped with processing tomatoes, a late-season crop commonly grown under deficit irrigation. First, I investigated soil physical and chemical properties over the course of the season as the soils were exposed to increasing water stress. Second, I investigated the spatial arrangement of microbial populations and variability of soil physicochemical properties as the wetting zone of the soil decreases throughout the season. The experiment occurred in two consecutive years, 2021 and 2022, in Five Points, California. Processing tomatoes were irrigated with a control treatment, or either low, medium or high water stress treatments, each replicated three times. Soil physical and chemical properties such as pH, electrical conductivity, microbial biomass carbon, nitrate nitrogen, ammonium nitrogen, and potentially mineralizable nitrogen were measured in both experiments four times throughout the season. Spatially, I analyzed phospholipid fatty acid groups in the soil at 10 cm, 25 cm, and 45 cm away from the irrigation emitter. In both years, tomato yields did not significantly differ between the deficit irrigation treatments. Nitrate-N decreased as the season progressed, while potentially mineralizable nitrogen and ammonium-N increased with distance from the irrigation emitter. The total concentration of bacteria decreased with distance from the irrigation emitter in the medium stress deficit irrigation treatment, but not substantially more so than in the other irritation treatments, and our measured concentrations did not fall below ranges of total bacteria found in previous literature. I conclude that deficit

irrigation does not significantly impact yield in processing tomatoes or reduce other soil properties such as pH, potentially mineralizable nitrogen, and microbial biomass carbon, sufficiently enough to affect soil health.

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Chapter 1

Introduction

1.1. Motivation

The demands of agricultural production are increasing exponentially alongside population rise (Organization for Economic Co-operation and Development, 2010) while the pressure to adapt to climate change-induced challenges is being placed on farmers. Developing farming practices that limit the use of diminishing resources like water, without sacrificing productivity, is one step toward adaptation to drought and increased water conservation (Ullah et al., 2017). California is the largest agricultural state in the United States, producing a third of the vegetables and over two-thirds of fruits and nuts for the country (Pathak et al., 2018), and is facing considerable aridification. One of the largest agricultural counties in the state, Fresno County, received only 50% of the historical average precipitation level and was classified as enduring extreme and exceptional drought during 2021 and 2022 (National Integrated Drought Information System, 2022). Arid regions, such as Fresno County, are working to develop irrigation management practices that allow them to maintain or increase the level of agricultural production to address diminishing groundwater tables and increasing salinity concentrations (Swain, 2015).

Deficit Irrigation (DI) practices have developed due to the concern about the future of water availability and were first explored by Chalmers et al., (1981) (Fereres and Soriano, 2007). Since then, DI has been used to increase the irrigation efficiency of agriculture and reduce water consumption (Geerts and Raes, 2009; Rodrigues and Pereira, 2009). Irrigation efficiency increases crop yield while decreasing the inputs, such as water. Deficit irrigation is the process of

irrigating the crop below the potential crop water requirement – the amount of water being lost through the process of evapotranspiration in a hypothetical, unstressed crop (Pereira and Alves, 2005). Deficit irrigation is commonly used for processing tomatoes, of which California is a major producer. In 2022, California was contracted to produce 9.5 million metric tons of processing tomatoes (United States Department of Agriculture, 2022). Deficit irrigation has allowed farmers to continue to keep up with processing tomato production despite water resource limitations (United States Department of Agriculture, 2022). Because of this major shift in California agriculture from surface irrigation, like sprinkler or furrow, to deficit irrigation via subsurface drip, it is important to understand not only how this irrigation technique is affecting soil health and microbial communities but also to help identify the most efficient watering amount and schedule.

Soil Health and Water

Environmental conservation has increased focus on soil health and biodiversity because of carbon storage capabilities (Smith, 2012) and the natural cycling of plant nutrients (Jacoby et al., 2017) that can improve agricultural production and microbial diversity of soil. Soil health is defined by the functional capability of soil to sustain natural productivity, promote plant and animal health, and maintain environmental quality within an ecosystem (Natural Resources Conservation Service, 2021). Low soil moisture, which can occur through deficit irrigation practices, is known to impose certain biological stresses on soil microorganisms and their communities (Schimel, 2018). Studying the effect of agricultural management practices on soil health can explain how soil microbial communities and structures will change because of intensive land use.

Water is an important factor in soil health because it allows for the mobilization of plant nutrients such as carbon, nitrogen, and potassium (Jacoby et al., 2017). Without proper irrigation, there is little solubilization of these nutrients and they are not available for plant uptake (Schimel, 2018). Microbial communities are also sensitive to changes in soil moisture and are important producers of soil organic matter and soil carbon (Paul, 1984). Soils with high amounts of soil carbon and soil organic matter have the potential to mitigate climate change through carbon sequestration (Jansson and Hofmockel, 2020; Smith, 2012). Because deficit irrigation waters a small portion of the soil when applied via subsurface drip irrigation, variables like soil carbon and soil organic matter can become scarce outside of the wetting zone (Carminati et al., 2010).

Spatial Effect of Irrigation on Microbial Communities

Very little is known about the effects of deficit irrigation spatially and temporally on soil health properties and microbial populations in conjunction with the effect on yields. Water stress is known to have negative effects on soil health and fertility, but subsurface drip (SDI) irrigation allows for a smaller portion of the soil to undergo these drastic changes in moisture less often than other irrigation techniques such as furrow irrigation (Brouwer and Heibloem, 1986; United States Department of Agriculture, 2022). But what is occurring in dry soils around the wetting zone is still unknown. For years, the soil past the SDI wetting zone remains dry, particularly in arid regions where there is little rainfall throughout the year (Hueso et al., 2012b). Microbial biomass and functions can deteriorate under the prolonged absence of sufficient moisture (Hueso et al., 2012b; Schimel, 2018). This project seeks to understand the effects of deficit irrigation on soil biogeochemistry and salinity.

Salinity can affect soil health properties and agricultural productivity (Luo et al., 2017). A previous study showed a negative correlation between electrical conductivity and yield in processing tomatoes (Shalhevet and Yaron, 1973). For every 2,000 µS/cm increase in electrical conductivity, there was a loss of 1 x 10^6 kg/ha in yield. The linear decrease in yield with an increase in electrical conductivity was due to a reduction in fruit size and weight and not a decrease in the number of processing tomatoes. Shalhevet and Yaron (1973) also demonstrated a significant decrease in yield with increased salinity. Salinity is an important factor for farmers to manage while irrigating with groundwater. However, salinity is not always increased from deficit irrigation practices, as salinity sometimes is more dependent on soil type than water stress (Hanson and Bendixen, 1995; Rath et al., 2017).

Chapter 2 examines soil physical and chemical properties over time and crop yield to four deficit irrigation treatments in processing tomatoes. Yield did not significantly differ between treatments, despite differences in volumetric water content that occurred throughout the season due to the deficit irrigation treatments. Variables such as nitrate nitrogen, pH, and microbial biomass carbon all declined over the course of the season in at least one instance of year, depth, and deficit irrigation treatments. Decreased pH, nitrate nitrogen, and microbial biomass carbon could cause challenges in agriculture by limiting soil health. Decreases in pH can reduce microbial activity and biomass, which can have cascading effects on nutrient cycling and soil fertility (Li et al., 2022; Ontman et al., 2023). Proper soil acidity (7.0 – 7.5) is required for denitrification, an important facet of the nitrogen cycle, meaning it is also important to prevent significant increases in soil pH (Li et al., 2022).

Chapter 3 investigates the spatial variability of soil physicochemical properties in addition to microbial communities' distribution under subsurface drip deficit irrigation. The experiment analyzed soil cores collected at three distances from the subsurface drip emitter at the end of a processing tomato growing season. Both potentially mineralizable nitrogen and ammonium-N increased with an increasing distance from the irrigation source. Spatial variability of bacterial communities like gram-positive, gram-negative, and total bacteria concentrations were also affected.

1.2. Objectives

Achieving a balance between supporting farm yields and water conservation is increasingly difficult. The first objective of this project was to compare four levels of deficit irrigation to find an optimum level that limits the negative effects on soil physicochemical and biological properties while maximizing yields.

The second objective was to measure the impacts of deficit and subsurface irrigation on soil ecology. Irrigating via a subsurface drip irrigation emitter minimizes the volume of soil being watered rather than practices irrigating from the topsoil and, therefore could cause a decrease in microbial activity and community size (Schimel, 2018).

Providing this case study will inform the adoption of water conservation practices and advance the understanding of management techniques practiced in agriculture and how they contrast with soil health goals.

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Chapter 2: The Effect of Deficit Irrigation on Soil Biogeochemistry in Processing Tomatoes

Abstract

Deficit irrigation and subsurface drip irrigation are irrigation management practices in the Central Valley of California, United States. Deficit irrigation supplies the crop with less water than the required amount, which can cause water losses to areas of the soil that are not directly in the rhizosphere. Using a field experiment, we measured soil physicochemical responses to deficit irrigation over the course of a processing tomato growing season. The experiment compared four deficit irrigation treatments: a control treatment, a low stress treatment, a medium stress treatment, and high stress treatment. Deficit irrigation decreased microbial biomass carbon and nitrate nitrogen in the soil throughout the season compared to microbial biomass carbon and nitrate nitrogen measurements quantified before deficit irrigation treatments began, indicating that deficit irrigation could be detrimental to the nutrient cycling processes carried out by soil microbes. Processing tomato yield was not significantly affected by the deficit irritation treatments.

2.1. Introduction

The increasing severity of drought in California is a pressing issue for not only the state's agricultural production and water resources but also poses a risk to the environment and human health (Swain, 2015). Diminishing groundwater levels and prolonged and intensified drought because of climate change threaten California's irrigated agricultural systems (Organization for Economic Co-operation Development, 2010). California's agriculture and food production industry provide 13.5% of food produced by the United States (United States Department of Agriculture, 2022). Within California, the Central Valley provides a significant amount of the food consumed by the United States (Marston and Konar, 2017a) and contains 8 of the top 10

agricultural counties in California (Jurgens et al., 2010). The Central Valley provides more than half of the fruits, vegetables, and nuts grown in the US (Johnson and Cody, 2015). However, the groundwater levels in this region are being increasingly depleted since drought increases both the need for groundwater use and limits groundwater recharge (Lu et al., 2019). Nearly half of the Central Valley's groundwater basins have been classified as critically over-drafted by the Sustainable Groundwater Management Act (SGMA) (Lu et al., 2019). Drought conditions lead to increased and unsustainable amounts of well drilling and groundwater extraction (Zou et al., 2018). Farmers and municipalities have had to rely more on groundwater during surface water deficits (Marston and Konar, 2017b). Water limitations call into question how farmers will adapt their agricultural practices, especially considering food demands are expected to double by 2050 (Organization for Economic Co-operation and Development, 2010). Fresno County is the largest producer of processing tomatoes in the United States, with 2.5 million metric tons produced in 2021 (United States Department of Agriculture, 2022) and deficit irrigation is an increasingly used irrigation method for processing tomatoes in the Central Valley. Processing tomatoes are used to create products such as tomato paste, canned tomato sauce, salsas, and other items and are popular crops in the Central Valley due to the Mediterranean climate, and their success in high temperatures (Hartz et al., 2008).

Deficit irrigation is a strategy used to maximize the efficiency of water used by crops to conserve water, to manage soil salinity and the cost of resources like money and fertilizers. The strategy involves intentionally irrigating the plant below its potential crop water requirements (Fereres and Soriano, 2007). Crop water requirements or potential crop evapotranspiration (ET_c) is defined as the amount of water, measured frequently by depth, required to meet water loss from evapotranspiration under optimal conditions (e.g., no diseases, uniform growth, favorable

soil conditions) (Brouwer and Heibloem, 1986). Crop type, growth stage, and climatic conditions affect the crop water requirement. The rate of evapotranspiration of a reference crop (ET_o) is multiplied by the specific crop coefficient (K_c) and used to calculate the ET_c (Pereira and Alves, 2005). Research shows that the adoption of deficit irrigation practices significantly increases water use efficiency (Lu et al., 2019) by about 10% (Cheng et al., 2021) – increasing the ratio of crop yielded per drop of water used. Processing tomatoes are known to benefit from the adoption of deficit irrigation, as deficit irrigation increases tomato quality indexes such as brix, firmness, and color (Rodriguez-Ramos et al., 2022).

However, there are potential tradeoffs with deficit irrigation. Water deficits can reduce total biomass (Fereres and Soriano, 2007b) and yields (Lu et al., 2019). Soil health properties may be negatively affected by limited exposure to water as there is a positive correlation between soil moisture and the carbon and nitrogen metabolic potential of bacterial communities (Li et al., 2019; Rodriguez-Ramos et al., 2022; Schimel, 2018). Soil health is defined by the United States Department of Agriculture as the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans (Natural Resources Conservation Service, 2021). Soil health is an important facet of agriculture to consider because of soil's capacity to mitigate climate change through carbon sequestration and storage (Smith, 2012) and the approximate 5 million hectares of agricultural land (Ritchie and Roser, 2013), globally, that could be used for carbon sequestration purposes. Healthy soils are important for ecosystem health by keeping land productive, supporting biodiversity, and providing clean air and water (Kinyangi, 2007). Healthy soils are necessary to support plant growth through "plant anchorage and nutrient supply, water retention and conductivity, support of soil food webs, and

environmental regulatory functions, such as nutrient cycling, source of microbial diversity and remediation of pollutants and sequestration of heavy metals" (Van Bruggen and Semenov, 2000).

It is imperative that soil fertility also be considered when adopting deficit irrigation practices to maintain, or even increase, agricultural productivity and soil health. The Food and Agriculture Organization defines soil fertility "as the ability of a soil to sustain plant growth by providing essential plant nutrients and favorable chemical, physical, and biological characteristics as a habitat for plant growth" (FAO, 2015). Adequate soil moisture allows for plant nutrients to be more mobile and can increase plant nutrient uptake and nutrient availability, as these nutrients travel in the soil through water-filled pore spaces. Soil moisture can also affect microbial groups that respire and build stable organic matter, which is an important component of soil's capacity to capture and store essential nutrients (Rodriguez-Ramos et al., 2022). Therefore, water is an important resource in creating the soil's physical conditions for plant growth and soil fertility. Soil health and fertility are also considered to be important from a regenerative context, in which soil is managed with practices that allow for higher nutrient availability and stable organic matter development (Prescott et al., 2021). A goal in managing agricultural soils is to reduce the use of agrochemical additives that can leach into and pollute groundwater (Kirchmann et al., 2002). Understanding how the practices used in agriculture affect soil health is a crucial step toward agricultural sustainability and adapting to a world in which water is a limited resource.

Past research has indicated that adopting deficit irrigation does not reduce microbial biomass up to a certain amount of water stress ($\%ET_c$, in this case), compared to lower $\%ET_c$ amounts (Rodriguez-Ramos et al., 2022). An experiment conducted at a processing tomato field

in Lemoore, CA observed a decrease in microbial biomass after 50% deficit irrigation compared to no effect on microbial biomass at 75% deficit irrigation through the entire season (Rodriguez-Ramos et al., 2022). In the experiment the deficit irrigation treatments were achieved by reducing irrigation time after crop establishment. Deficit irrigation can limit the plant availability and cycling of nutrients due to water stress-induced dormancy of microorganisms responsible for mineralization (Schimel, 2018). Deficit irrigation was shown to reduce soil CO_2 emissions and mineralization rates, and decrease β-glucosidase activity compared to full irrigation, reflecting lower microbial activities due to limited soil moisture (Bowles et al., 2016; Gao et al., 2020). However, these experiments did not look at intermediate deficit irrigation treatments that could provide results more desirable for conventional farmers, with less risk due to crop yield of negative impacts on soil health. Therefore, research discussing how deficit irrigation affects soil health and fertility is a relevant and impactful question.

The objective of this study was to compare deficit irrigation treatments, varying in $\%ET_c$ replaced, on processing tomato yields and important soil biological and physicochemical properties while utilizing water conservation practices. We hypothesized that under more water-restrictive deficit irrigation there would be a decrease in microbial biomass carbon, but little impact on crop yield and soil physicochemical properties, such as pH and electrical conductivity. We measured soil health indicators to provide insight into the nutrient availability in the soil, the microbial activity that turns over organic matter, and the physical properties that support plant growth.

2.2. Materials and Methods

2.2.1. Site description

A field experiment was carried out to assess the effects of deficit irrigation on processing tomato (*Solanum lycopersicum*) yields and soil biological and physicochemical properties. The experiment occurred over two years and two growing seasons (2021-2022) on a commercial production farm in Five Points, California (Figure 1). The tomato varieties SVTM9025 (2021) and SVTM9016 (2022) were chosen due to their resistance to *Fusarium falciforme*, a disease-causing pathogen, their uniformity, and their common use in the Central Valley region as late-season processing tomatoes. These tomato varieties are ideal for late-season growing and contain a higher °Brix content that allows sweeter flavor and easier peel-ability in tomato processing plants (Johnstone et al., 2005). Transplanting occurred on May 20, 2021, during the first year of this experiment. In year 1, the field was approximately 5,250 square meters, with replicates split into twelve 440 square meter sections. In year two, the experimental site was approximately 1.45 x 10^5 square meters with approximately 1.2 x 10^4 square meters allocated for each treatment block. In the second year of this experiment, transplanting occurred on May 1, 2022. The subsurface drip irrigation lines were installed in 2013 and 2014 at a depth of 30 cm for the first and second experimental sites, respectively. In both years, nitrogen fertilizers (CAN17 and UAN-32, J.R. Simplot, Boise, ID, USA) were applied through drip irrigation weekly from transplanting until deficit irrigation began.

The mean precipitation in Fresno County for the growing season for processing tomatoes, from May to September was 0.0 cm in 2021 and 0.1 cm in 2022, and the mean high temperature from May to September was 33.8 ºC for both 2021 and 2022 (National Weather Service, 2022). The experimental site changed between the two years due to regular field rotations. The site history for three prior years on the experimental sites is garbanzo beans in 2018, and processing tomatoes in 2019 and 2020 for the experimental site used in 2021. For the site used in 2022, the

site history was cotton grown in 2019, fallow in 2020, and onions in 2021. The soil in the first year is classified as an Aridisol, a common soil type in California. The composition of the soil is 88% Posochanet series, which indicates slow permeability and low to medium runoff with clay loam as the dominant textural class (National Cooperative Soil Survey, 2002). This, together with low precipitation rates typical of this region, leads to high concentrations of soluble salts and gypsum. The Posochanet soil type is commonly used to grow irrigated crops such as cotton, wheat, and seed alfalfa (National Cooperative Soil Survey, 2002). The second year of the experiment occurred in 85% Calflax soil, also an Aridisol, which in some cases is very deep and somewhat poorly drained and also was a clay loam soil (National Cooperative Soil Survey, 1999). The soils had low organic matter content and were sandy in the top 140 cm of soil according to estimates from the National Cooperative Soil Survey and qualitative observations in the field.

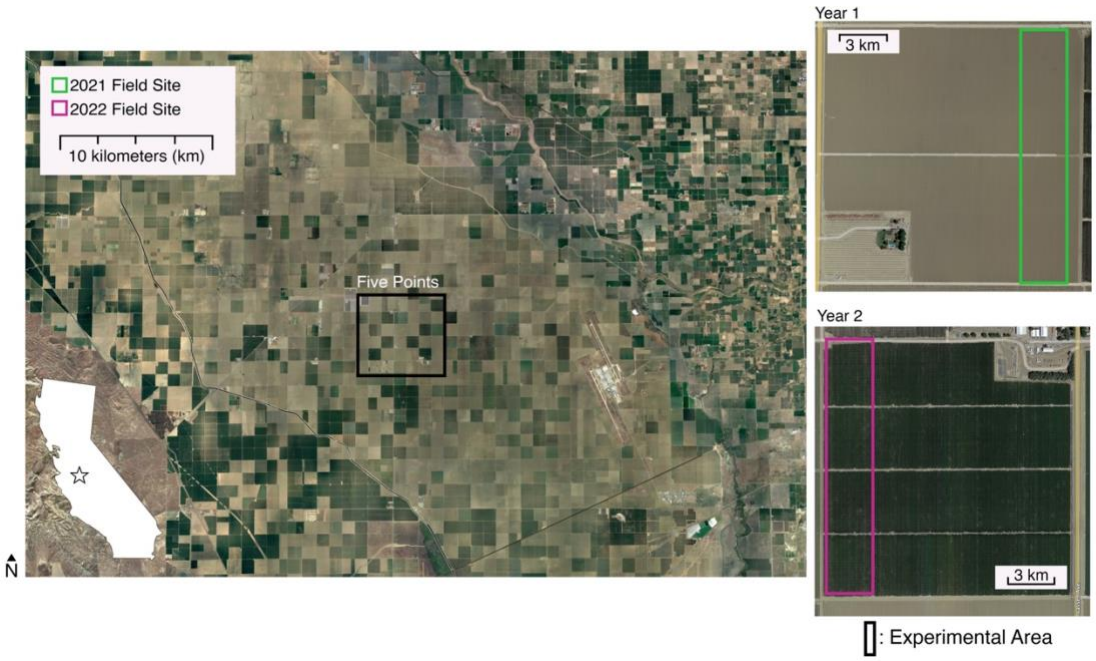

Figure 1. Aerial imagery from Google Earth on 5/5/2023 of the Central Valley and the city, Five Points, where the experiment took place. The 2021 (Top Right) and 2022 (Bottom Right) experimental sites.

2.2.2. Experimental design

We applied four irrigation treatments, which will be referred to as 'Control', 'Low', 'Medium', and 'High' treatments. The 'Control' treatment has some imposed stress, but is the grower standard treatment. The 'Low' treatment has the lowest level of imposed water stress of the three additional treatments, while the 'High' treatment has the highest level of imposed water stress (Table 1). The treatments were based on varying percentages of the total evapotranspiration rate (ET_o) obtained from the California Irrigation Management Information System (CIMIS) for this area as well as the crop coefficient (K_c) from CIMIS (*California Irrigation Management Information System*, 2023). These values are used to calculate the crop evapotranspiration (ET_c) in the specific location of our site using a formula noted below (1).

$$ET_o \times K_c = ET_c \quad (1)$$

Table 2.1

Deficit Irrigation Treatment Schedule

	Transplant	**Fruit Set**	**Midpoint**	**Until Harvest**	**Total Irr.**
	Day 0	Day 70-90	Day 95-100	Day 120	Full Season
Control	100% ET_c	~75% ET_c	~50% ET_c	No irrigation	813.82
Low	100% ET_c	~50% ET_c	~50% ET_c	No irrigation	756.73
Medium	100% ET_c	~50% ET_c	~37% ET_c	No irrigation	730.86
High	100% ET_c	~37% ET_c	~37% ET_c	No irrigation	701.34

The deficit irrigation schedule and the average of total irrigation (cm^3/cm^2*) for each treatment for both years.*

The treatments were applied via sub-surface drip irrigation and the amount of water changed throughout the season based on the processing tomato growth stages, 'fruit drop', and a midway point before mature fruiting and ripening begin. The four irrigation treatments were applied in a complete randomized block design, and each treatment was replicated three times in both years. In year one, the experimental site was sized at approximately 6.4 x 10^4 m^2 and the treatments replicates were organized randomly within blocks (a complete randomized block design), with 8 rows of tomatoes per treatment block and 96 total rows in the field (Figure 2).

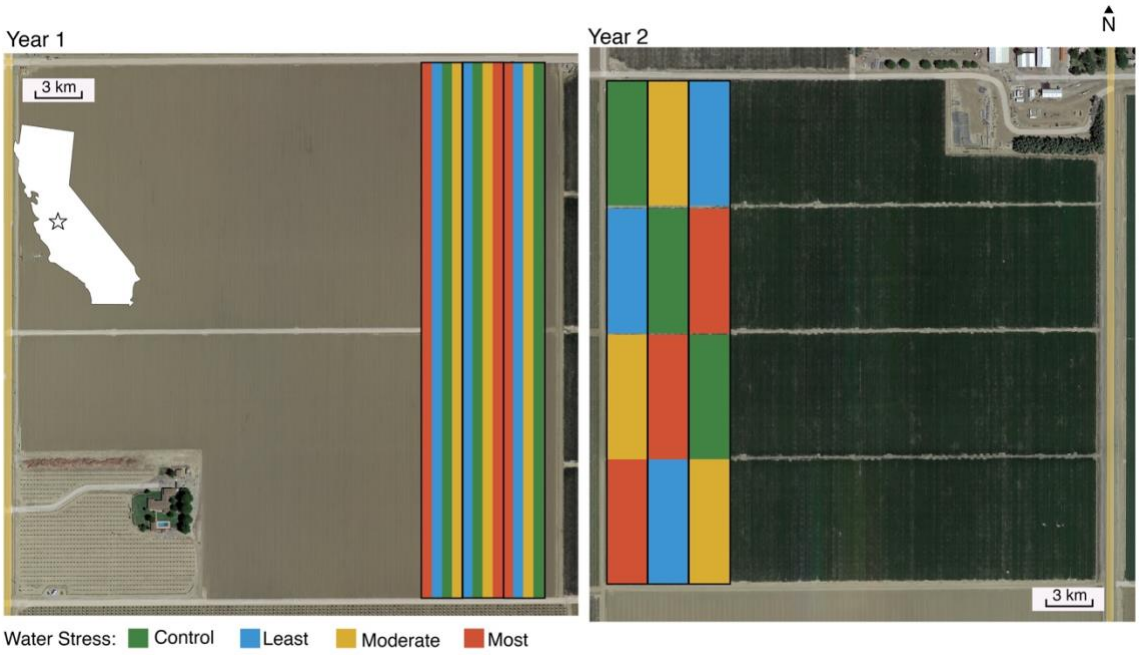

Figure 2. An aerial view, taken from Google Earth imagery on 5/5/2023, of the field site for 2021(right) and 2022 (left) with three treatment replicates indicated by color. Green (Control), Blue (Low), Yellow (Medium), and Red (High).

2.2.3. Volumetric Water Content and Electrical Conductivity Measurements

Soil frequency domain reflectometry probes (Teros 12, METER Group Inc, Pullman, Washington, USA) were placed 10 cm, 20 cm, and 40 cm into the soil bed in all four treatments in both years – 2 probes in the Control treatment, 2 probes in High Stress, 1 in Medium Stress,

and 1 in Low Stress in 2021 and 3 probes in each treatment in 2022. The probes were programmed to record soil moisture, soil temperature, and electrical conductivity every 5 minutes from a week before deficit irrigation began to harvest in 2021 due to project scheduling limitations and from transplanting until harvest in 2022. Irrigation in year 2 was staggered, with the north half of the field being irrigated on different days to the southern half of the field (Figure 1b).

2.2.4. Soil sampling and analyses

We sampled soils four times throughout the growing season to analyze soil responses to deficit irrigation. In year one, soil samples were collected 70, 95, 103 and 117 days after transplanting, to approximate soil responses at four DI irrigation stages. In year two, samples were collected 80, 93, 107, and 128 days after transplanting. The sampling dates were planned to occur at equally spaced intervals from the start of deficit irrigation to harvest: two samplings occurred before irrigation stopped for the season, and two samplings occurred while the plants were not being irrigated. On each sampling date, we took three soil cores at two depth intervals (0-10 cm and 10-20 cm) in the treatment block approximately at the center of the bed and composited into two samples (the soil from depth 1 and depth 2 of the treatment replicate). A total of 96 soil samples were collected each year. Soil samples were taken at two different depths due to the assumption that soils have higher organic matter and nutrients higher in the soil profile. All samples were taken with a 2.5 cm soil probe and stored in a plastic bag in an ice chest until transported to a conventional refrigerator then stored at 4ºC until analysis. The fresh soil samples were first sieved to 8mm and subsampled for different analyses. Subsamples for total carbon and nitrogen analysis were air-dried and sieved to 2 mm. Soil tests were done on all

96 samples, an upper depth and lower depth sample per block, in all 12 treatment replicates, four times throughout the season.

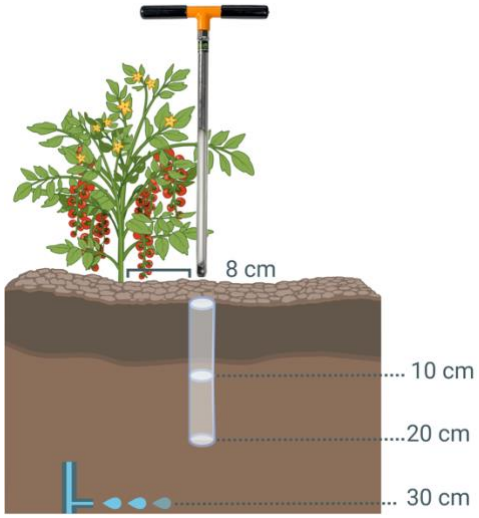

Figure 3. Soil sampling schematic used in year 1 and year 2 of the experiment. The subsurface drip emitter is installed at 30 cm depth. Soil cores were separated at the 10 cm mark to represent the soil at 0-10 cm and 10-20 cm sections.

2.2.5. Soil physicochemical properties

pH, Electrical Conductivity, and Gravimetric Water Content

In the lab, we weighed approximately 10 g of soil to put in an oven at 100ºC for a minimum of 24 hours until a constant weight was achieved for gravimetric water content analysis within 4 days of sampling. The difference in the fresh soil weight and oven-dried soil weight was recorded and the percentage of soil moisture was calculated using the equation below.

$$\frac{fresh\ soil\ weight - dried\ soil\ weight}{dried\ soil\ weight} \times 100 = Gravimetric\ Water\ Content\ (\%) \quad (2)$$

Soil pH and electrical conductivity (EC) were recorded for all samples using a pH and EC meter (Mettler Toledo, SevenCompact Duo) on soil solution, with a proportion of 1:2 (soil: water).

Carbon, Nitrate Nitrogen, and Ammonium Nitrogen

We measured carbon content in the soil as Microbial Biomass Carbon (MBC) with protocols adopted from Horwath and Paul (1994). Microbial Biomass Carbon is the carbon held within living microorganisms (Brady and Weil, 2020). A set of the collected samples were fumigated to kill microorganisms and release stored carbon, while another set was used as the control. We then extracted both the control and fumigated sets extracted using K_2SO_4. Blanks, standards, and soil extracts were run in a Shimadzu TOC-VCSH and combusted in order to quantify the dissolved organic carbon (DOC) concentration. Values were normalized and standardized in order to compare the fumigated and the non-fumigated samples. Then, results for the fumigated and control samples in mg of C/kg dry soil were subtracted from each other to calculate the amount of Carbon contained in microbial biomass. Nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N) concentrations were calculated in soil K_2SO_4 extracts. We measured nitrate-N concentrations in the soil samples using a colorimetric reaction using a reagent from a previous study (Doane and Horwath, 2003). Ammonium-N concentration was measured using the same colorimetric reaction by using different reagents on the control extracts. We calculated potentially mineralizable nitrogen by duplicating the soil samples and using one set as the control which was measured using colorimetry and using the other set as the counterparts that were incubated under anaerobic conditions for 7 days in the laboratory (Moebius-Clune, 2019). The difference in the ammonium-N produced in incubated samples versus the control samples

indicates the potential for mineralizing nitrogen in the soil and was found by calculating the difference between the control and the incubated concentration of ammonium-N. Each test was run with standards in a Synergy HTX Multi-Mode Reader (BioTek, Fisher Scientific) and using the Gen5 3.11 microplate data analysis software. We normalized the data points using the standard curve. Total carbon (C) and N were analyzed using the dry combustion method (Nelson and Sommers, 1996) on a Costech Elemental Combustion System (ECS 4010).

2.2.6. Crop Quality and Yield

Tomato yield was assessed approximately a week before commercial harvest in both experimental years. We harvested three 2-meter transects per treatment block and the total weight of marketable tomatoes was recorded using a field scale. We extrapolated the weight of tomatoes harvested within the 2-meter transect by multiplying the total area of the treatment plot. The tomatoes were harvested based on "marketable yield" requirements in consultation with our grower partner. Marketable processing tomatoes are considered any firm tomatoes with full or partial red hue.

2.2.8. Statistical analyses

We evaluated the effect of the irrigation treatments on the measured soil variables at different sampling times and depths using general linear models with irrigation treatment and sampling time as factors, separating the data by depth using the *lme4* package (v1.1-26; Bates et al., 2015) to perform an Analysis of Variance (ANOVA) (Girden, 1992) in R version 4.0.1 (R Core Team, 2022) using the RStudio graphic user interface (R Core Team, 2022.12.0). We used Scale-Location plots, Q-Q plots, and the Shapiro-Wilk test to assess the normality of the data. We then used histograms to decide if logarithmic transformations or square root transformations for non-normal data were necessary. We found that the data did not need any transformations in

order to move forward with the analysis. Tukey post hoc analysis was done using least-squares means in the R package *emmeans* (v2.30-0; Lenth, 2016). The effect of the irrigation treatments on the soil health indicators measured in this experiment was examined using Canonical Correspondence Analysis. We ran linear models and analysis of variance (ANOVA) in order to establish the treatment effects of the experiment. The p and F values from the ANOVA can be found in Table 1.1 and 1.2 for experimental years 1 and 2, respectively. The interaction between treatments (time and deficit irrigation treatment) and soil health indicators were calculated from data that were separated by the depth of soil, in order to avoid a three-way interaction and also because the effect of depth would override the effects of the treatments.

2.3. Results

2.3.1. Deficit Irrigation Effect on Soil Moisture

Sampling time significantly affected the gravimetric water content in the first year at both depths (Tables 2.2 and 2.3). Nonetheless, deficit irrigation treatment did not have a significant effect on gravimetric water content (GWC) in either year or depth. We did not visually observe variation or significant differences between the treatments' gravimetric water content values at each sampling point (Figure 4). However, soil moisture in the high stress treatment significantly decreased from deficit irrigation samplings to later samplings during the pre-harvest dry down period when irrigation was not occurring (Figure 5). In year 1, we saw a significant drop in soil moisture between the second and third sampling (Figure 5B). In year 2, the same drop occurred but between the third and fourth sampling nearest harvest (Figure 5D). In both years, the gravimetric water content was higher in the lower depth than in the upper depth.

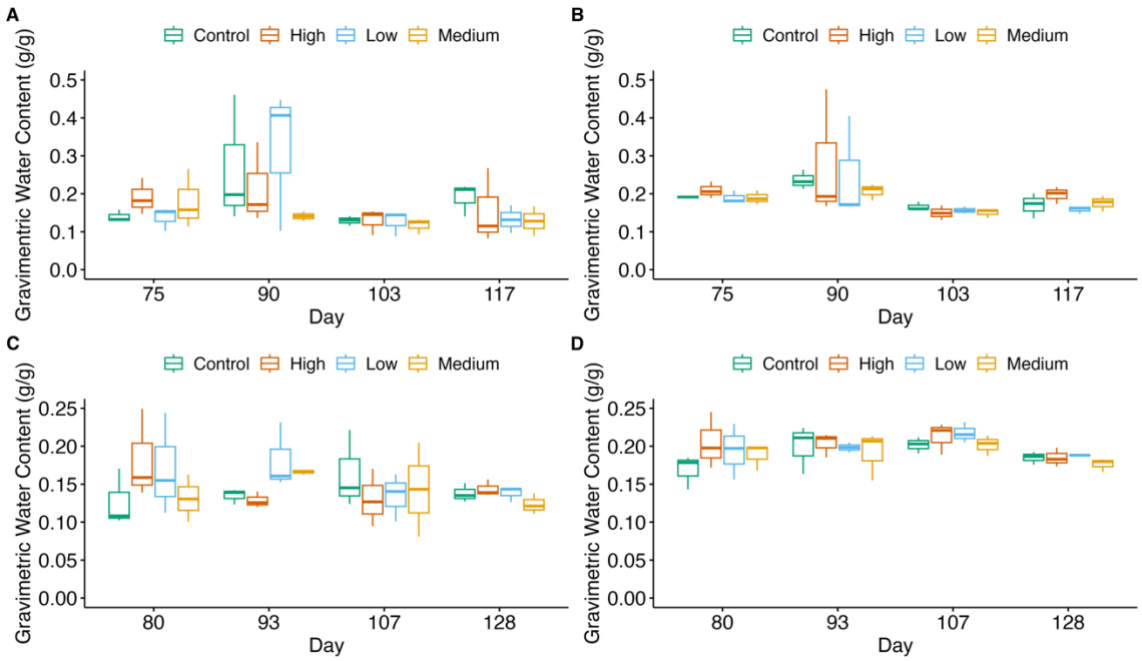

Figure 4. Gravimetric Water Content (GWC, g H_2O g^{-1} soil) grouped by sampling day for control, low, medium, and high water stress treatments. A) GWC in the 0-10 cm depth in Year 1, B) GWC in the 10-20 cm depth in Year 1, C) GWC in the 0-10 cm depth in Year 2, Graph D is GWC in the 10-20 cm depth in Year 2. No significant differences between treatments were found with 95% confidence.

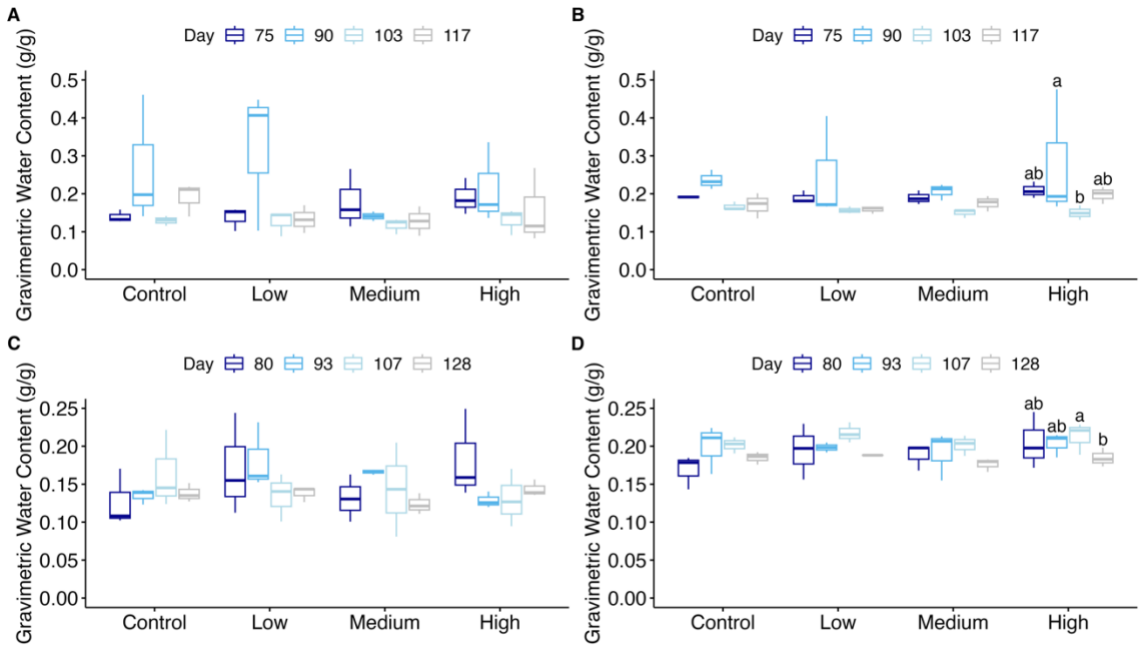

Figure 5. Gravimetric Water Content (GWC, g H_2O g^{-1} soil) grouped by treatment, comparing the effect of time on soil moisture. Graph A is GWC in the 0-10 cm depth in Year 1, Graph B is GWC in the 10-20 cm depth in Year 1. Graph C is GWC in the 0-10 cm depth in Year 2, Graph D is GWC in the 10-20 cm depth in Year 2. Different letters in the graph denote significant differences between sampling times within each treatment. The significance is $p<0.05$.

The volumetric water content data collected followed a more expected pattern according to imposed treatments. The control and low stress treatments had higher volumetric water content (VWC) than the medium and high water stress treatments in year 2, but not in year 1. In year 1, though the data were captured without replication, the data show that the control and the high stress treatments had consistently higher volumetric water content than the moderate and low stress treatments (Figure 6 and Figure 7). However, this trend was present before deficit irrigation treatments began, when all treatments were being irrigated equally.

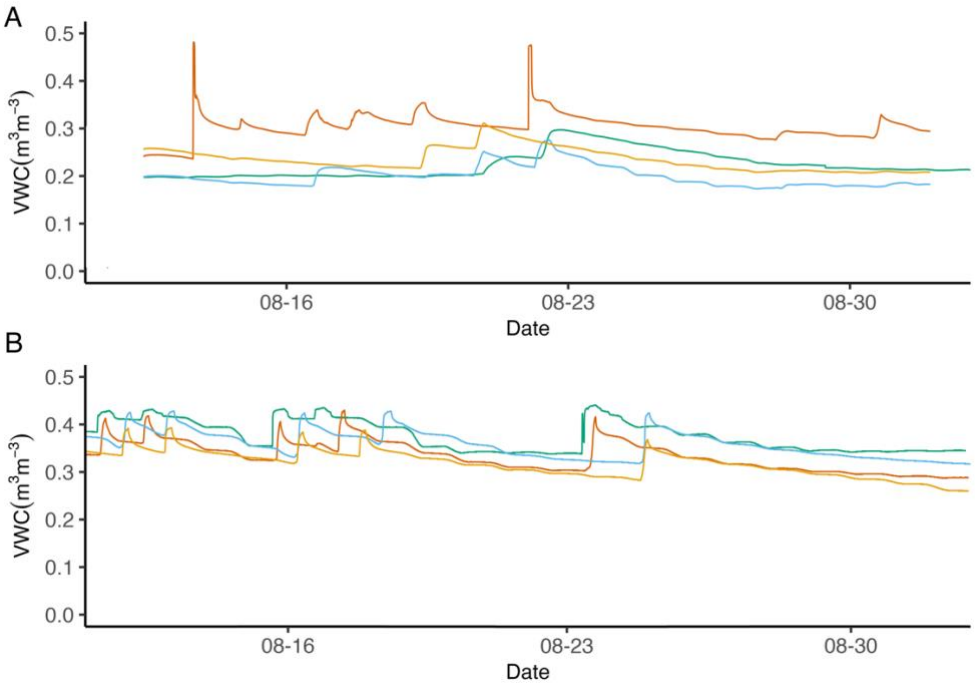

Figure 6. Volumetric water content (VWC, m^3m^{-3}) data at 20 cm depth in years 1 (A) and 2 (B). The data shown are segmented from August 15th – August 31st of each year. Peaks in VWC seen in the graph are indicators of irrigation events. The colors denote treatment, with green indicating control, blue indicating low, orange indicating moderate, and red indicating high-stress treatments.

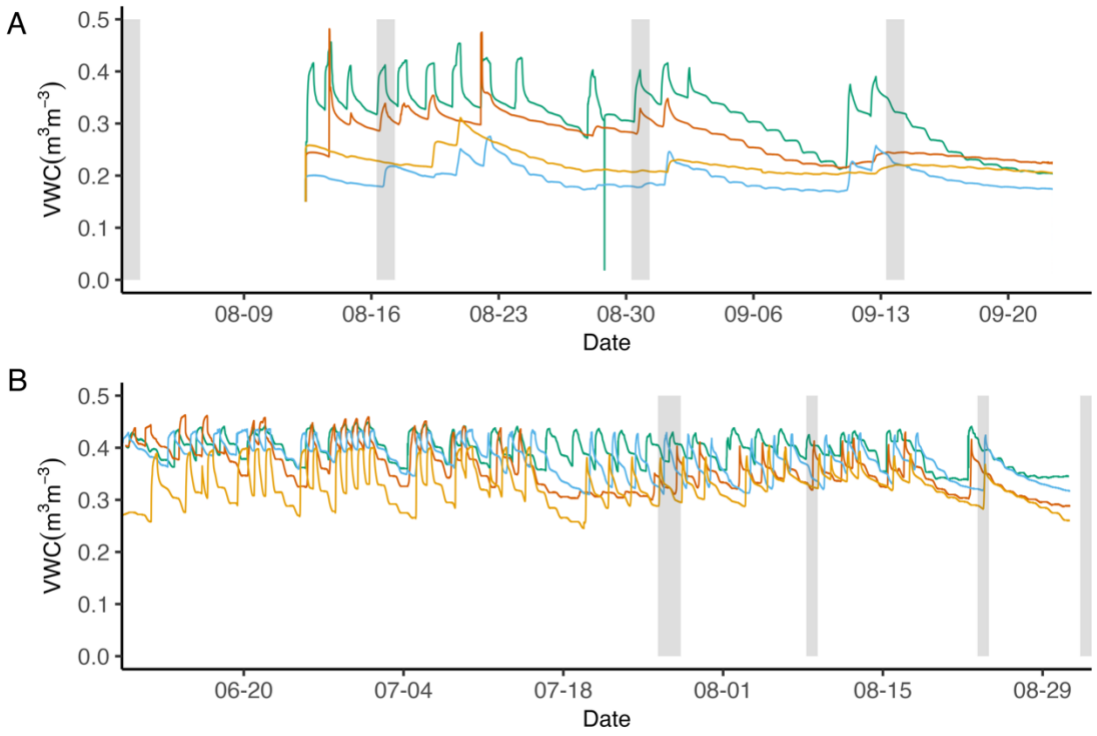

Figure 7. Volumetric water content (VWC, m^3m^{-3}) data at 20 cm depth in years 1 (A) and 2 (B) of this study. The grey areas indicate the dates soil samples were collected: August 2^{nd} – 4^{th}, August 16^{th} – 17^{th}, August 30^{th} – 31^{st}, and September 13^{th} – 15^{th} in 2021 and July 26^{th} – 27^{th}, August 8^{th} – 9^{th}, August 23^{rd} – 25^{th}, and September 12^{th} – 13^{th} in 2022.

2.3.2. Deficit Irrigation Effects on Soil Physical and Chemical Properties

An analysis of variance showed that treatments had a significant effect on soil pH in the lower depth of year 1 (Table 2.2). This was also seen when comparing the treatments between their sampling dates (Figure 9). The moderate and high stress were significantly different from each other between the second and last sampling, both with a decrease in pH value from the prior date to the following date (Figure 9B). When looking at the individual sampling points, there were no significant differences between any of the irrigation treatments on the sampling date (Figure 8). There was no significant difference in year 2 when comparing sampling dates within the treatments or the treatments within one sampling period.

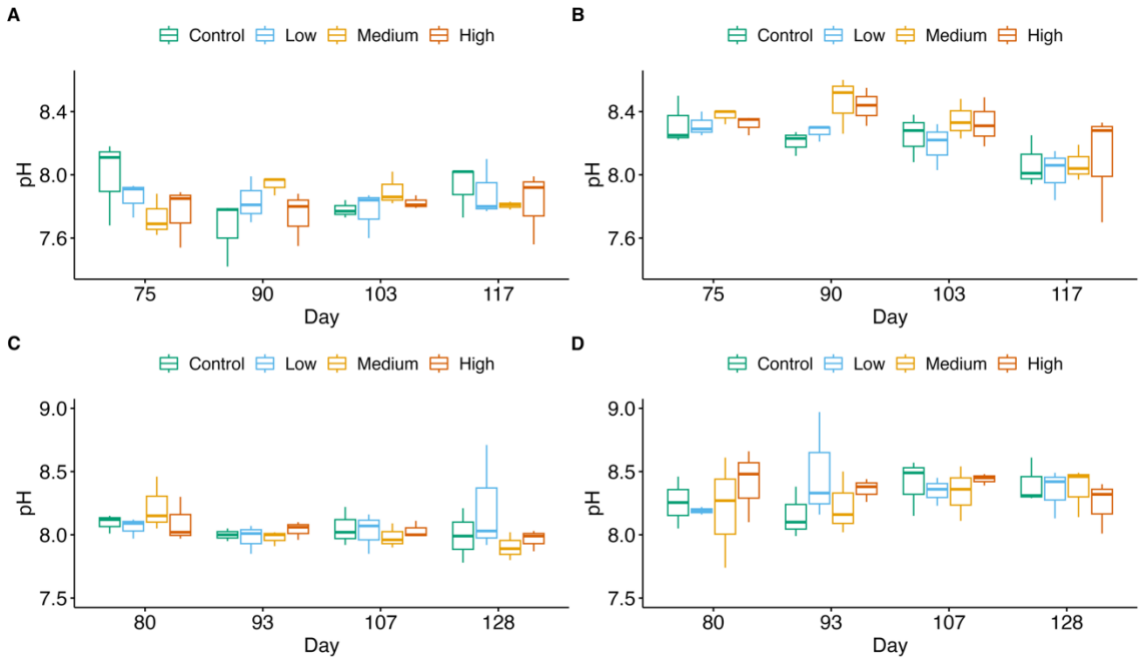

Figure 8. pH measured in soil samples collected at the trials over time and grouped by sampling date. Graph A is pH in the 0-10 cm depth in Year 1, Graph B is pH in the 10-20 cm depth in Year 1. Graph C is pH in the 0-10 cm depth in Year 2, Graph D is pH in the 10-20 cm depth in Year 2. No significant differences between treatments were found with 95% confidence.

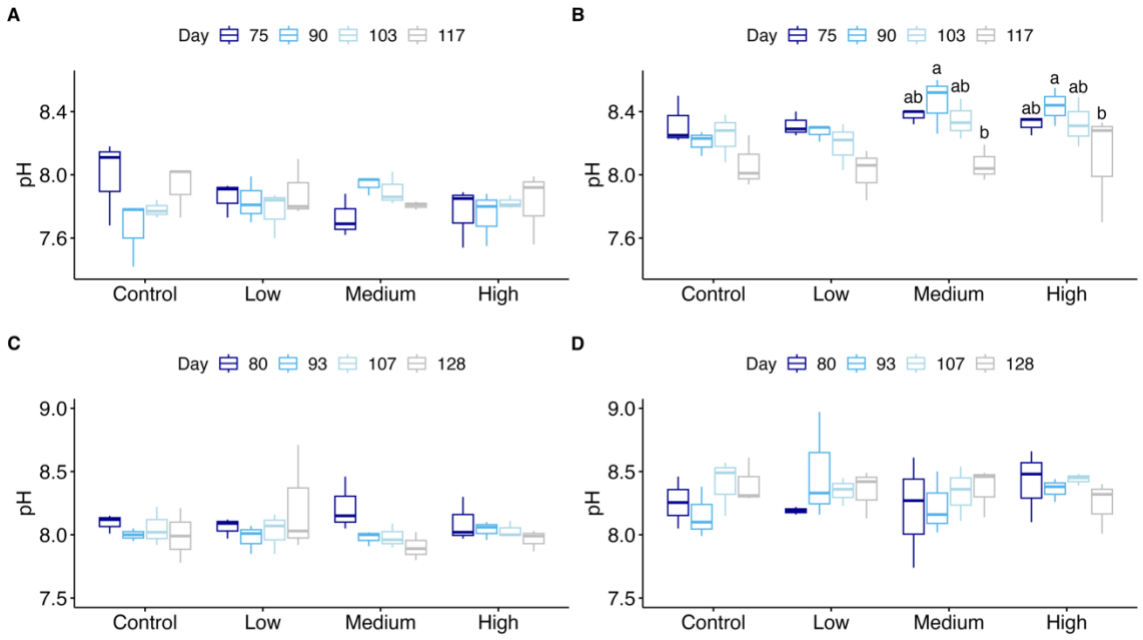

Figure 9. pH measured in soil samples collected over the season in year 1 and year 2, grouped by treatment to analyze the time effect. Graph A is pH in the 0-10 cm depth in Year 1, Graph B is pH in the 10-20 cm depth in Year 1. Graph C is pH in the 0-10 cm depth in Year 2, Graph D is pH in the 10-20 cm depth in Year 2. Different letters in the graph denote significant differences between sampling times within each treatment. The significance is $p<0.05$.

Although we observed a trend suggesting changes in electrical conductivity between irrigation treatments throughout the first year of the experiment, there were no significant differences when comparing the values of EC collected at different times. However, there was a significant effect in the first year in the second depth between the first and third sampling's control measurements and the final sampling's measurement (Figure 11B, Table 2.2). In the second year there were significant differences in the 10-20 cm depth both when comparing the treatments to each other but also the sampling times. The low stress was significantly different from the high stress treatment in the third sampling (Figure 10D, Table 2.3), and the third sampling's measurements were significantly higher than both the second and fourth sampling in the treatment with low water stress (Figure 11D). The ANOVA indicated a significant difference

due to the sampling time and irrigation treatment in the first year but neither had an effect in the second year.

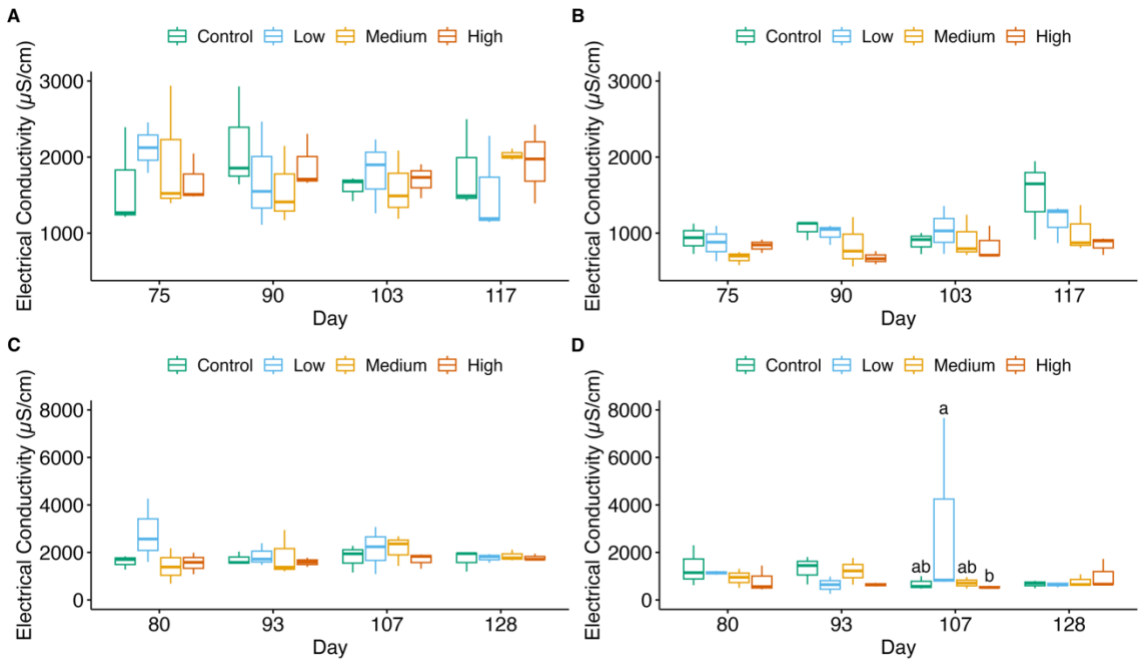

Figure 10. Electrical conductivity (EC, µS/cm) measured in the soil samples over the season in years 1 and 2 of the experiment in the upper and lower depths. Only year 1 depth 2 was significant. Graph A is EC in the 0-10 cm depth in Year 1, Graph B is EC in the 10-20 cm depth in Year 1. Graph C is EC in the 0-10 cm depth in Year 2, Graph D is EC in the 10-20 cm depth in Year 2. Different letters in the graph denote significant differences between treatments. The significance is $p<0.05$.

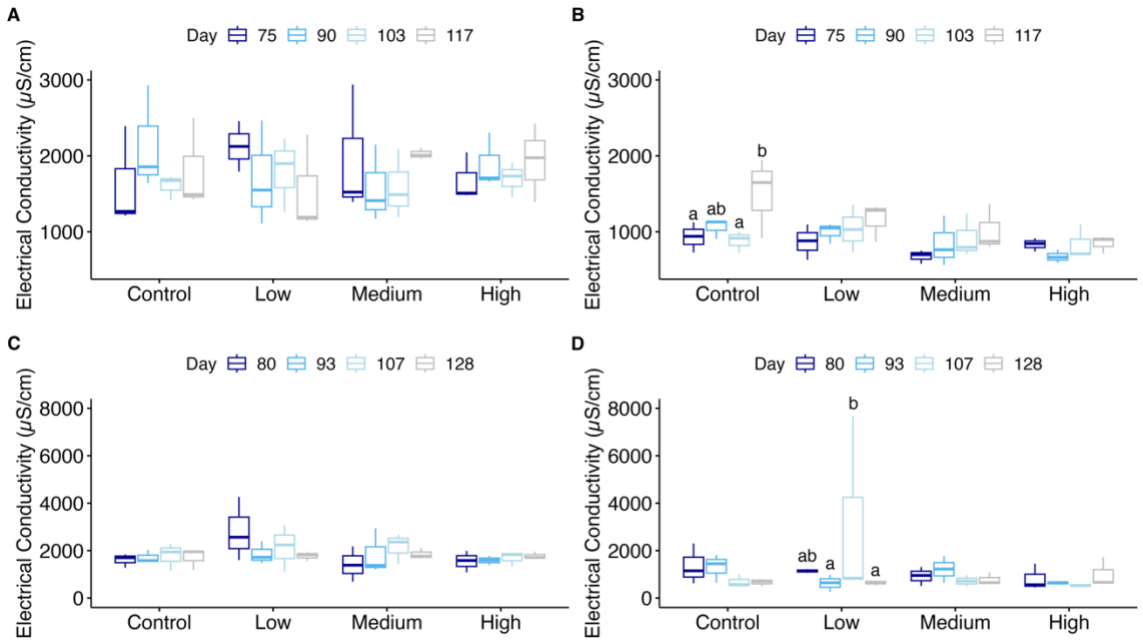

Figure 11. Electrical Conductivity (EC, µS/cm) measured in the soil samples grouped by treatment in order to analyze the sampling date effect. Graph A is EC in the 0-10 cm depth in Year 1, Graph B is EC in the 10-20 cm depth in Year 1. Graph C is EC in the 0-10 cm depth in Year 2, Graph D is EC in the 10-20 cm depth in Year 2. Different letters in the graph denote significant differences between sampling times within each treatment. The significance is $p<0.05$.

2.3.3. Deficit Irrigation Intensity Effect on Soil Microbial Ecology

Both the irrigation treatments and the sampling time had a significant effect on soil Microbial Biomass Carbon (MBC) for both depths in year 2 (Table 2.3). In year 1, the MBC in the upper depth (0-10 cm) significantly changed with sampling time (Table 2.2).

In the first year, there was a significant decline in MBC in the control treatment from the first to third sampling in the upper depth (0-10 cm) (Figure 13A). The MBC measured in the control treatment increased again at the final sampling time on day 117, despite decreasing significantly between day 75 and day 103 (Figure 13A). The medium stress treatment also followed this trend but was not significant (Figure 13A). In the second year, the moderate and

high stress treatment samples differed significantly in the upper depth at the first sampling but not after deficit irrigation treatments began.

In the second year, a significant decrease in microbial biomass carbon after deficit irrigation occurred in both depths. This occurred in depth 1 (0-10 cm) for the medium and high stress treatments, and in the depth 2 (10-20 cm) for the low stress treatment (Figure 13). In the upper depth, there was a significant decrease in the moderate and highest stress treatments and in the lower depth, the pattern is followed within all treatments, but significantly within the lower stress treatment.

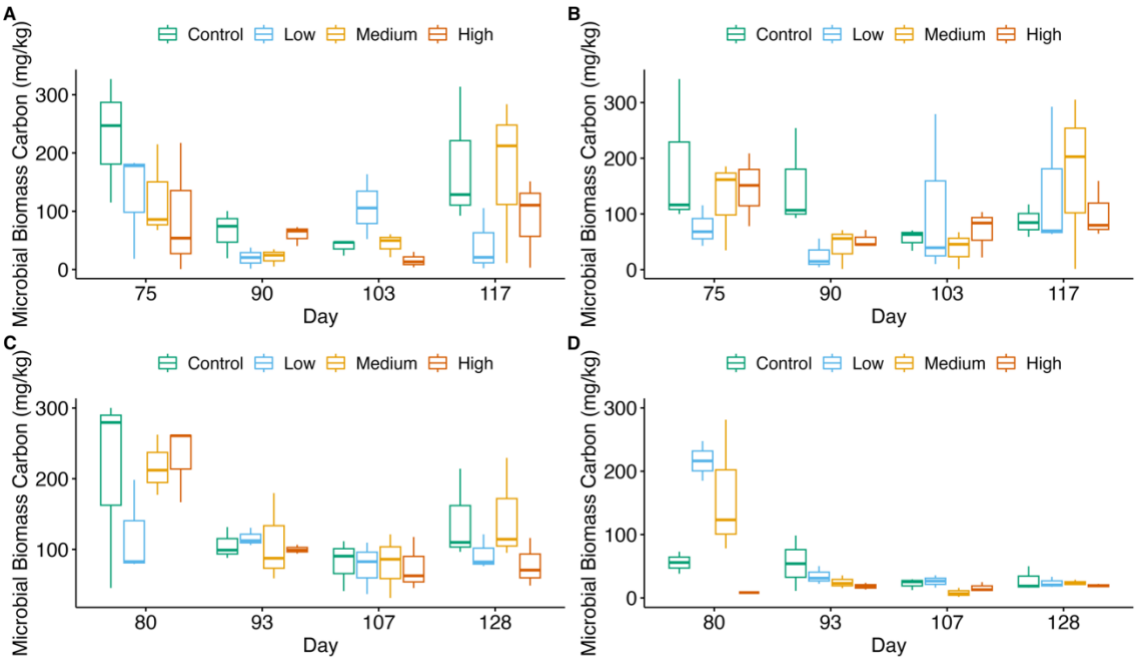

Figure 12. Microbial biomass carbon (MBC) (mg/kg of soil) measured in the soil samples grouped by sampling within each graph. Graph A is MBC in the 0-10 cm depth in Year 1, Graph B is MBC in the 10-20 cm depth in Year 1. Graph C is MBC in the 0-10 cm depth in Year 2, Graph D is MBC in the 10-20 cm depth in Year 2. No significant differences between treatments were found with 95% confidence.

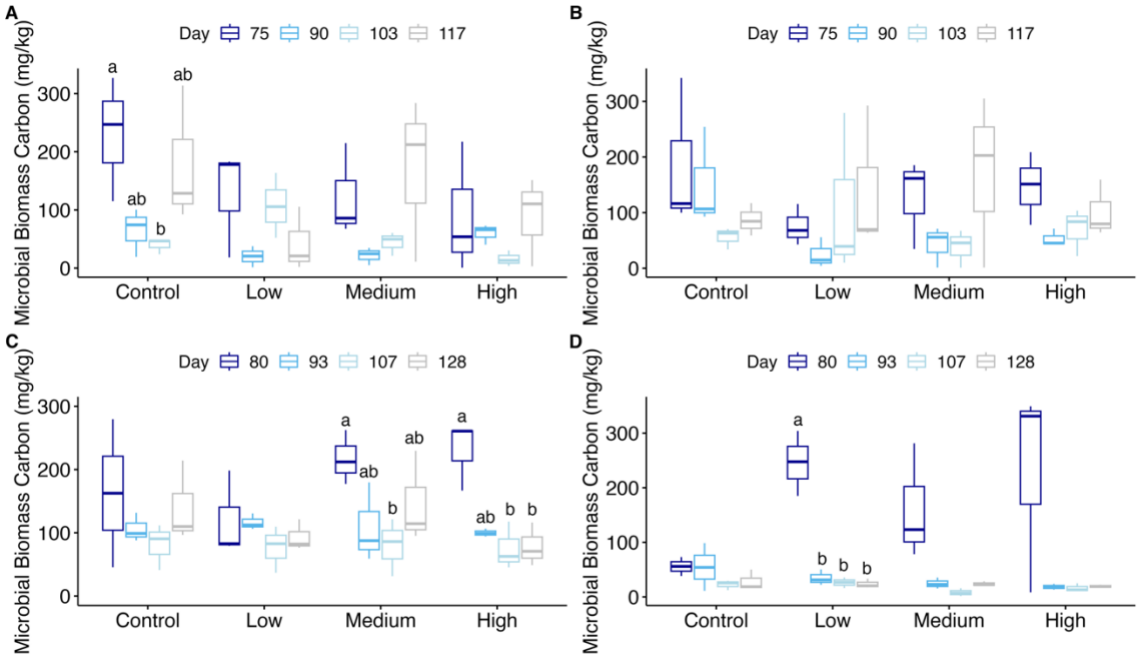

Figure 13. Microbial Biomass Carbon (MBC) (mg/kg of soil) measured in the soil samples and grouped by treatment within each graph. Graph A is MBC in the 0-10 cm depth in Year 1, Graph B is MBC in the 10-20 cm depth in Year 1. Graph C is MBC in the 0-10 cm depth in Year 2, Graph D is MBC in the 10-20 cm depth in Year 2. Different letters in the graph denote significant differences between sampling times within each treatment. The significance is $p<0.05$.

2.3.4. Deficit Irrigation Intensity Effect on Nitrogen Cycling

Potentially mineralizable nitrogen, nitrate-N, and ammonium-N concentrations were analyzed to study nitrogen cycling. The deficit irrigation treatments had limited effects on potentially mineralizable nitrogen (PMN). In the first year, the low stress treatment's initial PMN measurement had large variability. The concentration of potentially mineralizable nitrogen in the low stress treatment differed significantly from the control (Figure 14B). Potentially mineralizable nitrogen decreased from the first sampling to the third sampling in the low stress treatment (Figure 15B).

Sampling time had a significant effect on potentially mineralizable nitrogen (PMN) levels in the second depth of year 1 (Table 2.2) and in both depths in year 2 of the experiment (Table

2.3). In the first year, the trend varied, but in the second year, we saw a gradual decline in PMN as the treatments went from control to the high stress treatment at each sampling date. The control treatment, moderate and high stress treatment all experienced increases in PMN within its treatment grouping in the upper depth of year 2. There was a significant difference between the low stress treatment and all the others at the start of the experiment in year 1 at the lower depth, but that difference did not appear again during the first year.

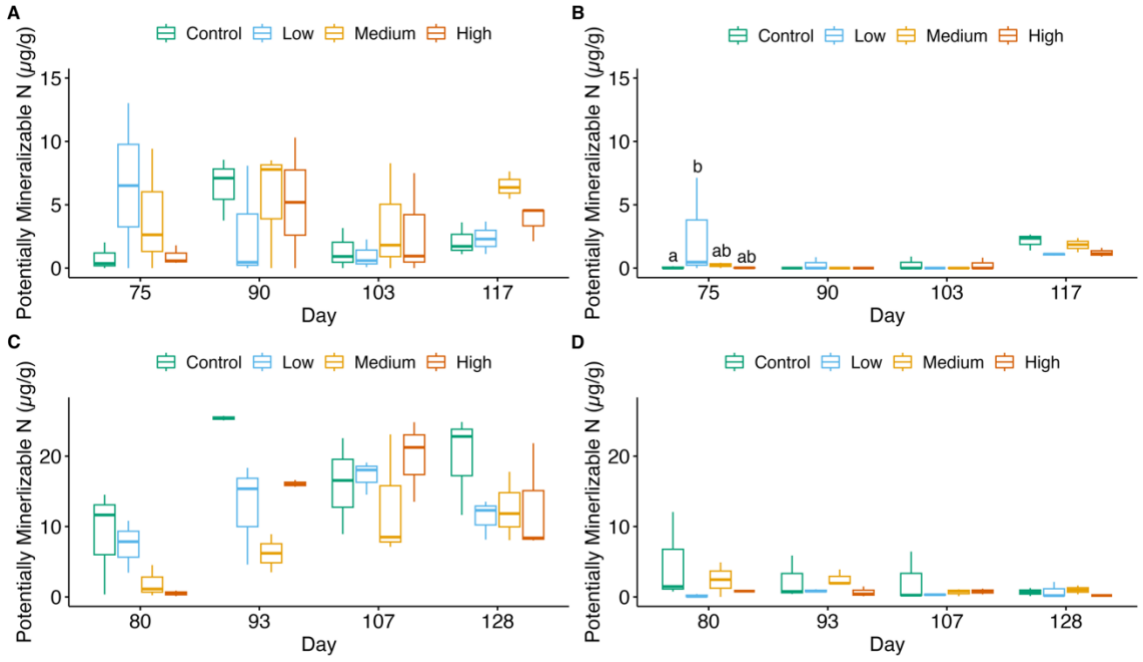

Figure 14. Concentration of Potentially Mineralizable Nitrogen (PMN, µg/g soil) in the soil samples presented separately by sampling date within each graph. Graph A is PMN in the 0-10 cm depth in Year 1, Graph B is PMN in the 10-20 cm depth in Year 1. Graph C is PMN in the 0-10 cm depth in Year 2, Graph D is PMN in the 10-20 cm depth in Year 2. Different letters in the graph denote significant differences between treatments. The significance is $p<0.05$.

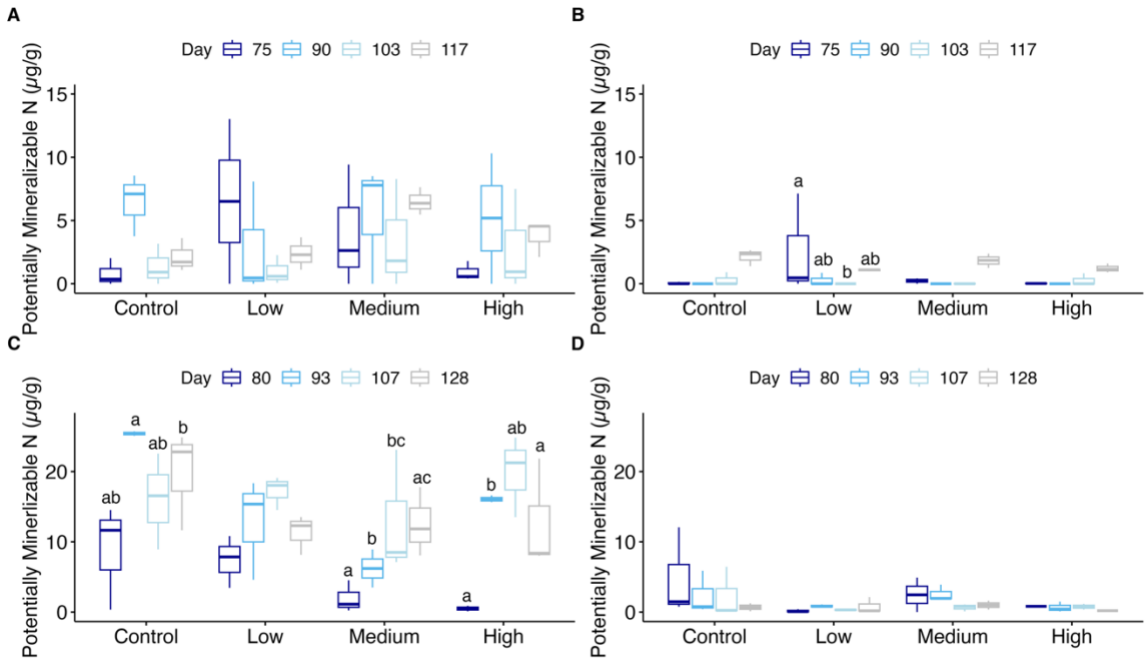

Figure 15. Concentration of Potentially Mineralizable Nitrogen (PMN, µg/g soil) in the soil samples presented separately by treatment within each graph. Graph A is PMN in the 0-10 cm depth in Year 1, Graph B is PMN in the 10-20 cm depth in Year 1. Graph C is PMN in the 0-10 cm depth in Year 2, Graph D is PMN in the 10-20 cm depth in Year 2. Different letters in the graph denote significant differences between sampling times within each treatment. The significance is $p<0.05$.

Nitrate nitrogen (NO_3-N) significantly differed between sampling times in the second year in the upper depth of the soil (Table 2.3). There were no other significant treatment effects observed in the experiment. In the first year, there was no DI treatment effect or sampling time effect on NO_3-N of a general decline at each time point aligning with water stress, and the data have a lot of variation and not a distinct trend. In the second year, there also were no significant differences apart from a significant difference between the first and third sampling in the high stress treatment in the upper depth in which the concentration of NO_3-N in the third sampling was significantly larger than the first sampling (Figure 1). There was initially a higher NO_3-N concentration in the low stress treatment soil that was consistent between the two depths.

In the second year, we see a repeating trend in both depths where NO_3-N increases after the first sampling (pre-deficit baseline), peaks at the second sampling, and then decreases throughout the season. This behavior was seen in all four treatments except the high stress treatment in the second depth, but the changes were not significant with 95% confidence.

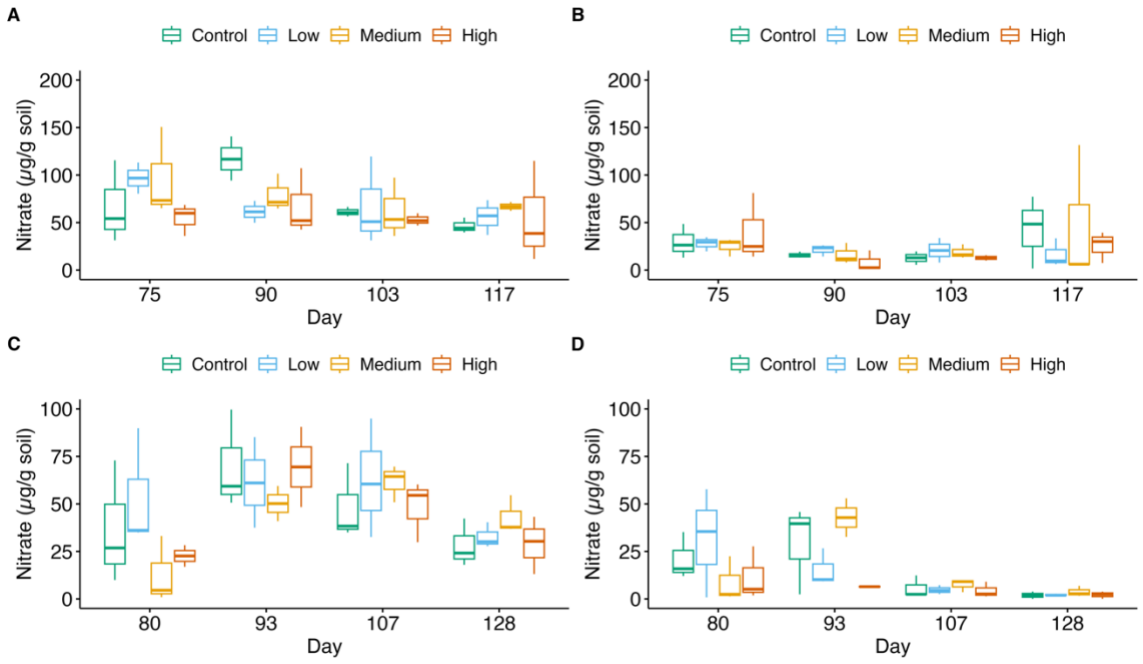

Figure 16. Nitrate nitrogen (NO_3^--N, µg/g soil) concentration measured in the soil samples collected on the four sampling dates throughout the season. Graph A is NO_3^--N in the 0-10 cm depth in Year 1, and Graph B is NO_3^--N in the 10-20 cm depth in Year 1. Graph C is NO_3^--N in the 0-10 cm depth in Year 2, and Graph D is NO_3^--N in the 10-20 cm depth in Year 2. No significant differences between treatments were found with 95% confidence.

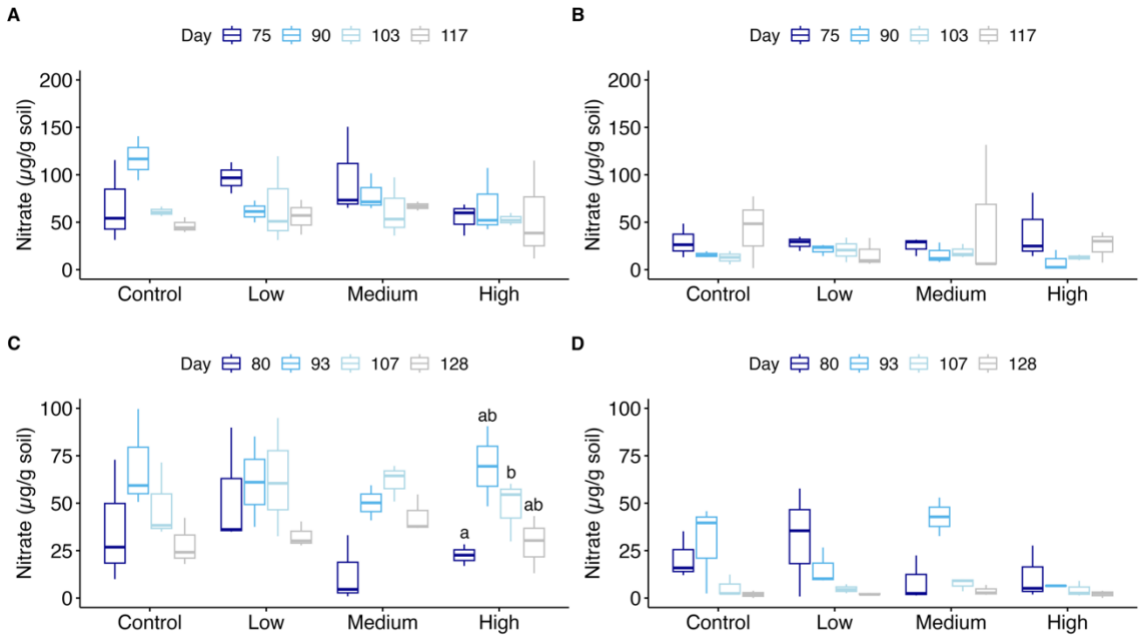

Figure 17. Nitrate nitrogen (NO_3^--N, µg/g soil) separated by treatment to understand the effect of sampling time. Graph A is (NO_3^--N) in the 0-10 cm depth in Year 1, Graph B is (NO_3^--N) in the 10-20 cm depth in Year 1. Graph C is (NO_3^--N) in the 0-10 cm depth in Year 2, Graph D is (NO_3^--N) in the 10-20 cm depth in Year 2. Different letters in the graph denote significant differences between sampling times within each treatment. The significance is $p<0.05$.

While there were significant differences in the ammonium nitrogen (NH_4^+-N) concentrations of the soil samples collected in both years at both depths, the trends that we see are variable. There was a significant difference between the control and the three other treatments at the baseline sampling. In the low stress treatment in the second year, the initial measurement was significantly lower than the third sampling (Figure 19D) and in the high stress treatment the second sampling was significantly higher than both the first and the last (Figure 19C). In the lower depth for year 2, the NH_4^+-N concentration in the low stress treatment's second sampling was significantly higher than the first and last sampling (Figure 19D). The NH_4^+-N concentration in the lower depth of the soil was all below 2 µg/g of soil in both experimental years. In the upper depth, while NH_4^+-N concentrations were higher, there were no

significant differences between deficit irrigation treatments or sampling time points excluding the trends noted above.

There was no significant effect of deficit irrigation treatments on NH_4^+-N concentrations diagnosed in the ANOVA, although there was a significant sampling time effect seen in the lower depth of year 1 (Table 2.2) and the upper depth of year 2 (Table 2.3). This was proven, when comparing each of the treatments at the same time point. There were no significant differences from post hoc analysis seen in these graphs (Figure 18). The sampling time treatment effect's significance was seen in the graphs (Figure 19). In year 1, we saw a significant decrease in NH_4^+-N from the first sampling to both the third and fourth sampling of the high stress treatment. In year 2, we saw the NH_4^+-N concentration drastically and significantly rise in the low stress treatment from the initial sampling to the second sampling in the upper depth and lower depths. Additionally, in the second depth, we also saw a significant decrease between sampling 2 and sampling 4 in the low stress treatment.

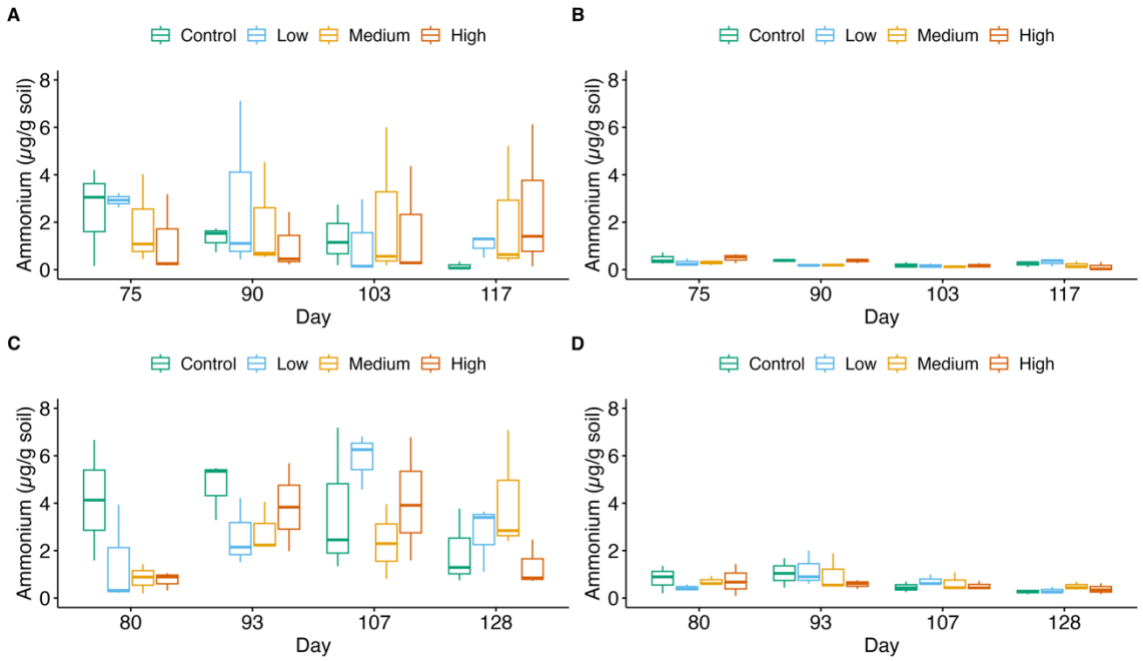

Figure 18. Ammonium nitrogen (NH_4^+-N, µg/g soil) concentration measured in the soil samples and represented by sampling date in each graph. Graph A is (NH_4^+-N) in the 0-10 cm depth in Year 1, and Graph B is (NH_4^+-N) in the 10-20 cm depth in Year 1. Graph C is (NH_4^+-N) in the 0-10 cm depth in Year 2, and Graph D is (NH_4^+-N) in the 10-20 cm depth in Year 2. No significant differences between treatments were found with 95% confidence.

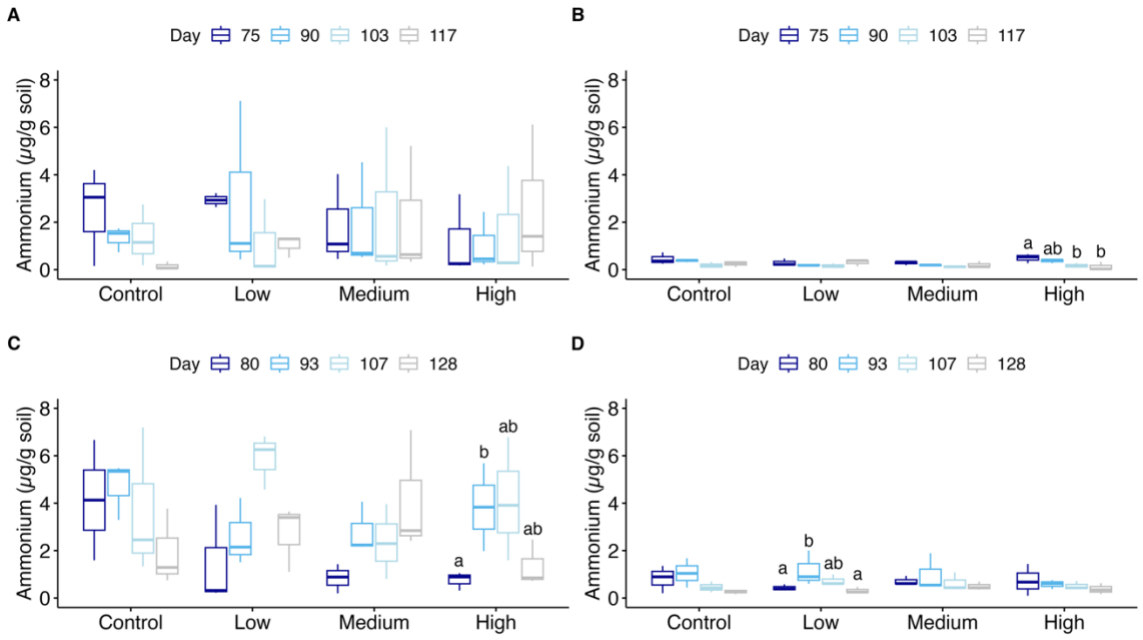

Figure 19. Ammonium nitrogen (NH_4^+-N, µg/g soil) concentration measured in the soil samples and represented by treatment. Graph A is NH_4^+-N in the 0-10 cm depth in Year 1, and Graph B is NH_4^+-N in the 10-20 cm depth in Year 1. Graph C is NH_4^+-N in the 0-10 cm depth in Year 2, and Graph D is NH_4^+-N in the 10-20 cm depth in Year 2. Different letters in the graph denote significant differences between sampling times within each treatment. The significance is $p<0.05$.

2.3.5. Deficit Irrigation Intensity Effect on Processing Tomato Yield

There was no significant treatment effect on marketable yield for either experimental year. The total water applied to each treatment in 2021 was 828 cm^3/cm^2 for the control treatment, 792 cm^3/cm^2 for the low stress treatment, 767 cm^3/cm^2 for the medium stress treatment, and 748 cm^3/cm^2 for the high stress treatment. In 2022, the total water applied for the control treatment was 799 cm^3/cm^2, 721 cm^3/cm^2 for the low stress treatment, 694.94 cm^3/cm^2 for the medium stress treatment, and 654 cm^3/cm^2 for the high stress treatment.

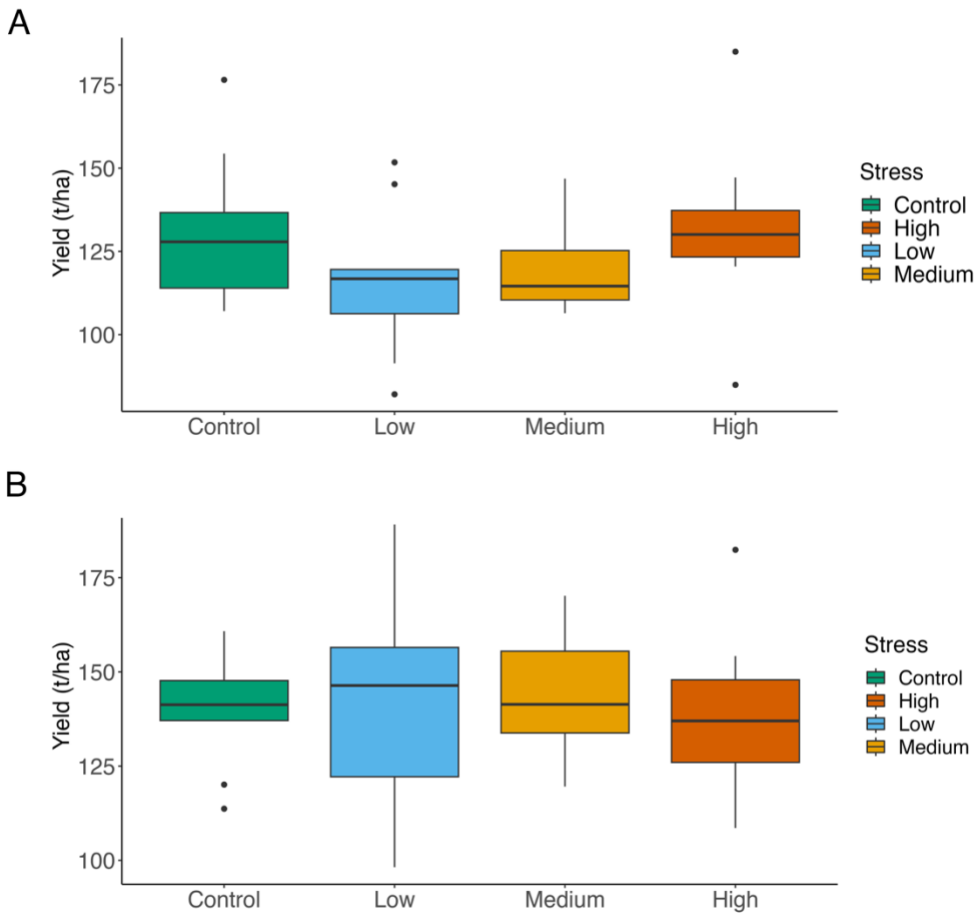

Figure 20. Boxplot of tomato yield (tonnes/hectare) collected in year 1 (top) and year 2 (bottom) from the different irrigation treatments.

Table 2.2. Year 1 F and p-values from ANOVA with soil depths analyzed separately

	Sampling				Treatment				Sampling x Treatment			
	0-10 cm		10-20 cm		0-10 cm		10-20 cm		0-10 cm		10-20 cm	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
GWC	3.41	0.02	5.12	0.007	0.19	0.90	0.49	0.69	0.38	0.93	0.22	0.98
pH	0.38	0.76	9.7	0.0002	0.34	0.79	1.45	0.29	1.27	0.28	0.43	0.90
EC	0.29	0.82	3.44	0.02	0.001	0.99	3.66	0.02	0.63	0.76	0.87	0.55
MBC	5.37	0.004	2.19	0.10	1.71	0.18	0.36	0.78	1.23	0.31	0.99	0.46
PMN	1.45	0.25	4.73	0.008	0.76	0.54	0.64	0.59	0.83	0.59	1.27	0.28
NO_3^--N	2.57	0.07	1.69	0.19	0.98	0.41	0.12	0.94	0.47	0.87	0.46	0.88
NH_4^+-N	0.26	0.84	6.74	0.001	0.15	0.92	1.71	0.24	0.65	0.73	1.14	0.37

GWC: gravimetric water content, EC: electrical conductivity, MBC: microbial biomass carbon, PMN: potentially mineralizable nitrogen

Table 2.3. Year 2 F and p-values from ANOVA with soil depths analyzed separately

	Sampling				Treatment				Sampling x Treatment			
	0-10 cm		10-20 cm		0-10 cm		10-20 cm		0-10 cm		10-20 cm	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
GWC	0.25	0.85	4.38	0.70	0.01	0.78	1.26	0.33	1.01	0.44	0.27	0.97
pH	1.07	0.37	0.77	0.52	0.06	0.97	0.85	0.49	0.43	0.90	0.65	0.73
EC	0.82	0.86	0.23	0.86	0.57	0.49	0.57	0.63	0.84	0.54	1.32	0.26
MBC	8.45	0.0002	11.40	0.00	0.73	0.50	0.19	0.89	0.77	0.63	0.63	0.75
PMN	18.3	>0.001	3.60	0.02	0.37	0.77	0.76	0.53	1.37	0.25	1.53	0.19
NO_3^--N	5.88	0.002	0.21	0.88	2.61	0.06	1.03	0.42	0.73	0.67	0.39	0.92
NH_4^+-N	3.03	0.04	2.60	0.07	0.90	0.48	0.44	0.72	2.05	0.07	0.81	0.60

GWC: gravimetric water content, EC: electrical conductivity, MBC: microbial biomass carbon, PMN: potentially mineralizable nitrogen

2.3.6. Correlations Between Soil Health Indicators

Although there was not a significant deficit irrigation treatment effect on any of the soil physical and chemical properties in the second year (or the first year excluding EC), there were correlating relationships between the soil health properties which were similar in both years (Figure 21). The direction of those correlations remained the same while the significance level varied. Electrical conductivity had a significant positive correlation with nitrate ntitrogen, ammonium nitrogen, and potentially mineralizable nitrogen (PMN) and a negative significant

correlation with pH and gravimetric water content (Figure 21). Nitrate nitrogen had a significant positive correlation with NH_4^+-N and PMN, and a significant negative correlation with pH. Ammonium nitrogen concentration was positively correlated with PMN and negatively correlated with pH and GWC. PMN was negatively correlated with pH and GWC.

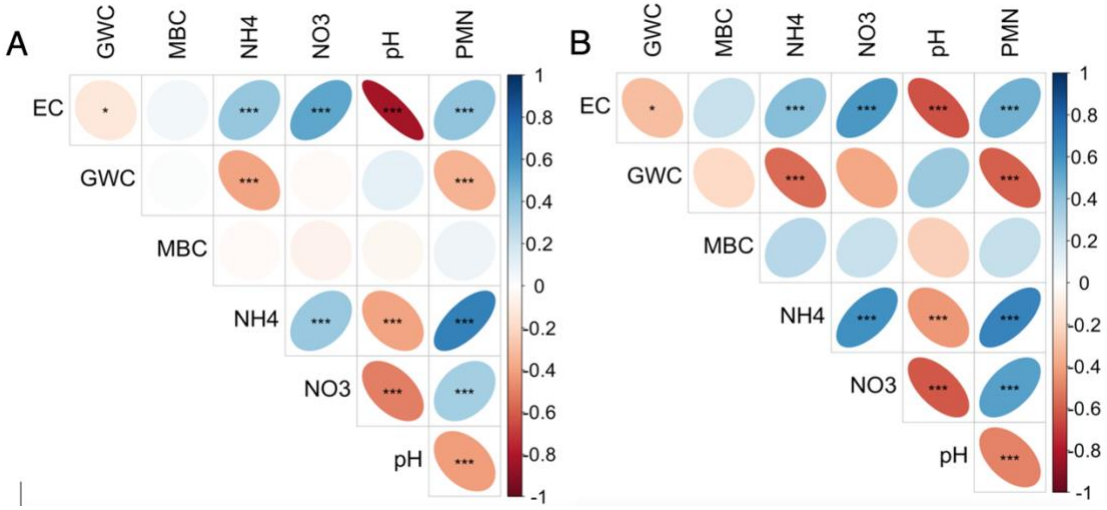

*Figure 21. A correlogram of Pearson's coefficients comparing soil health properties in year 1 (left) and year 2 (right). Only statistically significant correlations are shown with asterisks. (*** = ($p < 0.001$), ** = ($p < 0.01$), * = ($p < 0.05$)). The ellipse width indicates strength of correlation and the ellipse angle indicates the direction of the relationship.*

2.4. Discussion

2.4.1. Effect of Deficit Irrigation Intensity on Soil Physicochemical Properties

We hypothesized that the medium and high stress deficit irrigation treatments would cause a decrease in microbial biomass carbon, but not effect crop yield and soil physicochemical properties, such as pH and electrical conductivity. We found that few variables were affected in the same way between the upper and lower depths. Fertilization via fertigation – applied through the drip irrigation at a 30-cm depth – likely affected patterns observed in the lower depth versus the upper depth due to the proximity to the water and fertilizer source. We suggest that emitter depth limited significant variation between treatments in gravimetric water content in the upper

depth of both years by not providing much moisture in the upper 10 cm of the soil in any of the treatments. The volumetric water content ranges widely in the first year from 0.1 to 0.4 m^3/m^{-3}, but the data show that the moisture content for control and high stress treatments stayed within similar ranges throughout the season, while the low and moderate stress treatments stayed in similar ranges. It is unexpected that the control and high stress treatments would have more similar soil moistures than the control and low or the medium and high stress. The volumetric water content range is considered relatively dry for clay loam soils (Datta et al. 2018). The probes used to collect volumetric water content data only had about a week of lead time in the first year before deficit irrigation began. This could have prevented an appropriate assessment of the baseline of the treatments before the irrigation tapering took place. On top of that, there was little to no replication of the sensors in the treatments, meaning that the influence of a sensor's proximity to an emitter is more pronounced compared to in year 2. Gravimetric Water Content did not vary between irrigation treatments, but the Gravimetric Water Content did decrease over the season in the high stress treatment. The decrease in irrigation from 100% of crop evapotranspiration requirement at the beginning of the season to ~37% of crop evapotranspiration requirement at the start of deficit irrigation allows for significant contrasts in the gravimetric water content data while the other irrigation strategies did not.

In both years, gravimetric water content was negatively correlated with electrical conductivity. While there were no significant differences between the treatments in either year, there were significant decreases throughout the season, as the irrigation decreased across all treatments. Electrical conductivity increases as irrigation is limited, corresponding with the negative correlation between the gravimetric water content and electrical conductivity (Figure 21). This is expected, as water allows for the solubility of salts and the precipitation of these salts

can occur in arid regions and water-limited environments (Hanson and Bendixen, 1995). Our results suggest that deficit irrigation's effect on electrical conductivity is not detrimental to the productivity of processing tomatoes. Ideal electrical conductivity for tomatoes is approximately between 2000-5000 µS/cm (Dorai et al., 2001). Throughout the season in both years, the electrical conductivity measured by the frequency domain reflectometry was mostly within this range for all irrigation treatments in the upper 10 cm of the soil (1500-4000 µS/cm). Therefore, from this experiment, we can conclude that there is no negative impact on tomato yield from compounded salt stress using a tapered higher stress deficit irrigation treatment.

The pH of the soil decreased with increasing intensity of the deficit irrigation treatments. These observations in the experiment suggest that irrigation below 50% ET_c can significantly decrease the soil's pH by the end of the season. When plants experience water stress, the roots can close to prevent a drop in water potential around the roots and conserve water which can cause a buildup of carbon dioxide in soil (Schimel, 2018). Plant roots can also produce organic acids under water stress that can acidify the soil (Brady and Weil, 2020). The soil pH can also decrease as aluminum and hydrogen ions are released from soil particles and basic cations that provide buffering become less available (Siebielec et al., 2020). This is due to the ion exchange processes, in which charged soil particles cling onto molecules of the opposite charge, this problem can become exacerbated in the absence of water. As the pH changes in the soil and become more acidic, positively charged ions are released from soil particles and clay structures. Acidification is also a result of nitrification, the assimilation of ammonium to amino acids is also known to generate H^+ ions. The high stress deficit irrigation treatment had the largest decrease in pH, while the decreases in the other three treatments occurred in a similar gradual manner. The steep drop in pH occurred around the time irrigation ended for the remainder of the growing

season. Processing tomatoes thrive in soil with a pH ranging from 6.0-7.5 (Miyao et al., 2023). Slightly acidic soil is typically ideal for most crops, meaning that with crop rotations, a deficit irrigation treatment that lowered pH from 8.4 to 7.9 could be beneficial, as seen in our experiment.

A meta-analysis determined, by compiling data from 198 studies, that the soil net nitrogen mineralization rate increases with soil moisture (Jarvis et al., 2007; Li et al., 2019; Lupon et al., 2015) The decrease in ammonium-N that we observed in the second depth of the first year aligns with this trend, suggesting that the ammonium-N decreases in the high stress treatment due to the limited water being irrigated and also that ammonium-N is being taken up by the plant roots in the 10-20 cm range of depth. However, there was a negative correlation between ammonium-N (NH_4^+-N) and gravimetric water content that denotes trends seen in the second year of the experiment. This is consistent with our understanding that ammonium-N concentrations would decrease in areas where there is more uptake from the plants, and there would be more ammonium-N where there is more irrigation. In the second year, ammonium-N increased after 100% ET irrigation stops after day 93, indicating that with a lower gravimetric water content, ammonium-N increased initially between the first and second samplings on Day 80 and Day 93, respectively. Ammonium-N concentrations could be limited in the lower depths of the soil due to higher populations of microbes carrying out nitrification (Johnson et al., 2005).

Nitrate nitrogen concentrations did not vary between the deficit irrigation treatments in either year of the experiment, but like ammonium nitrogen, there was a significant increase in the upper depth of the high stress treatment in year 2 between the first and third sampling. Increases in nitrate nitrogen, ammonium nitrogen and potentially mineralizable nitrogen demonstrate the increase in nitrogen after fertilization during the first week of deficit irrigation. The measured

nitrate nitrogen values (0-150 µg/g soil) reach higher than ideal concentrations (10-50 µg/g soil) for agricultural soil, creating a risk of nitrate leaching (Koivunen and Horwath, 2005; Lazcano et al., 2015). There is a gradual decline in nitrate nitrogen observed graphically, though not significantly, throughout the season in the upper depths of the soil and the low amounts in the lower depth of the soil that is closer to the water source. With the observed increase in soil moisture in the lower depth, it is possible that accumulation of nitrate-N could be occurring there due to fertigation.

Significant positive correlations between ammonium nitrogen, nitrate nitrogen, nitrogen and potentially mineralizable nitrogen are consistent with observations from other row crop studies (Johnson et al., 2005; Li et al., 2019). The lack of correlation of any of these properties with microbial biomass carbon could argue that deficit irrigation did not impair nitrogen cycling processes that microorganisms carry out to mineralize ammonium and transform it into nitrate through the nitrification cycle (Johnson et al., 2005). However, the trends seen in our experiment appear to be consequences of fertigation occurring weekly at the beginning of the experiment and fertilization ending at 75-80 days, at which point most sources of nitrate sources decreased as fertigation ceased.

Microbial biomass carbon (MBC) generally decreased throughout the season in both depths in both years. Though these changes were only significantly different in some treatments and in some years, significant declines were observed in all deficit irrigation treatments at least once. Microbial biomass carbon declined in the control treatment in the upper depth of year 1, the moderate and high stress treatments in the upper depth of year 2, and the low stress treatment in the lower depth of year 2. Reduction in soil moisture can impair microbial metabolic capabilities (Carbone et al., 2011) and cause microorganisms to go dormant or die (Schimel,

2018). In Mediterranean climates, MBC values were found to range from 100-500 µg/g during the growing season of a vineyard (Bünemann et al., 2006) and 200-800 µg/g in a Mediterranean olive orchard. The values we observed in this experiment were on the lower end of those ranges at 100-300 µg/g due to the low microbial community concentration in the persistently dry Central Valley soils.

2.4.2. Deficit Irrigation Effect on Yield

Marketable yield was not significantly affected by any of the three deficit irrigation treatments, while a previous study shows that yield is impacted by deficit irrigation past 50% ET_c (Rodriguez-Ramos et al., 2022) and when irrigating within a 60-30% evapotranspiration range (Chen et al., 2013). Limitations in our yield methodology, too few repetitions and sampling bias, could also be a reason that significant differences in yield were not observed between treatments. Other studies rarely use the tapering strategy like we did in this experiment. Not decreasing irrigation until after fruit set allows the plants to receive irrigation during the most crucial period, as drought tolerance varies between growth stages for processing tomatoes. No difference in yield compared between all of the deficit irrigation treatments could suggest a benefit for tapering irrigation. Drought-tolerant stages typically include vegetative stages and late ripening stages (Geerts and Raes, 2009). Irrigating the processing tomatoes at 100% crop evapotranspiration requirement during the vegetative stages is adequate to prevent marketable yield losses.

2.5 Conclusion

This study provides insight into the response of several physicochemical soil properties to four deficit irrigation management strategies. With a lack of significant declines in soil

physicochemical properties or yield, the data suggest that even the highest intensity deficit irrigation treatments we studied do not impose measurable stress on the soil physicochemical properties measured in this experiment. Our results provide evidence that more conservative water use is possible without negative tradeoffs such as limited soil productivity or even significant declines in gravimetric water content. Significant declines of soil moisture occurred within all treatments, as the irrigation tapered, but the declines were not significantly different between the control and high stress treatments.

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Chapter 3: The Effect of Deficit Irrigation on the Spatial Arrangement of Soil Microbial Communities

Abstract

Subsurface drip irrigation and deficit irrigation are management techniques used in semi-arid regions such as the Central Valley in California to irrigate agricultural land and maintain agricultural productivity while limiting water use. Through subsurface drip irrigation a wetting zone can occur, as subsurface drip irrigation targets mainly the rhizosphere. The effect of limited soil moisture on the microbial population in the soil outside of the wetting zone is a relevant question when considering soil health. This experiment explores the spatial variability of soil physicochemical properties and microbial community distribution in response to the inconsistent wetting caused by subsurface drip irrigation. The experiment measured the soil response to four deficit irrigation treatments, a control, low, medium, and high stress deficit irrigation. There were limited physicochemical responses, apart from a decline in nitrate nitrogen and an increase in ammonium-N and potentially mineralizable nitrogen with distance from the irrigation source. Bacterial communities, such as gram-positive and gram-negative bacteria also decreased with distance from the irrigation source. Total living microbial biomass, microbial biomass carbon, and the fungi-to-bacteria ratio were not significantly affected by the deficit irrigation treatments. The deficit irrigation treatments did not significantly affect the yield in either year of the experiment.

3.1 Introduction

Irrigation is necessary for increasing crop yield and providing an adequate water supply for plant growth, particularly in places with scarce or irregular precipitation. In 2017, the Census of Agriculture reported that 5.4 million hectares of cropland in California are irrigated (Ritchie

and Roser, 2013). Drought and water resource issues are projected to limit food security in regions that rely heavily on irrigation (McEvoy et al., 2020). With irrigation management, farmers can manage the irrigation rate and reduce the risk of crop failure due to drought or overwatering. However, droughts have limited the availability of freshwater and groundwater used for irrigation (Quinteiro et al., 2018). Conventional irrigation practices are inefficient – water can be lost through evaporation, irrigating outside the root area, deep percolation, or surface runoff (Anyoji and Wu, 1994). Increasing the efficiency of irrigation used on farms can help to conserve water resources and support sustainable agriculture (Fereres and Soriano, 2007).

It is known that a lack of soil moisture can alter the soil's microbiome, which can impact soil health and ecosystem functioning (Li et al., 2019; Schimel, 2018). Water stress can cause microorganism dormancy or death (Schimel, 2018). While clay soils are more resistant to changes in soil moisture, as they have higher moisture retention, deficit irrigation still has the potential to alter microbial community structure, the composition and the abundance of soil microbial communities by decreasing the diversity of soil bacteria and fungi (Carbone et al., 2011; Schimel, 2018). Microbial function, responsible for the decomposition of organic matter and cycling of nutrients in soils, can also be affected by water stress (Johnson et al., 2005; Li et al., 2019). Nutrient cycling and decomposition of organic matter are essential for maintaining a healthy and fertile soil. Dry soils that lack fertility and structure provided by soil organic matter are more susceptible to soil erosion which can decrease agricultural productivity and dependence on agrochemical inputs like fertilizers and fumigants (Pimentel and Burgess, 2013). Current literature studying the impact of deficit irrigation on the soil microbiome suggests that along with the type of soil and vegetation, the content of soil organic matter is particularly important in the observed changes in soil structure, microbial activity and abundance, fluxes and carbon and

nitrogen cycling as a result of water stress (Siebielec et al. 2020). Research has found declines in soil organic matter with intensive agricultural land use by soil erosion and destruction of soil structures (Maikhuri, 2012), though this is not matched with declines in yield (Sojka and Upchurch, 1999) which causes soil organic matter content to not be a main concern for farmers.

When soil is under water stress, microbes can either die or go dormant (Schimel, 2018). When microbes die, they degrade and release plant nutrients like nitrogen, carbon, phosphates, and amino acids that support other living microbes (Lundquist et al., 1999). This death can cause a spike in these available nutrients, but the limited microbial abundance due to dormancy and microbial depth cannot continue to produce useful nutrients like nitrate, and in turn, soil fertility declines (Johnson et al., 2005; Li et al., 2019; Schimel, 2018). During drought, the rhizosphere becomes more populated by Actinobacteria and other Gram-positive species that replace the less drought-resistant Gram-negative taxa (Breitkreuz et al., 2021; Quach et al., 2022). This replacement can help support plant drought tolerance by improving soil structure and water-holding capacity with the production of polysaccharides (Miloševic et al. 2012). A decrease in microbial abundance can also alter microbial biomass carbon and nitrogen cycling. The Carbon to Nitrogen (C:N) ratio should remain ~24:1 for optimal plant growth (Brady and Weil, 2020). It has also been observed that fungal abundance and enzymatic activities and microbial respiration in soil-plant systems decrease under water stress (Carbone et al., 2011; Lundquist et al., 1999). Drought impacts microbial functions such as cycling of N via mineralization, nitrification, and decomposition (Johnson et al., 2005; Li et al., 2019; Schimel, 2018). Water stress in areas not within the wetting zone can suffer from a decrease in microbial activity.

In a subsurface drip system, the wetting pattern refers to the distribution of water in the soil surrounding the drip emitters. The wetting pattern is influenced by various factors such as

emitter spacing, lateral spacing of the crop rows and drip line, the discharge rate of emitters and system pressure. The size and shape of the wetting pattern in a subsurface drip system can vary depending on soil texture and climate. In a study comparing different soil textures, it was found that greater spreading of the wetting pattern occurred in loamy sand compared to sandy loam in both vertical and horizontal directions (Rasheed, 2020). Another study found that the wetting pattern in a subsurface drip system was spherical in shape (Monjezi et al., 2013). The wetting pattern has implications for the distribution of nutrients and microbial communities in the soil. During dry years, subsurface drip irrigation can lead to "drying and re-wetting" events in soils, which can flush out nitrate-N stored in soils and groundwater (Kaushal et al., 2008). Additionally, the wetting and drying cycles associated with deficit irrigation can affect microbial biomass carbon and electrical conductivity because they are primarily driven by soil moisture.

Subsurface drip irrigation causes inconsistent wetting at distances farther away from the irrigation emitter and constant wetness closer to the irrigation source, these changes in moisture are expected to consequently change biogeochemical properties such as microbial abundance and nutrient concentrations (Griffin, 2018). Previous research found that soil properties, like bacterial diversity, differed significantly with distance from the subsurface drip emitter (Quach et al., 2022). Organic carbon was found to be significantly higher in clay and clay loams soils within 15 cm from the irrigation emitter versus soils 45 cm away from the irrigation source (Quach et al., 2022) and significant differences in plant nutrients (NO_3^--N) with the distance from the drip irrigation emitter (Lazcano et al., 2015).

While there is research dedicated to microbial response to water stress, more information is needed to understand the combined effects of deficit irrigation and subsurface drip irrigation on soil health properties and the abundance of microbiota at different distances from the water

source. To maximize soil carbon sequestration and environmental conservation efforts, maximizing the abundance of microbes in soil is important to develop management practices that can mitigate any negative unintended consequences of crucial water conservation efforts.

The objective of this study is to measure soil physicochemical and biological properties in relation to subsurface drip and deficit irrigation and particularly as a function of proximity to the drip line (emitters). We hypothesized that soil physical properties will not be impacted by distance from the deficit irrigation emitters, but that microbial biomass, and associated microbial processes and metabolic by-products (such as nitrate-N) will decline with increasing distances away from the subsurface drip emitter due to limited access to water.

3.2 Materials and Methods

3.2.1. Site description

A field experiment was conducted to evaluate the effects of deficit irrigation on soil health in processing tomatoes (*Solanum lycopersicum*) in Five Points, CA. The experiment was conducted twice, in the years 2021 and 2022, roughly from May to August each year. The experiments were set up on a commercial agricultural farm with frequent crop rotation, and therefore the experiment was conducted on a different field each year. The field sites in 2021 and 2022 were approximately 2 km apart. In both years, the soils at the experimental sites were classed as aridisols (California Soil Resource Lab, 2022), a common soil type in California. Aridisols are commonly used to grow irrigated crops such as cotton, wheat, and alfalfa seed. The composition of the soil in 2021 was 88% Posochanet series, classified as a Fine-silty, mixed, superactive, thermic Sodic Haplocambid, which is indicative of slow permeability and low to medium water runoff in areas with low precipitation and drought, like the California Central Valley. In 2022, the soil was classified as 85% Calfax soil series, classified as a Fine-loamy,

mixed, superactive, thermic Sodic Haplocambid, which can be very deep and moderately drained soils. The soil texture at both experimental sites was classified as a clay loam in the top 20 cm, as reported by the UC Davis NRCS database for soil texture and class (California Soil Resource Lab, 2022).

The tomato varieties used in this study were SVTM9025 (2021) and SVTM9016 (2022) due to their popularity in the region. These tomato varieties are ideal for their late-season growing, high Brix^{o} content and easy peel-ability for industrial processing. In year one, the experimental site was sized at approximately 6.4 x 10^4 m^2 and the treatment replicates were organized randomly within blocks (a complete randomized block design), with 8 rows of tomatoes per treatment block and 96 total rows in the field. In year two, the experimental site was approximately 1.45 x 10^5 square meters with approximately 1.2 x 10^4 square meters allocated for each treatment block. Subsurface irrigation lines were installed throughout the entire field in 2013 (for the 2021 field) and 2014 (for the 2022 field) at a 30 cm depth.

The average precipitation in Central Valley during the months of the experiment is 0.07 cm for the month of July and 0.1 cm of rainfall in the month of August. The mean temperature during this season is 35.5^{o}C (National Weather Service, 2022).

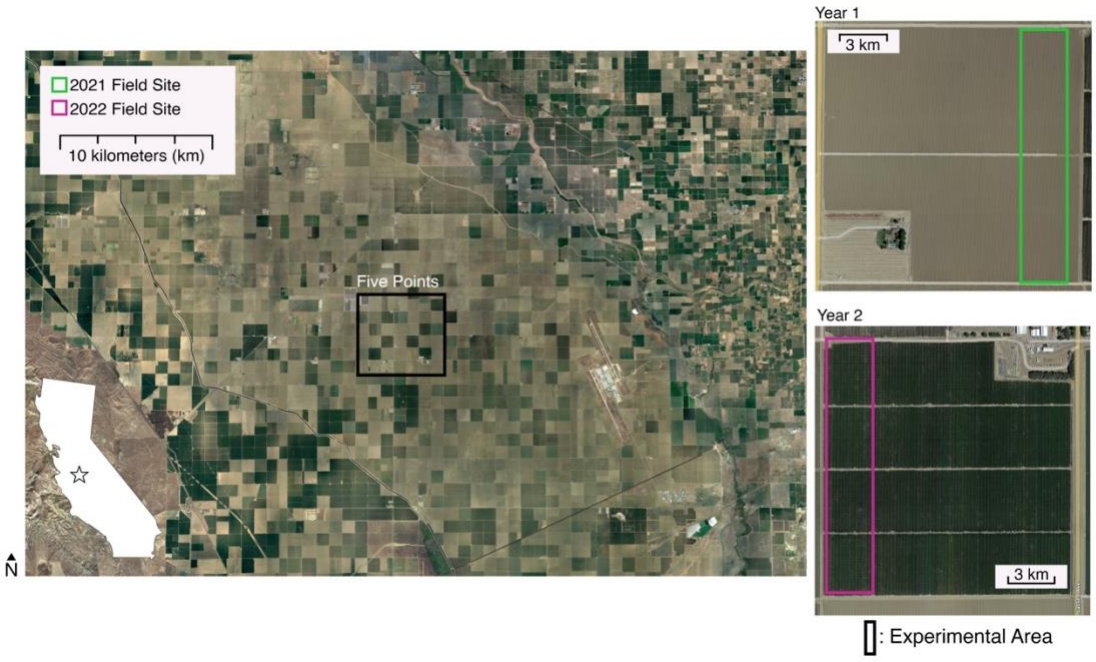

Figure 1. The site map of the surrounding area is shown on the left with the location of both experimental fields. The 2021 experimental site is identified by the green box and shown in detail in the top right and the 2022 experimental sight is identified by the pink box and shown in detail at the bottom right. Images from Google Earth taken 5/5/2023.

3.2.2. Experimental Design

Four irrigation treatments were applied in three blocks throughout the experimental plot (Figure 2). The four irrigation treatments are classified as the control treatment, which is conventionally at the farm, the low water stress (Low) treatment, decreasing the % ET_c directly from 100% to 50%, moderate water stress (Moderate), and the high water stress treatment (High), decreasing the % ET_c from 100% directly to 37%, the most water conservative approach to deficit irrigation in this study. The four irrigation treatments were tapered deficit irrigation treatments calculated using percent ET_o (reference evapotranspiration) (United States Department of Agriculture, 2022). The control treatment was based on the grower's standard and had a 75% ET_c from fruit set and was tapered to 50% ET_c at the "Midpoint" (Table 3.1). In comparison, the high stress treatment began with 37% ET_c at fruit set and continued until day

120 (Table 3.1). ET_o and the crop coefficient are used to calculate the estimated crop evapotranspiration (ET_c) in order to calculate the minimum requirements for irrigation.

Deficit irrigation occurred in a staggered approach via subsurface drip irrigation, typically the water limitations begin after the fruit set period and continue to decrease towards the end of the season. Therefore, differences between irrigation began approximately around day 80.

Table 3.1 Deficit Irrigation Treatment Schedule

	Transplant	**Fruit Set**	**Midpoint**	**Until Harvest**	**Total Irr.**
	Day 0	Day 70-90	Day 95-100	Day 120	Full Season
Control	100% ET_c	~75% ET_c	~50% ET_c	No irrigation	813.82
Low	100% ET_c	~50% ET_c	~50% ET_c	No irrigation	756.73
Medium	100% ET_c	~50% ET_c	~37% ET_c	No irrigation	730.86
High	100% ET_c	~37% ET_c	~37% ET_c	No irrigation	701.34

The deficit irrigation schedule and the average of total irrigation (cm^3/cm^2*) for each treatment for both years.*

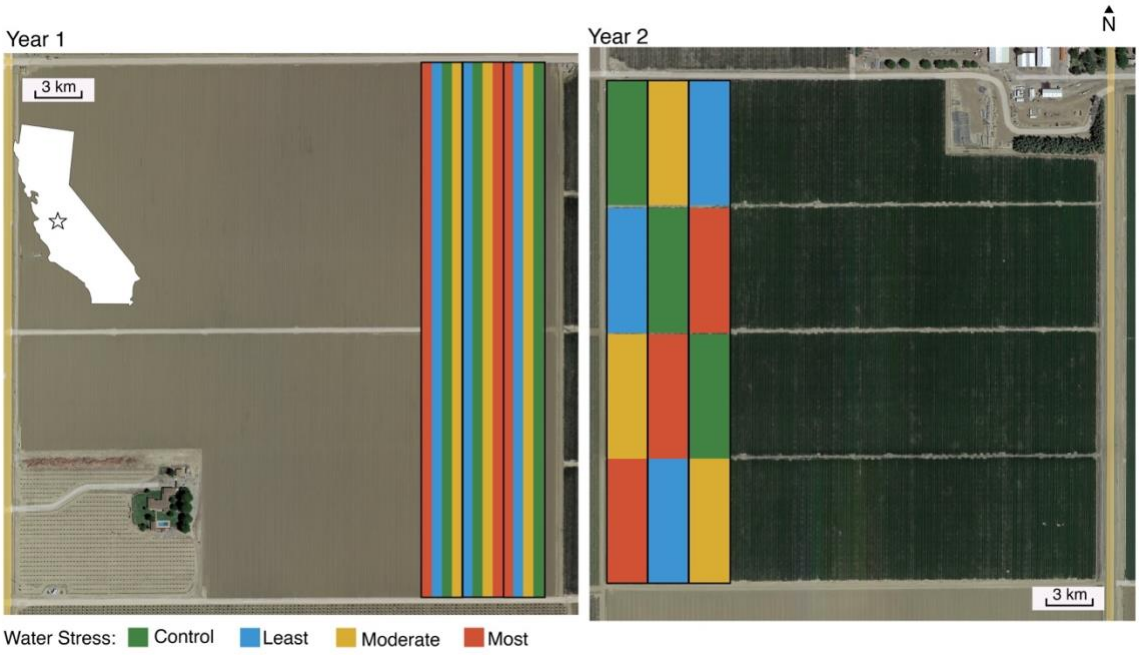

Figure 2. The block and treatment orientation in 2021 (right) and 2022 (left) with three treatment replicates indicated by color. Green (Control), Blue (Low), Orange (Moderate), and Red (High). Images from Google Earth taken 5/5/2023.

The irrigation treatments were applied in a complete randomized block design. In 2021, the study area was approximately 6.4 x 10^4 square meters and each treatment replicate had 8 rows of tomatoes resulting in 96 rows of the experimental plot. Transplanting occurred on May 20, 2021. The three previous crops grown at the 2021site were garbanzo beans (2018) and tomatoes (2019 and 2020). In 2022, the study area was approximately 1.45 x 10^5 square meters with approximately 1.2 x 10^4 m allocated for each treatment replicate. In 2022, transplanting occurred on May 1, 2022. The three previous crops grown at the 2022site were onions (2020 and 2021), and the site was fallow in 2019.

3.2.4. Soil sampling methods

To test the spatial variability of soil health properties under subsurface drip and deficit irrigation, soil was sampled at three distances (10 cm, 25 cm, and 45 cm) from the subsurface drip emitter

(approximately at the center of the bed and at a depth of 30 cm). In each treatment replicate, three plants were selected randomly and soil cores were taken at 10 cm, 25 cm and 45 cm away from the drip emitter. The soil cores were then split into upper and lower depths (0-10 cm and 10-20 cm) and bagged separately. Samples for each distance and depth within each plot were composited, meaning each treatment replicate had 6 soil samples (the upper depth at 10 cm, the lower depth at 10 cm, the upper depth at 25 cm, the lower depth at 25 cm, the upper depth at 45 cm and the lower depth at 45 cm). A total of 72 samples were collected each year.

Soil sampling was carried out twice over the growing season in each experiment. A baseline soil sampling occurred before deficit irrigation began on day 75 after transplanting in 2021 and day 85 after transplanting in 2022. This allowed for any pre-existing variability to be documented. A second sampling occurred to assess the soil health properties at harvest. The data presented in this study includes only the soil samples collected at the end of the season near harvest on day 117 and day 128 for 2021 and 2022, respectively.

The soil samples were taken with a 2.5 cm soil probe and stored in a 3.8 liter plastic zip lock bag in a cooler with ice until it could be transported back to the lab. Once in the lab samples were immediately processed and stored in a 4ºC refrigerator until analysis. Processing included wet sieving the soil to 8 mm and subsampling and freezing for phospholipid fatty acid (PLFA) testing. Subsamples for total carbon and nitrogen testing were set out to air dry and then sieved to 2 mm and then ground in a ball mill and gravimetric water content analysis was conducted within the first 72 hours of soil collection.

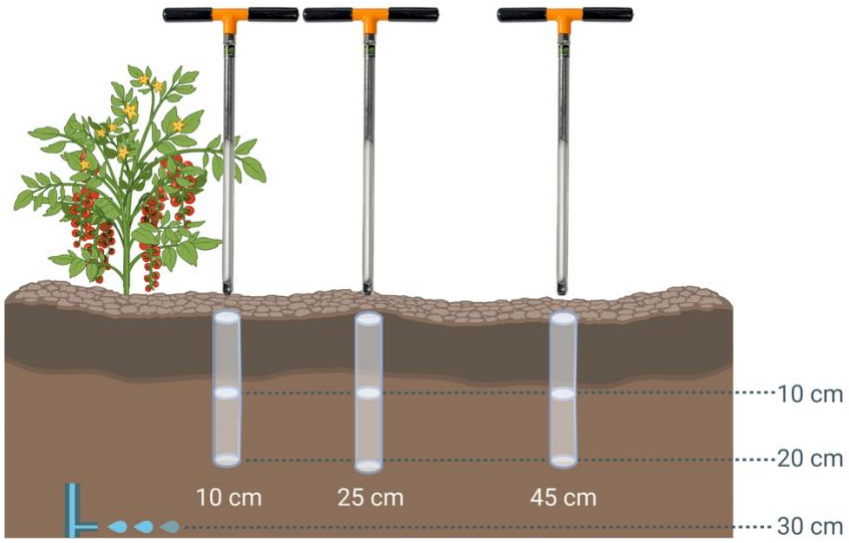

Figure 3. Soil sampling schematic in which soil probes were taken at 10 cm, 25 cm, and 45 cm indicated by the black tick marks. The soil cores were then split in half and the two depths were analyzed separately.

3.2.5. Analysis of soil health indicators

Soil Moisture, pH, and Electrical Conductivity

After sampling and processing, we weighed 10 g of each fresh soil sample and then dried the soil at 100ºC for at least 24 hours. The percent of soil moisture was then calculated from the difference between the wet weight of the soil and the dry weight. pH and electrical conductivity (EC) were measured using a pH and EC meter (Mettler Toledo, SevenCompact Duo) on a soil slurry of 10 g soil to 20 g of water. Volumetric water content was collected using a Teros 12 probe (METER Group Inc, Pullman, Washington, USA) every 10 cm to a depth of 70 cm.

Carbon, Nitrate Nitrogen and Ammonium Nitrogen

We measured soil carbon as microbial biomass carbon (MBC) and as total Carbon (C). MBC was measured using a fumigation-extraction protocol adapted from Horwath and Paul (1994). Briefly, for each sample two subsamples were prepared, with one counterpart undergoing

a 24-hour fumigation using chloroform ($CHCl_3$) to kill the soil microbes prior to extraction using K_2SO_4, while the other was extracted directly without fumigation. The extractions were then diluted and run in a Shimazdu TOC-VCSH and combusted along with blanks and standards in order to measure the concentration of dissolved organic C (DOC). The difference between the carbon content of the fumigated and the non-fumigated samples was the C (mg/kg soil) held in microbial biomass.

We measured nitrate nitrogen (NO_3^--N) and ammonium nitrogen (NH_4^+-N) concentration in the soil samples colorimetrically on K_2SO_4 extracts following Doane and Horwath (2003). Reagents were applied to the extracts and left to sit for 1 hour for nitrate nitrogen testing and 24 hours for ammonium nitrogen (NH_4^+-N) testing and then ran through a Synergy HTX Multi-Mode Reader (BioTek, Fisher Scientific) Machine. The soil were also duplicated and one set was incubated under anaerobic conditions for 7 days in the laboratory and then extracted using K_2SO_4 (Moebius-Clune et al. 2016). Potentially mineralizable nitrogen was calculated by taking the difference in ammonium nitrogen before and after incubation.

Phospholipid fatty acid analysis of soil microbial communities provides information on microbial biomass of the total community and of specific subgroups. 50 grams of the soil samples were subsampled and frozen in a -80ºC freezer until lab analysis for both years was complete. All subsampled soil was sent out for PLFA testing carried out by RegenAg Labs (Pleasanton, NE). Samples were extracted, fractionated, and transesterificated. Samples were then run on a 7890 gas chromatograph (GC; Agilent Technologies, Wilmington, DE, USA) equipped with a 7693 autosampler, split-spitless inlet, and flame ionization detector (FID). Agilent ChemStation and MIDI's Sherlock Software then assigned PLFAs to the following microbial groups: total living microbial biomass, total bacteria, gram-positive bacteria, gram-

negative bacteria, actinomycetes, arbuscular mycorrhizal fungi in ng per g of soil and the fungi:bacteria ratio.

Table 3.2 Measured Soil Physicochemical and Biological Properties

Physical Properties	Chemical Properties	Biological Properties
Gravimetric Water Content	pH	Microbial Biomass Carbon
Volumetric Water Content	Electrical Conductivity	Total Microbial Biomass
	Nitrate-N	Total Bacteria
	Ammonium-N	Gram + and Gram -
	Potentially Mineralizable N	Total Fungi
		Fungi:Bacteria

N: Nitrogen, Gram +: Gram-positive Bacteria, Gram -: Gram-negative bacteria

3.2.6. Crop quality and yield

Tomato yield was measured via 2-meter transect sampling, at three evenly-spaced locations per plot, which was linearly regressed to estimate the total tomato yield per treatment replicate. The yield sampling occurred a week before the farm's commercial harvest and was conducted in the middle and north and south sides of each treatment replicate. The total weight of the tomatoes collected in grams from 6 meters was measured, extrapolated, and converted to tonnes/hectare by multiplying the total area of the treatment plot.

3.2.7. Statistical analysis

The effect of the irrigation treatments and the distance from the SDI emitter were analyzed using the *lme4* package (v1.1-26; Bates et al., 2015) through Analysis of Variance (ANOVA) (Girden, 1992) for linear modeling in RStudio (R Core Team, 2022.12.0) and R version 4.0.1 (R Core Team, 2022). Block was used as a random effect in the linear model in

order to account for pre-existing spatial variability of the experimental plots. Soil physicochemical properties significantly varied between the two depths; therefore, treatment effects were individually assessed for each depth. Data diagnostics and normality testing were done using Scale-Location plots, histograms and Q-Q plots, while Shapiro-Wilks tests were used to supplement subjective qualifications of normality. Data transformations were not conducted on the collected data. Post-hoc analysis was conducted in R version 4.0.1 using the emmeans package. The effect of irrigation treatments and distance from irrigation source on microbial communities was analyzed using Principal Component Analysis (PCA) and Canonical Correspondence Analysis (CCA). P and F values from the ANOVA can be found in Table 2.1 and 2.2 for 2021 and 2022, respectively.

3.3 Results

3.3.1. Gravimetric Water Content

The deficit irrigation treatments did not have any significant impacts on gravimetric water content in year 1 of the experiment (Table 3.2), but the interaction between distance and treatment did have a significant effect on the soil moisture in year 2 (Table 2.2). Gravimetric water content was also significantly affected by the interactive effect of distance x treatment (Table 3.4) in the lower depth of year 1. Generally, soil moisture decreased with increasing distance from the irrigation emitter (Figure 4 and Figure 5). The gravimetric water content values differed significantly by distance in the second year in the high stress treatment in the 0-10 cm depth interval and for the control and low stress treatments in the 10-20 cm depth interval (Figure 5). Soil moisture was higher closer to the irrigation emitter, and decreased the farther away the samples were taken. In the high stress treatment and 0-10 cm soil depth interval there was a significant decrease in soil moisture measured at 10 cm in comparison to the soil measured

at 25 cm from the emitter (Figure 5). In the lower soil depth interval, there was a significant drop of 0.4 g of water/g of soil in soil moisture between the 10 cm from the emitter and the 25 cm from the emitter in the control treatment. For the low stress treatment there was a significant drop of 0.5 g of water/g of soil from the 25 cm point to the 45 cm point, there was no significant difference between the samples at 10 cm and 25 cm. The soil moisture was higher in the lower depth than the upper depth in the second year, but did not follow that same trend consistently in the first year (Figure 4), despite the drip line being at the same soil depth in both experimental sites.

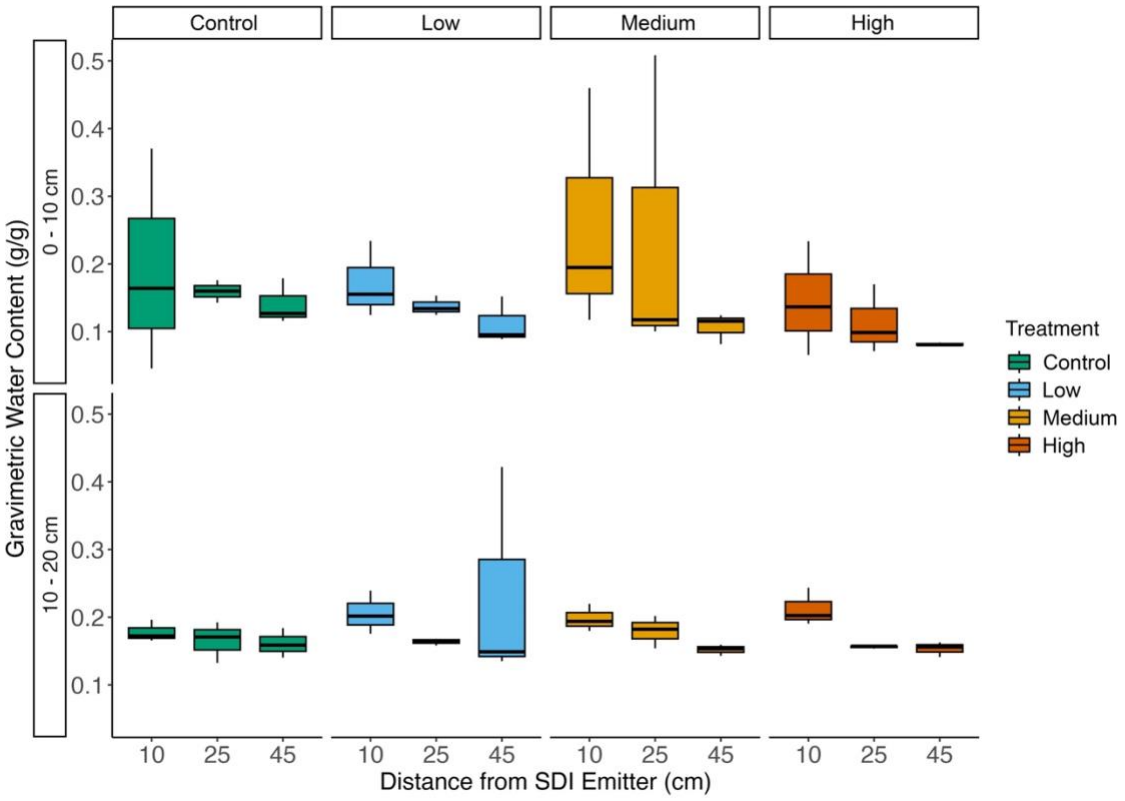

Figure 4. Gravimetric Water Content in g water/g soil in Year 1, separated by soil depths (0-10 cm and 10-20 cm). No significant differences between treatments were found with 95% confidence.

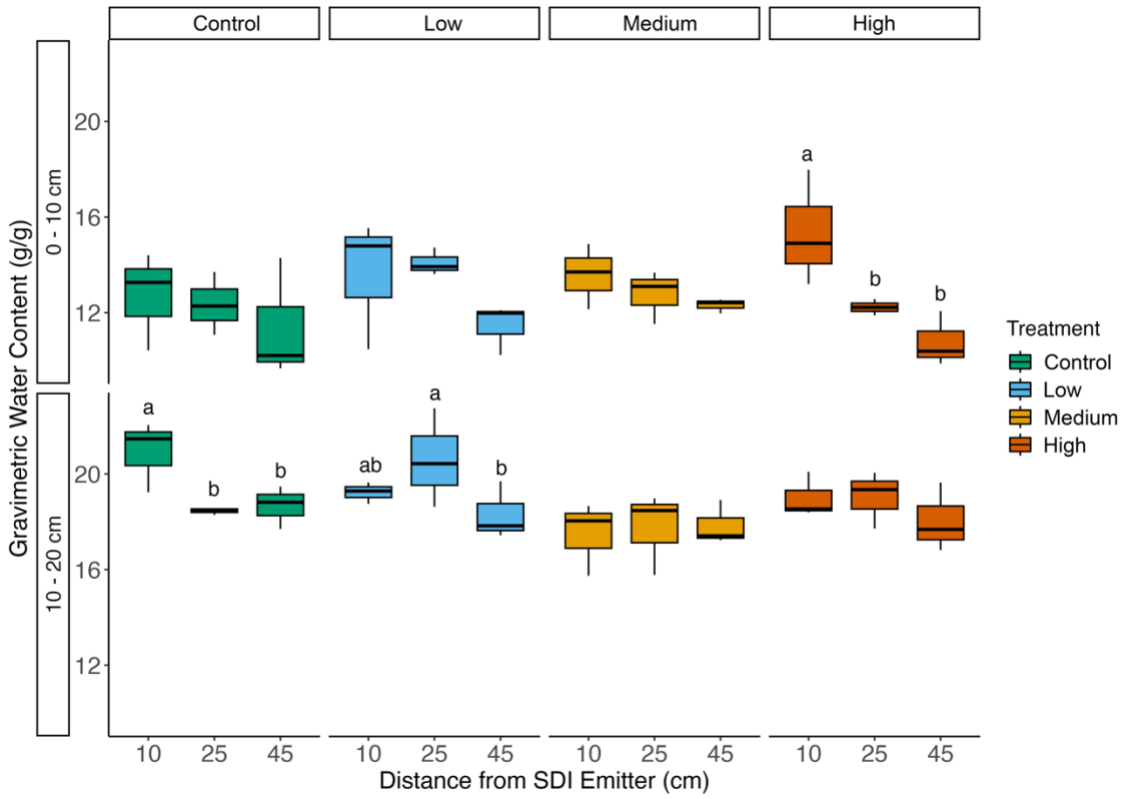

Figure 5. Gravimetric Water Content in g water/g soil in Year 2, separated by soil depths (0-10 cm and 10-20 cm). Different letters in the graph denote significant differences between treatments. Significance was found with 95% confidence.

3.3.2. Electrical Conductivity

Electrical Conductivity was higher in the upper depth of the soils in both years (Figure 6 and Figure 7). Nonetheless, there was no significant effect of the deficit irrigation treatments or the sampling distance on the soil electrical conductivity measured in either experimental year (Table 3.2 and 3.2). There were also no significant differences in electrical conductivity between the distances the soil samples were taken from. The trends are varied and insignificant for both years at both depths. The electrical conductivity was also on average 1 x 10^{-1} µS/cm smaller in year 2 than in year 1. The lower depth in the second year had much less variation in soil electrical conductivity.

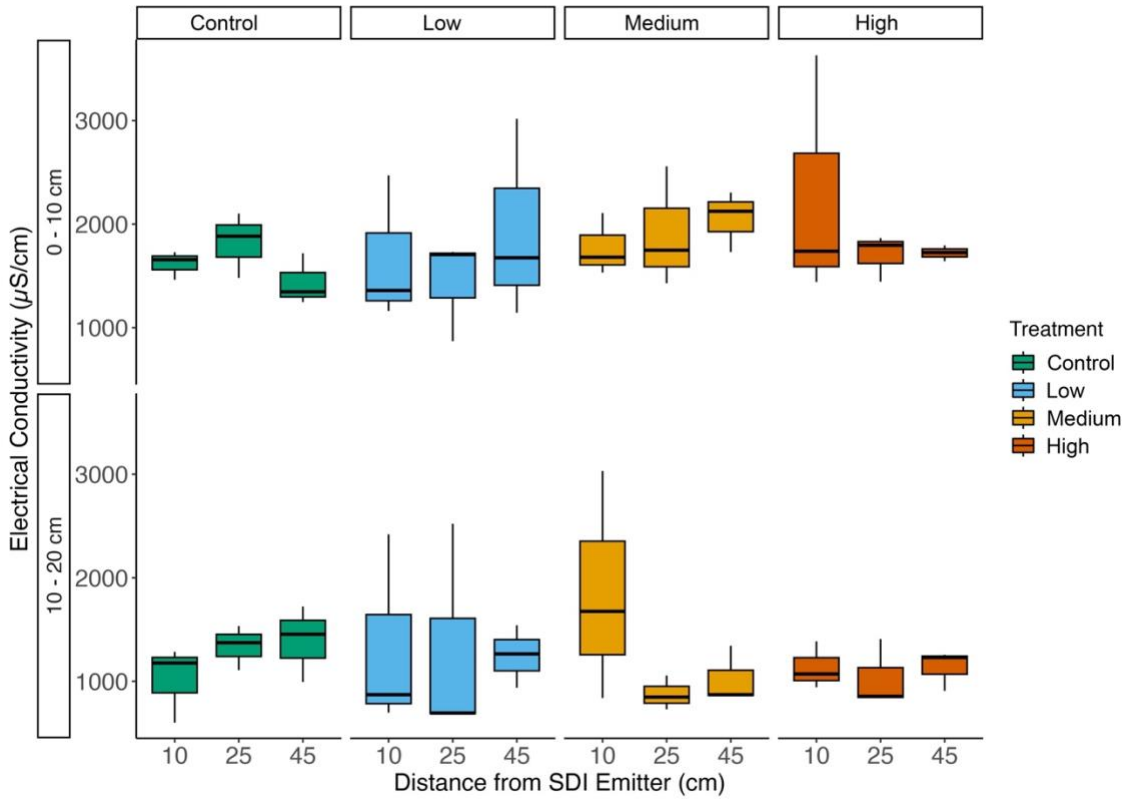

Figure 6. Soil electrical conductivity (µS/cm) at different distances away (10, 25, 45 cm) from the subsurface drip emitter in Year 1. No significant differences between treatments were found with 95% confidence.

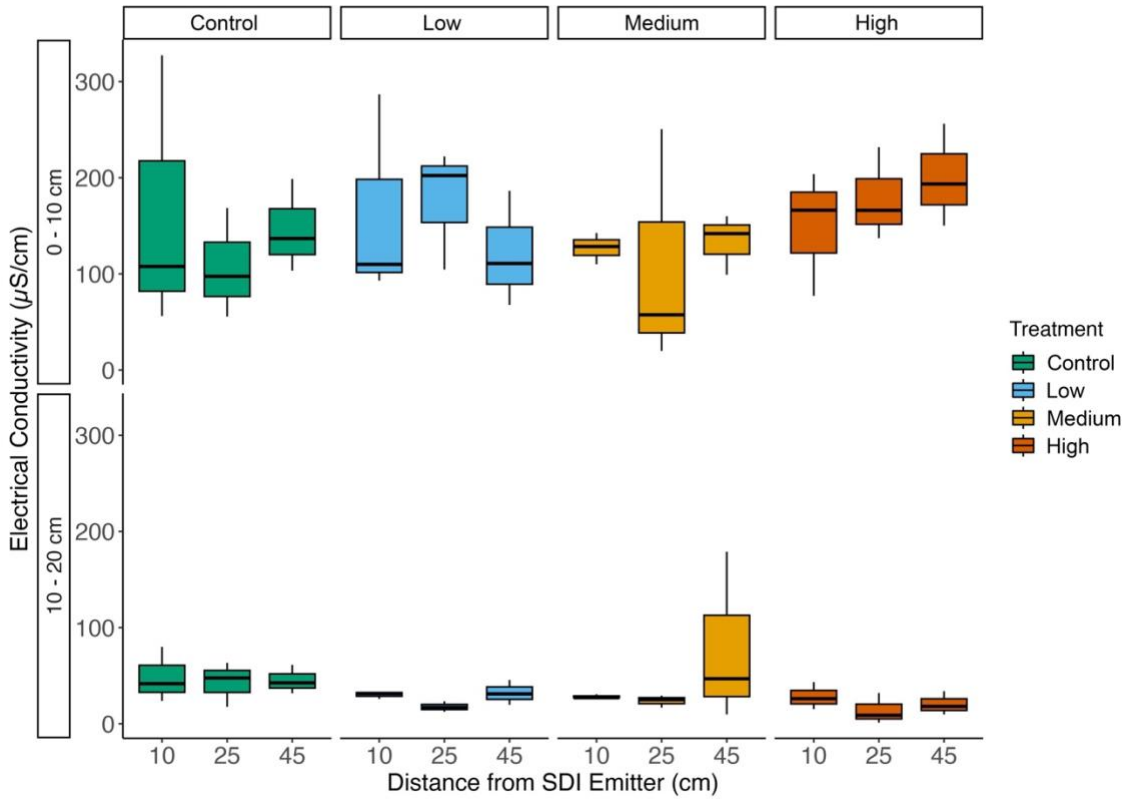

Figure 7. Soil electrical conductivity (µS/cm) at different distances away (10, 25, 45 cm) from the subsurface drip emitter in Year 2. No significant differences between treatments were found with 95% confidence.

3.3.3. pH

There was no significant impact of the deficit irrigation treatments on the pH of the soil in either of the depths in the two years of the study (Table 3.2 and 3.3). There was also no significant impact of the spatial distance on pH. In the second year, pH was higher in the lower depth than in the upper depth, while the pH values fell together in a larger range (Figure 8).

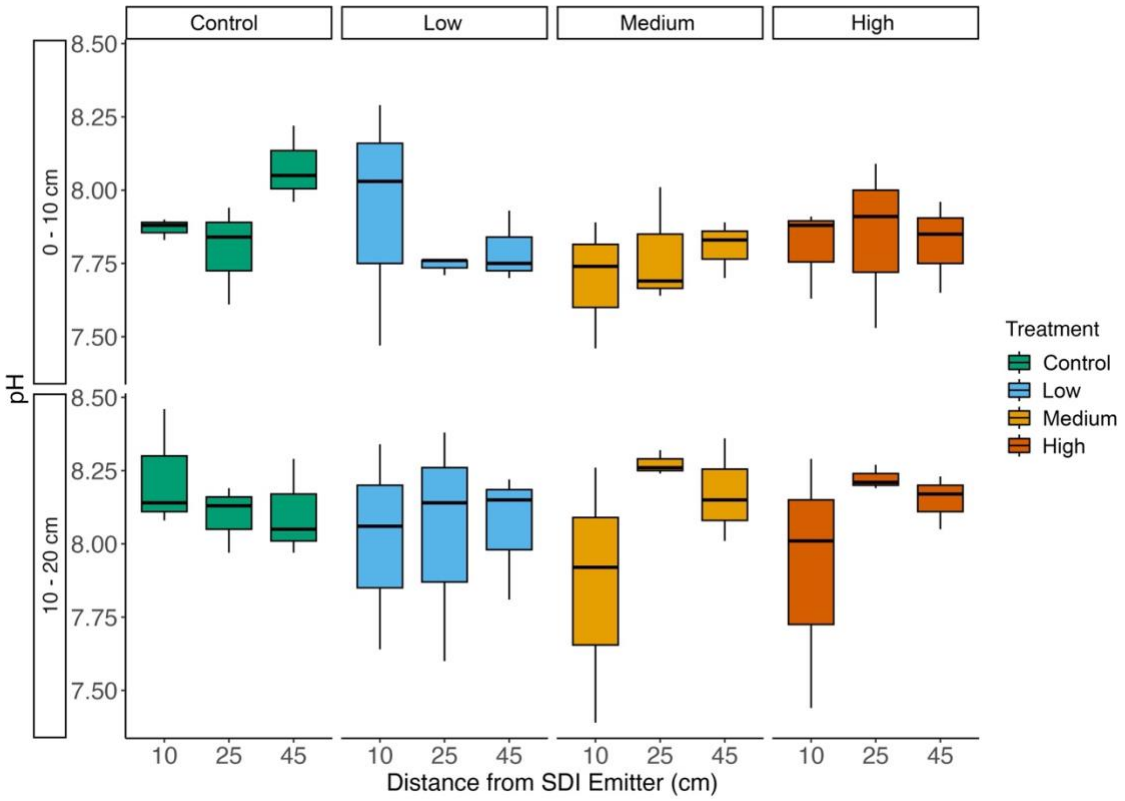

Figure 8. Soil pH measured at different distances from the drip emitter (10, 25, 45 cm) in the four deficit irrigation treatments in Year 1. pH measurements were separated by soil sample depths (0-10 cm and 10-20 cm). No significant differences between treatments were found with 95% confidence.

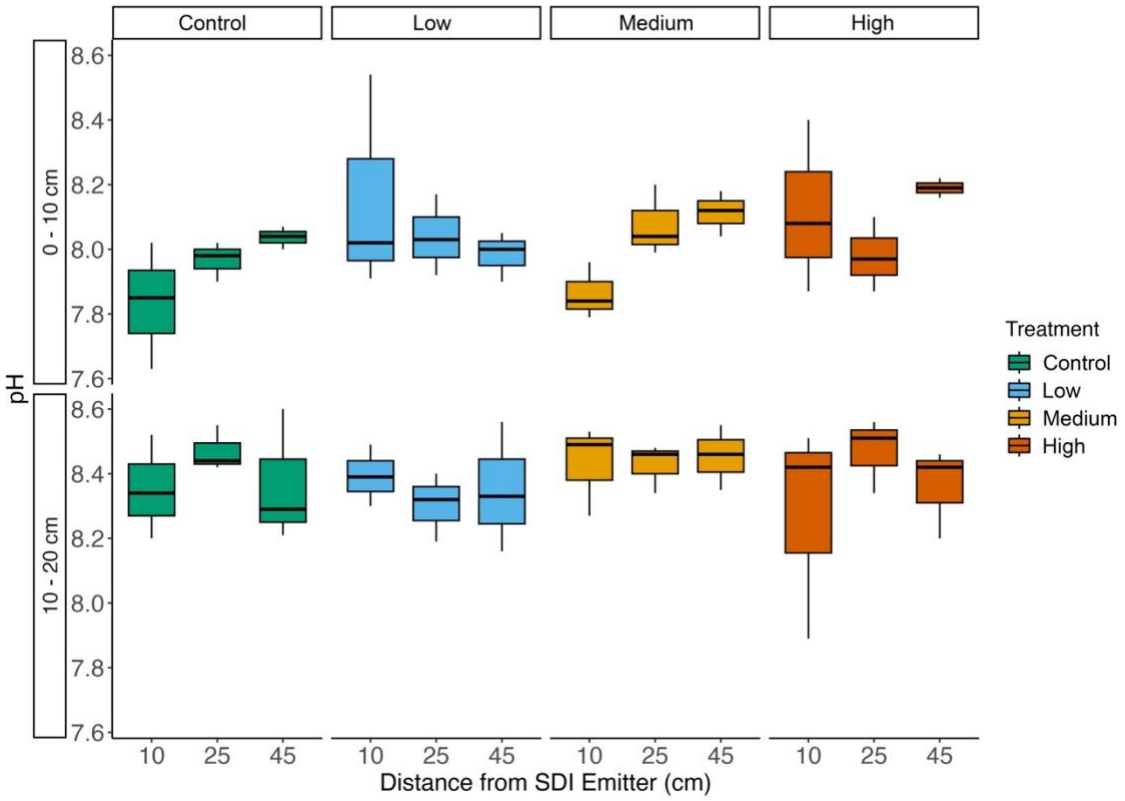

Figure 9. Soil pH measured at different distances from the subsurface drip emitter (10, 25, 45 cm) in four deficit irrigation treatments in Year 2. pH measurements were separated by soil sample depths of 0-10 cm and 10-20 cm. No significant differences between treatments were found with 95% confidence.

3.3.4. Nitrate Nitrogen

The deficit irrigation treatments resulted in significant differences in soil nitrate nitrogen (NO_3^--N) although this was only observed in year 1 (Table 3.2). Treatment also significantly affected NO_3^--N in the upper depth of year 1 (Table 3.2), indicated by the effect of distance in only two of the treatments, moderate and high stress. In year 1, there was a significant effect of sample distance on the NO_3^--N (µg NO_3^--N /g soil) concentrations in both depths, according to ANOVA (Table 3.2). There was a significant decrease of about 150 µg NO_3^--N /g soil between the 10 cm soil sample and the 25 and 45 cm soil samples in the high stress treatment in the upper depth (Figure 10). There was also a significant decrease of about 50 µg NO_3^--N/g soil between the 10 cm and 45 cm soil samples in the moderate stress treatment in the lower depth. The 25 cm

soil sample was not significantly different from the close or most distant measurements. The NO_3^--N concentrations between the two depths were within the same range in the first year (within a range of 0-100), but the lower depth in the second year was much lower compared to the upper depth in the first year, the lower depth did not reach about 10 µg/g, while the upper depth did not reach below 10 µg/g (Figure 11). Nitrate nitrogen was significantly impacted by distance in year 2 (Table 3.4), but significant differences were not shown using the Tukey's range test (Figure 11).

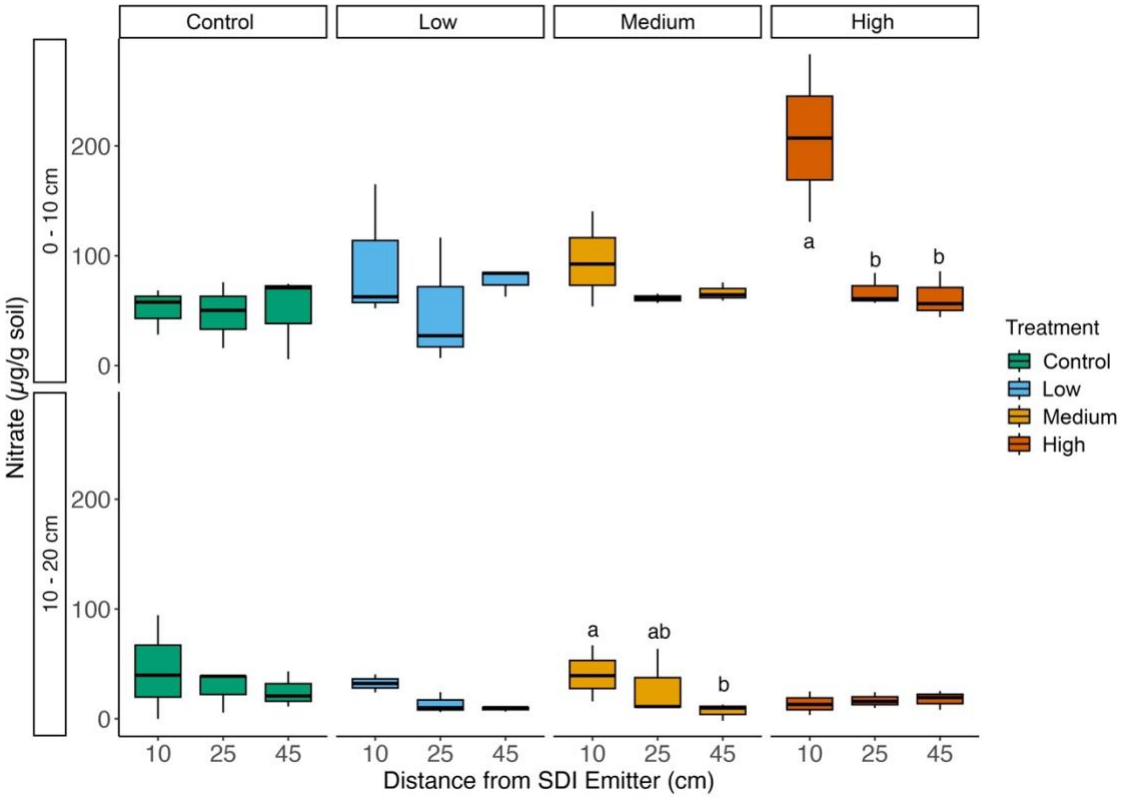

Figure 10. Nitrate-N concentration (µg/g soil) in the soil collected at different distances away from the subsurface drip emitter (10, 25, 45 cm) in Year 1 split into the upper (0-10 cm) and lower (10-20 cm) soil depths. Different letters in the graph denote significant differences between treatments. Significance was found with 95% confidence.

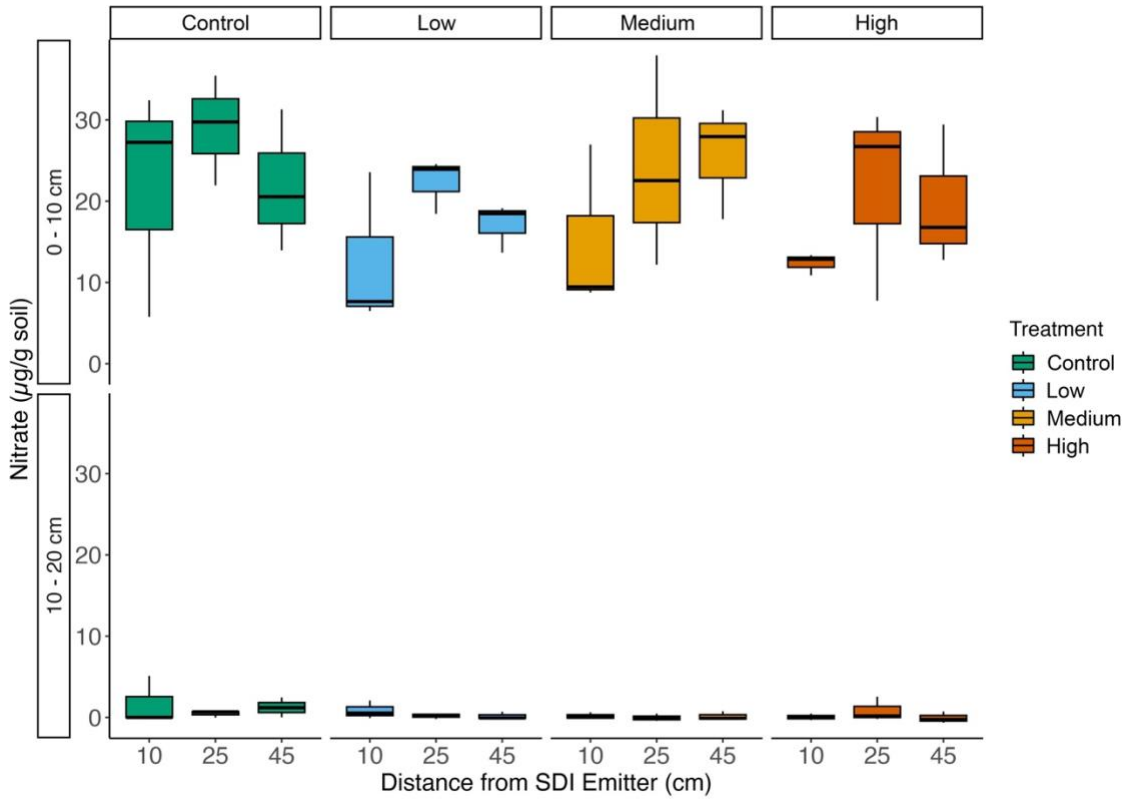

Figure 11. Nitrate-N concentration (µg/g soil) in the soil samples collected at different distances away from the subsurface drip emitter (10, 25, 45 cm) in Year 2 split into the upper (0-10 cm) and lower (10-20 cm) soil depths. No significant differences between treatments were found with 95% confidence.

3.3.5. Ammonium Nitrogen

Ammonium nitrogen (NH_4^+-N) observably increased in the medium and high stress deficit irrigation treatments in comparison to the control and low stress deficit irrigation treatments, though not with 95% confidence. In the second year, there was a significant increase in NH_4^+-N concentration of about 3 µg/g of soil between the 25 cm sample and the 45 cm sample in the low stress treatment in the upper depth. The NH_4^+-N concentration increases significantly from 25 cm to 45 cm, while the 10 cm distance NH_4^+-N concentrations did not significantly differ from the other locations in the treatment. In the second year, we did not see a significant effect of soil location nor irrigation treatment in the ANOVA (Table 3.4), but we did see a significance with posthoc analysis (Figure 13). In the first year, the effect of sample distance

significantly impacted the ammonium-N (NH_4^+-N) concentrations found in the soil sample in the upper depth according to the ANOVA (Table 3.3), however, there were no significant differences through posthoc analysis.

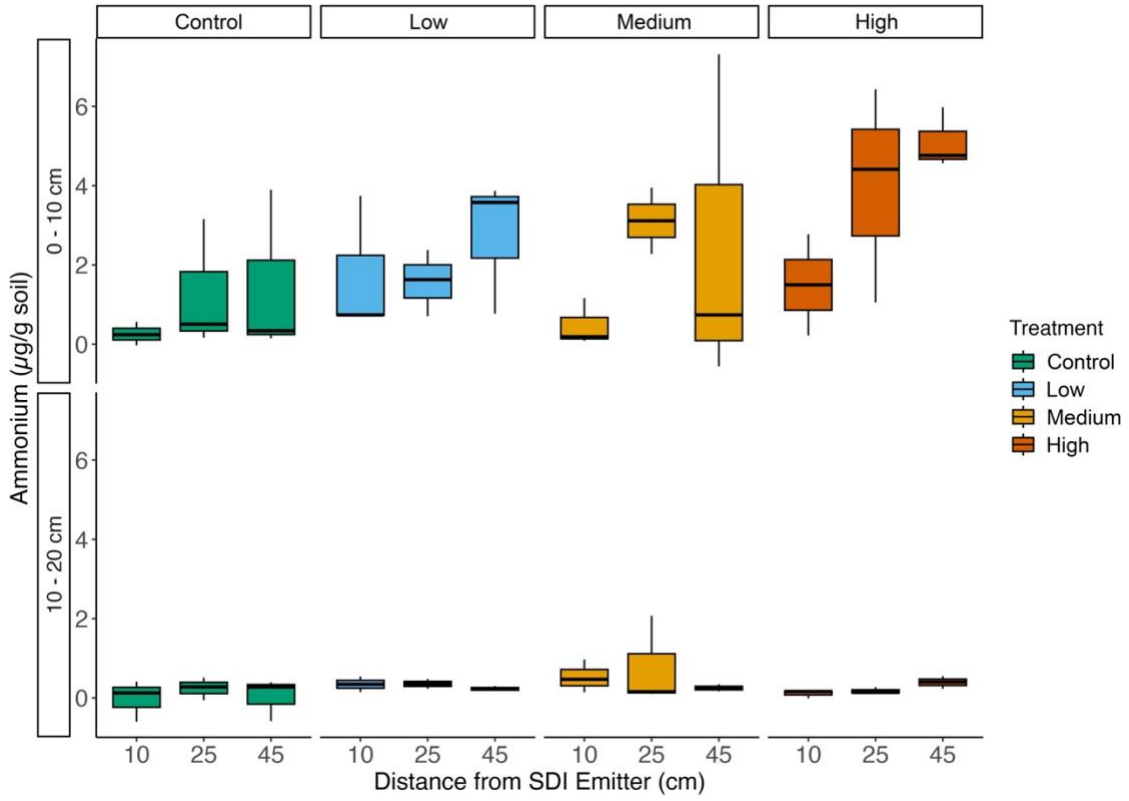

Figure 12. Ammonium-N concentration (µg/g soil) measured in the soil samples collected at three distances from the subsurface drip emitter, and separated into two depths (0-10 cm and 10-20 cm) in Year 1. No significant differences between treatments were found with 95% confidence.

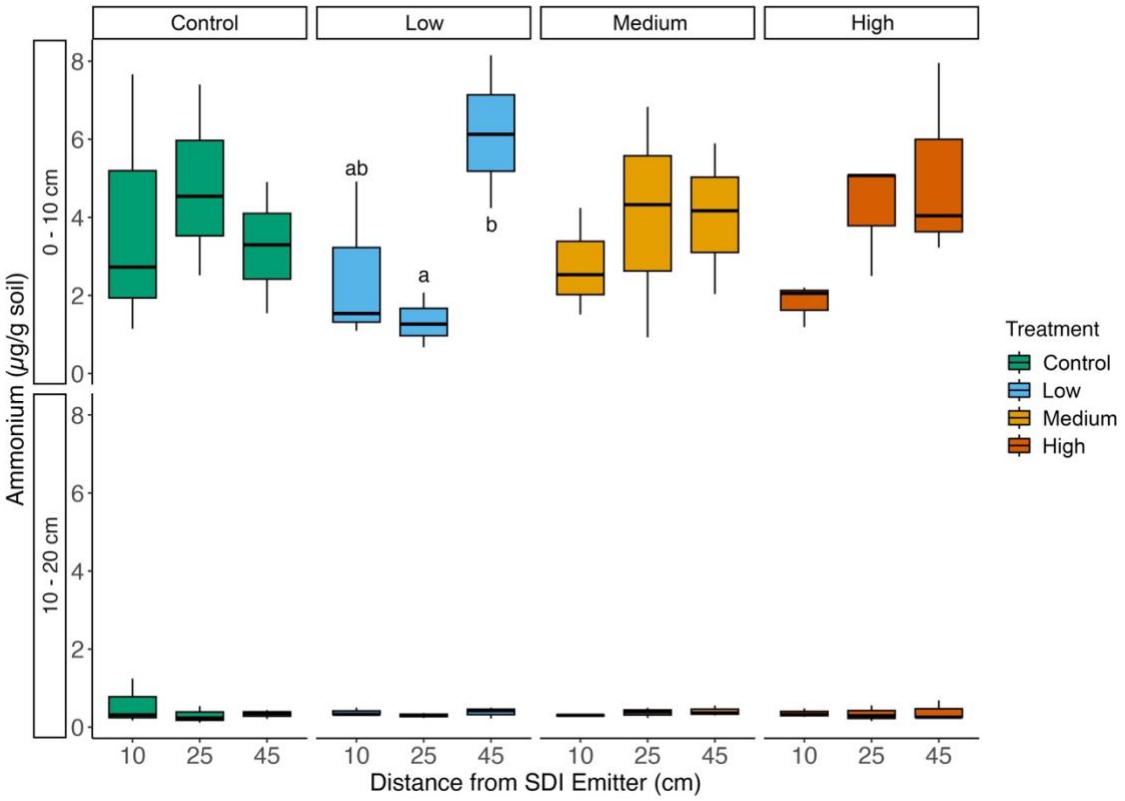

Figure 13. Ammonium-N (µg/g soil) measured at three distances from the subsurface drip emitter (10, 25, 45 cm), and separated into two depths (0-10 cm and 10-20 cm) in Year 2. Different letters in the graph denote significant differences between treatments. Significance was found with 95% confidence.

3.3.6. Potentially Mineralizable Nitrogen

The concentration of potentially mineralizable nitrogen (PMN) was generally higher (5-10 in year 1 and 20-60 µg/g in year 2) in the 0-10 cm soil depth interval and lower in the 10-20 cm depth interval, where the values hovered around 0 µg/g soil (Figure 14 and 15) in both years, but especially in the second year, where the data was up to 40 µg/g higher than the PMN concentration in the lower depth. Furthermore, within each depth, we also found significant effects of the sampling distances (Table 3.3 and 3.3). Concentrations of PMN significantly differed in the low stress treatment between the samples taken at 10 cm and 45 cm, with the 25 cm samples not significantly differing. The concentration of PMN increased by about 7 µg/g as the samples got farther away from the irrigation emitter. In the second year in the lower depth,

we see a significant difference again between 10 cm and 45 cm in the control treatment. However, in this year and depth, the PMN concentration decreased by about 5 µg/g (Figure 15) although there was no significance in the ANOVA for year 2 (Table 3.4).

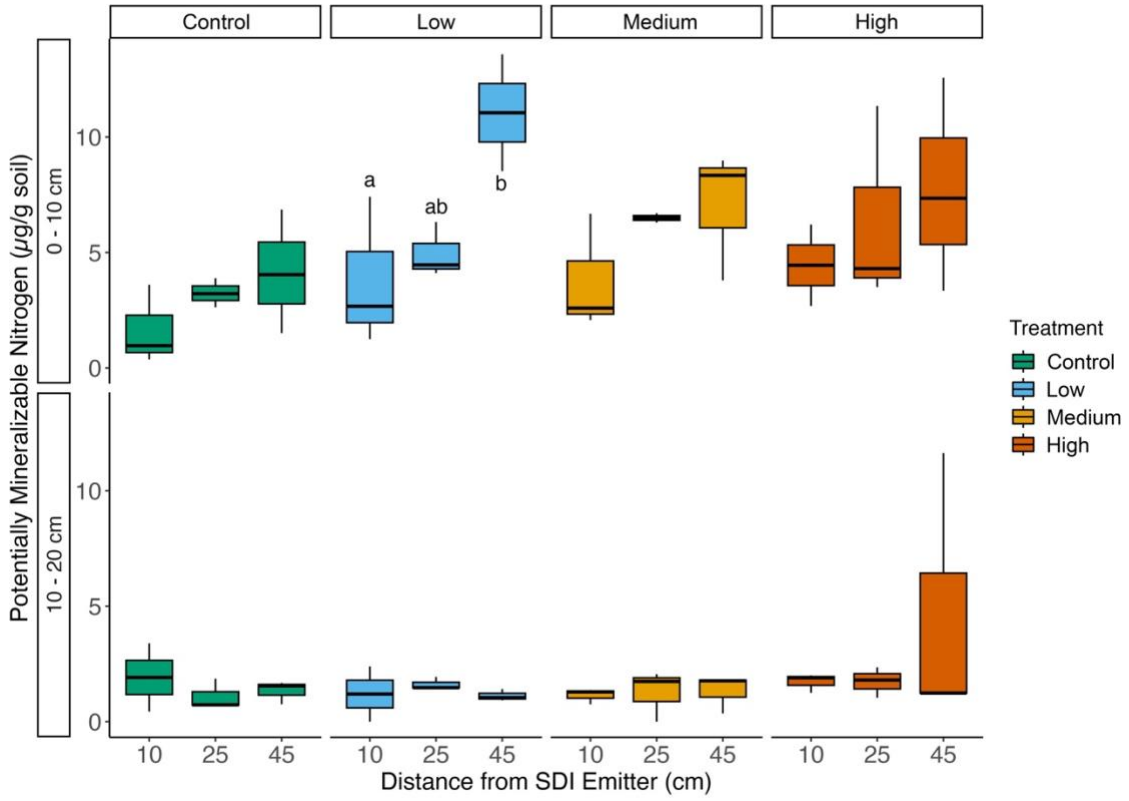

Figure 14. Potentially Mineralizable Nitrogen (PMN) concentration (µg/g soil) in soil samples collected at different distances from the subsurface drip emitter (10, 25, 45 cm) at two soil depths – 0-10 cm and 10-20 cm in Year 1. Different letters in the graph denote significant differences between treatments. Significance was found with 95% confidence.

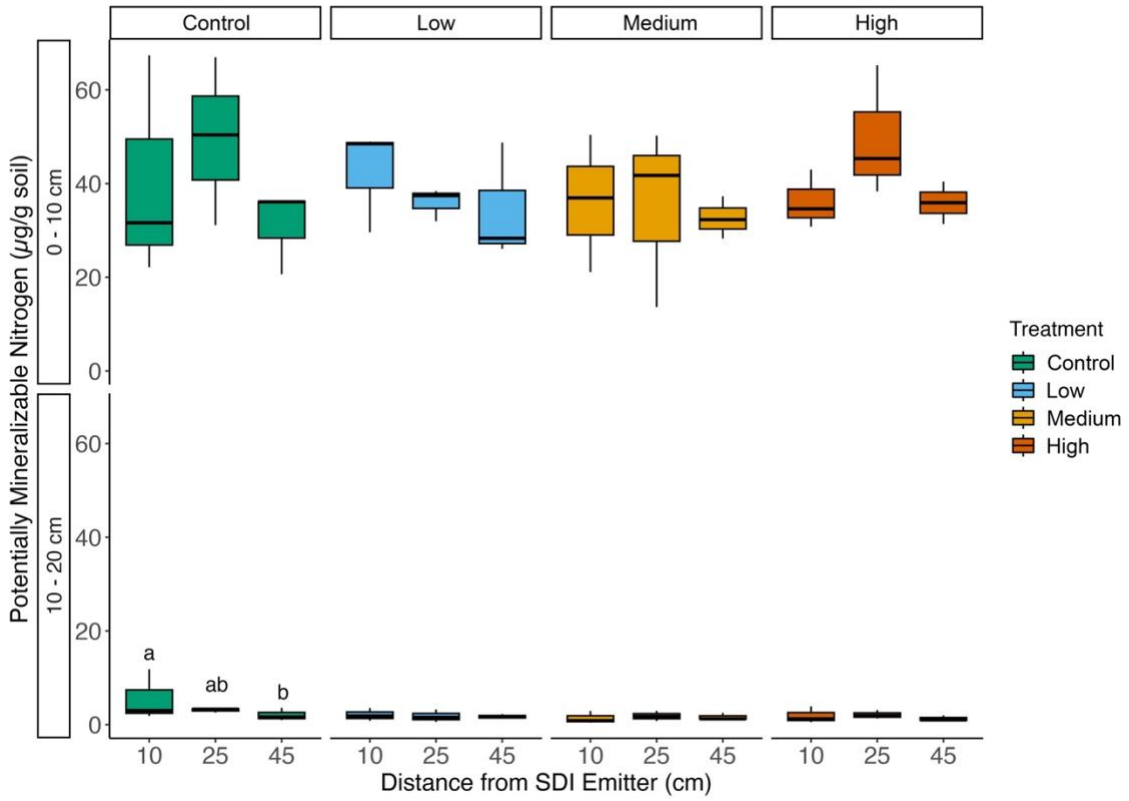

Figure 15. Potentially Mineralizable Nitrogen (PMN, µg/g soil) at different distances from the subsurface drip emitter (10, 25, 45 cm) at two soil depths – 0-10 cm and 10-20 cm in Year 1. Different letters in the graph denote significant differences between treatments. Significance was found with 95% confidence.

3.3.7. Microbial Biomass Carbon

The deficit irrigation treatments did not have a significant effect on the soil microbial biomass in both years of the study (Figures 16 and 17) (Table 3.3 and 3.3). The distance from the drip emitter also had no significant impact on the Microbial Biomass Carbon measured. There were no significant differences in the microbial biomass spatially, by depth or distance from the subsurface drip emitter in either year of the experiment.

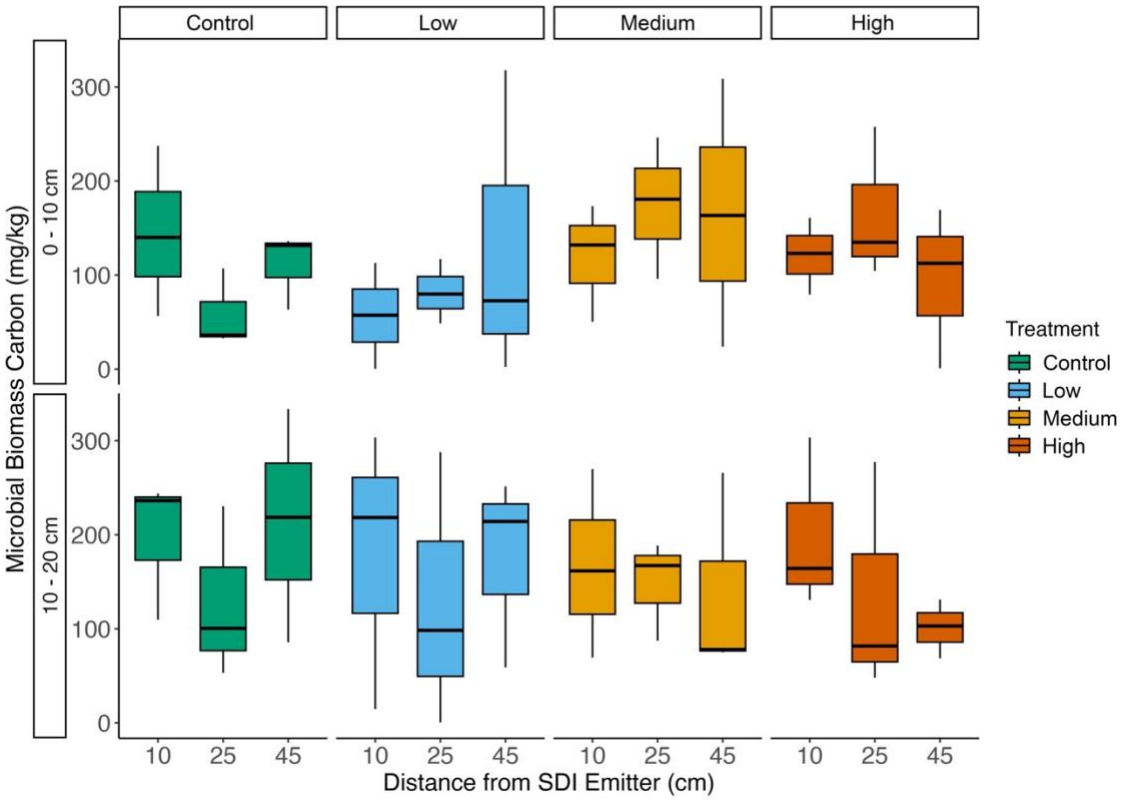

Figure 16. Microbial Biomass Carbon (MBC) at three distances away from the subsurface drip emitter (10, 25, 45 cm) at two depths (0-10 cm and 10-20 cm). No significant differences between treatments were found with 95% confidence.

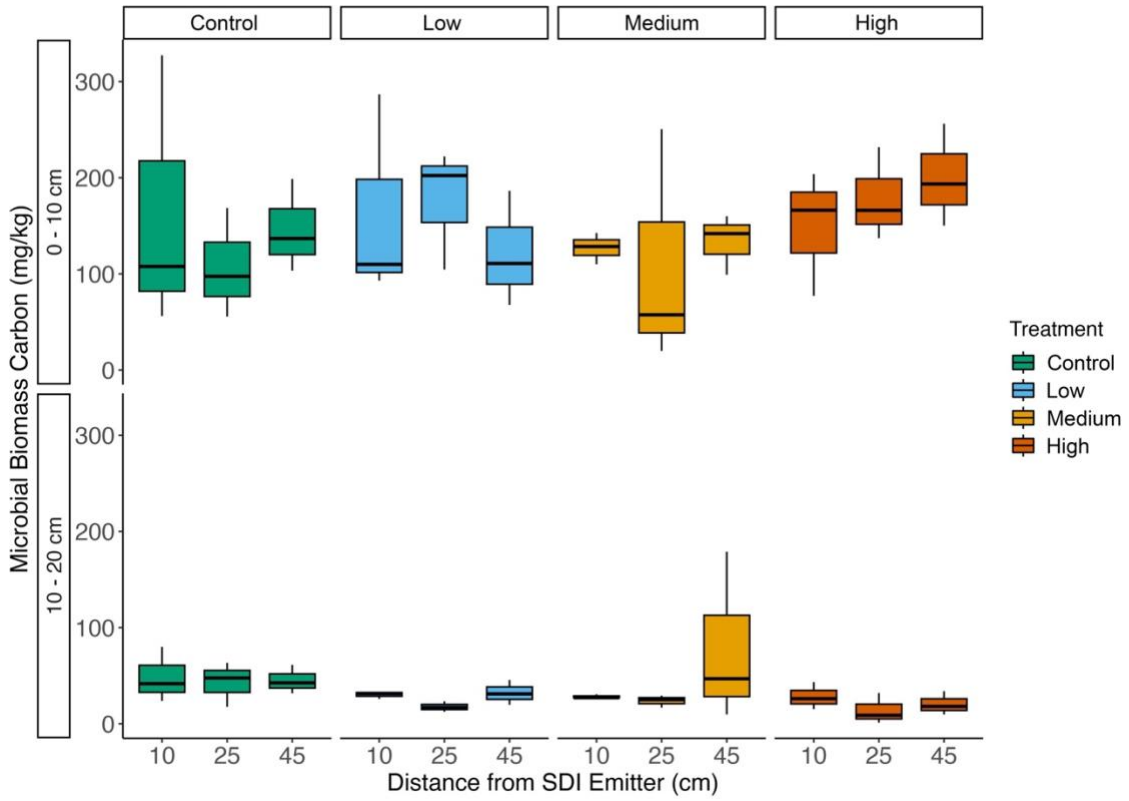

Figure 17. Microbial Biomass Carbon (MBC) at three distances away from the subsurface drip emitter distributing four different deficit irrigation treatments in Year 2. The samples were split into two depths (0-10 cm) and (10-20 cm). No significant differences between treatments were found with 95% confidence.

3.3.8. Total Microbial Biomass

Total living microbial biomass is a measure in mass of the living component of soil organic matter. There was no significant treatment effect of sample distance or deficit irrigation treatments in either year (Figures 18 and 19, Table 3.3 and 3.3).

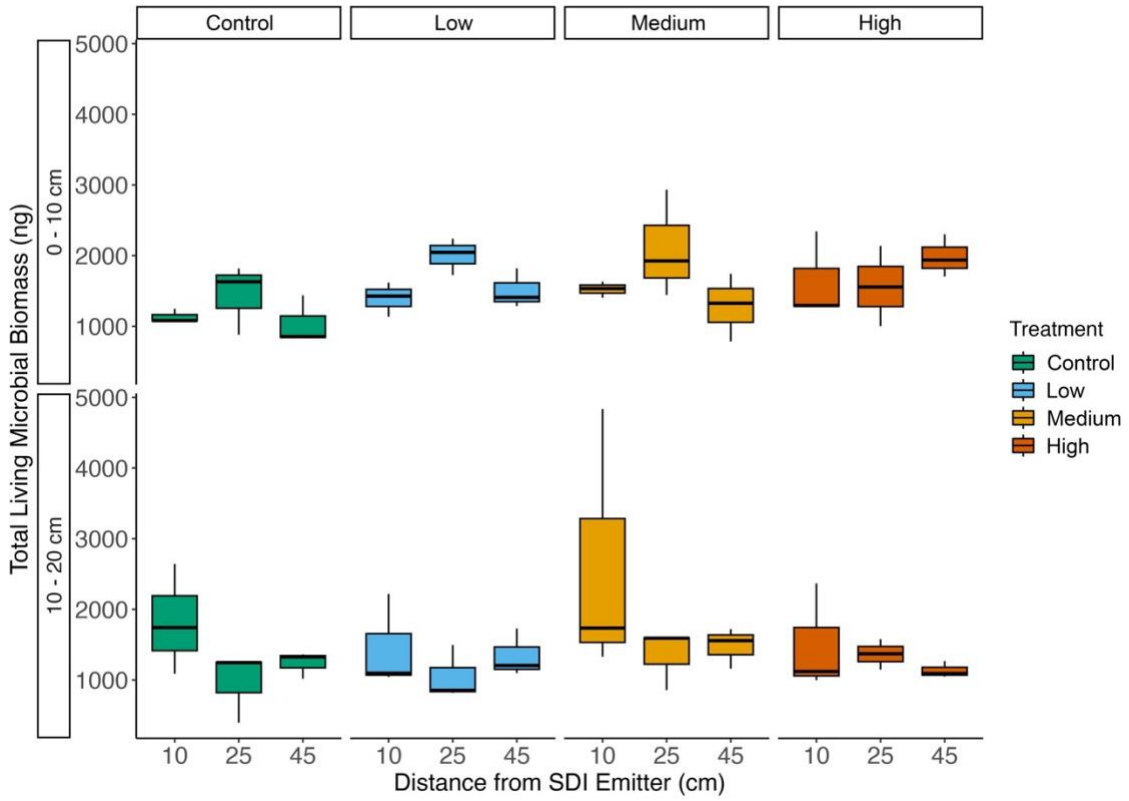

Figure 18. Total Living Microbial Biomass (ng/g soil) at three distances away from the subsurface drip emitter (10, 25, 45 cm) in year 1. No significant differences between treatments were found with 95% confidence.

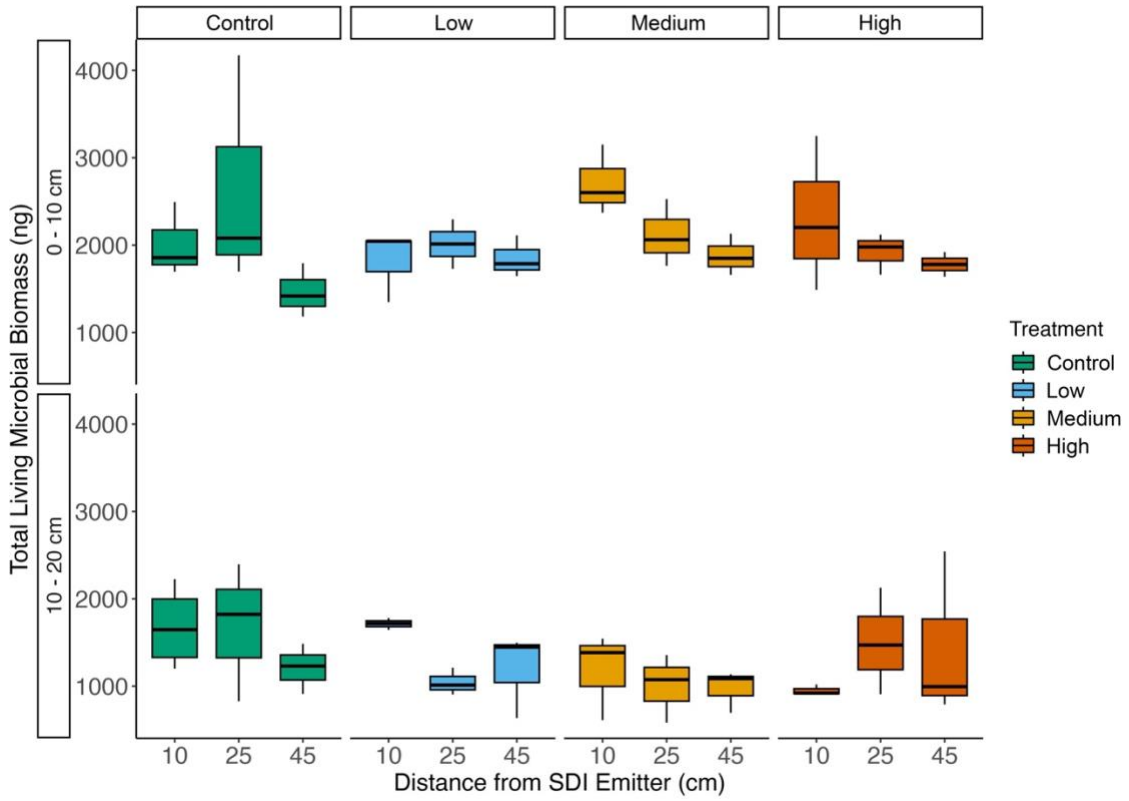

Figure 19. Total Living Microbial Biomass (ng/g soil) at three distances away from the subsurface drip emitter which was distributing four deficit irrigation treatments in year 2. No significant differences between treatments were found with 95% confidence.

3.3.9. Total Bacteria

There was a significant treatment effect of sample distance on the total bacterial biomass in year 1 (Tables 3.2). In the first year, the moderate stress treatment had a significant decrease in total bacterial biomass in the lower depth between the 10 cm soil sample and the 45 cm soil sample (Figure 20). In the second year, the moderate stress treatment experienced a decrease between the 10 cm and 45 cm soil samples in the upper depth (Figure 21).

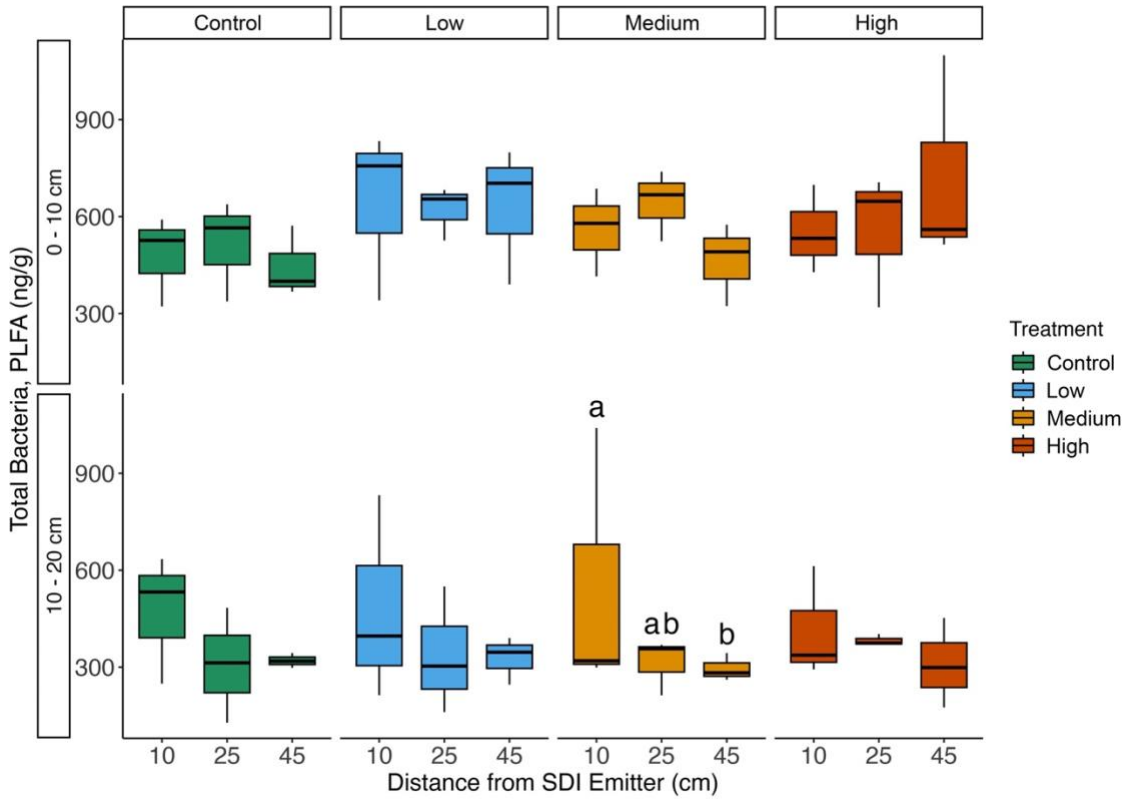

Figure 20. Total Bacterial Biomass (ng/g soil) in the soil at three distances from the drip irrigation emitter under four deficit irrigation treatments in Year 1 at two different depths (0-10 cm and 10-20 cm). Different letters in the graph denote significant differences between treatments. Significance was found with 95% confidence.

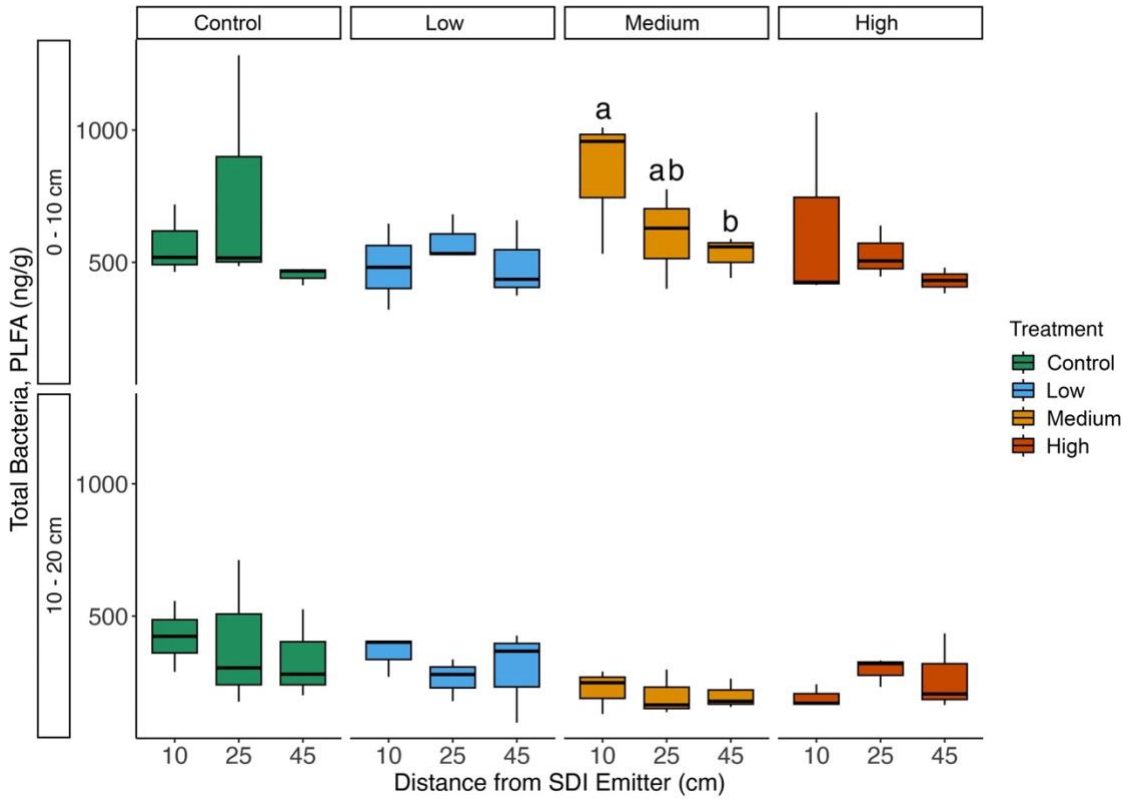

Figure 21. Total Bacterial Biomass (ng/g soil) in the soil at three distances from the drip irrigation emitter under four deficit irrigation treatments in Year 2 at two different depths (0-10 cm and 10-20 cm). Different letters in the graph denote significant differences between treatments. Significance was found with 95% confidence.

3.3.9. Gram-Positive Bacteria

Gram-Positive Bacteria were found to be significantly affected by the sampling distance in the lower depth of the first year and in the upper depth of the second year (Table 3.3 and Table 3.4). The abundance of Gram-positive bacteria decreased with increasing distance from the subsurface drip emitter in the lower depth of the moderate treatment in year 1 (Figure 22), the same trend occurred in year 2 (Figure 23), although the decrease was not statistically significant using the Tukey's range test. The concentration of gram-positive bacteria dropped about 50 ng/g within the 35 cm between the 10 cm sample and the 45 cm sample.

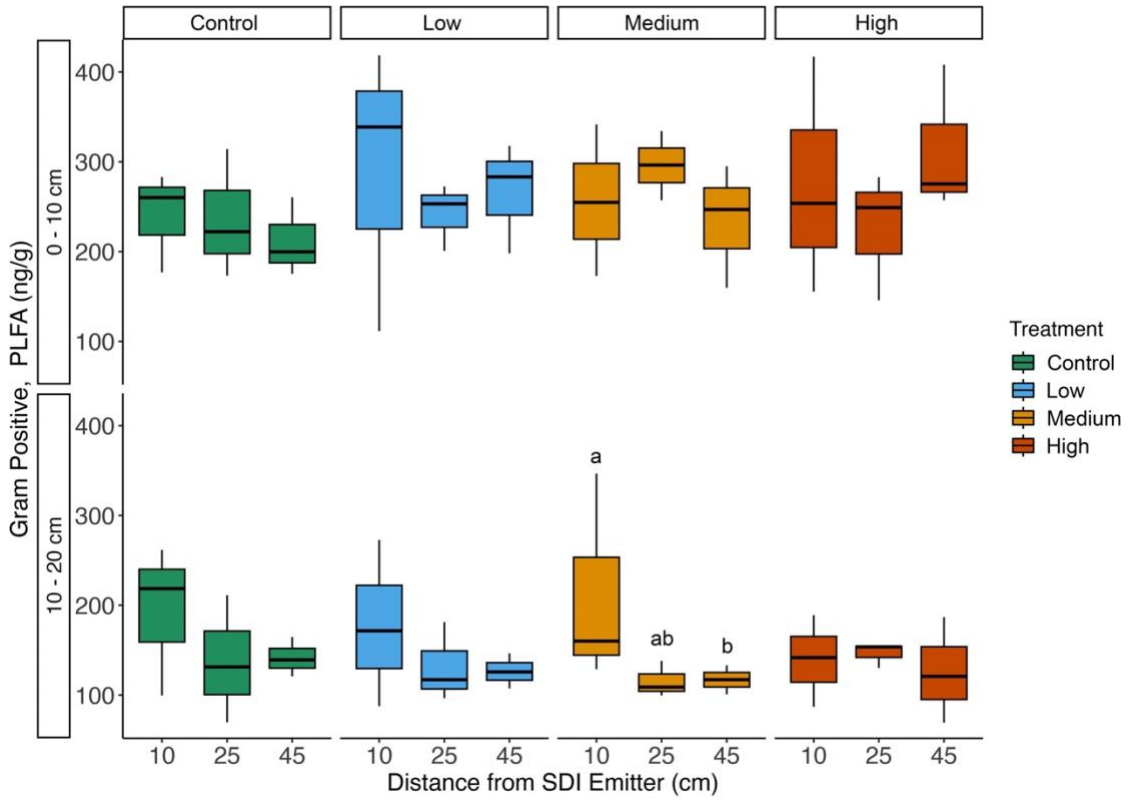

Figure 22. Gram-Positive Bacteria (ng/g) in the soil at three distances from the drip irrigation emitter under four deficit irrigation treatments in Year 1 at two different depths (0-10 cm and 10-20 cm). Different letters in the graph denote significant differences between treatments. Significance was found with 95% confidence.

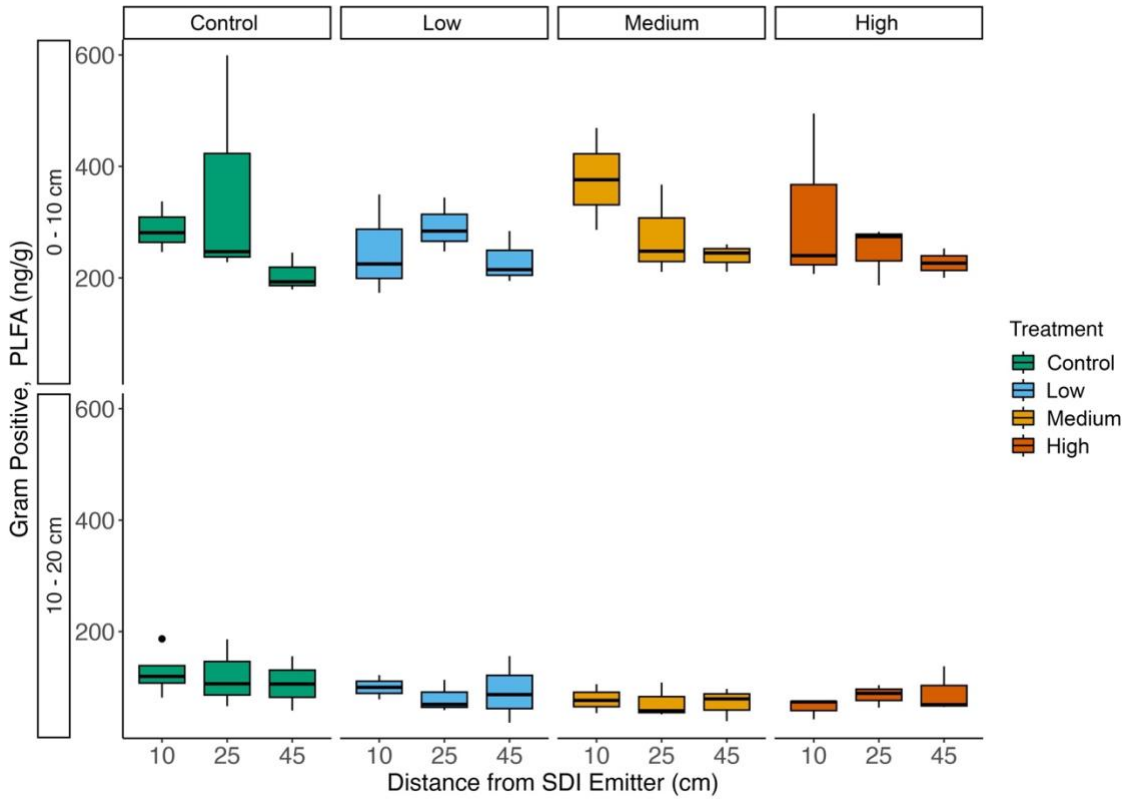

Figure 23. Gram-Positive Bacteria (ng/g) in the soil at three distances from the drip irrigation emitter under four deficit irrigation treatments in Year 2 at two different depths (0-10 cm and 10-20 cm). No significant differences between treatments were found with 95% confidence.

3.3.10. Gram-Negative Bacteria

The concentration of Gram-Negative Bacteria found at the 10 cm distance significantly differed from the concentration at the 45 cm distance in the first year of the experiment (Table 3.3), however, post-hoc analysis showed that there were only significant differences between the distances in the second year at the upper depth (Figure 25). There was a significant decrease of about 200 ng/g between the sample taken at 10 cm and the sample taken at 45 cm, while the sample taken at 25 cm did not significantly differ from either (Figure 25).

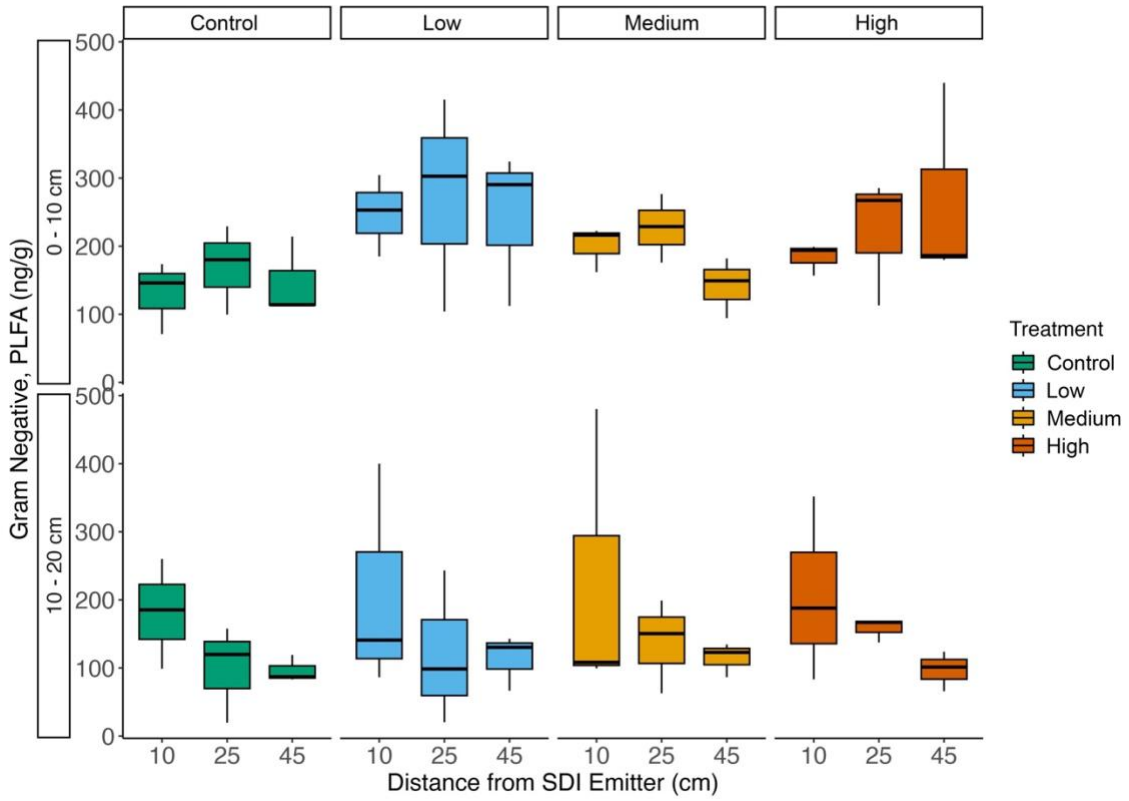

Figure 24. Abundance of Gram-Negative Bacteria in the soil at three distances from the drip irrigation emitter under four deficit irrigation treatments in Year 1 at two different depths (0-10 cm and 10-20 cm). No significant differences between treatments were found with 95% confidence.

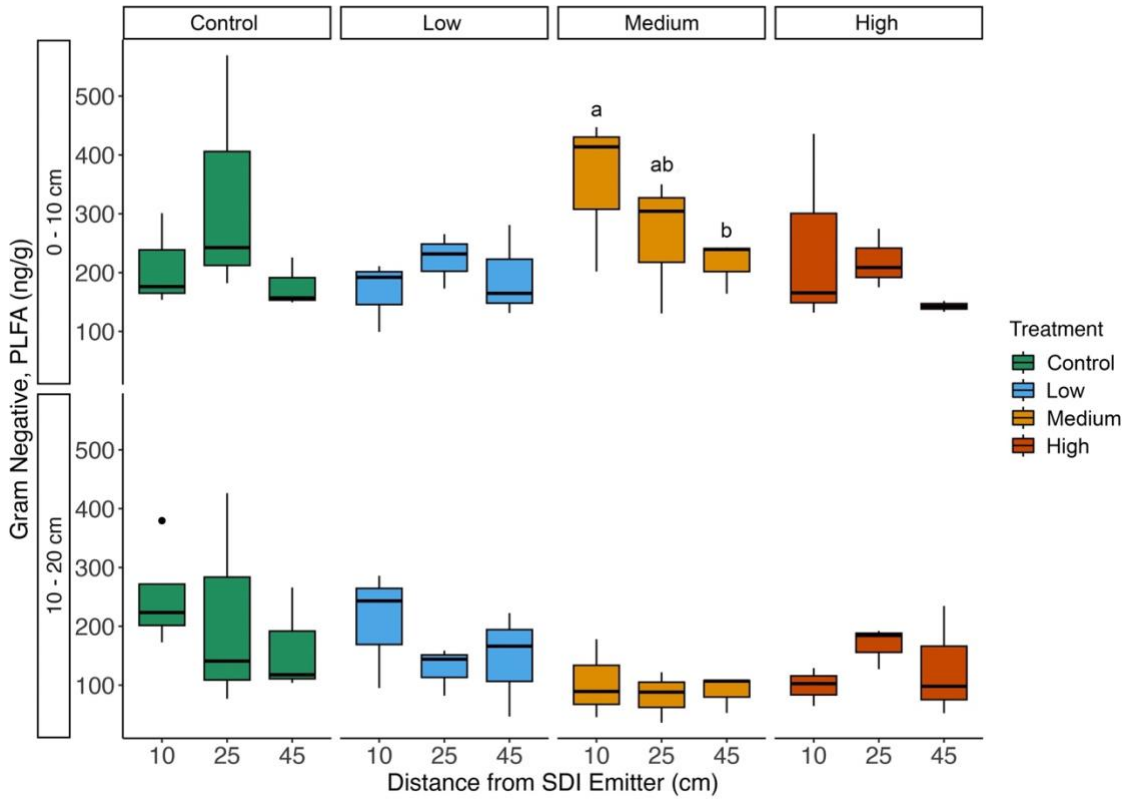

Figure 25. Abundance of Gram-Negative Bacteria in the soil at three distances from the drip irrigation emitter under four deficit irrigation treatments in Year 2 at two different depths (0-10 cm and 10-20 cm). Different letters in the graph denote significant differences between treatments. Significance was found with 95% confidence.

3.3.11. Actinomycetes

There is no evidence that distance or deficit irrigation treatment had a significant treatment effect on Actinomycetes in either year of the experiment (Figure 26 and 27, Table 3.3 and Table 3.4).

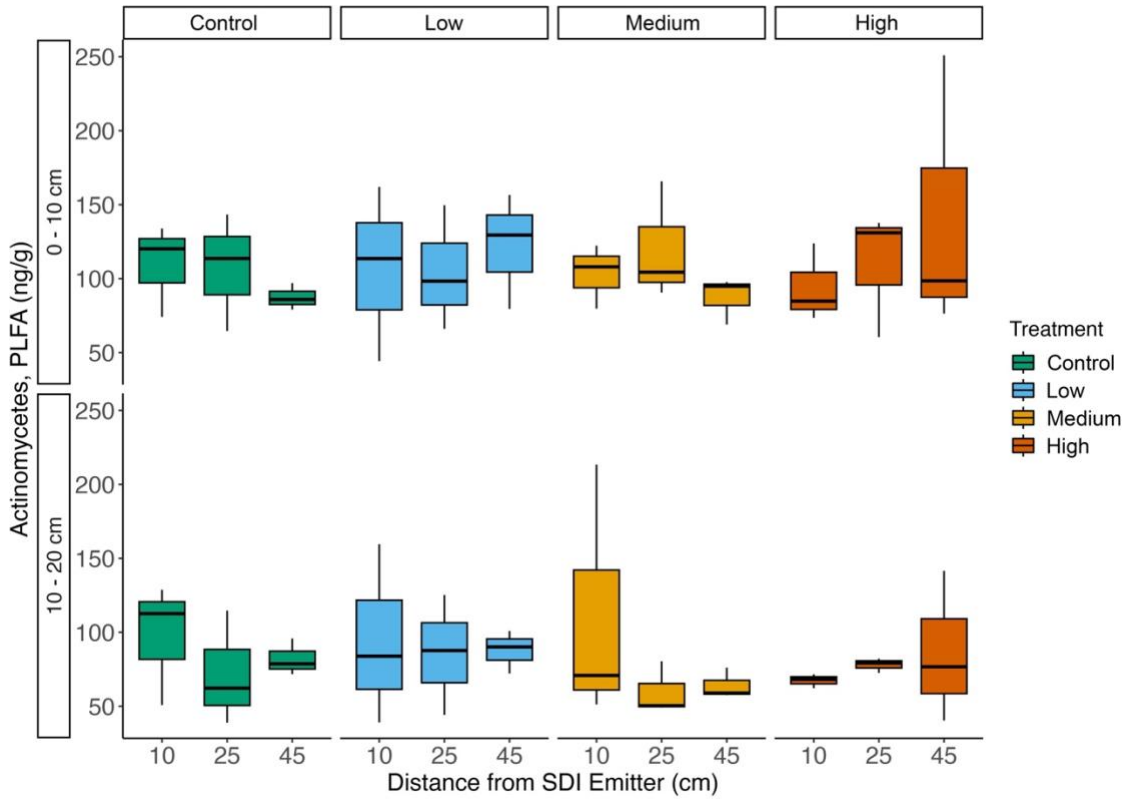

Figure 26. Abundance of Actinomycetes in the soil at three distances from the drip irrigation emitter under four deficit irrigation treatments in Year 1 at two different depths (0-10 cm and 10-20 cm). No significant differences between treatments were found with 95% confidence.

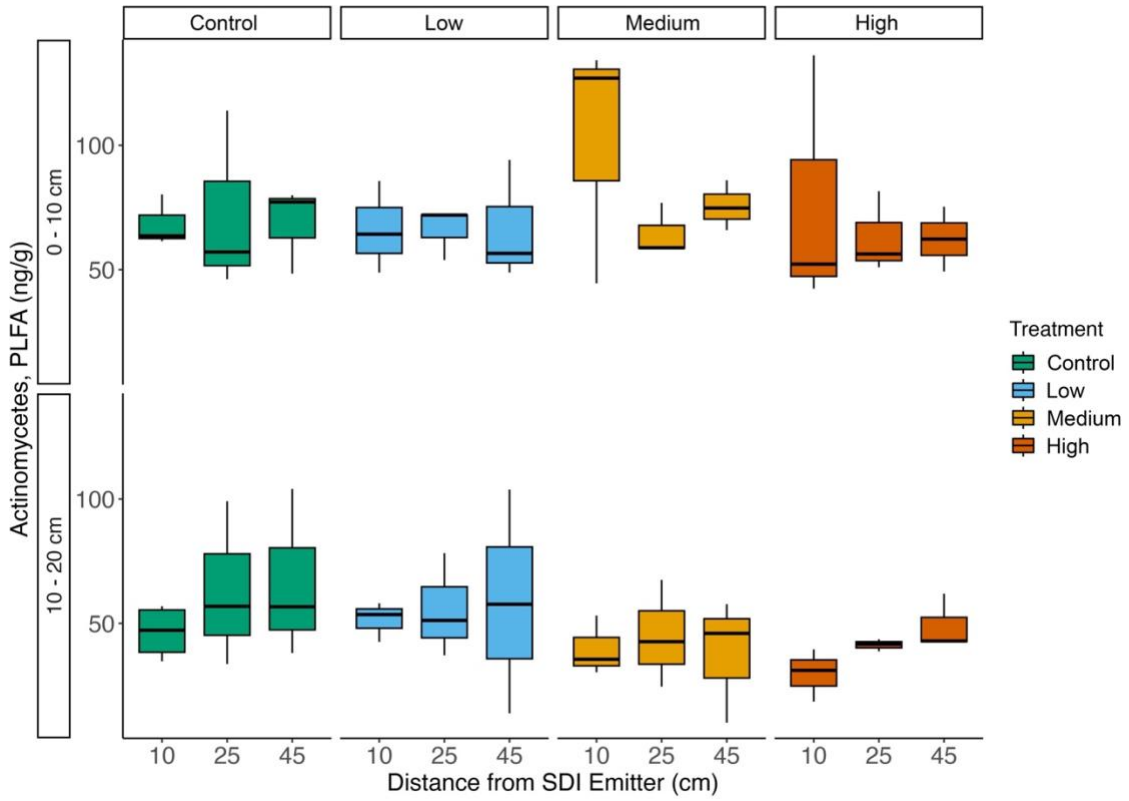

Figure 27. Abundance of actinomycetes in the soil at three distances from the drip irrigation emitter under four deficit irrigation treatments in Year 2 at two different depths (0-10 cm and 10-20 cm). No significant differences between treatments were found with 95% confidence.

3.3.12. Total Fungi

Distance and deficit irrigation did not have a significant effect on Total Fungi biomass in either year of the experiment (Figure 28 and 29).

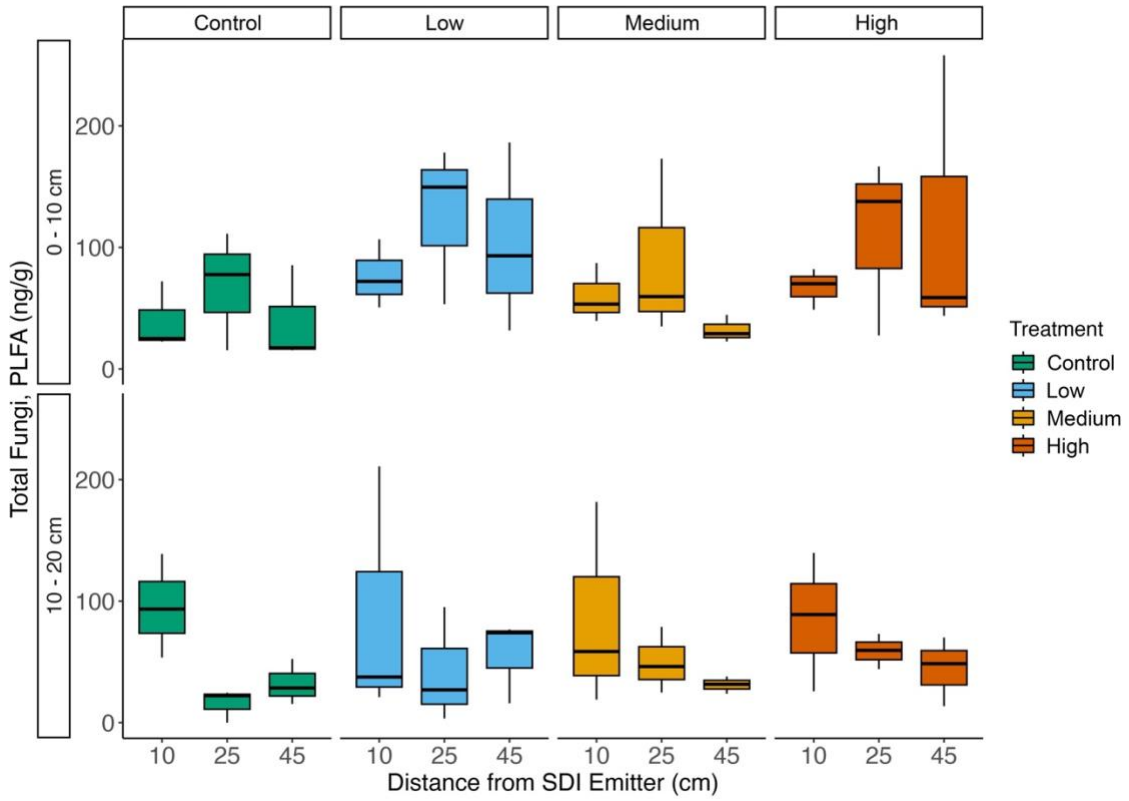

Figure 28. Fungal Biomass in the soil at three distances from the drip irrigation emitter under four deficit irrigation treatments in Year 1 at two different depths (0-10 cm and 10-20 cm). No significant differences between treatments were found with 95% confidence.

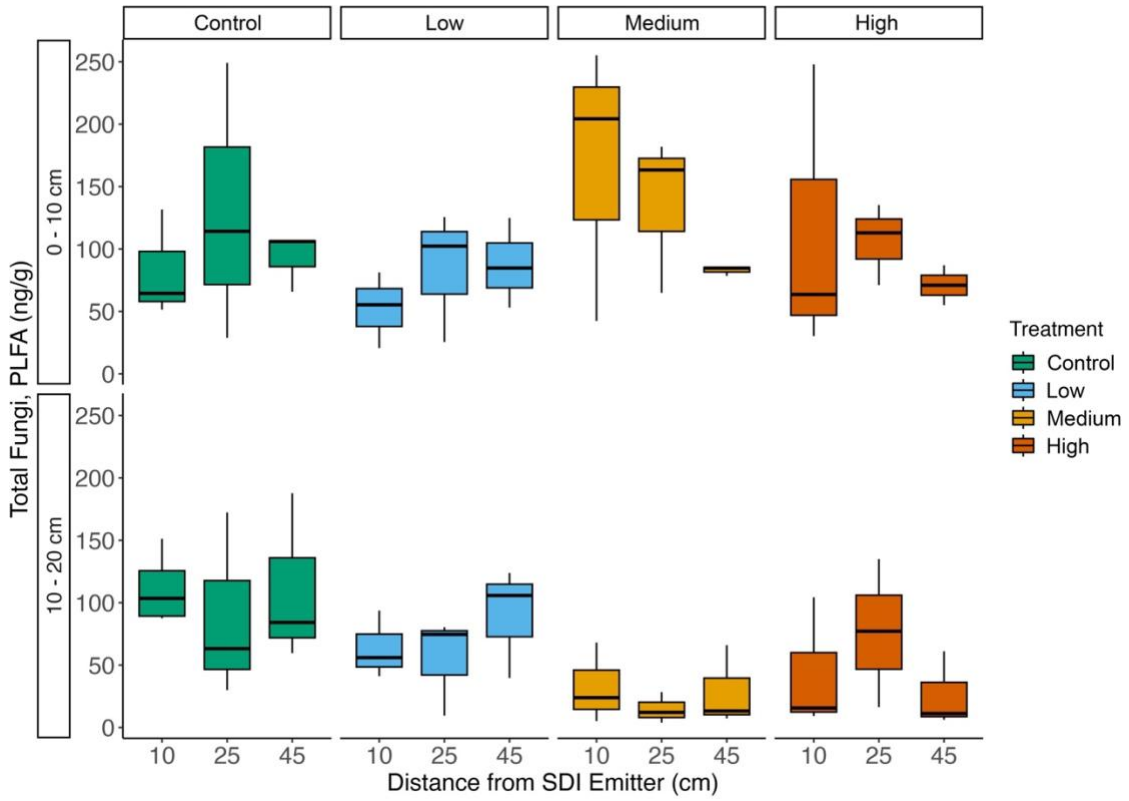

Figure 29. Fungal Biomass in the soil at three distances from the drip irrigation emitter under four deficit irrigation treatments in Year 2 at two different depths (0-10 cm and 10-20 cm). No significant differences between treatments were found with 95% confidence.

3.3.13. Arbuscular Mycorrhizal Fungi

Arbuscular Mycorrhizal Fungi were not significantly affected by the distance of the soil samples or the deficit irrigation treatments in either year of the experiment (Figure 30 and 31, Table 3.3 and Table 3.4).

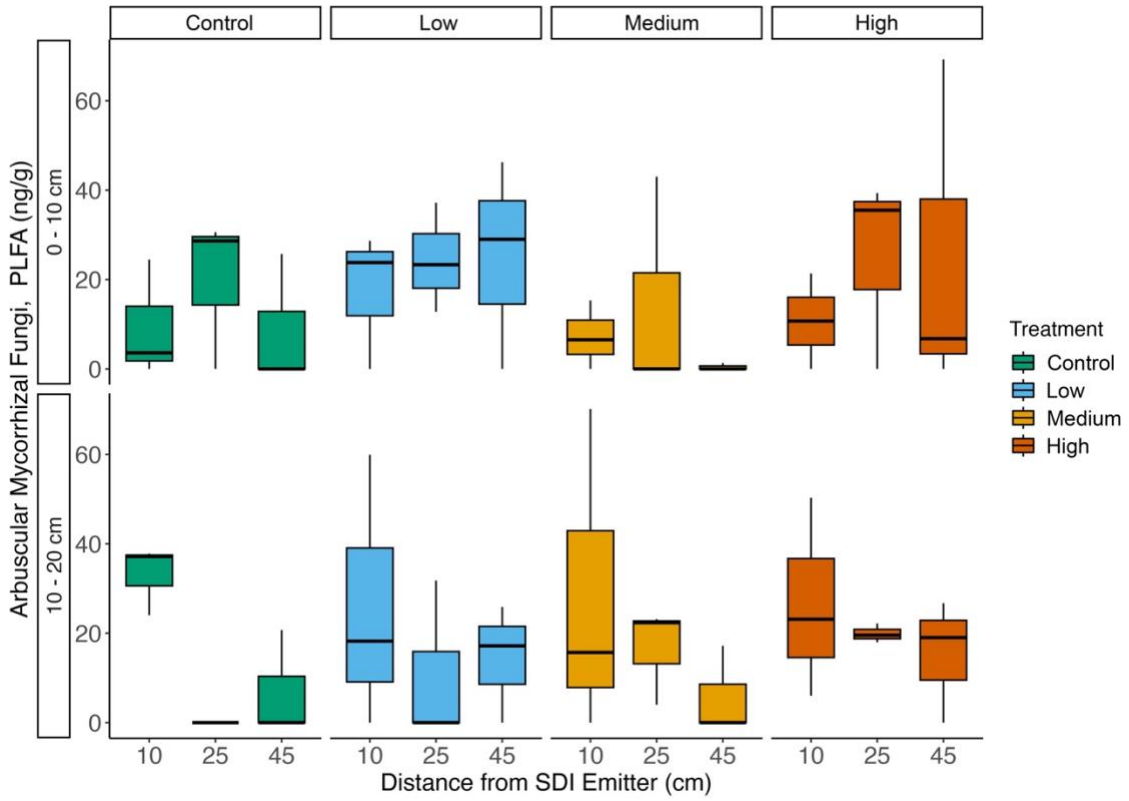

Figure 30. Arbuscular Mycorrhizal Fungi in the soil at three distances from the drip irrigation emitter under four deficit irrigation treatments in Year 1 at two different depths (0-10 cm and 10-20 cm). No significant differences between treatments were found with 95% confidence.

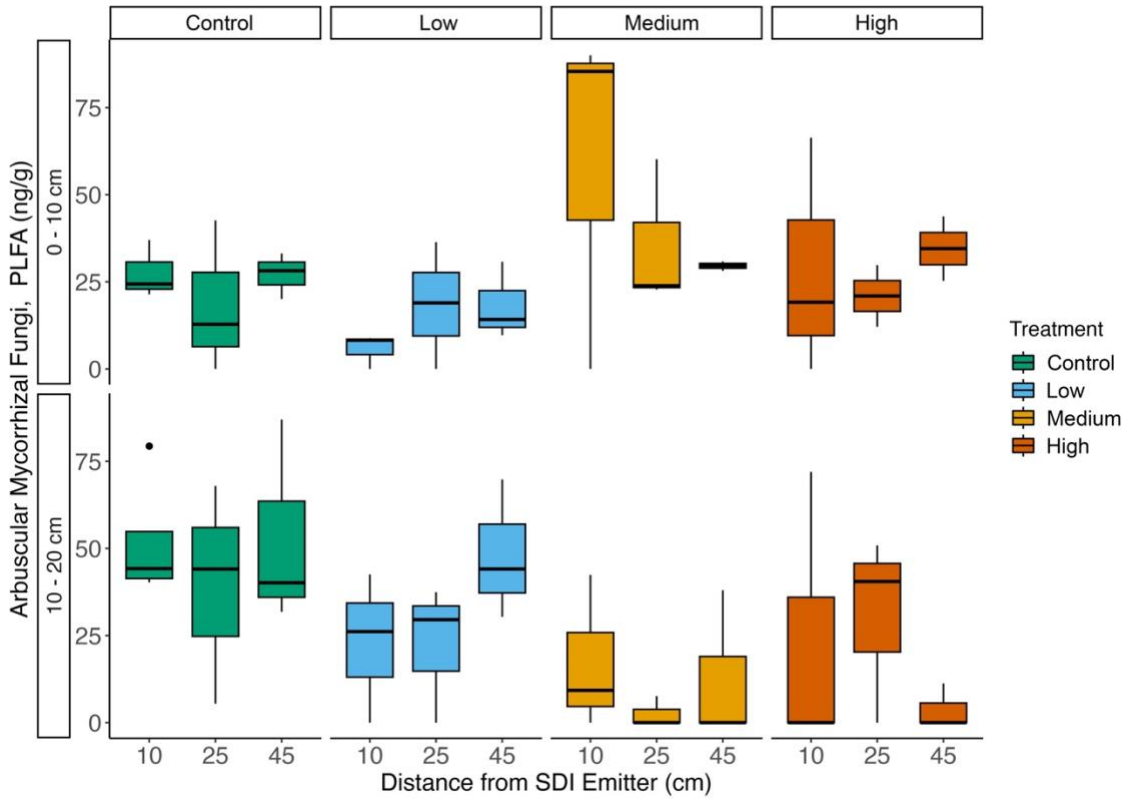

Figure 31. Arbuscular Mycorrhizal Fungi in the soil at three distances from the drip irrigation emitter under four deficit irrigation treatments in Year 2 at two different depths (0-10 cm and 10-20 cm). No significant differences between treatments were found with 95% confidence.

3.3.14. Fungi:Bacteria

The data do not suggest that the deficit irrigation treatment or the distance of the soil samples had any treatment effect on the Fungi to Bacteria ratio at either depth in both experimental years (Figure 32 and 33, Table 3.3, and Table 3.4).

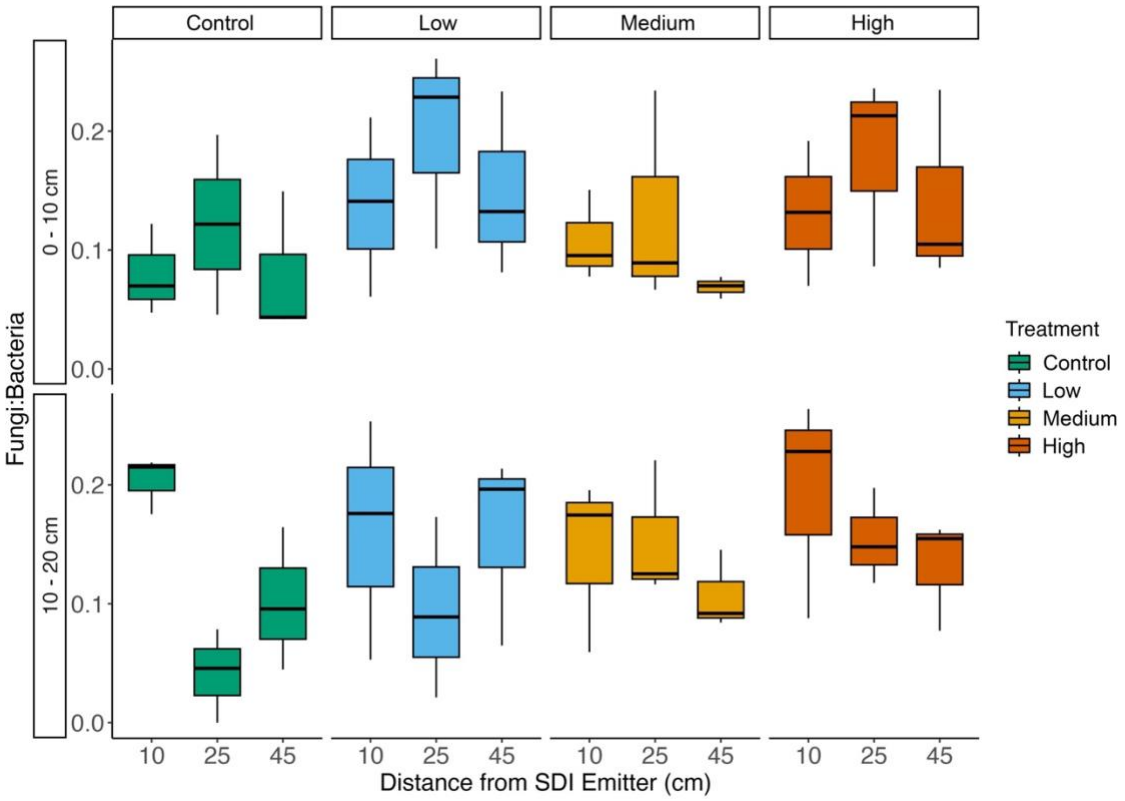

Figure 32. Fungi:Bacteria in the soil at three distances from the drip irrigation emitter under four deficit irrigation treatments in Year 1 at two different depths (0-10 cm and 10-20 cm). No significant differences between treatments were found with 95% confidence.

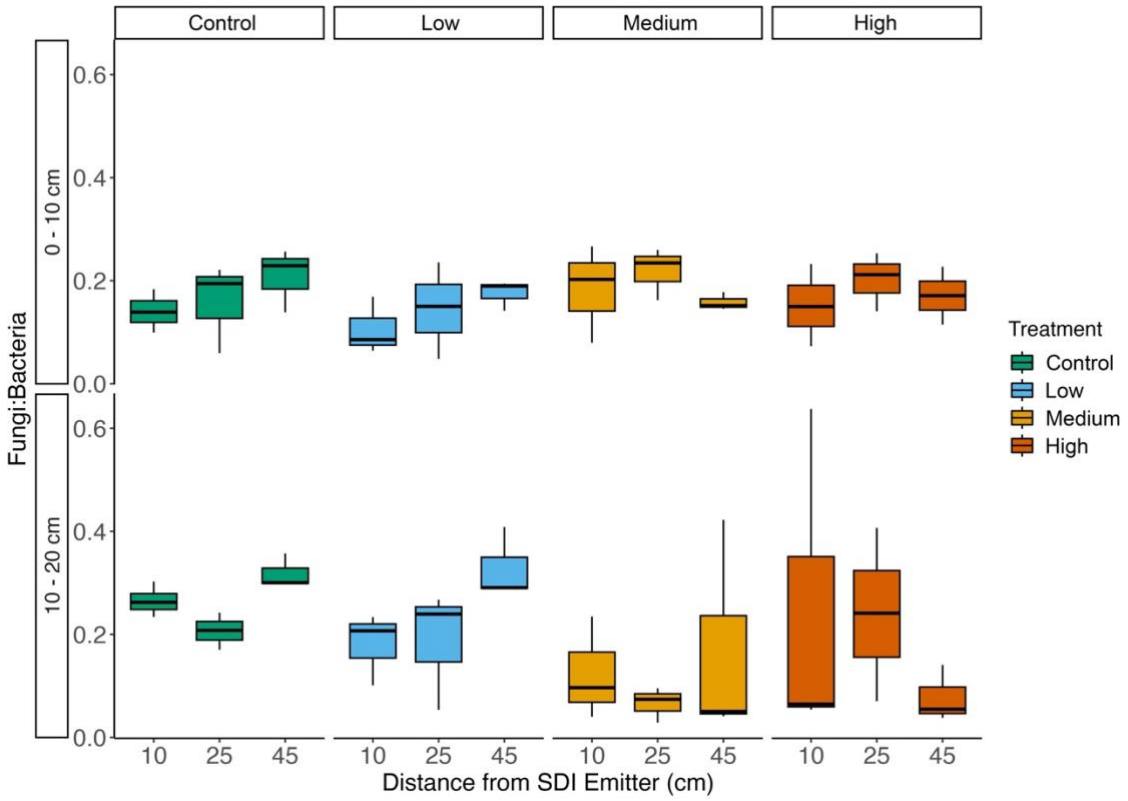

Figure 33. Fungi:Bacteria in the soil at three distances from the drip irrigation emitter under four deficit irrigation treatments in Year 2 at two different depths (0-10 cm and 10-20 cm). No significant differences between treatments were found with 95% confidence.

3.3.15. Crop Yield

There were no significant differences in yield between the four deficit irrigation treatments in either year of the experiment (Figure 34). The total water estimates applied to each treatment in 2021 are 828 cm^3/cm^2 for the control treatment, 792 cm^3/cm^2 for the low stress treatment, 766 cm^3/cm^2 for the medium stress treatment, and 748 cm^3/cm^2 for the high stress treatment. In 2022, the total water estimate for the control treatment was 799 cm^3/cm^2, 721 cm^3/cm^2 for the low stress treatment, 694 cm^3/cm^2 for the medium stress treatment, and 654 cm^3/cm^2 for the high stress treatment.

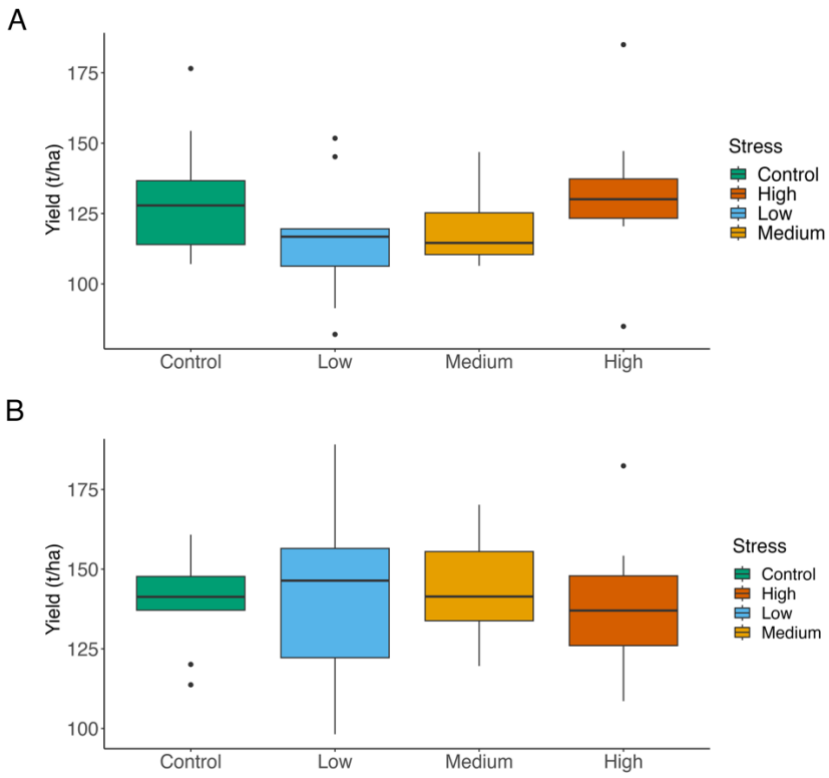

Figure 34. Processing tomato yield (tonnes/ha) extrapolated from three 2-meter transect samplings per treatment replicate in year 1 (A) and 2(B) of the study. No significant differences between treatments were found with 95% confidence.

Table 3.3 Year 1 ANOVA Table showing the F-ratio and p-values for the distance treatment, the deficit irrigation treatment, and the interactive treatment effect of both distance and irrigation

	Sampling				Treatment				Sampling x Treatment			
	0-10 cm		10-20 cm		0-10 cm		10-20 cm		0-10 cm		10-20 cm	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
GWC	*2.32*	*0.88*	*1.64*	*0.22*	*1.08*	*0.42*	*0.53*	*0.67*	*0.3*	*0.92*	*0.94*	*0.48*
EC	*0.12*	*0.88*	*0.55*	*0.58*	*0.5*	*0.68*	*0.11*	*0.94*	*0.69*	*0.65*	*1.29*	*0.31*
pH	*0.72*	*0.5*	*1.93*	*0.17*	*0.6*	*0.62*	*0.13*	*0.93*	*0.92*	*0.49*	*1.28*	*0.31*
MBC	*0.09*	*0.91*	*1.03*	*0.37*	*0.91*	*0.44*	*0.1*	*0.95*	*0.71*	*0.64*	*0.39*	*0.87*
PMN	*5.42*	*0.12*	*0.45*	*0.64*	*2.76*	*0.06*	*1.12*	*0.36*	*0.57*	*0.74*	*0.66*	*0.68*
NO_3^--N	*6.29*	*0.01*	*4.28*	*0.03*	*2.7*	*0.01*	*0.59*	*0.63*	*2.06*	*0.11*	*1.03*	*0.44*
NH_4^+-N	*4.07*	*0.04*	*0.62*	*0.56*	*1.49*	*0.29*	*1.1*	*0.39*	*0.56*	*0.74*	*0.53*	*0.77*
TLMB	*0.79*	*0.45*	*2.65*	*0.08*	*1.78*	*0.23*	*1.09*	*0.40*	*0.93*	*0.48*	*0.31*	*0.93*
TB	*0.93*	*0.40*	*4.99*	*0.01*	*0.59*	*0.64*	*0.12*	*0.94*	*0.67*	*0.66*	*0.31*	*0.93*
Gram +	*2.25*	*0.12*	*4.96*	*0.01*	*0.28*	*0.84*	*0.12*	*0.94*	*0.53*	*0.78*	*0.44*	*0.85*
Gram -	*0.15*	*0.86*	*7.12*	*0.01*	*1.65*	*0.26*	*0.15*	*0.92*	*0.55*	*0.76*	*0.29*	*0.94*
Actino	*0.38*	*0.69*	*0.33*	*0.72*	*0.19*	*0.90*	*0.11*	*0.95*	*1.41*	*0.23*	*0.45*	*0.83*
TF	*0.05*	*0.95*	*3.49*	*0.04*	*0.65*	*0.60*	*0.06*	*0.98*	*0.95*	*0.47*	*0.30*	*0.94*
AMF	*0.08*	*0.92*	*5.16*	*0.01*	*0.12*	*0.95*	*0.49*	*0.69*	*0.29*	*0.94*	*0.23*	*0.97*
F:B	*0.37*	*0.69*	*2.22*	*0.12*	*0.51*	*0.69*	*0.01*	*0.99*	*0.65*	*0.69*	*0.61*	*0.72*

GWC: gravimetric water content (g H_2O/g soil), EC: electrical conductivity (µS/cm), MBC: microbial biomass carbon (g/g soil), PMN: potentially mineralizable nitrogen (g/g soil), N: Nitrogen, TLMB: total living microbial biomass (ng/g), TB: total bacteria (ng/g), Actino: Actinomycetes (ng/g), TF: total fungi (ng/g), AMF: arbuscular mycorrhizal fungi (ng/g), F:B: fungi to bacteria ratio

Table 3.4 Year 2 ANOVA Table showing the F-ratio and p-values for the distance treatment, the deficit irrigation treatment, and the interactive treatment effect of both distance and irrigation

	Sampling				Treatment				Sampling x Treatment			
	0-10 cm		10-20 cm		0-10 cm		10-20 cm		0-10 cm		10-20 cm	
	F	p	F	p	F	p	F	p	F	p	F	p
GWC	*6.51*	*0.01*	*3.19*	*0.06*	*0.55*	*0.65*	*1.84*	*0.21*	*1.13*	*0.37*	*2.79*	*0.04*
EC	*2.6*	*0.1*	*1.21*	*0.31*	*0.39*	*0.75*	*0.67*	*0.57*	*0.41*	*0.85*	*0.69*	*0.65*
pH	*0.99*	*0.39*	*0.43*	*0.65*	*1.40*	*0.32*	*0.36*	*0.77*	*1.63*	*0.2*	*0.6*	*0.72*
MBC	*0.04*	*0.95*	*1.54*	*0.24*	*0.80*	*0.5*	*1.21*	*0.36*	*0.42*	*0.85*	*0.89*	*0.52*
PMN	*2.95*	*0.08*	*1.44*	*0.26*	*0.19*	*0.89*	*1.19*	*0.37*	*1.00*	*0.45*	*1.02*	*0.44*
NO_3^--N	*3.98*	*0.03*	*0.42*	*0.66*	*0.77*	*0.53*	*0.99*	*0.44*	*0.28*	*0.93*	*0.65*	*0.68*
NH_4^+-N	*3.45*	*0.05*	*0.43*	*0.65*	*0.08*	*0.96*	*0.06*	*0.97*	*2.16*	*0.1*	*0.53*	*0.77*
TLMB	*2.65*	*0.08*	*0.05*	*0.96*	*0.32*	*0.81*	*0.52*	*0.68*	*1.33*	*0.26*	*0.71*	*0.6*
TB	*2.90*	*0.06*	*0.09*	*0.91*	*0.75*	*0.55*	*0.17*	*0.91*	*1.14*	*0.35*	*0.68*	*0.66*
G +	*3.56*	*0.04*	*0.07*	*0.93*	*0.36*	*0.77*	*0.20*	*0.89*	*0.80*	*0.57*	*0.54*	*0.77*
G -	*2.25*	*0.12*	*0.29*	*0.75*	*0.77*	*0.54*	*0.28*	*0.83*	*1.42*	*0.22*	*0.72*	*0.63*
Actino	*1.30*	*0.28*	*1.03*	*0.36*	*0.79*	*0.49*	*0.18*	*0.90*	*0.72*	*0.62*	*0.35*	*0.90*
TF	*0.54*	*0.59*	*0.04*	*0.96*	*0.41*	*0.74*	*0.67*	*0.59*	*1.19*	*0.32*	*0.28*	*0.94*
AMF	*0.03*	*0.97*	*0.02*	*0.98*	*0.99*	*0.44*	*0.78*	*0.53*	*0.76*	*0.60*	*0.24*	*0.95*
F:B	*0.90*	*0.41*	*0.13*	*0.87*	*0.38*	*0.76*	*0.88*	*0.48*	*0.87*	*0.52*	*0.66*	*0.68*

GWC: gravimetric water content (g H_2O/g soil), EC: electrical conductivity (µS/cm), MBC: microbial biomass carbon (g/g soil), PMN: potentially mineralizable nitrogen (g/g soil), N: Nitrogen, TLMB: total living microbial biomass (ng/g), TB: total bacteria (ng/g), G+: Gram + bacteria, G-: Gram – bacteria (ng/g), Actino: Actinomycetes (ng/g), TF: total fungi (ng/g), AMF: arbuscular mycorrhizal fungi (ng/g), F:B: fungi to bacteria ratio

3.4 Discussion

In both years of the experiment, soil physicochemical and biological properties did change with distance from the subsurface drip emitter, however, marketable tomato yield was not negatively impacted by any of the deficit irrigation treatments. Deficit irrigation treatment did not significantly decrease the abundance of any microbial group or any physicochemical properties apart from nitrate nitrogen (NO_3^--N) in the first year.

Marketable yield was not affected under deficit irrigation treatments above 75% ET_c as seen in other research (Lu et al., 2019; Rodriguez-Ramos et al., 2022), which creates evidence that the staggered deficit irrigation schedule used in this experiment prevents major yield losses by irrigating fully throughout the vegetative state (Geerts and Raes, 2009). Soil physicochemical properties such as pH and electrical conductivity were also unaffected by the deficit irrigation treatments and the spatial distribution of their samples within the soil.

There is evidence that the nitrogen cycle was affected by the distance from the subsurface drip emitter and also that the nitrogen supplied via fertigation to the soil affected the ammonium-N concentrations in the soil. Microbial communities carry out nitrogen-cycling processes like mineralization and nitrification (Johnson et al., 2005; Li et al., 2018), and these processes can be limited in dry areas because water is necessary to transport these nutrients and microorganisms in the soil (Schimel, 2018). In the first year, nitrate nitrogen concentrations declined in the moderate and high stress deficit irrigation treatments, both of which reached irrigation treatments of ~37% ET_c. Microbial Biomass Carbon and Total Living Biomass were both not significantly affected by distance from the subsurface drip emitter or the deficit irrigation treatments. Additionally, ammonium-N and potentially mineralizable nitrogen (PMN) concentrations increased significantly in the low stress treatment in the first year. Unfortunately, the PLFA

method does not distinguish nitrifying bacteria specifically so it was not possible to relate abundance of nitrifiers to other soil parameters.

The abundance of Gram-Negative, Gram-Positive, and Total Bacteria declined with distance from the subsurface drip emitter. Because soil moisture can influence the abundance of microorganisms in the soil, the distance from the irrigation source can play a critical role in the activity and presence. Interestingly, though Gram-Positive bacteria are thought to be more drought tolerant than Gram-Negative bacteria (Quach et al., 2022), both groups declined significantly between the 10 cm and 45 cm soil samples. Bacterial communities are more sensitive to drought than fungal communities (Preece et al., 2019), which could indicate why we see evidence that bacterial community concentrations decreased while fungal communities were not significantly disturbed by limited water. Total bacteria concentrations also declined as a result of distance from the irrigation source in the medium but not high stress treatment. This could be due to human or sampling error including sampling location bias, or due to the deficit irrigation treatments not differing drastically in comparison to other studies testing deficit irrigation effects.

Microbial community concentrations were consistently lower in the lower (10-20 cm) than upper depths (0-10 cm) of the soil, despite their having more access to water in the lower depth. Limited organic matter at this soil depth was noted as a reason for lower microbial biomass populations in the lower depths of soil (Flynn et al., 2021). Bacterial communities in this experiment were more negatively affected by water stress than fungal communities. This is consistent with other research that found that the Fungi:Bacteria ratio increased at %50 ET_c, but stayed the same under %100 ET_c and %75 ET_c, suggesting bacterial abundance declined while fungal abundance likely remained unaffected (Rodriguez-Ramos et al., 2022).

The sites of both experiments have been managed for several years with some degree of deficit irrigation and tapering. Microbial community, structure, and size can be reduced in response to water stress (Hueso et al., 2012). Previous adaptations of microbial communities to limited nutrient cycling and deficit irrigation practices may explain why we did not observe a drop in microbial biomass in our treatments.

Deficit irrigation can be a successful management technique for water conservation, as well as maintaining agricultural production. However, there are responses in the microorganism community that could limit the capacity for soil to support long-term soil health (Natural Resources Conservation Service, 2021). Future research should be done to understand the capacity of agricultural fields in arid regions to sequester carbon under inconsistent wetting patterns.

3.5 Conclusions

Soil health and biological activity are important facets of agricultural production and environmental conservation, as intensive agriculture can strip soils of their physical structure and organic nutrients. Understanding the effects of farming management practices that consider soil health while also adapting to the climatic and demand challenges is a focus of this paper. While soil microbial abundances and plant nutrients declined with distance from the irrigation source, the declines were not significantly larger in the medium or high stress deficit irrigation treatments compared to the control. We expected that with a larger wetting bulb in treatments with higher inputs of irrigation water, one would see greater benefits for soil health indicators further from the SDI emitter. The experiment showed evidence that wetting bulb caused by subsurface drip irrigation affects bacterial community group abundance, but further research should be done to explore the impacts of persistent water stress outside of the wetting bulb on the capacity of the soil to sequester carbon.

An argument can be made that when deficit irrigation is necessary, the high stress treatment would not create any drawbacks that the control stress or low stress treatments would not, apart from nitrate nitrogen concentrations at 45 cm. Evidence shows that implementing more conservative deficit irrigation treatments does not limit agricultural productivity or soil physiological properties, but could limit biological properties, in Central Valley soils. However, irrigating more efficiently using less water is a necessary step towards adapting to the water crisis in California.

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Chapter 4

Conclusion

4.1. Conclusion

The Central Valley of California is a major agricultural region that faces increasing pressure to conserve water resources. Deficit irrigation is a water conservation practice that has increased in use due to the threat of drought, but its long-term impact on soil health has received little attention. The deficit irrigation treatments tested in this project did not differ from each other to the extent they differ in other studies. Our project found no evidence that deficit irrigation tapering strategies with a minimum of ~37% ET_c reduced yield of processing tomatoes or negatively impact soil physicochemical properties, but more research should be done to determine how spatial variability of biological properties affects the soil within the context of soil health and carbon sequestration.

In Chapter 2, I found that there were no impacts on soil physicochemical properties from the varying intensities of the deficit irrigation treatments. Though there were significant effects on the soil's pH, microbial biomass carbon, potentially mineralizable nitrogen, nitrate nitrogen, and ammonium nitrogen over the course of the season, the changes were more due to the tapering method and period of no irrigation after the midpoint. As the season progressed, deficit irrigation levels decrease and eventually stopped fully for the remainder of the season. This experiment show evidence that the responses I observe are due to the tapering and last of irrigation in the last stage of the growing season than due to the $\%ET_c$. Microbial biomass, nitrate-N, ammonium-N and potentially mineralizable nitrogen on the other hand, were limited in response to water stress, meaning that the capacity for the soil using deficit irrigation in the Central Valley to sustain nitrogen cycling and microbial activity is limited. In Chapter 3, using

spatial samples, I found little impact of deficit irrigation treatments on pH, electrical conductivity, microbial biomass carbon, and nitrate nitrogen. However, soil biology, particularly bacterial abundance, was impacted by deficit treatments, and the impacts were a function of distance from the drip line. Total bacteria, gram-positive, and gram-negative bacteria decreased with distance away from the deficit irrigation subsurface emitter. I am not certain what are the impacts of these changes on ecosystem services. Further research should investigate if carbon sequestration potential might be reduced by the lower microbial abundance, and how the persistently dry soil past the wetting zone affects the goals of achieving regenerative soils.

This thesis contextualized the effects of deficit irrigation within the content of soil physicochemical properties and microbial community distribution. The findings suggest that deficit irrigation as an agricultural practice does not necessarily reduce agricultural productivity. Further research should explore specific concentrations of nitrifiers, gene sequencing, and functions of the microbial communities in the Central Valley in order to understand how soil health and function are affected by water stress. An unexpected result was that gravimetric water content did not act as a proxy for soil moisture and did not respond to the deficit irrigation treatments in this experiment. Measuring soil bulk density and texture could provide insights into this. Additionally, testing the salinity of the irrigated water could also provide further insight into soil physicochemical properties and microbial community changes, as groundwater for irrigation can increase in salt as the water table gets lower through the season.

While there are small effects on microbial communities, somewhat limiting the capacity of soils to sequester carbon, the benefit of increasing the efficiency of water use to farmers and to the environment is more valuable considering the drought challenges California is facing. In a time where water is limited, deficit irrigation is not a management practice to shy away from.

Appendix A.1. R Code

Calling Data

```{r}
#year1
correlationy1 = read_excel("correlation graphs.xlsx", .name_repair = "universal")
depth1 = filter(correlationy1,
        Depth == "D1", Distance == "SC")

depth2 = filter(correlationy1,
        Depth == "D2", Distance == "SC")
controly1 = filter(depth1,
            Treatment == "Control")

depth1$Sampling <- as.factor(depth1$Sampling)
depth2$Sampling <- as.factor(depth2$Sampling)
depth1$Treatment <- factor(depth1$Treatment, levels=c("Control", "Low", "Medium",
"High"))
depth2$Treatment <- factor(depth2$Treatment, levels=c("Control", "Low", "Medium",
"High"))

#view(controly1)
#year 2 correlations and significance

correlationy2 = read_excel("correlations year 2.xlsx", .name_repair = "universal")

d1 = filter(correlationy2,
          Distance == "SC",Depth == "D1")
d2 = filter(correlationy2,
          Distance == "SC", Depth == "D2")

d1$Day <- as.factor(d1$Day)
d2$Day <- as.factor(d2$Day)
d1$Treatment <- factor(d1$Treatment, levels=c("Control", "Low", "Medium", "High"))
d2$Treatment <- factor(d2$Treatment, levels=c("Control", "Low", "Medium", "High"))
#view(d2)
```

Creating Individual Graphs separated by Year for pH as an example

```{r}
#linear function

ph1 <- lmer(pH ~ Sampling * Treatment + (1|Row), depth1)
p1 <- lmer(pH ~ Sampling * Treatment + (1|Sampling) + (1|Row) + (1|Block), depth1)

ph2 <- lmer(pH ~ Sampling * Treatment+ (1|Row), depth2)

#testing normality
anova(ph1)
anova(ph2)
pls205_diagnostics(ph1)
pls205_diagnostics(ph2)
hist(residuals(ph1))
hist(residuals(ph2))

#shapiro-wilk
shapiro.test(depth1$pH)

#results: p = 0.7179 (greater than 0.05, normality is not rejected - normal)

shapiro.test(depth2$pH)

#results: p = 0.0946 (greater than 0.05, normal!)

#transformations
#no transformations needed

#anova
anova(ph1)
anova(p1) #testing iff block makes a difference
anova(ph2)
```

Creating Individual Graphs

```{r}
#post-hoc
ph1cy1 <- ggboxplot(depth1, x = "Sampling", y = "pH",
         color = "Treatment", palette = c("Control" = "#009E73",
                                          "Low" = "#56B4E9",
                                          "Medium" = "#E69F00",
                                          "High" = "#D55E00"),
         ylab = "pH", xlab = "Day")+
    ylim(7.4, 8.6)+
    labs(color = NULL)+
  theme(axis.text.x=element_text(size=15),
        axis.text.y=element_text(size=15),
        axis.title.y = element_text(size=15),
        axis.title.x=element_text(size=15),
        legend.title = element_text(size=13),
        legend.text = element_text(size=13))

ph1cy1
#ggsave("ph1cy1.jpg", width = 10, height = 7, units = c("in"), dpi = 600)

ph2cy1 <- ggboxplot(depth2, x = "Sampling", y = "pH",
         color = "Treatment", palette = c("Control" = "#009E73",
                                          "Low" = "#56B4E9",
                                          "Medium" = "#E69F00",
                                          "High" = "#D55E00"),
         ylab = "pH", xlab = "Day")+
    ylim(7.4, 8.6)+
    labs(color = NULL)+
  theme(axis.text.x=element_text(size=15),
        axis.text.y=element_text(size=15),
        axis.title.y = element_text(size=15),
        axis.title.x=element_text(size=15),
        legend.title = element_text(size=13),
        legend.text = element_text(size=13))

ph2cy1
#ggsave("ph2cy1.jpg", width = 10, height = 7, units = c("in"), dpi = 600)


ph1by1 <- ggboxplot(depth1, x = "Treatment", y = "pH",
         color = "Sampling", palette = c("75" = "darkblue",
                                          "90" = "#56B4E9",
                                          "103" = "lightblue",
                                          "117" = "grey"),
         ylab = "pH", xlab = " ")+
```

```
   ylim(7.4, 8.6)+
   labs(color = "Day")+
  theme(axis.text.x=element_text(size=15),
        axis.text.y=element_text(size=15),
        axis.title.y = element_text(size=15),
        axis.title.x=element_text(size=15),
        legend.title = element_text(size=13),
        legend.text = element_text(size=13))+
  scale_x_discrete(limits = c("Control","Low","Medium", "High"))

ph1by1
#ggsave("ph1by1.jpg", width = 10, height = 7, units = c("in"), dpi = 600)

ph2by1 <- ggboxplot(depth2, x = "Treatment", y = "pH",
          color = "Sampling", palette = c("75" = "darkblue",
                                          "90" = "#56B4E9",
                                          "103" = "lightblue",
                                          "117" = "grey"),
          ylab = "pH", xlab = " ")+
   ylim(7.4, 8.6)+
   labs(color = "Day")+
  theme(axis.text.x=element_text(size=15),
        axis.text.y=element_text(size=15),
        axis.title.y = element_text(size=15),
        axis.title.x=element_text(size=15),
        legend.title = element_text(size=13),
        legend.text = element_text(size=13))+
  scale_x_discrete(limits = c("Control","Low","Medium", "High"))

ph2by1
#ggsave("ph2by1.jpg", width = 10, height = 7, units = c("in"), dpi = 600)

#no post hoc needed because there is no significance

#tukey
#calling ph data to make graph
phstat <- (subset(correlationy1, select = c("Sampling", "Treatment", "Row", "Distance",
"Depth", "pH")))

#separating by sampling because there used to be a treatment effect
phstat_d175 <- filter(phstat, Depth == "D2", Distance == "SC", Sampling == "75")

phanova_d175 <- aov(pH ~ Treatment, data = phstat_d175)

phtukd175 <- TukeyHSD(phanova_d175)
print(phtukd175)
```

```
phstat <- (subset(correlationy1, select = c("Sampling", "Treatment", "Row", "Distance", "Depth", "pH")))

#filtering out the lower depth of the single core samples
phstat_d195 <- filter(phstat, Depth == "D2", Distance == "SC", Treatment == "Control")

phanova_d195 <- aov(pH ~ Treatment, data = phstat_d195)

phtukd195 <- TukeyHSD(phanova_d195)
print(phtukd195)

#filtering out the lower depth of the single core samples
phstat_d1103 <- filter(phstat, Depth == "D2", Distance == "SC", Sampling == "103")

phanova_d1103 <- aov(pH ~ Treatment, data = phstat_d1103)

phtukd1103 <- TukeyHSD(phanova_d1103)
print(phtukd1103)

phstat <- (subset(correlationy1, select = c("Sampling", "Treatment", "Row", "Distance", "Depth", "pH")))

#filtering out the lower depth of the single core samples
phstat_d1117 <- filter(phstat, Depth == "D2", Distance == "SC", Sampling == "117")

phanova_d1117 <- aov(pH ~ Treatment, data = phstat_d1117)

phtukd1117 <- TukeyHSD(phanova_d1117)
print(phtukd1117)
```

Code for Pairwise Comparisons

```{r}
#Depth 1
#simpler linear model without Sampling as random effect
mod3 <- lmer(pH ~ Sampling * Treatment + (1|Row), depth1)
summary(mod3)

#useful plots to make to know what the data looks like
plot(mod3)
plot(allEffects(mod3))

#emmeans
modem3 <- emmeans(mod3, c("Treatment", "Sampling"))
modem3
```

```
#sampling 1
contrast(modem3, list(conVsLeast_75 = c(1,-1,0,0,0,0,0,0,0,0,0,0,0,0,0,0)))
contrast(modem3, list(conVSmod_75 = c(1,0,-1,0,0,0,0,0,0,0,0,0,0,0,0,0)))
contrast(modem3, list(conVSmost_75 = c(1,0,0,-1,0,0,0,0,0,0,0,0,0,0,0,0)))
contrast(modem3, list(leastVSmost_75 = c(0,1,0,-1,0,0,0,0,0,0,0,0,0,0,0,0)))
contrast(modem3, list(modVSmost_75 = c(0,0,1,-1,0,0,0,0,0,0,0,0,0,0,0,0)))
contrast(modem3, list(leastVSmod_75 = c(0,1,-1,0,0,0,0,0,0,0,0,0,0,0,0,0)))

#sampling 2
contrast(modem3, list(conVsleast_90 = c(0,0,0,0,1,-1,0,0,0,0,0,0,0,0,0,0)))
contrast(modem3, list(conVsmod_90 = c(0,0,0,0,1,0,-1,0,0,0,0,0,0,0,0,0))) #significantly
different
contrast(modem3, list(conVsmost_90 = c(0,0,0,0,1,0,0,-1,0,0,0,0,0,0,0,0)))
contrast(modem3, list(leastVSmost_90 = c(0,0,0,0,0,1,0,-1,0,0,0,0,0,0,0,0)))
contrast(modem3, list(modVSmost_90 = c(0,0,0,0,0,0,1,-1,0,0,0,0,0,0,0,0)))
contrast(modem3, list(leastVSmod_90 = c(0,0,0,0,0,1,-1,0,0,0,0,0,0,0,0,0)))

#sampling 4
contrast(modem3, list(conVsleast_103 = c(0,0,0,0,0,0,0,0,0,0,0,0,1,-1,0,0)))
contrast(modem3, list(conVsmod_103 = c(0,0,0,0,0,0,0,0,0,0,0,0,1,0,-1,0)))
contrast(modem3, list(conVsmost_103 = c(0,0,0,0,0,0,0,0,0,0,0,0,1,0,0,-1)))
contrast(modem3, list(leastVSmost_103 = c(0,0,0,0,0,0,0,0,0,0,0,0,0,1,0,-1)))
contrast(modem3, list(modVSmost_103 = c(0,0,0,0,0,0,0,0,0,0,0,0,0,0,1,-1)))
contrast(modem3, list(leastVSmod_103 = c(0,0,0,0,0,0,0,0,0,0,0,0,0,1,-1,0)))

#sampling 3
contrast(modem3, list(conVsleast_103 = c(0,0,0,0,0,0,0,0,1,-1,0,0,0,0,0,0)))
contrast(modem3, list(conVsmod_103 = c(0,0,0,0,0,0,0,0,1,0,-1,0,0,0,0,0)))
contrast(modem3, list(conVsmost_103 = c(0,0,0,0,0,0,0,0,1,0,0,-1,0,0,0,0)))
contrast(modem3, list(leastVSmost_103 = c(0,0,0,0,0,0,0,0,0,1,0,-1,0,0,0,0)))
contrast(modem3, list(modVSmost_103 = c(0,0,0,0,0,0,0,0,0,0,1,-1,0,0,0,0)))
contrast(modem3, list(leastVSmod_103 = c(0,0,0,0,0,0,0,0,0,0,1,-1,0,0,0,0)))

#testing the time effect
contrast(modem3, list(con75VScon90 = c(1,0,0,0,-1,0,0,0,0,0,0,0,0,0,0,0)))
contrast(modem3, list(con90VScon103 = c(0,0,0,0,1,0,0,0,-1,0,0,0,0,0,0,0)))
contrast(modem3, list(con103VScon117 = c(0,0,0,0,0,0,0,0,1,0,0,0,-1,0,0,0)))
contrast(modem3, list(con117VScon90 = c(0,0,0,0,1,0,0,0,0,0,0,0,-1,0,0,0)))
contrast(modem3, list(con75VScon103 = c(1,0,0,0,0,0,0,0,-1,0,0,0,0,0,0,0)))
contrast(modem3, list(con75VScon117 = c(1,0,0,0,0,0,0,0,0,0,0,0,-1,0,0,0)))

contrast(modem3, list(least75VSleast90 = c(0,1,0,0,0,-1,0,0,0,0,0,0,0,0,0,0)))
contrast(modem3, list(least90VSleast103 = c(0,0,0,0,0,1,0,0,0,-1,0,0,0,0,0,0)))
contrast(modem3, list(least103VSleast117 = c(0,0,0,0,0,0,0,0,0,1,0,0,0,-1,0,0)))
contrast(modem3, list(least117VSleast90 = c(0,0,0,0,0,1,0,0,0,0,0,0,0,-1,0,0)))
contrast(modem3, list(least75VSleast103 = c(0,1,0,0,0,0,0,0,0,-1,0,0,0,0,0,0)))
```

```
contrast(modem3, list(least75VSleast117 = c(0,1,0,0,0,0,0,0,0,0,0,0,0,-1,0,0)))

contrast(modem3, list(least75VSleast90 = c(0,0,1,0,0,0,-1,0,0,0,0,0,0,0,0,0)))
contrast(modem3, list(least90VSleast103 = c(0,0,0,0,0,0,1,0,0,0,-1,0,0,0,0,0)))
contrast(modem3, list(least103VSleast117 = c(0,0,0,0,0,0,0,0,0,0,1,0,0,0,-1,0)))
contrast(modem3, list(least117VSleast90 = c(0,0,0,0,0,0,1,0,0,0,0,0,0,0,-1,0)))
contrast(modem3, list(least75VSleast103 = c(0,0,1,0,0,0,0,0,0,0,-1,0,0,0,0,0)))
contrast(modem3, list(least75VSleast117 = c(0,0,1,0,0,0,0,0,0,0,0,0,0,0,-1,0)))

contrast(modem3, list(least75VSleast90 = c(0,0,0,1,0,0,0,-1,0,0,0,0,0,0,0,0)))
contrast(modem3, list(least90VSleast103 = c(0,0,0,0,0,0,0,1,0,0,0,-1,0,0,0,0)))
contrast(modem3, list(least103VSleast117 = c(0,0,0,0,0,0,0,0,0,0,0,1,0,0,0,-1)))
contrast(modem3, list(least117VSleast90 = c(0,0,0,0,0,0,0,1,0,0,0,0,0,0,0,-1)))
contrast(modem3, list(least75VSleast103 = c(0,0,0,1,0,0,0,0,0,0,0,-1,0,0,0,0)))
contrast(modem3, list(least75VSleast117 = c(0,0,0,1,0,0,0,0,0,0,0,0,0,0,0,-1)))

#Depth 2
#simpler linear model without Sampling as random effect
mod4 <- lmer(soil.moisture ~ Sampling * Treatment + (1|Row), depth2)
summary(mod4)

#useful plots to make to know what the data looks like
plot(mod4)
plot(allEffects(mod4))

#emmeans
modem4 <- emmeans(mod4, c("Treatment", "Sampling"))
modem4

#sampling 1
contrast(modem4, list(conVsLeast_75 = c(1,-1,0,0,0,0,0,0,0,0,0,0,0,0,0,0)))
contrast(modem4, list(conVSmod_75 = c(1,0,-1,0,0,0,0,0,0,0,0,0,0,0,0,0)))
contrast(modem4, list(conVSmost_75 = c(1,0,0,-1,0,0,0,0,0,0,0,0,0,0,0,0)))
contrast(modem4, list(leastVSmost_75 = c(0,1,0,-1,0,0,0,0,0,0,0,0,0,0,0,0)))
contrast(modem4, list(modVSmost_75 = c(0,0,1,-1,0,0,0,0,0,0,0,0,0,0,0,0)))
contrast(modem4, list(leastVSmod_75 = c(0,1,-1,0,0,0,0,0,0,0,0,0,0,0,0,0)))

#sampling 2
contrast(modem4, list(conVsleast_90 = c(0,0,0,0,1,-1,0,0,0,0,0,0,0,0,0,0)))
contrast(modem4, list(conVsmod_90 = c(0,0,0,0,1,0,-1,0,0,0,0,0,0,0,0,0)))
contrast(modem4, list(conVsmost_90 = c(0,0,0,0,1,0,0,-1,0,0,0,0,0,0,0,0)))
contrast(modem4, list(leastVSmost_90 = c(0,0,0,0,0,1,0,-1,0,0,0,0,0,0,0,0)))
contrast(modem4, list(modVSmost_90 = c(0,0,0,0,0,0,1,-1,0,0,0,0,0,0,0,0)))
contrast(modem4, list(leastVSmod_90 = c(0,0,0,0,0,1,-1,0,0,0,0,0,0,0,0,0)))

#sampling 4
```

```
contrast(modem4, list(conVsleast_103 = c(0,0,0,0,0,0,0,0,0,0,0,0,1,-1,0,0)))
contrast(modem4, list(conVsmod_103 = c(0,0,0,0,0,0,0,0,0,0,0,0,1,0,-1,0)))
contrast(modem4, list(conVsmost_103 = c(0,0,0,0,0,0,0,0,0,0,0,0,1,0,0,-1)))
contrast(modem4, list(leastVSmost_103 = c(0,0,0,0,0,0,0,0,0,0,0,0,0,1,0,-1)))
contrast(modem4, list(modVSmost_103 = c(0,0,0,0,0,0,0,0,0,0,0,0,0,0,1,-1)))
contrast(modem4, list(leastVSmod_103 = c(0,0,0,0,0,0,0,0,0,0,0,0,0,1,-1,0)))

#sampling 3
contrast(modem4, list(conVsleast_103 = c(0,0,0,0,0,0,0,0,1,-1,0,0,0,0,0,0)))
contrast(modem4, list(conVsmod_103 = c(0,0,0,0,0,0,0,0,1,0,-1,0,0,0,0,0)))
contrast(modem4, list(conVsmost_103 = c(0,0,0,0,0,0,0,0,1,0,0,-1,0,0,0,0)))
contrast(modem4, list(leastVSmost_103 = c(0,0,0,0,0,0,0,0,0,1,0,-1,0,0,0,0)))
contrast(modem4, list(modVSmost_103 = c(0,0,0,0,0,0,0,0,0,0,1,-1,0,0,0,0)))
contrast(modem4, list(leastVSmod_103 = c(0,0,0,0,0,0,0,0,0,0,1,-1,0,0,0,0)))

#testing the time effect
contrast(modem4, list(con75VScon90 = c(1,0,0,0,-1,0,0,0,0,0,0,0,0,0,0,0)))
contrast(modem4, list(con90VScon103 = c(0,0,0,0,1,0,0,0,-1,0,0,0,0,0,0,0)))
contrast(modem4, list(con103VScon117 = c(0,0,0,0,0,0,0,0,1,0,0,0,-1,0,0,0)))
contrast(modem4, list(con117VScon90 = c(0,0,0,0,1,0,0,0,0,0,0,0,-1,0,0,0)))
contrast(modem4, list(con75VScon103 = c(1,0,0,0,0,0,0,0,-1,0,0,0,0,0,0,0)))
contrast(modem4, list(con75VScon117 = c(1,0,0,0,0,0,0,0,0,0,0,0,-1,0,0,0)))

contrast(modem4, list(least75VSleast90 = c(0,1,0,0,0,-1,0,0,0,0,0,0,0,0,0,0)))
contrast(modem4, list(least90VSleast103 = c(0,0,0,0,0,1,0,0,0,-1,0,0,0,0,0,0)))
contrast(modem4, list(least103VSleast117 = c(0,0,0,0,0,0,0,0,0,1,0,0,0,-1,0,0)))
contrast(modem4, list(least117VSleast90 = c(0,0,0,0,0,1,0,0,0,0,0,0,0,-1,0,0)))
contrast(modem4, list(least75VSleast103 = c(0,1,0,0,0,0,0,0,0,-1,0,0,0,0,0,0)))
contrast(modem4, list(least75VSleast117 = c(0,1,0,0,0,0,0,0,0,0,0,0,0,-1,0,0)))

contrast(modem4, list(least75VSleast90 = c(0,0,1,0,0,0,-1,0,0,0,0,0,0,0,0,0)))
contrast(modem4, list(least90VSleast104 = c(0,0,0,0,0,0,1,0,0,0,-1,0,0,0,0,0)))
contrast(modem4, list(least104VSleast117 = c(0,0,0,0,0,0,0,0,0,0,1,0,0,0,-1,0)))
contrast(modem4, list(least117VSleast90 = c(0,0,0,0,0,0,1,0,0,0,0,0,0,0,-1,0)))
contrast(modem4, list(least75VSleast103 = c(0,0,1,0,0,0,0,0,0,0,-1,0,0,0,0,0)))
contrast(modem4, list(least75VSleast117 = c(0,0,1,0,0,0,0,0,0,0,0,0,0,0,-1,0)))

contrast(modem4, list(least75VSleast90 = c(0,0,0,1,0,0,0,-1,0,0,0,0,0,0,0,0)))
contrast(modem4, list(least90VSleast103 = c(0,0,0,0,0,0,0,1,0,0,0,-1,0,0,0,0)))
contrast(modem4, list(least103VSleast117 = c(0,0,0,0,0,0,0,0,0,0,0,1,0,0,0,-1)))
contrast(modem4, list(least117VSleast90 = c(0,0,0,0,0,0,0,1,0,0,0,0,0,0,0,-1)))
contrast(modem4, list(least75VSleast103 = c(0,0,0,1,0,0,0,0,0,0,0,-1,0,0,0,0)))
contrast(modem4, list(least75VSleast117 = c(0,0,0,1,0,0,0,0,0,0,0,0,0,0,0,-1)))
```

Creating Facet Graphs

```{r}
```

```
#ph
ph <- ph1cy1 + ph2cy1 + ph1cy2 + ph2cy2
ph
ggsave("ph.jpg", width = 12, height = 7, units = c("in"), dpi = 600)

phb <- ph1by1 + ph2by1 + ph1by2 + ph2by2
phb
ggsave("phb.jpg", width = 12, height = 7, units = c("in"), dpi = 600)
```

Visualizing Yield

```{r}
#calling yield data
yield1 = read_excel("yield data.xlsx", sheet = "Year 1", .name_repair = "universal")
yield2 = read_excel("yield data.xlsx", sheet = "Year 2", .name_repair = "universal")

#year 1
#linear model
y1 <- lm(yield ~ Treatment, yield1)
view(yield)
#diagnostics
pls205_diagnostics(y1)
hist(yield1$yield)
shapiro.test(yield1$yield)

#results: p = 0.2036, normal!!

#anova
anova(y1) #no significant difference

#year 2
#linear model
y2 <- lm(Yield ~ Treatment, yield2)

#diagnostics
pls205_diagnostics(y2)
hist(yield2$Yield)
shapiro.test(yield2$Yield)

#results: p = 0.9938, normal!

#anova
anova(y2) #no significant difference
```

Yield Post Hoc Analysis

```{r}
yieldaov1 <- aov(Yield ~ Treatment, data = yield2)
summary(yieldaov1) #significant results, need to perform Tukey
yieldtukey1 <- TukeyHSD(yieldaov1) #performing Tukey posthoc
print(yieldtukey1)
cld.ML <- multcompLetters4(yieldaov1, yieldtukey1) #get letters needed for plots
print(cld.ML)

yieldaov1 <- aov(yield ~ Treatment, data = yield1)
summary(yieldaov1) #significant results, need to perform Tukey
yieldtukey1 <- TukeyHSD(yieldaov1) #performing Tukey posthoc
print(yieldtukey1)
cld.ML <- multcompLetters4(yieldaov1, yieldtukey1) #get letters needed for plots
print(cld.ML)
```

Pairwise Correlation Graphs

```{r}
na.omit(year1corr)
str(correlationy1)

year1corr <- subset(correlationy1, select = -c(Sampling, Treatment, Row, Distance,
Depth, inorganic.N, Category, Block, gwc))
colnames(year1corr) <- c("EC", "pH", "GWC", "MBC", "PMN", "NO3", "NH4")
Data1m <- rcorr(as.matrix(year1corr))
Data1m

cor(year1corr, use = "complete.obs")

#Use these lines of code below - THIS IS THE CORRECT CODE)#
corrplot(cor(year1corr, use = "complete.obs"),
        method = "number",
        number.font = 440,
        number.cex = 0.6,
        type = "upper" # show only upper side,
)

ellipse1 <- corrplot(cor(year1corr, use = "complete.obs"),
            p.mat = testRes$p, method = 'ellipse',
            order = 'alphabet', diag = FALSE,
            type = 'upper', tl.col = 'black',
            sig.level = c(0.001, 0.01, 0.05),
            pch.cex = 1.5,
            tl.cex = 2,
            cl.cex = 1.7,
```

```
            insig = 'label_sig',
            pch.col = 'black')

#Additional Code that plots all your parameters against each other#
plot (year1corr, main="Biological Soil Health Indicators for Year 1")
cor(year1corr)


year2corr <- subset(correlationy2, select = -c(Sampling, Treatment, id, Plot, Section,
Distance, Depth, inorganic.N, Block, gwc, Day))
str(correlationy2)
colnames(year2corr) <- c("GWC", "EC", "pH", "MBC", "NO3", "PMN", "NH4")

Data2m <- rcorr(as.matrix(year1corr))
Data2m

cor(year2corr, use = "complete.obs")

#Use these lines of code below - THIS IS THE CORRECT CODE)#
corrplot(cor(year2corr, use = "complete.obs"),
        method = "number",
        number.font = 440,
        number.cex = 0.6,
        type = "upper" # show only upper side,
)
testRes = cor.mtest(year2corr, conf.level = 0.95)
ellipse2 <- corrplot(cor(year2corr, use = "complete.obs"),
            p.mat = testRes$p,
            method = 'ellipse',
            order = 'alphabet',
            diag = FALSE,
            type = 'upper',
            tl.col = 'black',
            sig.level = c(0.001, 0.01, 0.05),
            pch.cex = 1.50,
            tl.cex = 2,
            cl.cex = 1.7,
            insig = 'label_sig',
            pch.col = 'black')

#Additional Code that plots all your parameters against each other#
plot (year2corr, main="Biological Soil Health Indicators for Year 2")
cor(year2corr)

ellipse1
ellipse1 + ellipse2 + plot_layout(ncol=2)
```

```
ggsave("trendgraph1.jpg", ellipse1, dpi = 600)
```

Volumetric Water Content over Time Graphs

```{r}
#YEAR 1 SOIL MOISTURE
dfc1 <- read_excel("FP_Soil_METER_2021_n.xlsx", sheet = "SCT_C1_R") # read excel file
dfc2 <- read_excel("FP_Soil_METER_2021_n.xlsx", sheet = "SCT_C2_R") # read excel file
dfl1 <- read_excel("FP_Soil_METER_2021_n.xlsx", sheet = "SCT_L1_R") # read excel file
dfm1 <- read_excel("FP_Soil_METER_2021_n.xlsx", sheet = "SCT_M1_R") # read excel file
dfh1 <- read_excel("FP_Soil_METER_2021_n.xlsx", sheet = "SCT_H1_R") # read excel file
dfh3 <- read_excel("FP_Soil_METER_2021_n.xlsx", sheet = "SCT_H2_R") # read excel file

data2 <- rbind(dfc1, dfl1, dfm1, dfh1)
data2$Treatment <- as.factor(data2$Treatment)

str(data2)

#data_avg <- data %/%
 # group_by(Timestamps, Treatment) %/%
 # summarize_at(vars(SM_20), list(name = mean, sd))

#data2$Timestamp <- as.POSIXct(data$Timestamp, format = "%Y-%m-%dT%H%M",
                      #tz = "UTC") # convert TIMESTAMP column in dataframe to POSIXct datetime format
is(data2$Timestamp, "POSIXct") # check and see if its really in POSIXct

sm1 <- ggplot(data2, aes(x = Timestamp, y = SM_20, color = Treatment))+
  theme_classic(base_size = 18)+
  #theme(axis.text.x=element_blank())+ #remove x axis labels, keep this hashed out for the upper graph?
  labs(x = "Date", color = "Treatment")+
  ylab(bquote('VWC'(m^3*m^-3)))+
  theme(plot.title = element_text(hjust = 0.5))+
  geom_line(aes(color=Treatment))+
  scale_color_manual(values = c("Control" = "#009E73", "Low" = "#56B4E9", "Moderate" = "#E69F00", "High" = "#D55E00"))+
  theme(legend.position = "none")+
  scale_x_datetime(labels=date_format("%m-%d", tz = "UTC"),
```

```
                date_breaks = "1 week",
                expand = c(0,0))+
  scale_y_continuous(limits = c(0,1), breaks = seq(5, 40, by = 5), expand = c(0,0))+
  ylim(0,0.5)+
  annotate("rect", xmin = as.POSIXct("2021-08-02 00:00:00"), xmax =
as.POSIXct("2021-08-03 00:00:00"), ymin = 0, ymax = .5, alpha = 0.2)+
  annotate("text", x = as.POSIXct("2021-08-02 12:00:00"), y = 36, label = "Sampling 1")+
  annotate("rect", xmin = as.POSIXct("2021-08-16 00:00:00"), xmax =
as.POSIXct("2021-08-17 00:00:00"), ymin = 0, ymax = .5, alpha = 0.2)+
  annotate("text", x = as.POSIXct("2021-08-16 00:00:00"), y = 36, label = "Sampling 2")+
  annotate("rect", xmin = as.POSIXct("2021-08-30 00:00:00"), xmax =
as.POSIXct("2021-08-31 00:00:00"), ymin = 0, ymax = .5, alpha = 0.2)+
  annotate("text", x = as.POSIXct("2021-08-30 00:00:00"), y = 36, label = "Sampling 3")+
  annotate("rect", xmin = as.POSIXct("2021-09-13 00:00:00"), xmax =
as.POSIXct("2021-09-14 00:00:00"), ymin = 0, ymax = .5, alpha = 0.2)+
  annotate("text", x = as.POSIXct("2021-09-13 00:00:00"), y = 36, label = "Sampling 4")+
  guides(color = guide_legend(override.aes = list(linewidth = 3)))+
  theme(axis.text.x=element_text(size=15),
        axis.text.y=element_text(size=15),
        axis.title.y = element_text(size=15),
        axis.title.x=element_text(size=15),
        legend.title = element_text(size=13),
        legend.text = element_text(size=13))
sm1
png(filename = "soilmy1.png", res = 300, height = 1080, width = 3840)
fig
dev.off()

sm1_n + sm2_n + plot_layout(ncol=1)
ggsave("vwc20.jpg", width = 10, height = 7, units = c("in"), dpi = 600)

```