

DISSERTATION

THE IMPACTS OF DEFICIT IRRIGATION ON CROP PRODUCTION
AND SUSTAINABLE SOIL MANAGEMENT

Submitted by

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ABSTRACT

THE IMPACTS OF DEFICIT IRRIGATION ON CROP PRODUCTION AND SUSTAINABLE SOIL MANAGEMENT

Growing issues of water scarcity around the planet highlight a need for more efficient use of agricultural water. Deficit irrigation (DI) offers a promising option to reduce water use with relatively small impacts on crop yield, when properly managed. However, the impacts of DI management on above and belowground crop growth and the interactions between plants and soil are complex and need further study. There are concerns that DI, because it often reduces crop biomass, could reduce soil carbon (C) stocks, and negatively impact soil processes related to soil health. Additionally, DI alters soil moisture conditions with significant implications for soil C turnover and for the movement, transformation, and fate of soil nitrogen (N). At the same time soil N could buffer crops from water stress. Therefore, the goal of this research was to examine the potential impacts of DI on crop production and water stress and implications for soil C and N dynamics.

Chapter 2 explores the effect of DI on maize above and belowground growth, soil microbial community composition, soil aggregation as well as soil C concentrations in surface soils (0-20 cm) and at depth (40-60 cm). Deficit irrigation increased root length density in deep soils (40-60 cm), with a trend towards higher soil C in treatments with the most root growth. Deficit irrigation also reduced total microbial biomass in the surface layer and led to shifts in microbial community composition. While aggregation and soil C were not strongly impacted by DI here, increased root growth under DI could eventually increase soil C and benefit a range of

soil health related parameters, which are advantageous for crop production in water-limited systems.

Chapter 3 quantifies greenhouse gas emissions from DI compared to full irrigation and suggests that DI can reduce both N_2O and CO_2 . While this is a promising result, we also found that yields were reduced under DI, such that yield-scaled emissions were higher under DI compared to FI. The tradeoff between reducing emissions at the cost of reducing yield is important to recognize in the development of more sustainable agricultural practices. An additional important observation in this study was that emissions from this drip-irrigated maize system appeared to be much lower than from sprinkler or furrow irrigated maize systems reported elsewhere in the Great Plains.

Chapter 4 sought to elucidate the impact of DI on the fate of N and the interactions between water and N in a drip-irrigated maize system. Yield and the amount of N at the end of the growing season in the harvested material vs. N lost via N_2O emissions or remaining in the soil. Deficit irrigation reduced grain yield compared to full irrigation was quantified. Less N was taken up by maize under DI, leaving more residual nitrate in the soil at the end of the growing season, which is vulnerable to subsequent loss via leaching or emissions. While DI reduced consumptive water use in this experiment, yields were also reduced, thereby reducing water use efficiency. Overall, the findings of this study suggest that farmers should apply less fertilizer when utilizing DI.

Chapter 5 examines the impact of DI and N level on above and belowground growth of five different sorghum genotypes in a greenhouse experiment. We found that DI led to an increase in root biomass allocation for all the sorghum genotypes, and that a low N treatment further increased root biomass allocation and specific root length (SRL) compared to a high N

treatment under DI. Importantly, increasing root biomass allocation did not decrease aboveground biomass which is a common tradeoff in drought-stressed agriculture.

In summary, this research indicates that DI alters crop growth in important ways beyond just grain yield. Deficit irrigation can increase maize and sorghum root growth, which has important implications for water and nutrient acquisition and for building soil C. This finding is especially significant in semi-arid systems, where maintaining and building soil C presents a significant challenge for long term soil health. We also showed that DI can be used to reduce greenhouse gas emissions, but it is important to note that such management can also reduce yield. Overall, this research will help inform farmers and policymakers in making decisions around the adoption of DI practices. Most importantly, this work suggests that proper implementation of DI offers promise to maintain crop growth with less water and that doing so could maintain or increase soil C stocks and would require less N fertilizer application compared to full irrigation.

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CHAPTER 1: INTRODUCTION

Irrigated agriculture is critical to meeting global food and fuel demand, currently producing roughly 40% of the world's food and fiber on just 17% of the farmland (Evans and Sadler, 2008). Growing uncertainty in water supply is one of the most significant future challenges facing both irrigated and rainfed agricultural systems. In the Great Plains and the Western US, variable snowpack, higher temperatures, altered precipitation patterns, rising municipal and industrial water demand, and groundwater depletion are all straining irrigation water supplies (Kukul and Irmak, 2018; Robertson et al., 2018; Scanlon et al., 2012; Warziniack and Brown, 2019; Wienhold et al., 2018; Zhao et al., 2017). Therefore, irrigation strategies that allow for continued crop production with less water are needed. Deficit irrigation (DI) represents a promising alternative to switching crops or implementing rotational fallow when insufficient water is available to meet full crop water demands (Fereris and Connor, 2004; Geerts and Raes, 2009). Deficit irrigation involves applying water below the full crop-water requirement, allowing some water stress to onset and lowering overall consumptive water use (via reduced evapotranspiration). Although crop yield typically has a linear response to water applied, DI that is optimized with strategic timing (usually during the water-stress resistant late-vegetative and maturation growth stages) can create a curvilinear water production function for some crops like maize, which may make DI agronomically and economically viable for producers (Comas et al., 2019; Manning et al., 2018; Trout and DeJonge, 2017; Trout and Manning, 2019).

While water use and yield under DI have received considerable attention, the impacts of DI on soil health, including C and N dynamics, remain poorly understood (Comas et al., 2019; Payero et al., 2009, 2006). Elucidating the impacts of DI on belowground processes is crucial

because changes to soil health can impact crop production and overall agroecosystem sustainability in the long term. Also, soils in semi-arid environments are particularly vulnerable to soil health changes because they are often highly water and C-limited (Miner et al., 2020; Peterson et al., 2020). For example, crops usually respond to DI by reducing vegetative growth, resulting in reduced biomass C input to the soil. However, the increased root growth under DI could supplement or replace aboveground C inputs when it comes to maintaining soil organic carbon (SOC). Root C is more protected from decomposition by soil aggregates and mineral particles, which may improve the stability and longevity of SOC compared to aboveground inputs, which are incorporated into the soil more slowly and are more labile in nature (Rasse et al. 2005; Kong and Six 2010). Additionally, if roots reach deep depths, SOC turnover occurs more slowly than at the surface (Rasse et al., 2006). Thus, differences in rooting under DI, especially in deeper soil horizons, could significantly impact SOC accumulation.

The maintenance and accrual of SOC in agricultural systems is crucial for long-term sustainability and offers numerous benefits for soil health, and is a fundamental driver of global C cycling (Follett, 2001; Power, 2010). Specifically, plant C inputs provide the energy to sustain soil biological activity. Soil organic C and microbial exudates tend to increase soil aggregation, which benefits water infiltration and soil water holding capacity (Álvaro-Fuentes et al., 2009; Blankinship et al., 2016; Franzluebbers, 2002). Therefore, assessing the potential impact of DI on above and belowground C inputs to the soil and overall effects on soil health is essential for evaluating the impact of DI on the viability of DI in the long term.

Beyond potential impacts on soil health, water management plays a vital role in the fate of N fertilizers by altering N movement, uptake, and transformations in the soil (Barakat et al., 2016; Chilundo et al., 2016; Rimski-Korsakov et al., 2009; Sanchez-martin et al., 2008). The

ability of roots to take up N is dependent on the water availability (Ercoli et al., 2008), such that lower soil moisture conditions under DI could potentially hinder N uptake. Nitrogen is a crucial building block for plant growth and is needed in large amounts to maintain optimum photosynthetic capacity, so any barriers to N uptake are of concern. Plants grown under DI are likely to have lower plant N uptake than fully irrigated plants because of reduced vegetative biomass. However, even with decreased biomass, plants grown under DI can maintain grain yield similar to fully irrigated plants (Comas et al., 2019; Zhang et al., 2019). Therefore, recommendations for N application rates developed under full irrigation scenarios (and without differentiating the demand for N in the biomass vs. the grain) may not be optimal under DI. Therefore, an improved understanding of DI impacts on N demand and uptake is needed to inform N fertilizer management under DI.

While N fertilizers have made it possible to increase yields, excess fertilizers lost from farms have created a wide range of negative consequences for the environment (Robertson and Vitousek, 2009). Fertilizer N lost from the field via runoff or leaching can degrade surface and groundwater supplies (Galloway et al., 2003). Deficit irrigation reduces soil moisture, so it is likely to reduce leaching and runoff, at least during the growing season when DI is in effect (Barakat et al., 2016). Another important pathway of N lost from the field is via N₂O emissions. Fertilizer-derived N₂O represents 35% of anthropogenic N₂O emissions (Davidson, 2009), and reducing greenhouse gas emissions from agriculture is a critical agricultural sustainability goal (Davidson et al., 2014; Paustian et al., 2016). It is difficult to predict what effect DI will have on N₂O emissions since the microbial-driven pathways that produce N₂O occur under a wide range of soil moisture conditions, and soil drying does not necessarily prevent N₂O emissions (Bateman and Baggs, 2005; Homyak et al., 2017; Zhu et al., 2013).

Greater root growth under can DI potentially increases access to soil nutrients. Managing for increased root growth has been proposed as a strategy to improve water and nutrient use efficiency in cropping systems and reduce NO₃⁻ leaching (Garnett et al., 2009). However, it remains unclear if increased root growth under DI is a reliable strategy for improving N uptake when N movement is limited in water-limited scenarios. Management practices such as DI and selecting cropping varieties with more extensive root systems could complement efforts to reduce inorganic fertilizer additions. Root proliferation in capturing immobile and organic sources of N, in addition to competing with microbes for N availability, is likely critical (Hodge 2004). Therefore, it is essential to understand how DI impacts the fate of N in agroecosystems since DI has the potential to alter the form and size of N losses from the soil.

This dissertation sought to understand how DI impacts crop growth, N dynamics, soil C, and related soil health parameters. The research utilizes DI and varying N fertilization levels on maize grown in field experiments and sorghum grown in a greenhouse experiment. The following questions are addressed in the four main chapters:

1. How does DI impact above and belowground growth, and what are the potential implications on soil organic carbon, aggregation, and soil microbial communities?
2. How does DI impact soil CO₂ and N₂O emissions, and does DI represent a viable climate-smart option for irrigated maize in the High Plains region of Colorado?
3. How do irrigation and N application rates interact to influence N uptake, yield, and N losses?
4. How does DI and N level impact the above and belowground growth and plant N use efficiency of different sorghum varieties with varying levels of drought stress tolerance?

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CHAPTER 2: DEFICIT IRRIGATION DRIVES MAIZE ROOT DISTRIBUTION AND SOIL MICROBIAL COMMUNITIES WITH IMPLICATIONS FOR SOIL CARBON DYNAMICS¹

2.1 Introduction

Water supply for irrigation is the limiting factor in semiarid production systems around the world and availability of this key resource is declining (Chartzoulakis and Bertaki 2015; Smidt et al. 2016). Reliance on irrigated agriculture, which already provides >40% of food and fiber, will intensify as global food demand continues to rise and the frequency and intensity of drought makes rain-fed agriculture more challenging (Evans & Sadler, 2008). Deficit irrigation (DI) represents a promising alternative to traditional irrigation management and can help sustain crop production in times of water scarcity, while reducing overall crop water usage (Fereres and Soriano, 2007; Geerts and Raes, 2009). Deficit irrigation consists of water application below the full crop-water requirement, which can be applied strategically during key crop growth stages to save water but minimize yield losses (Comas et al., 2019) and close the yield gap relative to fully irrigated crops (Chilundo et al. 2017). While water use and yield of cropping systems under DI have received attention (Nesmith and Ritchie 1992; Payero et al. 2006, 2009), the impacts of DI on roots and soil properties remain poorly understood.

Water stress under DI can increase relative and absolute root growth, which enables the exploration of moisture deeper in the soil profile (Sampathkumar et al., 2012; Comas et al., 2013). Deep, dense root systems not only allow plants to better mine soil for water but are also likely to affect overall C inputs and soil moisture dynamics, with implications for soil structure

¹ Flynn, N.E., Comas, L.H., Stewart, C.E., Fonte, S.J., 2021. Deficit irrigation drives maize root distribution and soil microbial communities with implications for soil carbon dynamics. *Soil Sci. Soc. Am. J.* 85, 412-422.

(Angers and Caron, 1998), and soil organic carbon (SOC) stabilization (Rasse et al., 2006), as well as microbial abundance and community structure (Fuchslueger et al. 2014).

The maintenance or accrual of SOC in agricultural systems is fundamental to ensuring the long-term productivity of soils. Soil organic carbon offers numerous benefits for soil nutrient and water dynamics and is a key driver of global C cycling (Follett, 2001). Because DI typically results in smaller aboveground biomass production, there is some concern for diminished capacity of agricultural systems under DI to build SOC (Rawls et al., 2003). However, increased root growth under DI and the recalcitrant nature of root C may be of greater importance to SOC stocks than aboveground residue (Rasse et al. 2005; Kong and Six 2010). The physiochemical protection by interactions with soil aggregates and mineral particles may also contribute to a longer residence of root C (Rasse et al., 2005) and disproportionate contribution of root C in forming stable SOC (Kong and Six, 2010). Additionally, SOC turnover occurs more slowly at depth than at the surface (Rasse et al., 2006). Thus, differences in rooting architecture under DI, especially in deeper soil horizons, could have significant impacts on SOC accumulation.

Soil microbial communities are key drivers of plant productivity due to their impacts on a variety of soil processes, including C turnover and regulating the availability of nutrients that are essential to plant growth (Van Der Heijden et al., 2008). Soil microbial communities are also valuable indicators of change in the soil environment due to their rapid growth and sensitivity to temperature, moisture and resource availability (Entry et al., 2008). Deficit irrigation and associated impacts on roots are likely to affect soil microbial communities in numerous ways via changes in the quantity and quality of C inputs, alterations to habitat, as well as via impacts on water availability. Furthermore, we note that community composition and abundance of microorganisms in deeper in soil layers (i.e., below 20 cm), has received much less attention than

surface soils, yet is of particular interest because of microbial involvement in C sequestration and nutrient cycling (Fierer et al., 2003; Roosendaal et al., 2016).

Finally, soil aggregation creates habitat for soil organisms and facilitates the physical stabilization of SOC (Briar et al., 2011; Kong et al., 2005). Soil aggregates can be formed via the root and fungal hyphae entanglement with soil particles (Angers and Caron, 1998), and through the addition of extracellular microbial and root excretions that bind soil particles together (Czarnes et al., 2000; Morel et al., 2017). While DI is likely to affect aggregation, and aggregate-associated C storage, due to effects on root growth and microbial biomass and community structure, the combined impacts of DI and altered rooting activity on aggregate formation are poorly understood.

In this study, we examined the impacts of DI after 4 years in a field experiment in Northeastern Colorado, a region where irrigated agriculture plays an important role in the local economy, but faces considerable water supply challenges (CWCB, 2011). We examined root density, soil C and N concentrations, microbial biomass and communities, and water stable aggregation at two soil depths (0-20 and 40-60 cm) within four levels of DI management, ranging from 40 to 100% of evapotranspiration (ET) needs during late vegetative and maturation stages. We hypothesized that DI would result in greater root growth at depth (> 40 cm); and that the combined effect of greater root-derived C, enhanced aggregation, lower water availability, and altered microbial biomass and community structure would lead to greater C accumulation at depth relative to the fully watered treatment.

2.2 Methods

Study Site and Experimental Design

Research was conducted at the USDA-ARS Limited Irrigation Research Farm (LIRF) near the city of Greeley in Northern Colorado, USA (40° 26' 57" N, 104° 38' 12" W). This site is located at an elevation of 1427 m and receives on average 215 mm of precipitation during the maize cropping season (May – October) and 335 mm annually (PRISM 2015). Soils are predominately Olney fine sandy loam (fine-loamy, mixed, superactive, mesic Ustic Haplargids) with Otero sandy loam (coarse-loamy, mixed, superactive, calcareous, mesic Aridic Ustorthents) in small areas, with an average pH of 8.2 (Trout and DeJonge, 2017).

This study focused on four treatments within a larger study that began in 2012 to optimize irrigation scheduling based on crop growth stage. The study examined DI with varying levels of water stress imposed during the late vegetative (~V10-V19) and maturation growth stages (R4-R6) (Comas et al., 2019). The four irrigation treatments measured in this study correspond to target percent of maximum crop evapotranspiration (ET) goal during late vegetative (~V10-V19) and maturity growth stages (R4-R6), respectively: 1) 100/100, aiming to apply 100% of calculated ET from planting until maturation; 2) 65/65, targeting 65% of maximum ET in the two stages, 3) 40/80, targeting 40% of maximum ET during the late vegetative and 80% during the maturation stage, and 4) 40/40, targeting 40% of maximum ET during the two stages. Irrigation amounts (Table 1.1) were determined throughout the season based on target ET levels as determined by water balance using neutron soil moisture meter and time domain reflectometer. Additional details of the soil water content measurements are given by Trout and Bausch (2017).

Each treatment was replicated four times in a randomized complete block design. Individual treatment plots were 9 m wide and 43 m long, containing 12 rows of maize that were spaced 0.76 m apart. This study took place during the second maize year of a two-year sunflower

(Helianthus) - maize (*Zea mays*) rotation, strip-tilled once annually in the spring and crop residue was retained during the four years of the experiment prior to sampling in this study. Flow-monitored irrigation was delivered by surface drip and precipitation was measured by an on-site weather station. A liquid starter fertilizer of 28 kg N ha⁻¹ was applied at planting on June 3, followed by 168 kg N ha⁻¹ applied in four irrigation events in July. The irrigation water, containing approximately 25 ppm N, resulted in an additional 38-121 kg N ha⁻¹ to total 205-290 kg N ha⁻¹, depending on the treatment. Despite these differences, yield was not N-limited in any of the treatments.

Root measurements

To assess root growth, acrylic minirhizotron tubes (5 cm inner diameter) were installed in Spring of 2015, approximately two weeks after seedling emergence. Two tubes per plot were placed between plants, 10 cm from the planting row and 3 m apart, near the center of each plot. Tubes were inserted an angle of 30° from vertical (such that both tube ends were same horizontal distance from the maize row), sealed at the bottom, and the exposed end was wrapped with two layers of vinyl tape to block light. The top of each tube was capped with a rubber stopper and shielded with an aluminum can and rubber skirt cut from a tire inner tube (both painted white). A minirhizotron digital camera was used to collect images during the growing season through fixed windows in the tube to a depth of 60 cm. Root length per viewing area was measured using Rootfly 2.0.2 (Clemson University, Clemson, SC, USA). The average root length density for each treatment in both depths at the completion of the late vegetative and maturation stages were determined. Observed root density was expressed as length of root per viewing area of minirhizotron windows (mm root cm⁻²) (Johnson et al. 2001).

Soil Sampling and Plant Biomass

Soil samples were collected in the R5 growth stage between Sept 9 and 14 of 2015, 2.7 m from the border (next to the fourth row) of the plot to avoid edge effects. This timing coincides with the second set of root measurements. Two soil cores (8 cm diameter) per treatment plot were taken to a depth of 60 cm using a pneumatic post driver. Upon removal, cores were divided into three depths of 0-20, 20-40 and 40-60 cm, sealed in plastic bags and kept cool for transfer to the lab. Subsequent analyses were performed on the 0-20 and 40-60 cm layers only, as preliminary data (not shown) revealed the greatest DI imposed differences on root density occur at these depths. Shortly after returning to the lab, field moist soils were gently broken by hand along natural planes of weakness to pass through an 8-mm sieve, to homogenize soil and remove large rocks and plant material. For analysis of microbial communities, a representative subsample of 50 g of the field-moist soil, from both soil depths, was collected immediately after passing through the 8 mm sieve. This soil was handpicked to remove all identifiable plant material, frozen at -22 °C, and then freeze-dried for subsequent analysis of microbial communities. The remainder of the 8-mm sieved soil was air-dried for elemental analyses and aggregate fractionation via wet-sieving.

At plant maturity (R6) in 2015, the average aboveground dry biomass (kg ha^{-1}) of each treatment was determined by averaging the shoot dry weight of five plants from each replicate and multiplying by stand density (plants ha^{-1}).

Elemental analyses

Soil samples from each depth were dried and ground prior to analysis of total soil C and N using a Europa Scientific C-N analyzer with a Solid/Liquid Preparation Module (Dumas combustion sample preparation system) mass spectrometer (Europa Scientific Ltd., Crewe, England). Organic C was determined after the removal of carbonates by acid fumigation (Harris

et al., 2001). Inorganic C was calculated by taking the difference between total and organic C. Soil organic C, soil inorganic C (SIC), total C (TC) and N (TN) were expressed as a concentration (g C kg⁻¹ soil).

Aggregate fractionation

Subsamples of the air-dried soil (40g) were wet-sieved using methods adapted from Elliott (1986). Soil was placed on top of a 2 mm sieve and submerged in deionized water for 5 min for slaking. The sieve was then moved gently up and down (in and out of the water) 50 times per minute, for two minutes. Soil remaining on the 2 mm sieve after 2 min was then rinsed into a pre-weighed aluminum pan for drying. This process was repeated with a 250 µm and a 53 µm sieve to generate four aggregate fractions: large macroaggregates (>2000 µm), small macroaggregates (250 – 2000 µm), microaggregates (53 – 250 µm), and silt and clay fraction (< 53 µm). The four fractions were then dried in an oven at 60 °C to determine the proportion of whole soil mass in each fraction. Mean weight diameter (MWD), an indicator of aggregate stability, was calculated with the following equation:

$$\text{MWD} = \sum_i P_i S_i \quad [1]$$

where S_i is the average diameter (µm) for particles in the i^{th} fraction and P_i is the proportion of the whole soil in the same fraction (van Bavel, 1950).

Microbial analysis

Phospholipid fatty-acids (PLFAs) are the main structural component of the phospholipids in microbial cell tissues and rapidly degrade in the soil, and therefore, can be used as indicators of the microbial community. The high throughput method developed by Buyer and Sasser (2012) was used to analyze PLFAs from freeze-dried soils at MIDI labs (MIDI, Inc., Newark, DE, USA). Extracted samples were analyzed with gas chromatography performed on an Agilent

6890N gas chromatograph (Agilent Technologies, Wilmington, DE, USA). PLFAs were identified using MIDI PLFAD1 calibration mix and MIDI Sherlock Software version 6.2B (MIDI, Inc., DE, USA). The 23 dominant PLFAs were split into major PLFA types for analysis (see details in Table 1.2).

Statistical analyses

Means of root density, SOC, SIC, soil N, MWD, total biomass and relative abundance of microbial groups were compared using ANOVA with irrigation treatment (categorical variable) and soil depth as fixed effects and block as a random effect followed by a Tukey's post-hoc test. Root density was analyzed separately for the two growth stages. Results were considered statistically significant when $P < 0.05$. Pearson correlation analyses were performed to assess the relationship between the aforementioned variables with total water applied for each irrigation treatment (as a continuous variable). Correlation analyses were performed separately on each soil depth. Microbial community similarity was visualized using non-metric multi-dimensional scaling (NMDS) analysis with 23 identified PLFAs. Permutational multivariate analysis of variance (PERMANOVA) was used to test for statistically significant variance between groups. Data analyses were performed in R v.3.6.2.

2.3 Results

Precipitation and Irrigation

Total precipitation during the 2015 cropping season (May - October) was 231 mm. With additional water applied via irrigation, the total water applied to the 100/100 treatment was 621 mm. The 40/80, 65/65, and 40/40 treatments received 70, 64, and 52% compared to the 100/100 treatment, respectively (Table 2.1).

Aboveground vegetative biomass and maize root density

Average aboveground vegetative biomass (leaves and stems) at maturity of the 100/100 treatment was 8905 kg ha⁻¹ and was reduced by 47, 37 and 47% in the 40/80, 65/65, and 40/40 treatments, respectively (Table 2.1). At maturity, the 100/100 treatment had fewer maize roots (mm roots per cm² viewing area) than the DI treatments at both measured depths (0-20 and 40-60 cm), although this was only statistically significant at the 40-60 cm depth (Fig. 2.1). Root growth was similar among the three DI treatments at each depth. Root growth in the 100/100 treatment was significantly less at the 40-60 cm depth compared to the surface (0-20 cm) layer (Fig. 2.1).

Soil C and N concentrations and soil aggregation

There were no significant differences among irrigation treatments in SOC, TC, or TN concentrations at either soil depth (Table 2.1). There was a significant difference in SIC among treatments in the 0-20 cm depth with higher SIC observed in the 65/65 and 40/40 treatments than in the 100/100 and 40/80. While not significantly different ($P > 0.05$), SOC concentration in the 0-20 cm soil depth tended to be higher under full irrigation and declined with total water applied. At the 40-60 cm depth, SOC tended to be higher under the most water-limited treatment (40/40) followed by the 100/100, 40/80 and 65/65 treatments. Soil SOC and TN concentration was significantly lower in the 40-60 cm depth in all treatments (Table 2.1). The relative contribution of SIC to TC was greater at the 40-60 cm depth (58-74% of TC was SIC) than in the 0-20 cm depth (14-25 % of TC was SIC) in all the treatments.

In the 0-20 cm soil depth, aggregate stability (MWD) generally decreased with more irrigation such that 100/100 and 40/80 treatments displayed significantly lower MWD than the 65/65 treatment (Table 2.1). Aggregate stability had a positive relationship with maturation stage root density in the 0-20 cm depth ($r^2=0.32$, $P < 0.01$). Aggregate stability was also greater in the

lower soil depth within the 100/100 and 40/80 treatments, but not the 65/65/ or 40/40 treatments (Table 2.1).

Soil PLFA biomass and community composition

Total PLFA biomass and biomass of all individual PLFA groups was reduced by deficit irrigation in the 0-20 cm depth (Table 2.2). Within the 40-60 cm depth, total bacterial PLFAs were decreased by DI, but there were no treatment differences among irrigation treatment for fungal PLFAs (saprotrophic and AMF) at this depth (Table 2.2). The biomass of all microbial groups was also significantly lower in the 40-60 cm depth compared to the 0-20 cm depth within all irrigation treatments (differences not shown in Table 2.2). A shift in microbial community composition is indicated in the NMDS ordination of the 0-20 cm depth more so than the 40-60 cm depth (Fig. 2.2). In the 0-20 cm depth, the treatments are roughly separated in order of irrigation water applied during the late vegetative stage. Results from PERMANOVA analysis show a significant distinction between microbial community structures in both soil depths ($P < 0.01$). This difference appears to be largely driven by the relative abundance of AMF fungi, which was significantly higher in the 100/100 treatment compared to all the DI treatments in both soil depths. In the 0-20 cm depth the relative abundance of AMF fungi was 5.5%, 4.9%, 4.8% and 4.6% in the 100/100, 65/65, 40/80 and 40/40 treatments, respectively. In the 40-60 cm depth, relative abundance of AMF fungi was 3.3%, 2.5%, 2.7% and 2.9% in the 100/100, 65/65, 40/80 and 40/40 treatments, respectively. There were no significant differences in relative abundance among the other PLFA groups (data not shown).

2.4 Discussion

Improved understanding of DI impacts on SOC and overall soil functioning is critical for evaluating the potential of this management strategy to contribute to agricultural sustainability in

the long-term. Our findings indicate that DI can have important impacts on several belowground processes related to C cycling and accumulation in soils. We found that DI significantly increased maize rooting density in the 40-60 cm soil depth, but decreased surface microbial biomass, and AMF biomarkers. Impacts of DI on soil structure and SOC were less clear, at least within the 4 years that treatments were in place.

Deficit irrigation significantly reduced aboveground biomass (kg ha^{-1}) and increased maize root density (mm cm^{-2}) relative to full irrigation. While some have reported both decreased maize shoot and root growth under DI (e.g., Benjamin et al. 2014), others have found that moderate decreases in soil moisture from DI can lead to deeper maize root distribution and greater root density in the soil, especially when differences in soil moisture occur during the vegetative growth stages (Sampathkumar et al., 2012; Comas et al., 2013; Chilundo et al., 2017). Our findings confirm the results of other DI studies (Sampathkumar et al., 2012; Comas et al., 2013) indicating that maize root growth increases under DI during the late vegetative growth stage and that these differences in root growth persist until the end of the growing season. This deep, dense rooting system likely enables plants under DI to explore a greater soil volume and increase water extraction.

In irrigated cropping systems, large aboveground biomass production can contribute to building SOC, yet losses through increased decomposition may counteract potential gains (Gillabel et al., 2007; Deneff et al., 2008). In a previous DI experiment by Blanco-Canqui et al. (2010), surface SOC concentration (0-10 cm) increased with increasing water applied, mirroring plant productivity. In the 0-20 cm depth of our study, despite much higher aboveground biomass production in the fully irrigated treatment, no equivalent increase in SOC was observed. This could be because belowground C inputs, via increased rooting density, compensated for lower

aboveground C inputs under DI. Although we did not differentiate between root and shoot C in this study, roots are known to be important for sequestering C in agricultural systems (Rasse et al. 2005). Any C inputs in the higher irrigation treatments would also have been subjected to increased soil moisture that would likely enhance decomposition of new and/or existing soil organic matter pools (Stewart et al., 2017). Together, our observations of plant inputs, water availability, total PLFA biomass indicate that factors controlling C cycling are elevated under full irrigation (100/100) compared to DI, with strong potential for influencing SOC dynamics. The results of our 4-year study indicate that SOC was not diminished under DI, but that increased root growth under deficit could benefit SOC accumulation, especially in deeper soil layers. We note that the DI effects may be somewhat muted in this study due to the high sand content (> 70%), which is generally associated with lower capacity to stabilize C on mineral surfaces and reduced potential for physical stabilization in soil aggregates (Six et al., 2002). Further research is needed to understand the sources of SOC (shoot, root, microbial) under DI and if DI systems can be managed to favor SOC accumulation and benefit soil function and crop production in the long-term.

Microorganisms are known to respond quickly to environmental change. Our study provides evidence that DI could create long-lasting effects on soil microbial communities. The microbial communities in our study experienced frequent drying and wetting events and this cycle is more severe under stronger deficits. The stress of dry conditions in addition to osmotic stress caused by frequent rewetting events could have led to the decreased PLFA biomass under DI observed in this study (Sawada et al., 2017; Sun et al., 2017). Total PLFA biomass mirrors the level of water application during the late vegetative growth stage (the first of two imposed water deficit periods) more than the amount of total water applied. This may suggest that short

periods of stress have strong influence on the microbial community compared to the normal conditions which were otherwise consistent for all treatments during the remainder of the year. This is demonstrated by the 65/65 treatment which has higher total PLFA biomass than the 40/80 treatment at the end of the growing season (when we soil sampled) despite having received a lower total amount of water compared to 40/80 treatment. Microbial biomass was also impacted by soil depth and was lower in the 40-60 cm depth (40-60 cm) in all treatments compared to the 0-20 cm depth. This result is not surprising because soil microorganisms at deeper depths are often resource-limited (Fierer et al., 2003).

Shifts in microbial community composition have been previously observed in drought studies (e.g., Fierer et al., 2003; Fuchslueger et al., 2014). We found that, all microbial groups had reduced absolute abundance under DI compared to full irrigation (100/100 treatment). When compared on a relative abundance basis, the DI treatments had significantly fewer AMF biomarkers compared to full irrigation. This is in contrast to other studies that have found fungi to be either unaffected or relatively tolerant to dry soils (Hueso et al. 2012; Drenovsky et al. 2010). The reduced presence of AMF under DI is concerning because of the positive effect AMF are known to have on maize drought tolerance (Begum et al., 2019). These results suggest that under DI, not only is the size of the microbial community diminished but the beneficial capacity of that community may be reduced as well.

Maize plants have been shown to adjust their root exudation to modify their surrounding soil microbial community and plants in general are thought to increase rhizodeposition under drought conditions (Quiroga et al., 2017; Williams et al., 2020). In this study, we did not measure rhizodeposition, but maize root length density, which is often correlated to rhizodeposition, was not well related total PLFA biomass or any particular PLFA group. It is

possible that root exudates remained inaccessible due to diffusion limitations (Naylor and Coleman-Derr, 2018) or that irrigation amount outweighed any added benefit of increased root density (such as exudates or habitat) in the DI treatments. Our results suggest that irrigation treatment and within-year early season soil moisture deficit appear to have greater influence on microbial communities than root growth. It should be noted that our findings come from a single timepoint at the end of 4 years of irrigation differences.

Soil inorganic carbon made up a large portion of total soil carbon (58-74% in the 40-60 cm soils) and we suspect that this is associated with the lithology of the region. Additional carbonates can be added via irrigation water carrying high concentrations of Ca and dissolved HCO_3^- and CO_2 ions which precipitate carbonates when exposed to elevated temperatures and high pH (Entry et al., 2004). In the 0-20 cm depth, greater SIC was observed in the drier treatments, perhaps because carbonates were less easily leached to lower depths under the dry conditions. Given that SIC is the dominant form of C at depth in this system, is influenced by irrigation regime, and generally has a longer residence time than SOC, this C pool should be monitored because of its potential importance to net C sequestration in agricultural systems (Monger et al., 2015).

Soil aggregation is a function of soil texture, SOC content, positive cations such as Ca and Mg, in addition to root growth and microbial mucilage (Angers and Caron, 1998; Czarnes et al., 2000; Morel et al., 2017). Our results partially support our hypothesis that DI would increase soil aggregation (MWD), as aggregation was significantly higher under the 65/65 treatment compared to the 100/100 treatment in the 0-20 cm depth. Soil inorganic carbon, which can help to cement soil particles, was higher in the 65/65 compared to the 100/100 treatment and could be at least partly responsible for the observed differences in aggregation between treatments.

Interestingly the mechanism by which DI improved aggregation seem to be lost beyond a medium deficit (65/65). Elevated C inputs from roots and shoots can also improve aggregation (Kong et al., 2005), but we doubt that this mechanism had much of an effect on aggregation in this study because of the limited differences in SOC observed. Further research is needed to understand impacts of DI on soil aggregation formation under different farm management practices and soil types given that these factors are likely to supersede impacts of DI.

2.5 Conclusion

Agricultural management strategies like DI offer important water-saving opportunities, but also affect key belowground properties that contribute to the long-term agroecosystem productivity. Our study indicates that DI has potential to alter C cycling because of changes to shoot and root inputs, water availability, and the size and composition of soil microbial communities. The net impact of DI on SOC, is not fully elucidated by this study, but will be important to monitor because of the important role SOC has on multiple soil functions. Drier soils had fewer beneficial AMF biomarkers, suggesting an unfavorable shift in microbial community composition on DI. Soil aggregation showed relatively minimal effects of DI. Further studies should examine these effects throughout the season and for longer time-frames to further elucidate the complex interactions between plant productivity and soil C cycling. Efforts to optimize water-saving technologies using DI in arid regions can improve some soil quality metrics.

Table 2.1 Mean water applied, aboveground vegetative biomass, total carbon, organic carbon, inorganic carbon, total nitrogen, and aggregation (mean weight diameter) at two depths and within four irrigation treatments. Different lower-case letters indicate significant ($P < 0.05$) differences between treatments within each soil depth, while upper-case letters (in the 40-60 cm depth) indicate significant difference between depths within a treatment.

	Depth (cm)	100/100	65/65	40/80	40/40
		Mean \pm SE			
Total Water Applied (mm)		621	398	434	323
Aboveground Biomass (kg ha ⁻¹)		8905 \pm 254 a	5644 \pm 406 b	4762 \pm 347 c	4767 \pm 210 c
Total Carbon (mg kg ⁻¹ soil)	0-20	910 \pm 20	940 \pm 50	910 \pm 20	940 \pm 50
	40-60	1070 \pm 60	890 \pm 270	810 \pm 140	1050 \pm 180
Inorganic Carbon (mg kg ⁻¹ soil)	0-20	140 \pm 30aA	240 \pm 60 bA	140 \pm 30 aA	240 \pm 40 bA
	40-60	750 \pm 60 B	780 \pm 260 B	490 \pm 140 B	520 \pm 200 B
Organic Carbon (mg kg ⁻¹ soil)	0-20	780 \pm 30 A	700 \pm 30 A	760 \pm 30 A	700 \pm 30A
	40-60	320 \pm 20 B	270 \pm 30 B	320 \pm 20 B	370 \pm 50B
Total Nitrogen (mg kg ⁻¹ soil)	0-20	80 \pm 4A	70 \pm 3 A	80 \pm 4 A	70 \pm 3 A
	40-60	40 \pm 3 B	40 \pm 4 B	40 \pm 3 B	30 \pm 5 B
Soil Aggregation (MWD) (μ m)	0-20	396 \pm 36 aA	512 \pm 104 b	396 \pm 35 aA	488 \pm 66 ab
	40-60	519 \pm 81 B	518 \pm 127	527 \pm 66 B	455 \pm 89

Note: The four irrigation treatments correspond to target percent of maximum crop evapotranspiration (ET) goal during late vegetative and maturity growth stages, respectively: 1) 40/40, targeting 40% of maximum ET during the two stages; 2) 65/65, targeting 65% of maximum ET in the two stages, 3) 40/80, targeting 40% of maximum ET during the late vegetative and 80% during the maturation stage, and 4) 100/100, aiming to apply 100% of calculated ET from planting until maturation.

Table 2.2 Mean values of absolute biomass of PLFA types (nmol PLFA g⁻¹ Soil).

Means of identified biomarkers (n=8)					
Biomarkers	Depth (cm)	100/100	65/65	40/80	40/40
		Mean ± SE (nmol g ⁻¹)			
Saprotrophic Fungi ^b	0-20	8.8 ± 0.4 a	6.5 ± 0.2 b	3.3 ± 0.1 c	3.8 ± 0.2 c
	40-60	1.6 ± 0.2	1.6 ± 0.5	0.6 ± 0.1	1.3 ± 0.2
AMF ^c	0-20	4.2 ± 0.1 a	2.9 ± 0.1 b	1.5 ± 0.1 c	1.6 ± 0.1 c
	40-60	0.6 ± 0.05	0.3 ± 0.04	0.2 ± 0.02	0.4 ± 0.09
Total Bacteria ^d	0-20	40.6 ± 1.3 a	30.9 ± 1.2 b	16.6 ± 0.6 c	19.2 ± 0.8 c
	40-60	9.5 ± 0.8 a	6.4 ± 0.8 ab	4.1 ± 0.4 b	7.6 ± 1.4 ab
Iso and Anteiso ^e	0-20	15.3 ± 0.5 a	11.5 ± 0.4 b	6.4 ± 0.2 c	7.3 ± 0.3 c
	40-60	3.5 ± 0.3 a	2.3 ± 0.3 ab	1.6 ± 0.2 b	2.8 ± 0.5 ab
10-Methyl ^f	0-20	9.9 ± 0.3 a	7.8 ± 0.3 b	4.20 ± 0.1 c	5.0 ± 0.2 c
	40-60	3.0 ± 0.3 a	1.9 ± 0.3 bc	1.2 ± 0.1 c	2.3 ± 0.5 ab
Cyclopropyl ^g	0-20	6.8 ± 0.2 a	5.1 ± 0.2 b	2.7 ± 0.1 c	3.3 ± 0.2 c
	40-60	1.5 ± 0.1 a	1.1 ± 0.1 ab	0.7 ± 0.1 b	1.2 ± 0.2 ab
General ^h	0-20	21.1 ± 0.5 a	15.7 ± 0.6 b	8.8 ± 0.2 c	10.3 ± 0.4 c
	40-60	4.7 ± 0.2 a	4.5 ± 0.4 a	2.4 ± 0.2 b	4.6 ± 0.6 a
Total Biomass ⁱ	0-20	74.7 ± 2.2 a	56.0 ± 2.1 b	30.3 ± 1.0 c	35.0 ± 1.4 c
	40-60	16.4 ± 1.0 a	12.9 ± 1.4 ab	7.3 ± 0.6 b	14.0 ± 2.1 a

Note: The four irrigation treatments correspond to target percent of maximum crop evapotranspiration (ET) goal during late vegetative and maturity growth stages, respectively: 1) 40/40, targeting 40% of maximum ET during the two stages; 2) 65/65, targeting 65% of maximum ET in the two stages, 3) 40/80, targeting 40% of maximum ET during the late vegetative and 80% during the maturation stage, and 4) 100/100, aiming to apply 100% of calculated ET from planting until maturation. Different letters indicate significant (P <

0.05) differences between treatments within each soil depth. All differences between the 0-20 and 40-60 cm depth are significant within treatment. Soils were sampled in September during the maize growth stage R5.

^aSaprotrophic fungi fatty acids: Sum of 18:2w6c and 18:1w9c (Frostegård et al. 2010 and Kaiser et al. 2010).

^bArbuscular Mycorrhizal fungi (AMF) fatty acid: Sum of 16:1w5c (Olsson et al. 1997).

^cTotal Bacteria fatty acids: Sum of general bacterial fatty acids (15:0, 17:0, i17:0, 16:1w7c and 16:1w9c) in addition to Iso and Anteiso, 10-Methyl, Cycloprophyl fatty acids. (Frostegård and Bååth 1996)

^dIso- and Anteiso-fatty acids (gram positive): Sum of i14:0, i15:0, a15:0, i16:0, and a17:0 (Kaneda 1991 and Zelles 1999).

^e10-Methyl fatty acids (actinomycetes): Sum of 10Me16:0, 10Me17:0, and 10Me18:0 (Kroppenstedt 1992 and Zelles 1999)

^fCycloprophyl fatty acids (gram negative bacteria): Sum of cy17:0w7 and cy19:0w7c (Zelles 1999)

^gGeneral PLFAs (widespread among organisms): 16:0, 18:0, 20:0 and 18:1w7c (Bossio et al. 1998)

^hTotal Biomass: Sum of Saprotrophic fungi, AMF, total bacterial, and general PLFAs.

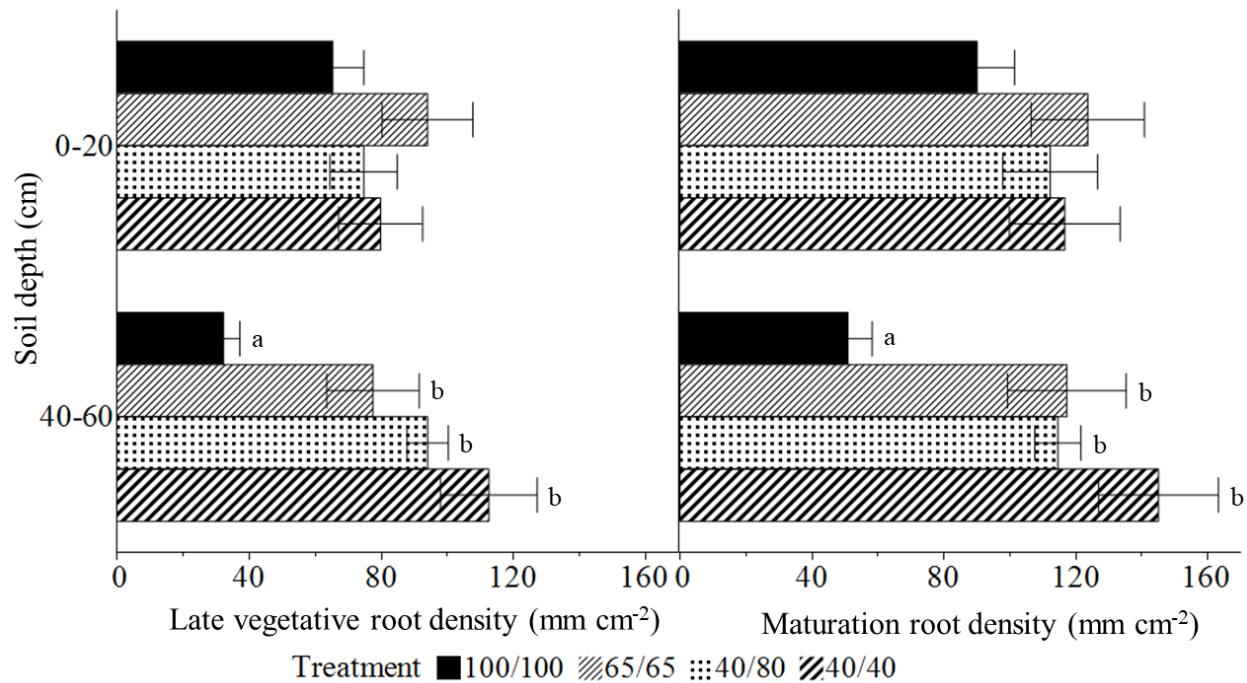


Figure 2.1 Maize root growth per minirhizotron camera viewing area (mm cm^{-2}). The four treatments correspond to target percent of maximum crop evapotranspiration (ET) during late vegetative and maturity growth stages, respectively: 1) 40/40, targeting 40% of maximum ET during the two stages; 2) 65/65, targeting 65% of maximum ET in the two stages, 3) 40/80, targeting 40% of maximum ET during the late vegetative and 80% during the maturation stage, and 4) 100/100, aiming to apply 100% of calculated ET from planting until maturation. Different letters indicate significant difference ($P < 0.05$) between treatments within depth. Error bars represent the standard error of the mean.

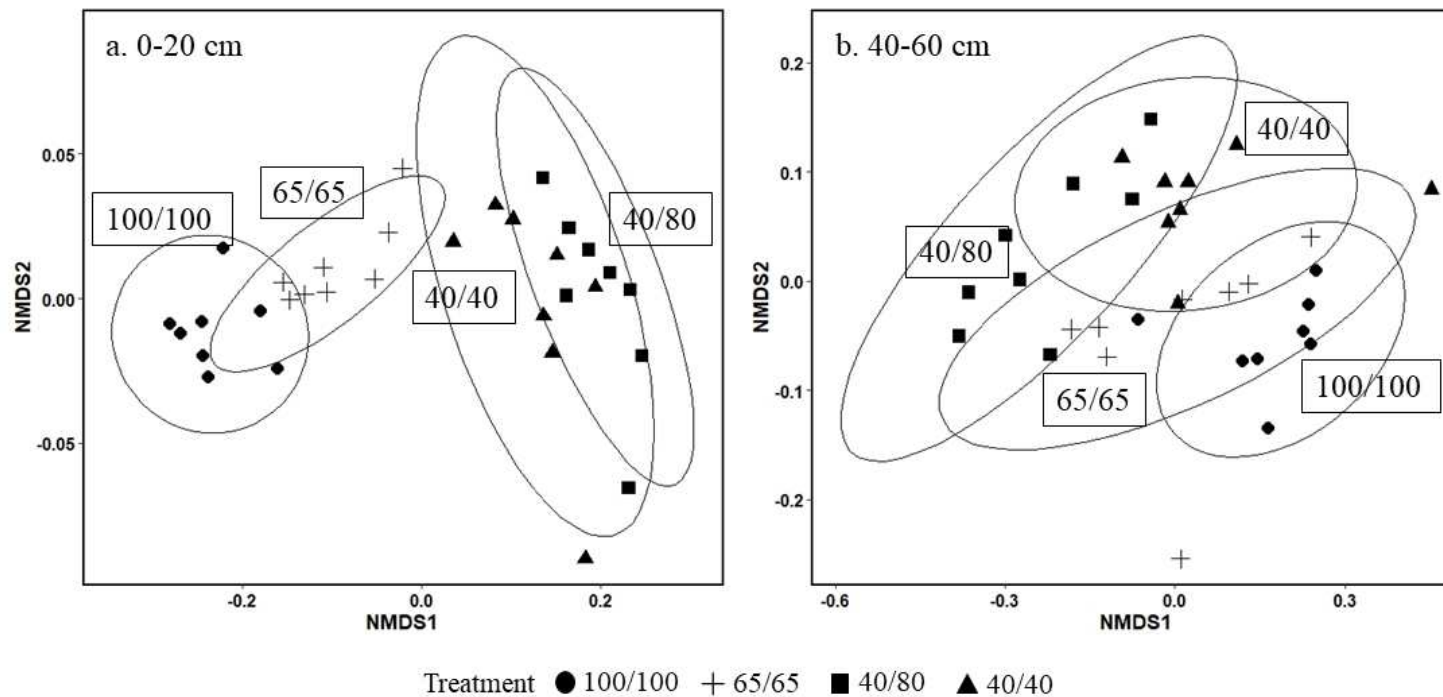


Figure 2.2 Analysis of microbial community composition using Non-metric Multidimensional Scaling (NMDS) with PLFA extracted from soil samples collected from 0-20 cm (panel a) and 40-60 cm (panel b) soil depth from four different irrigation treatments. The four treatments correspond to target percent of maximum crop evapotranspiration (ET) during late vegetative and maturity growth stages, respectively: 1) 40/40, targeting 40% of maximum ET during the two stages; 2) 65/65, targeting 65% of maximum ET in the two stages, 3) 40/80, targeting 40% of maximum ET during the late vegetative and 80% during the maturation stage, and 4) 100/100, aiming to apply 100% of calculated ET from planting until maturation.

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CHAPTER 3: DEFICIT IRRIGATION IMPACTS ON GREENHOUSE GAS EMISSIONS UNDER DRIP-FERTIGATED MAIZE IN THE GREAT PLAINS OF COLORADO²

3.1 Introduction

Agricultural soils are a major source of anthropogenic greenhouse gas (GHG) emissions, and thus of key interest to mitigate climate change (Davidson et al., 2014). Crop production in the Great Plains of the US is vulnerable to higher temperatures, altered precipitation patterns (Wienhold et al., 2018; Kukul and Irmak, 2018), rising municipal and industrial water demand, and groundwater depletion (Warziniack and Brown 2019; Scanlon et al. 2012). However, agricultural management practices that precisely apply water and N fertilizer can both increase crop water productivity (CWP) and reduce agricultural contributions to GHG emissions (Lipper et al. 2014).

Regulated deficit irrigation (DI) applies irrigation below evapotranspiration (ET) demands of the crop at targeted growth stages, which can reduce irrigation water use with minimal yield losses (Comas et al., 2019, Fereres & Soriano 2007). For example, Comas et al. (2019) found that partial-season DI for maize applied during the late vegetative stage followed by full irrigation (FI), or nearly FI during the rest of the season, consistently resulted in yields similar to FI, while saving 15-17% in water use via ET compared to FI. Regulated DI is therefore a promising strategy for continued crop production in drier climates or agricultural systems with limited irrigation supplies.

² Flynn, N.E., Stewart, C.E., Comas, L.H., Del Grosso, S.J., Schnarr, C., Schipanski, M.E., von Fischer, J.C., Stuchiner, E.R., Fonte, S.J., in press. Deficit irrigation impacts on greenhouse gas emissions under drip-fertigated maize in the Great Plains of Colorado. JEQ.

Reduced soil moisture under DI is likely to decrease GHG emissions compared to FI, as soil microorganisms are responsible for most GHG and are sensitive to changes in soil moisture and its subsequent impacts on soil temperature and redox status. Specifically, the respiration of carbon dioxide (CO₂) by soil microbes and plant roots, and loss of nitrous oxide (N₂O) from the nitrification pathway tends to increase with soil moisture up to about 60% of water-filled pore space (WFPS) (Bateman & Baggs, 2005; Zhu et al., 2013). Above this threshold, anaerobic conditions begin to limit CO₂ respiration and nitrification and begins to favor denitrification, where N₂O is also a byproduct (Linn and Doran 1984). A meta-analysis on drought manipulations found that reducing precipitation lowered (N₂O) emission in 37 field studies across different biomes (Homyak et al 2017). Deficit irrigation uses less water and will therefore create a drier soil environment during portions of the growing season, yet, the reduction of GHG emissions under DI remains poorly studied.

How irrigation and fertilizer are applied to the field also impacts GHG emissions. Drip fertigation enables doses of water and fertilizer to be timed with plant demand and applied close to the root zone which can increase N uptake and reduce N losses to the environment. As a result, drip irrigation has been shown to reduce N₂O emissions in comparison to sprinkler and furrow irrigation (Guardia et al. 2017; Sanchez-Martin et al., 2010). While emissions from drip irrigation under select vegetable crops has been measured, emissions from drip-irrigated maize have seldom been quantified (Guardia et al. 2017; Tian et al. 2017) and therefore the impact of drip-irrigated maize on GHG emissions is still poorly understood (Sapkota et al. 2020).

Deficit irrigation and drip fertigation have great potential to reduce GHG emissions, but their effectiveness has not yet been measured in a maize system. The goal of this study was to quantify and compare the GHG emissions from DI and FI in a drip-fertigated maize system in

the Great Plains region of Colorado. We tested the hypotheses that: a) DI would decrease GHG emissions and that b) DI based on growth stage would minimize yield impacts of reduced irrigation and result in increased CWP and reduced yield-scaled emissions compared to FI.

3.2 Methods

Study site and experimental design

This study was conducted at the USDA-ARS Limited Irrigation Research Farm, located near the city of Greeley in Northern Colorado, USA (40° 26' 57" N, 104° 38' 12" W) at an elevation of 1427 m. This site receives 335 mm of rainfall annually on average, 75% of which falls from May to October. During the growing season (May to October), average maximum and minimum daily temperatures are 27 and 10 °C, respectively. Soils at this site have been managed for 100+ years and are dominated by Olney fine sandy loam with Otero sandy loam in some areas, with an average pH of 7.8 (Trout & Bausch, 2017) and topsoil soil organic carbon (SOC) concentration of < 1%.

The plots used in this study were established in 2011 as part of an experiment to examine the effects of DI in a maize (*Zea mays* L.) – sunflower (*Helianthus annuus* L.) rotation under surface drip irrigation. Each treatment was replicated four times in a randomized complete block design. Individual treatment plots were 9 m wide and 44 m long, containing 12 rows of maize that were spaced 0.76 m apart. Fields were managed using strip-tillage. Approximately 25% of crop residue was retained with the remainder carried away by wind. After seedling emergence each year, surface irrigation drip lines were installed next to each maize row.

To study the impact of DI on GHG emissions we measured N₂O and CO₂ emissions from two DI treatments and FI. Data collection took place in 2014 and 2016, during the maize phase of the rotation. Planting and harvest occurred in the first week of May and October, respectively,

in both years. We attempted to create a gradient with extreme, moderate, and full ET treatments in each year. Target ET levels determined using a water balance approach (Comas et al., 2019). Compared to total water applied, ET is more easily comparable across seasons and environments. In 2014, the large amount of precipitation limited our ability to control the irrigation treatments and resulted in only slight DI. The irrigation treatments are thus labeled based on the actual ET achieved by each treatment during the late vegetative and late maturation growth stages (Table 3.1). To avoid impacts on yield, full ET was supplied during the more sensitive early vegetative and reproductive growth stages in all the treatments in both years. Irrigation was applied weekly, or as-needed, and amounts were determined throughout the season based on target ET levels and accounted for soil water availability. There is very little leaching in this drip irrigated system since irrigation amounts are adjusted based on precipitation amounts and soil moisture is measured a least twice a week with neutron probe measurements. Precipitation and air temperature were measured by an on-site weather station.

A starter fertilizer of 17 kg ha⁻¹ N fertilizer (urea ammonium nitrate, UAN 32%) was applied at planting in both years. Five split-applications, about 25-30 kg ha⁻¹, of UAN fertilizer were applied via drip fertigation from V5 to V15. The total amount of N applied to the irrigation treatments differed because of high nitrate concentrations in the irrigation water (Table 1). The difference in N applied between treatments was amplified in 2016 when there was higher dependence on the irrigation water and less precipitation.

GHG sampling

The type of gas sampling chamber used in the two years differed due to equipment availability. In 2014, rectangular aluminum anchors (80.5 cm x 43 cm, 0.346 m²) were placed across the row to integrate row and inter-row space with a single chamber per field replicate. In

2016, two 20.3 cm diameter circular polyvinyl chloride anchors were staggered in the row to best capture the same spatial variability as the previously used chamber. One was placed 3.5 cm from the drip line, while the other was placed directly in the middle of the interrow space. Both types of anchors were inserted to a depth of 10 cm. Lids with an airtight gasket were placed on top of anchors at the time of GHG sampling.

Measurement of trace gas emissions were divided by growth stage in 2014 as follows: the early vegetative stage (planting - V5) was 6/10/14 - 6/16/14, the late vegetative stage (V5-VT) was 6/17-14 - 7/30/14, the reproductive stage (VT- R2) was 7/31/14 - 8/14/21 and the maturation stage (R2-R6) was 8/15/14-9/25/21. In 2016, emissions were divided by growth stage as follows: the early vegetative stage (planting - V5) was 5/7/23- 6/23/16, the late vegetative stage (V5-VT) was 6/24/16 - 7/24/16, the reproductive stage (VT- R2) was 7/25/16-8/11/16 and the maturation stage (R2 - R6) was 8/12/16-9/29/16. Sampling began one month earlier in 2016 compared to 2014 in an effort to observe more of the early season emissions. Samples were collected approximately twice a week during periods of frequent irrigation and less often when fertigation ended (exact dates are points on Fig. 1 and 3). Gas samples were taken in the morning between 8:00 h and 12:00 h, to approximate average daily flux and minimize effects of diurnal variation (Nichols et al., 2016; Mosier 1981). During GHG sampling, chambers were airtight-sealed and gas samples were collected 4 times at 15 min intervals (including time 0 min), for a total of 45 minutes of deployment. Samples were collected with 35 mL polypropylene syringes and immediately transferred to a 12 mL evacuated glass exetainer fitted with a screw cap and rubber butyl septum (Exetainer vial from Labco Limited, High Wycombe, Buckingham-shire, UK). Internal chamber temperature was measured using thermocouple wires installed in chamber lids with an airtight seal and used to calculate gas concentrations. Samples were analyzed within two

weeks of collection with an automated gas chromatograph equipped with an electron capture detector (Varian model 3800, Varian Inc., Palo Alto, CA).

Flux Calculation

Because N₂O and CO₂ emissions were low, we used linear regression of gas concentration vs. time to estimate gas flux. Linear regression has been shown to help avoid over estimation of fluxes when emission rates are low (Nichols et al., 2016; Parkin & Venterea, 2010).

To compare the effect of DI on GHG emissions, cumulative N₂O and CO₂ emissions for each growth stage (early and late vegetative, reproduction, and maturation) were calculated separately. To compare the season cumulative effect of DI management, cumulative emissions were calculated for the 2014 (6/10/2014-/10/14/2014) and 2016 (4/21/2016 - 10/11/2016) season. Cumulative fluxes for each individual soil chamber were calculated from the sum of measured and interpolated values of daily fluxes. Interpolated values for non-measured days were determined by linear interpolation using this equation from Hoefft et al. (2012):

$$\text{Flux} = F1 + (F2-F1/D2-D1)$$

Where F1 is the measured gas flux on the closest day before the day which requires interpolation, F2 is the measured gas flux on the closest day after the date of the computed flux. D1 is the day of the growing season on which F1 was measured, D2 is the day of the growing season on which F2 was measured. Total CO₂ equivalents were calculated as the sum of CO₂ and N₂O emissions converted to their CO₂ equivalent. To calculate the CO₂ equivalent of N₂O emissions, N₂O emissions were multiplied by 298 based on the 100-year global warming potential of N₂O (IPCC, 2006; Millar et al., 2010).

Soil moisture, temperature and N measurements

Soil moisture, temperature and N concentration were paired with each chamber location in 2016 to evaluate drivers of GHG emissions. Volumetric water content at each chamber location was measured at the time of gas sampling at a depth of 6 cm with a Decagon GS3 soil moisture sensor and used to calculate water-filled pore space (WFPS = volumetric water content/soil porosity). Soil temperature data was collected at each chamber location at the time of gas sampling with a 10 cm temperature probe. Soil samples for soil nitrate and ammonium analysis were collected on five dates (May 5th, May 11th, June 15th, July 14th and August 16th) during the 2016 growing season. For soil nitrate and ammonium sampling, four soil cores from each plot (16 plots total) were collected to a depth of 10 cm, near the anchors, at locations equivalent to the center of each anchor (19 and 38 cm from the maize row). Soil nitrate and ammonium content was determined by extracting 5 g of homogenized fresh soil with 25 mL of 1 M KCl solution. Nitrate and ammonium concentrations were measured with colorimetry on a Shimadzu UV-Vis Spectrophotometer.

Plant Sampling

At plant maturity (R6), the average aboveground dry biomass (kg ha⁻¹) of each treatment was determined by averaging the shoot dry weight of five plants from each plot and multiplying by stand density (plants ha⁻¹). Yield was determined by collecting ears via hand harvest in a yield area 2 rows wide 5 m long that was within the gas sampling plots but did not disturb the gas sampling area. Grain was removed from ears, weighed and adjusted to 15.5% moisture, and divided by harvest area to determine yield on kg ha⁻¹ basis.

Calculations

Crop water productivity (CWP) of each irrigation treatment was calculated as:

$$CWP = \text{kg maize grain mm}^{-1} ET$$

Emissions factor is the ratio of N₂O emissions to total N fertilizer applied. Normally, emissions factor (EF) is calculated by subtracting the emissions of a control treatment from the treatment emissions. Because this experiment didn't have a 0 N treatment, a modified emissions factor was calculated as:

$$EF = (kg\ N_2O\text{-}N\ ha^{-1}\ season^{-1} / kg\ N\ applied\ season^{-1}) * 100$$

where kg N applied is the total of N fertilizer applied and NO₃⁻ inherently present in irrigation water.

The impact of irrigation and fertilizer management on GHG emissions must also be weighed against yield impacts because nutrient and water stress can constrain plant growth. Yield-scaled emissions allow for the assessment of yield and GHG emission trade-offs.

Yield-scaled emissions were calculated as:

$$Yield\text{-}scaled\ emissions = kg\ N_2O\text{-}N\ ha^{-1} / Mg\ grain\ ha^{-1}$$

Statistical analysis

Data for the two years were analyzed independently because precipitation and treatments were different between years, rather than using time as a random effect in our analysis. Treatment effects on cumulative N₂O and CO₂ emissions were assessed using separate analysis of variance (ANOVA) for each of the four growth stages and season total. The N₂O and CO₂ emissions data were checked for normality and homogeneity of variance and adjusted as needed with log transformations to meet ANOVA assumptions. Within year treatment differences of measured (yield, biomass) and calculated variables (cumulative emissions, CWP, EF, Yield-scaled emissions) were analyzed using ANOVA, and post hoc comparisons (Tukey-Kramer HSD test) were used to correct for multiple comparisons. Differences were considered significant if $P \leq 0.10$. The relationship between daily N₂O and CO₂ emissions across treatments with soil

temperature, WFPS, $\text{NH}_4^+\text{-N}$, and $\text{NO}_3^-\text{-N}$ concentration was tested using Pearson's correlations. All analyses were performed using R version 4.0.3.

3.3 Results

Environmental conditions

The 2014 growing season began with a wet spring and a total of 301 mm of precipitation from May thru October (Table 3.1). The 2016 growing season was drier with 165 mm precipitation received May thru October. Both seasons had yield-diminishing weather events (early freeze in 2014 and hail in 2016) that resulted in a 25% yield reduction compared to normal years at this field site. Average maximum and minimum air temperatures from May to October were 26.0 and 8.6 °C, and 26.9 and 8.8 °C in 2014 and 2016, respectively. In 2016, when drivers of ghg production were measured, soil WFPS varied widely, from 15-75%, and was between 30% and 65% for most of the season in all the treatments.

N₂O emissions

For both years, season total N₂O emissions were reduced one third to half by DI compared to FI (Table 3.2). Three-quarters of season total N₂O emissions occurred during the early and late vegetative growth stages. The largest difference in N₂O emissions between DI and FI occurred during the late vegetative growth stage. Nitrous oxide emissions rates were between 0 and 50 $\mu\text{g N m}^{-2} \text{ hr}^{-1}$ for all measured dates except for two dates in 2014 that had high peaks above 90 $\mu\text{g N m}^{-2} \text{ hr}^{-1}$ (Fig. 3.1 and 3.2). Soil temperature and soil $\text{NO}_3\text{-N}$ concentration had the strongest correlations with daily N₂O emission rate (Table 3.3). Soil temperature in 2016 had a positive relationship that explained 12% of the variation in daily N₂O emission rate. Soil $\text{NO}_3\text{-N}$ concentration, measured in 2016, had a negative relationship that explained 28% of the variation in daily N₂O emission rate.

CO₂ emissions

Season total CO₂ emissions were reduced 30% by extreme DI in 2016 but not by moderate or mild DI in 2014 (Table 3.2). Two thirds of season total CO₂ emissions occurred during the early and late vegetative growth stages. Extreme DI reduced CO₂ emissions compared to FI in all 2016 growth stages but only significantly in the 2016 early vegetative stage and the 2014 reproductive stage. Soil CO₂ emissions varied between 10 and 140 mg C m⁻² hr⁻¹ over the course of the 2014 and 2016 growing seasons (Fig. 3.1 and 3.2). Extreme DI reduced cumulative CO₂ equivalents (CO₂ and CO₂ equivalents of N₂O summed) by 30% compared to FI in 2016. Correlation analyses showed that soil temperature had a significant, positive relationship that explained 32% of the variation in daily CO₂ emissions (Table 3.3).

DI impact on yield, crop water productivity, EF, and yield-scaled emissions

In 2016, moderate and extreme DI reduced maize yield by 30% and 32%, respectively, and reduced vegetative biomass by 38% and 45%, respectively (Table 3.4), while in 2014, the relatively mild levels of DI achieved did not significantly impact yield or biomass. Crop water productivity (kg grain mm⁻¹ ET) was similar among the three irrigation treatments within each year but was higher in 2014 compared to 2016. Emissions factor and yield-scaled emissions were significantly decreased by mild DI in 2014 (Table 3.4). Mild DI reduced the EF by an average of 34% and yield-scaled emissions (kg N₂O-N Mg⁻¹ grain) by an average of 50%. There was no difference in EF or yield-scaled emissions between treatments in 2016.

3.4 Discussion

Summary

We found that DI reduced N₂O and CO₂ emissions in an irrigated maize system depending on the level of DI imposed and annual weather. In comparison to FI, mild DI reduced

N₂O emissions by 50% in 2014 and extreme DI reduced CO₂ emissions by 15% in 2016.

Interestingly, total N₂O emissions, EF, and yield-scaled emissions from this high-frequency surface drip fertigation system were 1/10 of those observed in other maize systems with sprinkler irrigation systems (Adviento-Borbe et al. 2007, Halvorson et al. 2016, Jin et al., 2017).

Deficit irrigation reduced N₂O and CO₂ emissions

Deficit irrigation reduced total N₂O emissions in both years of study. This confirms our hypothesis and previous findings that limited irrigation reduces N₂O emissions from maize systems (Liu et al., 2011; Ning et al., 2019; Tian et al., 2017). In this experiment, it's difficult to discern if the reduction in N₂O is solely due to DI because the varying N levels create a confounding effect. However, comparing the effect of irrigation on EF (the percentage of fertilizer N applied that is emitted on-site as N₂O) offers a way to account for this complication and is an important metric for comparing agricultural practices (Lesschen et al., 2011; Lokupitiya & Paustian, 2006) In 2014, DI reduced total N₂O emissions *and* EF so we may more strongly conclude that DI impacted N₂O producing processes. However, in 2016, DI reduced total N₂O emissions but *not* EF. In this case, we cannot be certain if the difference in total N₂O emissions is due to DI or due to the difference in total N applied. Another caveat is that background N₂O emissions are likely different due to irrigation treatment but our experiment did not include a zero N treatment. The inconsistent result between years is notable because mild DI reduced N₂O emissions in 2014 but the effect of more extreme DI in 2016 is less clear. However, GHG emissions are known to be highly variable between years (Philibert et al., 2012; Roelandt et al., 2005). In addition, N₂O emissions were also likely impacted by differences in weather between years wherein 2014 received more precipitation than 2016. Despite the variability

caused by weather and N application in this study, differences in EF support the conclusion that DI management can reduce N₂O emissions.

Deficit irrigation reduced CO₂ emissions in the second year of study and appear to be indirect, wherein the reduction in CO₂ under DI management was offset from the periods during which DI was imposed. Specifically, in 2016, CO₂ emissions were significantly lower under extreme DI compared to FI during the early vegetative stage. While there was no difference in water applied during this stage, the difference in CO₂ emissions could be due to the difference in C substrate availability. The quantity of biomass produced has been shown to be a key determinant of CO₂ emission in maize systems (Guo et al., 2021; Han et al., 2007). Typically, FI produces more shoot biomass than DI, and assuming that maize stover is retained in the field, FI, therefore, provides more substrate for microbial respiration when spring moisture and higher temperatures return. Differences in available soil C were likely greater in 2016 than in 2014 due to more time in the experiment. Root growth, the decomposition of roots, and root exudates also contribute to CO₂ emissions (Ding et al., 2007) but this study was not set up to differentiate sources of CO₂. Overall, we may conclude that DI can reduce CO₂ emissions, but similar to N₂O emissions, the effect is variable annually and may be especially sensitive to the amount of carbon substrate available which can change with the length of time DI treatments have been in effect.

Despite irrigation treatments impacting total GHG emissions, WFPS was not significantly related to either N₂O or CO₂ daily emissions. This is a common finding in GHG studies because even in relatively dry soils, soil moisture and redox status of the soil microenvironment can vary substantially in space and time and obscure the relationship between GHG emissions and WFPS with measurements in the field setting (Sihi et al., 2020). Not surprisingly, soil N₂O and CO₂ were both positively correlated with increasing soil temperature

since microbial processes that produce N_2O and CO_2 are known to be limited by cool soil temperatures (Singh and Gupta, 1977; Smith et al., 1998). Soil moisture, and temperature can also have synergistic and antagonistic effects that impact GHG emissions which we did not analyze here.

Typically, N applied also has direct impact on N_2O emissions. Ammonium and nitrate, the main components of inorganic fertilizer are the key substrate for nitrification and denitrification pathways, respectively. However, in this study, there was a negative correlation between N_2O emissions and soil NO_3^- -N concentration. This is in contrast to most studies where N_2O emissions increase with soil NO_3^- -N concentration (Abalos et al., 2014, Smith et al., 1998). The minimal or negative relationship between N_2O emissions and NO_3^- -N in this study could be a superficial phenomenon but suggests N_2O emissions were controlled more by other abiotic interacting factors such as labile carbon, soil moisture, soil pH, or soil temperature (Pilegaard et al., 2006; Schindlbacher et al., 2004; Zhang et al. 2021). In addition, the dry soil conditions in the DI plots could have resulted in the accumulation of soil NO_3^- -N in the DI plots since DI could have reduced plant N uptake (Pandey et al., 2000) and limited microbially-mediated N mineralization processes (Barakat et al., 2016). A buildup of NO_3^- -N in the DI plots paired with lower N_2O emissions would have contributed to the negative correlation between NO_3^- -N and N_2O emissions.

Impact of deficit irrigation on crop water productivity and yield-scaled emissions

The goal of DI is to maintain high yields while reducing irrigation water use. Where producers are not limited by pumping capacity or timing of irrigation, DI based on growth stage, which was used in this study, is preferred over continuous DI throughout the growing season to avoid water-stress during the growth stages that most impact pollination and grain filling (Comas

et al., 2019; Zhang et al., 2019). This growth-stage based DI implementation has been shown to maintain yields at a similar level to FI while increasing CWP (Comas et al., 2019). In this study, only the mild DI achieved in 2014 maintained yield at the same level as FI, while moderate and extreme DI in 2016 resulted in significant yield reductions. Mitigating yield impacts with growth-stage based DI is challenging because maize is very sensitive to water stress (Payero et al., 2006). Growth-stage based DI relies on plants to compensate for drought during the early season with water applied later in the season but the freeze and hail that occurred in 2014 and 2016, respectively could have hampered yield recovery. The extent of irrigation water savings was largely dependent on annual precipitation and amount of irrigation needed to meet crop ET targets. For example, irrigation water applied was about a third higher in 2016 compared to 2014 due to differences in annual precipitation. However, in terms of ET, or consumptive water use, there was not a large difference between 2014 and 2016. Mild DI resulted in small ET savings with little impact on yield resulting in an increased CWP under DI compared to FI, though not significantly different. However, moderate and extreme DI created greater ET savings that were accompanied by roughly proportional reductions in yield resulting in little to no benefit for CWP under DI compared to FI. Our results demonstrate the sensitivity of corn grain yield to DI, even with strategic growth-stage-based deficits, which may assist irrigation water managers to make decisions about water use in relation to yield goals. Finding the right level of DI that improves CWP in comparison to FI will depend on ET-based irrigation scheduling tools that utilize local climate conditions, annual weather, and ability to precisely manage the timing of deficits.

Yield-scaled emissions are a useful metric for evaluating the tradeoff between GHG emissions and food production. It is desirable to simultaneously reduce agriculture's impact on the environment (by decreasing GHG emissions) and maintain or increase yield to meet rising

food demand (Horton et al., 2021; Robertson & Vitousek, 2009). In this study, only mild DI reduced yield-scaled emissions. Moderate and extreme DI also decreased N₂O emissions but were accompanied by decreased yield. This is not surprising given that yield-scaled emissions are often lowest when crops were grown close to their yield potential, when water and N needs are met, but not applied in excess (van Groenigen et al. 2010).

Low emissions from surface drip-fertigated maize system

The use of high-frequency drip fertigation can reduce soil GHG emissions compared to more common types of irrigation, such as sprinkle or furrow, because 1) N supply becomes more synchronous with plant demand, and 2) soil moisture is reduced both spatially and temporally, both of which limit soil microbial processes that produce CO₂ and N₂O emissions (Barakat et al., 2017, Bronson et al., 2018, Li et al., 2020, Sanchez-Martin et al., 2008). In this study, a small amount of starter fertilizer N was applied at planting. The rest of the fertilizer was applied via low-dose fertigation, which generates relatively low emissions because N supply is synchronized with plant demand. Water filled pore space varied widely during the growing season in response to the frequent wet-dry cycles of this drip-fertigated system, but long periods of high soil moisture that promote GHG producing processes were rare (Barakat et al. 2016). These low moisture conditions under drip fertigation resulted in lower emissions and especially lower peaks compared to sprinkler irrigated systems (Adviento-Borbe et al. 2007, Halvorson et al. 2016). We found that soil N₂O and CO₂ emissions from this surface drip fertigated maize field were a fraction of the emissions from other maize systems in the region. For example, our N₂O emissions from the FI treatment were one-tenth to one-half of the emissions observed in other sprinkler irrigated maize systems in eastern Nebraska (Adviento-Borbe et al., 2007, Jin et al., 2017). Emissions of CO₂ from our FI were also 50% of that reported from sprinkler irrigated

maize systems (Adviento-Borbe et al. 2007, Halvorson et al. 2016, Jin et al., 2017). The soils at this field site have very low soil C (<1% SOC) in the surface layer (Flynn et al. 2021), which limits our ability to infer the impacts of DI in agricultural settings with higher SOC.

The default EF provided by the Intergovernmental Panel on Climate Change (IPCC, 2007) of this irrigated maize system is 1%, but in this study, our estimates were much lower (0.07 – 0.17 %). The IPCC empirical estimate assumes the emissions are driven entirely by N inputs, and some studies have shown a linear relationship between fertilizer N inputs and N₂O emissions (Grace et al. 2011). However, others have shown the response to be non-linear, with some exhibiting saturating at high levels of fertilizer input and others showing an exponential response (Kim et al., 2013; Philibert et al., 2012). In our system, there was a non-positive relationship between N₂O and soil NO₃⁻ or NH₄⁺ concentration. This provides support for the idea that a generalized EF may not be appropriate for all systems. Irrigation technique or precipitation are among the most important factors controlling the magnitude of EFs and drip-irrigated systems have a high potential for mitigation. For example, a meta-analysis derived a mean EF of 0.51% for drip vs 0.91 for sprinkle irrigated Mediterranean systems (Cayuela et al., 2017). Nitrogen fertilizer supplied via fertigation has also been shown to reduce EFs (Maharjan et al., 2013; Tian et al., 2017). Ecosystem models that take into account more than N inputs, such as DayCent (Del Grosso et al., 2009), which has the capacity to represent more complex plant-soil system N cycling processes, may do a better job, in comparison to the default IPCC method, in this instance.

Irrigation management strategies that impact GHG emissions can also impact yield. Yield-scaled emissions in this drip fertigated maize system were about one tenth of other sprinkler irrigated maize systems in the literature (Guardia et al. 2017, Jin et al., 2017). While

irrigation delivery system was not explicitly considered in our experimental design, our results suggest there may be potential for drip fertigation to reduce GHG emissions.

3.5 Conclusion

Irrigation management that precisely controls the amount, location, and timing of water and N fertilizer application has the potential to minimize GHG emissions. We found that DI is effective at reducing N₂O and CO₂, but that the level of reduction depends on the level of DI imposed and annual weather. Deficit irrigation also reduced ET, or consumptive water use. However, only mild DI improved CWP, while moderate and extreme DI did not improve CWP compared to FI due to impacts on yield. Surface drip fertigation used in this study resulted in very low GHG emissions and EF and had yield-scaled emissions that were only one-tenth of those observed from other sprinkler irrigated maize systems. These results suggest the potential of DI and surface drip fertigation systems to mitigate GHG emissions from irrigated agricultural systems.

Table 3.1 Precipitation, irrigation, total water applied, total evapotranspiration (ET), percent of full ET met during the vegetative and maturation corn growth stages due to deficit irrigation management, and total N applied (as fertilizer and in irrigation water) in the 2014 and 2016 growing season.

Year	Irrigation Treatment	Precipitation (mm)	Irrigation (mm)	Total water applied (mm)	ET (mm)	ET % of full Veg/Mat	N applied (kg ha ⁻¹)
2014	Full	301	349	650	565	100	255
	Mild 1	301	239	540	523	90/90	197
	Mild 2	301	156	457	472	90/80	172
2016	Full	165	549	714	698	100	323
	Moderate	165	323	488	502	69/80	252
	Extreme	165	258	423	459	54/68	205

Table 3.2 Mean total emissions (\pm standard error) of N₂O (g N ha⁻¹) and CO₂ (kg C ha⁻¹) in each growth phase and season total in 2014 and 2016. Season totals for CO₂ equivalents incorporate both N₂O and CO₂ season total emissions. Irrigation treatment differed by level of irrigation applied during the late vegetative and maturation growth stages (see Table 1 for details). Different letters indicate significant differences between irrigation treatments ($P < 0.10$).

Year	Irrigation Treatment	Flux	Early Veg.	Late Veg.	Reproduction	Maturation	Season Total	Season Total
2014	Full	N ₂ O	13 (7) a	432 (44) a	56 (30) a	64 (15) ab	565 (10) a	
2014	Mild 1	N ₂ O	10 (2) a	188 (32) b	24 (8) a	30 (9) b	250 (46) b	
2014	Mild 2	N ₂ O	15 (5) a	179 (32) b	4 (2) a	85 (1) a	283 (36) b	
2016	Full	N ₂ O	90 (6) a	100 (12) a	27 (9) a	30 (5) a	245 (27) a	
2016	Moderate	N ₂ O	91 (9) a	82 (13) a	20 (7) a	18 (4) b	211 (23) ab	
2016	Extreme	N ₂ O	81 (15) a	34 (4) b	23 (5) a	9 (1) b	146 (21) b	
								CO ₂ -C eq.
2014	Full	CO ₂	92 (13) a	820 (25) a	238 (10) ab	302 (2) a	1452 (24) a	1621 (21) a
2014	Mild 1	CO ₂	106 (6) a	776 (43) a	312 (31) a	389 (49) a	1585 (104) a	1660 (93) a
2014	Mild 2	CO ₂	90 (6) a	740 (49) a	212 (37) b	345 (75) a	1387 (155) a	1472 (144) a
2016	Full	CO ₂	509 (36) a	516 (29) a	193 (12) a	301 (25) a	1520 (99) a	1593 (91) a
2016	Moderate	CO ₂	418 (33) ab	392 (132) a	184 (7) a	310 (13) a	1420 (62) a	1483 (55) a
2016	Extreme	CO ₂	296 (35) b	360 (42) a	149 (17) a	256 (26) a	1061 (120) b	1105 (121) b

Table 3.3 Pearson correlation matrix (r values) among measured variables in 2016 at the limited irrigation research farm near Greeley, Colorado. Measured variables include daily N₂O and CO₂ flux data, daily soil moisture (percent water-filled pore space, WFPS), daily soil temperature, and inorganic nitrogen to a 10 cm depth at five points during the growing season. A * indicates significant at $P < 0.10$.

	WFPS	Soil Temperature (°C)	NH ₄ -N (mg kg ⁻¹ soil)	NO ₃ -N (mg kg ⁻¹ soil)
N ₂ O	0.003	0.12*	0.08	-0.28*
CO ₂	0.01	0.32*	-0.11	0.075

Table 3.4 Mean yield, biomass, crop water productivity (CWP, kg grain mm⁻¹ ET), emission factor (EF, kg N₂O-N emissions lost kg⁻¹ N applied *100), yield-scaled emissions (YS emissions, kg N₂O-N Mg⁻¹ grain) for the 2014 and 2016 maize growing season. Irrigation treatments are designated by either full irrigation or severity of deficit irrigation during the late vegetative and maturation stages. Different letters indicate significant differences between irrigation treatments within a year.

Year	Treatment	Yield (kg ha ⁻¹)	Biomass (kg ha ⁻¹)	CWP (kg mm ⁻¹)	Emissions Factor	YS Emissions (kg N ₂ O-N Mg ⁻¹ grain)
2014	Full	11679 (80) a	7506 (352) a	20.7 (0.1) a	0.22 (0.01) a	0.048 (0.001) a
2014	Mild 1	11017 (422) a	6444 (447) a	21.1 (0.8) a	0.13 (0.02) b	0.023 (0.004) b
2014	Mild 2	11111 (172) a	7041 (391) a	23.5 (0.4) a	0.16 (0.02) ab	0.026 (0.004) b
2016	Full	11132 (348) a	5964 (375) a	15.9 (0.5) a	0.08 (0.01) a	0.022 (0.002) a
2016	Moderate	7724 (266) b	3718 (216) b	15.4 (0.5) a	0.07 (0.01) a	0.027 (0.003) a
2016	Extreme	6458 (564) b	3313 (294) b	14.1 (1.2) a	0.08 (0.01) a	0.025 (0.005) a

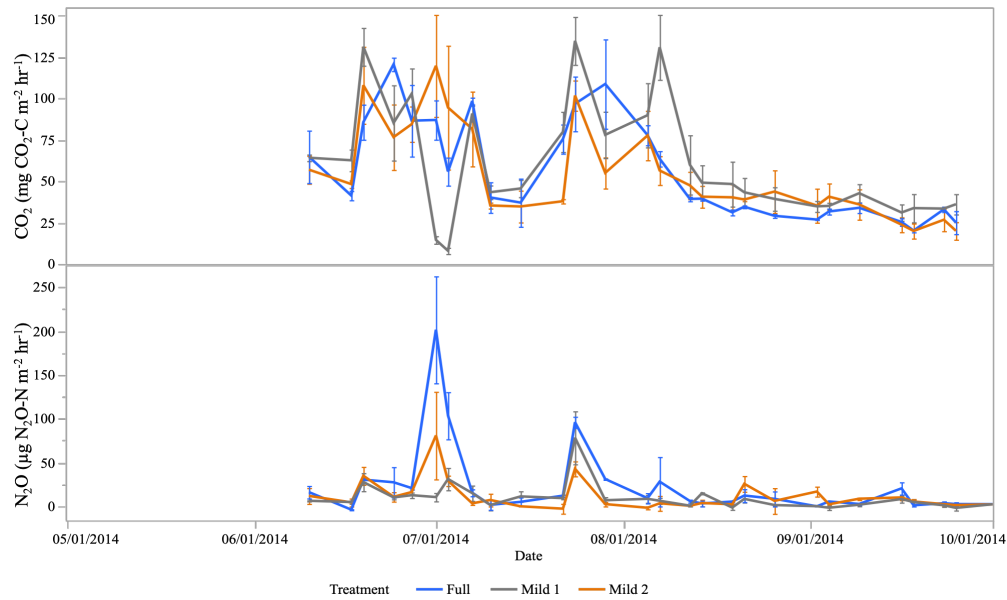


Figure 3.1 N₂O emissions ($\mu\text{g N}_2\text{O-N m}^{-2} \text{hr}^{-1}$), and b) CO₂ emissions ($\text{mg CO}_2\text{-C m}^{-2} \text{hr}^{-1}$) during the 2014 growing season at the limited irrigation research farm near Greeley, Colorado. Irrigation treatments are Full (total evapotranspiration demands met) or deficit irrigation (Mild 1 and Mild 2).

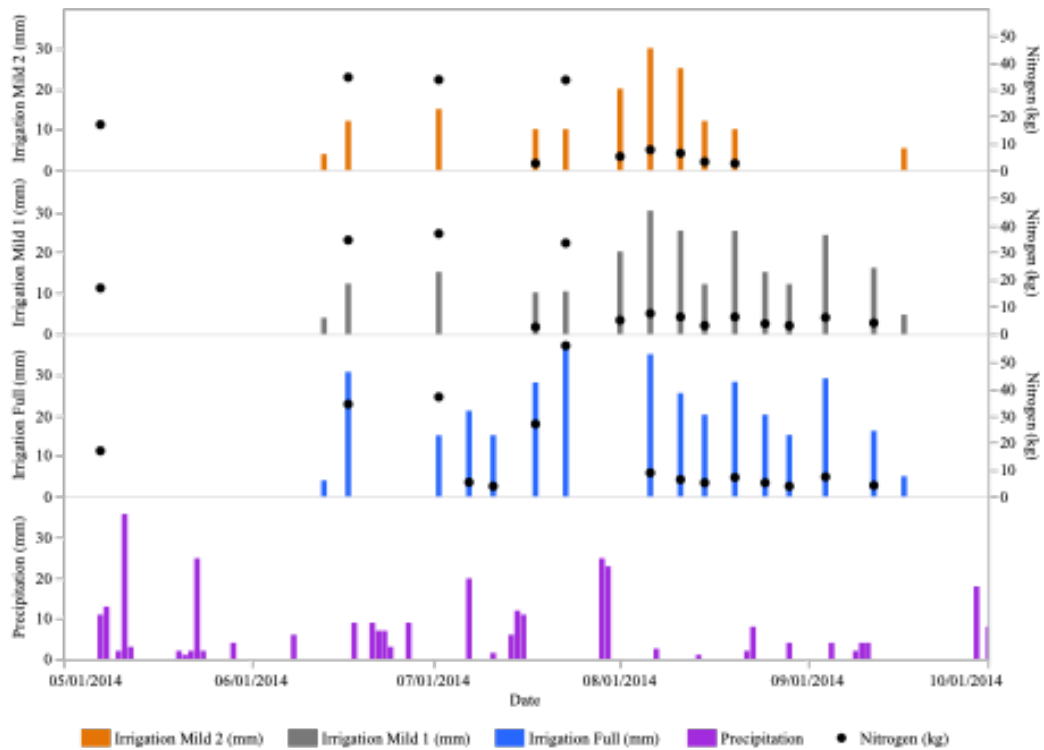


Figure 3.2 Precipitation (mm) and irrigation (mm) denoted by bars and axis on the left and nitrogen (kg) additions denoted by dots with axis on the right. Irrigation treatments are Full (total evapotranspiration demands met) or deficit irrigation (Mild 1 and Mild 2) in 2014 at the limited irrigation research farm near Greeley, Colorado.

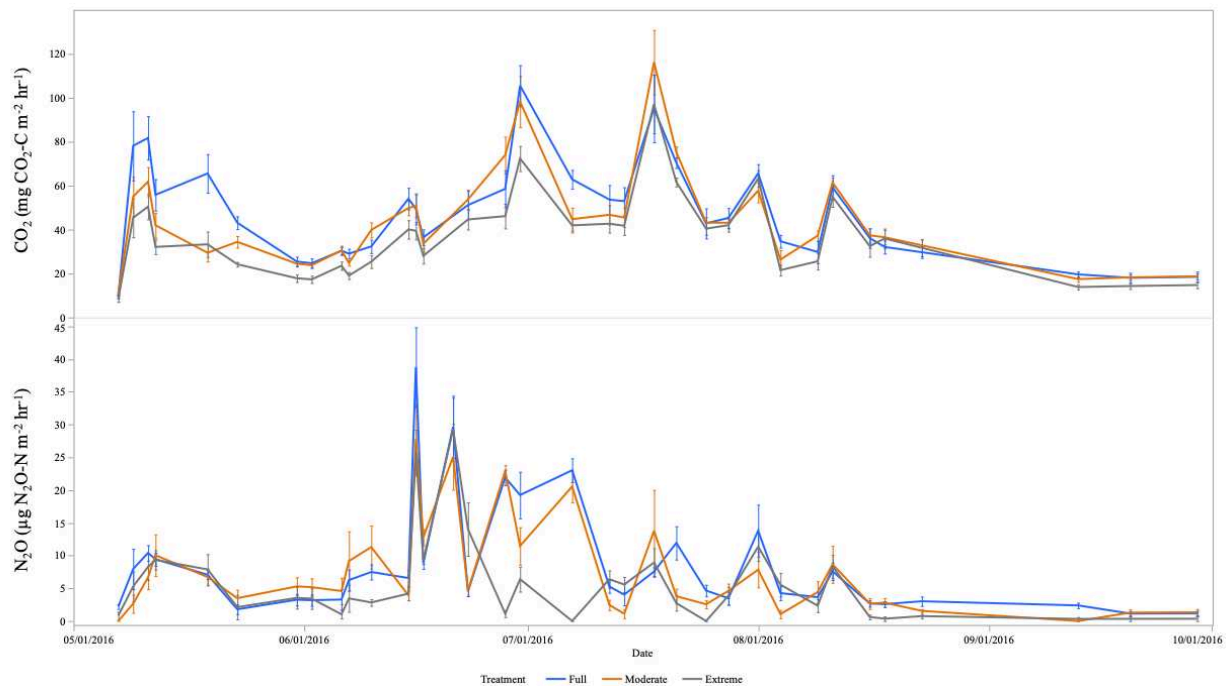


Figure 3.3 N₂O emissions ($\mu\text{g N}_2\text{O-N m}^{-2} \text{hr}^{-1}$) and CO₂ emissions ($\text{mg CO}_2\text{-C m}^{-2} \text{hr}^{-1}$) during the 2016 growing season at the limited irrigation research farm near Greeley, Colorado. Irrigation treatments are Full (total evapotranspiration demands met) or deficit irrigation (Mild 1 and Mild 2).

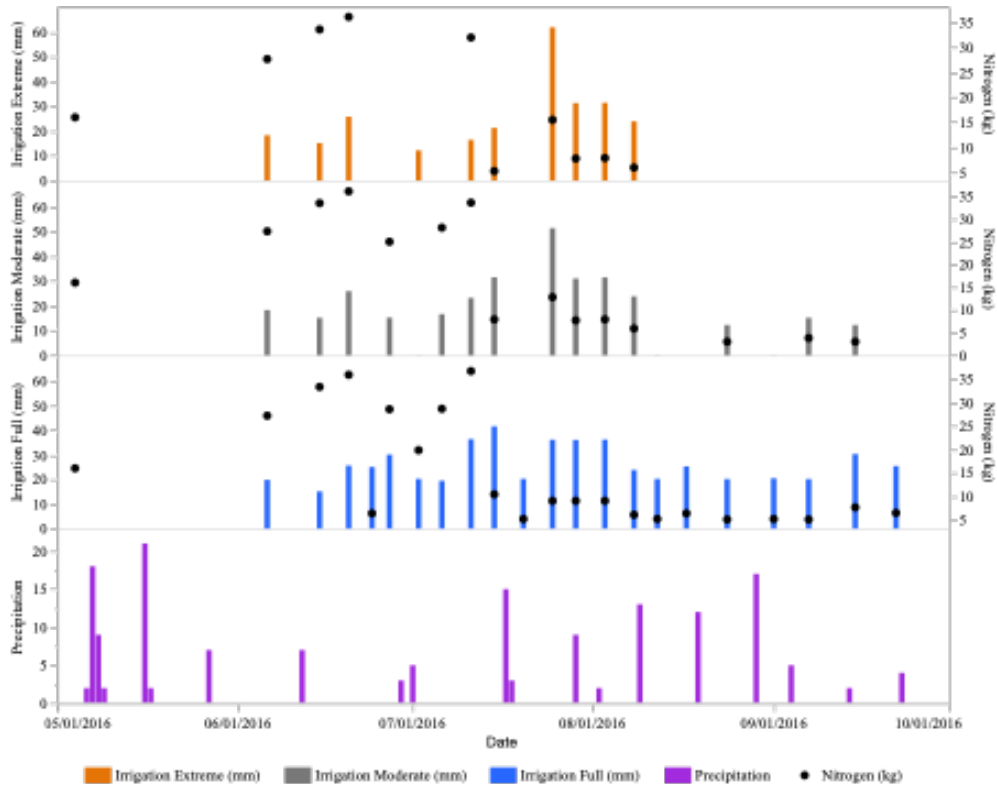


Figure 3.4 Precipitation (mm) and irrigation (mm) denoted by bars and axis on the left and nitrogen (kg) additions denoted by dots with axis on the right. Irrigation treatments are full evapotranspiration demands met (Full) or deficit irrigation (Moderate and Extreme) in 2016 at the limited irrigation research farm near Greeley, Colorado.

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CHAPTER 4: DEFICIT IRRIGATION REDUCED MAIZE N UPTAKE UNDER DRIP IRRIGATION IN A SEMI-ARID SYSTEM

4.1 Introduction

Maize (*Zea mays*) is a globally important crop and is often limited by water and nitrogen (N) supply. In eastern Colorado, maize is the dominant irrigated crop (Derner et al., 2015). However, groundwater depletion, variable snowpack, and increasing urban and industrial water demands are straining water supplies throughout the region (Kukul & Irmak, 2018; Scanlon et al., 2012; Warziniack & Brown, 2019; Wienhold et al., 2018). Decreasing water availability combined with increasing food demand suggests a great need to improve agricultural water use efficiency (Wallace, 2000). In addition, there's a concurrent need to optimize nitrogen (N) inputs due to the high fertilizer costs and multiple negative environmental impacts associated with fertilizer use, including water quality degradation and climate change (Robertson & Vitousek, 2009). Since water and N management challenges frequently co-occur and interact in their impacts on crop growth and the environment, studies that consider these factors simultaneously offer great promise for improving resource use efficiency and overall agricultural sustainability.

Water-saving strategies like deficit irrigation (DI) have the potential to increase water productivity (WP) or yield per unit of water used (Feres & Soriano, 2007; Geerts & Raes, 2009). Improving WP with DI usually relies on regulated or growth-stage based DI wherein the amount of water applied is adjusted depending on the yield-sensitivity of each growth stage rather than even distribution of DI throughout the growing season (Comas et al., 2019; Feres & Soriano, 2007; Geerts & Raes, 2009). For maize, utilizing DI during the vegetative growth stage followed by full irrigation during anthesis and grain-filling stages appears to be the best strategy for improving the amount of grain produced per unit of water applied (Comas et al., 2019). In

practice, maximizing yield with DI requires a great deal of control over the timing and degree of water stress, however, more research is needed to understand the impacts of DI on plants and subsequent effects on the agroecosystem.

The optimal N fertilizer rate under DI irrigation is uncertain because of the interactive effect of water and N on plant growth, plant N demand, and plant N uptake. For example, plants grown under DI management usually have smaller vegetative biomass (Çakir, 2004), which reduces plant demand for N. Belowground, the ability of roots to take up N is dependent on water availability (Ercoli et al., 2008), so lower soil moisture under DI could potentially hinder N uptake. Significant efforts have been made to optimize N fertilizer management of water-limited maize (Ashraf et al., 2016; Eissa & Roshdy, 2019; Hammad et al., 2017; Kirda et al., 2005; Mansouri-far et al., 2010), and findings to date suggest that when farmers cannot meet the total water demand of maize, less N is needed to achieve maximum growth and yield. Given that DI generally decreases vegetative biomass and yield, it is likely that at least somewhat less N is needed to meet maximum yield under DI. However, determining the optimal N application for maize under DI may not be so straightforward, as some studies suggest a positive response of maize grain yield and water use efficiency to additional N applied during water stress (Di Paolo & Rinaldi, 2008; Eissa & Roshdy, 2019; Li et al., 2019; Pandey et al., 2000). Therefore, it's unclear how to optimize the joint reduction in water and N fertilizer rate under DI management.

When evaluating DI impacts on crop growth and N demand, it is critical to understand the benefits of N fertilizer for supporting yield vs. the potential environmental consequences of N loss. Water management plays a vital role in the fate of N fertilizers by altering N movement and transformations in the soil (Barakat et al., 2016; Chilundo et al., 2016; Rimski-Korsakov et al., 2009; Sanchez-martin et al., 2008). For example, soil moisture changes under DI can impact

N₂O producing processes, and DI can decrease N₂O emissions compared to FI (Chapter 3). However, if plants take up less N under DI, the amount of residual N increases which could also lead to greater gaseous losses of N and NO₃⁻ loss from the agricultural field via leaching, which has negative consequences for water quality. Therefore, it is critical to understand DI impacts the fate of N in agroecosystems since DI has the potential to alter the form and size of N losses from the soil.

To improve our ability to predict optimal water and N additions, we established a field experiment where maize was grown under DI and full irrigation (FI) with three N fertilizer application rates. The goals of this study were to: 1) improve our understanding of how DI and N rate influence maize yield as well as water and N productivity, and 2) understand the implications of DI for N movement and loss from soils (above and belowground N uptake, N leaching, and N₂O emissions) in a subsurface drip-irrigated maize system. We hypothesized that 1) DI would increase WP but reduce vegetative biomass so that 2) N uptake would be reduced under DI compared to FI and that less N uptake would result in 3) increased residual soil N paired with greater potential for N loss.

4.2 Methods

Study Site and Experimental Design

This experiment was conducted at the USDA-ARS Limited Irrigation Research Farm near Greeley, in Northern Colorado, USA (40° 26' 57" N, 104° 38' 12" W). The site is located in a semi-arid climate at an elevation of 1427 m and receives, on average, 215 mm of precipitation during the growing season (May – October) and 335 mm annually. Soils are predominately Olney fine sandy loam with Otero sandy loam in small areas, with an average pH of 8.2. The

field used for this study previously had maize, under drip irrigation with uniform irrigation treatment, and was strip-tilled annually.

In 2018, a field experiment with two irrigation and three N fertilizer levels was established to investigate the combined impact of DI and N fertilization rate on maize crop growth and N dynamics (Table 3.1). The DI treatment achieved 75% of crop evapotranspiration (ET) during the late vegetative (~V7-VT) growth stage and 100% of ET for all other growth stages (Figure 3.1). This treatment was selected because it was previously shown to generate significant water savings without reducing yield (Comas et al., 2019). The FI treatment received 100% of ET during the entire growing season. Application rates for N treatments were based on local recommendations for irrigated maize and existing soil N (~20 kg ha⁻¹) and percent soil organic matter in the surface layer (0-30 cm depth) (Davis & Westfall, 2014). Based on this recommendation, a medium rate was set at 200 kg N ha⁻¹, and low and high levels of N application were determined by subtracting or adding ~70 kg ha⁻¹ from this recommended rate. All irrigation and fertilizer treatment combinations were applied within a randomized split-plot design, with each treatment being present in four replicate blocks. Irrigation was applied to the main plots, and N treatments were applied to sub-plots within each irrigation plot. Sub-plots were 9 m wide and 20 m long, each containing 12 rows of maize planted at a density of 84,000 seeds ha⁻¹, with 0.76 m spacing between rows.

Before planting, the field was strip-tilled. An equal amount of starter fertilizer (14 kg N ha⁻¹) was injected into rows with seeds at planting. Subsequent N fertilizer was added by side-dressing at the V4 stage (approximately four weeks after planting) to create fertilizer treatment differences (Table 3.1). Groundwater used for irrigation at the site contains 30 ppm NO₃⁻, so additional side-dressed fertilizer N was adjusted based on expected N supplied via irrigation.

Flow-monitored sub-surface drip lines were used to deliver irrigation throughout the season, based on target ET levels as determined by water balance using neutron probe and time domain reflectometry. An on-site weather station measured precipitation and allowed calculation of local ET.

Plant and soil measurements

Plants were sampled in late September (at physiological maturity, R6) to assess aboveground biomass and N uptake. Five plants, located in a row with no skips or doubles, were cut at ground level in each plot, separated into stalks, leaves, and ears. Leaves were scanned with a leaf area meter (LI-3100C; LI-COR, Lincoln, Nebraska, USD) to calculate the total leaf area per plant. All plant components were oven-dried at 65 °C and weighed. Aboveground vegetative biomass included all aboveground parts (e.g., stalks, cobs, husks) except grain. Representative sub-samples of each oven-dried component were ground for subsequent elemental analyses. Grain yield was determined at plant maturity (R6) by collecting ears by hand from an area within the center of the plots, two rows wide and 5 m long. Grain was separated from the ears, weighed separately, and corrected for moisture content.

End-of-season soil cores (6.35 cm diameter) were collected in October 2018 to determine root biomass (kg ha^{-1}) and soil N concentration at four depths (0-30, 31-60, 61-90, 91-120 cm). Four soil cores were collected with a Giddings probe in each plot: two in-row cores and two inter-row cores. Visible roots from each depth increment were removed by hand-picking from samples. Collected roots were then oven-dried, weighed, and sub-replicates averaged for each plot. Row and in-row soil samples from each plot were composited within each depth, air-dried, and sieved to 2 mm. Stalks, leaves, grain, and root subsamples were analyzed for total C and N using a combustion analyzer (LECO Tru-SPEC, St. Joseph, MI). To determine soil NO_3^-

concentration at each depth, a 5 g of subsample homogenized air-dried soil was extracted with 25 mL of 1 M KCL solution and measured with colorimetry (Doane & Horwath, 2003) with a UV-Vis Spectrophotometer (Shimadzu, Kyoto, Japan).

Greenhouse Gas Measurements

Measurements of N₂O emissions began in early May and ended in late September 2018. Samples were collected approximately twice a week during periods of frequent irrigation and less often after irrigation ended. Gas samples were collected in the morning between 8:00 h and 12:00 h to approximate average daily flux and minimize the effects of diurnal variation (Hutchinson & Mosier, 1981; Nichols et al., 2016). During gas sampling, chambers were vented for atmospheric pressure and deployed for 45 min. Gas samples were collected starting at 0, 15, 30, and 45 min with 35 mL polypropylene syringes and immediately transferred to a 12 mL evacuated glass exetainer fitted with a screw cap and rubber butyl septum (Exetainer vial from Labco Limited, High Wycombe, Buckingham-shire, UK). Internal chamber temperature was measured using thermocouple wires installed in chamber lids with an airtight seal and used later to calculate gas abundance in the chamber. Samples were analyzed within two weeks of collecting with an automated gas chromatograph (Varian model 3800, Varian Inc., Palo Alto, CA) equipped with an electron capture detector.

Analysis and calculations

Leaf area index (LAI) was calculated as the total leaf area per ground surface area based on the average of five plants sampled. Harvest index was calculated by dividing the oven-dry grain biomass by total aboveground plant oven-dry biomass (grain and vegetative biomass). Crop water productivity was calculated at grain yield divided by total ET used. Evapotranspiration was calculated using weather station data and a water balance approach

described in Trout & DeJonge (2017). Nitrogen productivity was calculated as grain yield divided by total N applied (fertilizer and N in the irrigation water). Total N uptake (kg ha^{-1}) was calculated by multiplying the oven-dry biomass of each plant component by its corresponding N concentration and then summing up all aboveground parts. To examine total N recovery and account for the slightly different N application amount for each N level with the two irrigation treatments, we divided the total amount of N uptake by the total amount of N applied (fertilizer and N in the irrigation water).

Greenhouse gas calculation

Because N_2O emissions were low, we used linear regression to estimate gas fluxes to avoid overestimating fluxes (Nichols et al., 2016; Parkin & Venterea, 2010). Cumulative fluxes for each soil chamber were calculated from the sum of measured and interpolated values of daily fluxes. Interpolated values for non-measured days were determined by linear interpolation using this equation from Hoefl et al. (2012): $\text{Flux} = F1 + (F2-F1/D2-D1)$. Where F1 is the measured gas flux on the closest day before the day that requires interpolation, F2 is the measured gas flux on the closest day after the date of the computed flux. D1 is the day of the growing season on which F1 was measured, D2 is the day of the growing season on which F2 was measured.

The emissions factor (EF) was calculated to estimate the percent of N applied lost as N_2O emissions. Typically, EF is calculated by subtracting the emissions of a control treatment from the treatment emissions. Because this experiment didn't have a 0 N treatment, a modified emissions factor was calculated as $EF = (\text{kg } \text{N}_2\text{O-N } \text{ha}^{-1} \text{ season}^{-1} / \text{kg N applied season}^{-1}) * 100$.

Statistical Analysis

Analysis was conducted on 21 plots of the original 24 plot experiment due to irrigation line failure. This resulted in four replicates of each N level under DI and three replicates of each

N level under FI. We assessed the effect of irrigation, N, and the interaction of irrigation and N using two-way ANOVA. Irrigation treatment was treated as a categorical variable, and N level was treated as a continuous variable. Root mass and residual NO_3^- were assessed separately at each depth they were measured. Response variables were checked for normality and homogeneity of variance. Post hoc mean comparisons (Tukey-Kramer HSD test) were conducted to assess treatment differences. Analyses were performed using R version 4.0.3.

4.3 Results

Water and nitrogen productivity

During the four-week late vegetative period, deficit irrigation achieved 75% of full ET, which, with full irrigation applied during the remaining season, resulted in 91% of total full ET for the growing season and 88% of full yield (Fig. 4.2 and 4.3a). In a previous DI study at the same research location, a DI treatment that achieved 72% and 71% of full ET in the late vegetative and maturation growth stages, respectively and which resulted in 86% of growing season full ET and 89% of the full yield that was not significantly different from full yield (Fig. 4.2, Comas et al. 2019).

There was no change in WP under DI compared to FI (Table 4.2). Nitrogen level did not impact yield or WP. Nitrogen productivity was 1.5 to 2 times higher for the low N than for the high N treatment under both FI and DI. The overall effect of DI across N treatments reduced N productivity by 9% (Table 4.2), although the effect was only marginally significant ($p = 0.08$).

Aboveground vegetative and root biomass

Deficit irrigation significantly reduced total aboveground vegetative biomass by 22% and leaf area by 12 % compared to FI (Figure 4.3b). Accordingly, the harvest index increased by 5%

under DI, relative to FI (Figure 4.3c). Nitrogen level did not have a significant effect on biomass or harvest index.

About 50% of root biomass was in the top 0-15 cm of the soil, 20% in the 15-30 cm, 20% in the 30-60 cm, and 10% in 60-90 cm (Table 4.3), with no roots below 90 cm. Irrigation and N treatments did not influence root biomass except for the 60-90 cm soil depth, where there was a significant interaction between treatments such that root biomass increased with greater N input under FI, and the opposite trend was observed under DI (Table 4.3).

Plant N uptake

Deficit irrigation significantly reduced maize N uptake by 10% compared to FI (Fig. 4.4). Among the measured plant components, N uptake under DI was 11-16% lower in the vegetative tissue and grain than FI, but there was no difference in N uptake in the root tissue. Despite total N uptake generally being lower under DI, N concentration in the vegetative tissues tended to be higher under DI (Table 4.4). Stalk and root N concentrations were 17 and 20% higher, respectively, under DI compared to FI. Although not significantly different under either irrigation treatment, grain N concentration tended to increase with N applied. Deficit irrigation reduced the overall proportion of applied N recovered in the maize plants across N treatments (Table 4.2), although the effect was only marginally significant ($P=0.06$). Plant N recovery was 7% higher under FI than DI and was 1.5 to 2 times higher in the low N relative to the high N treatment under both FI and DI.

Residual soil NO₃⁻ and N₂O emissions

Deficit irrigation increased residual soil NO₃⁻ by 22-35% in the 30-60 and 60-90 cm soil depths (Figure 4.5). Total residual soil NO₃⁻ did not otherwise differ between treatments. There were no significant treatment effects on cumulative N₂O emissions (Table 4.2) though the

highest peaks in N₂O emissions occurred under FI with high and medium N rates (Figure 4.6). As a result of little to no difference in N₂O emissions between treatments, the emissions factor (i.e., N₂O emissions per unit N applied) increased with lower rates of N (Table 4.2).

4.4 Discussion

DI impact on yield, water, and N productivity

Applying DI during the late vegetative growth stage reduced maize yield compared to FI and did not significantly improve WP. This is contrary to our hypothesis and previous studies that have demonstrated increased WP with regulated DI of maize (Comas et al., 2019; Fereres & Soriano, 2007; Geerts & Raes, 2009). Improving maize WP with DI is challenging because maize is susceptible to water stress, and maize yield is often linearly related to ET (Pandey et al., 2000; Payero et al., 2006). Using DI only during the late vegetative stage, as we did in this study, is thought to be the ideal stage to reduce irrigation since it's less yield-sensitive than early vegetative or anthesis and grain-filling (Çakir, 2004; Kirda, 1999) and doing so can create a curvilinear water response function (Comas et al., 2019; Trout & DeJonge, 2017), which leads to an increase in WP. However, the yields in this study were lower and appeared to be more severely impacted by DI than in previous studies at this location (Comas et al. 2019).

Water availability during the vegetative stage is a crucial determinant of leaf area and photosynthetic capacity, which strongly influences yield potential (Geerts & Raes, 2009). In our study, leaf area was reduced under DI compared to FI, which could have partly caused the reduced grain yield we observed. Interestingly, harvest index, a measure of how efficiently carbon synthesized by the crop is allocated to grain versus vegetative biomass, was higher under DI than FI. This more efficient use of synthesized carbon represents a potentially desirable drought-tolerant trait, but overall, grain yield was still compromised by DI. While our results do

not support the idea that DI improves WP, we agree with other studies that suggest more research is needed to discern how and when DI can result in improved WP for maize (Payero et al., 2006; Rudnick et al., 2017). This could be done by narrowing in on ways to maintain leaf area under temporary water stress or focusing on site-specific climate and economic considerations (Rudnick et al., 2017).

The N fertilizer rates used in this study were based on commonly used rate calculators but our data suggest the rates here may have been high or excessive. Nitrogen is needed in large amounts by maize plants, so when N fertilizer prices are low, some farmers apply N fertilizers in excess as a form of yield insurance. Luxury N uptake has been shown to improve leaf longevity and lead to greater biomass and minimize the impact of water stress during maize grain filling (Nasielski et al., 2019). However, excess N can have a negative effect on yield. For example, too much N in maize leaves has been shown to negatively impact photosynthetic parameters and increase plant sensitivity to drought stress (Song et al., 2019). Excessive N rates have also been shown to restrict root growth and function and negatively impact crop growth (Chen et al., 2015; Liu et al., 2017). Nitrogen rate did not have a significant impact on yield under either irrigation treatment, but it is notable that yield did not increase with increasing N, and that yield was lowest with the highest N rate under DI and trended downward from the middle N rate under FI.

Nitrogen productivity, or the amount of grain produced per unit N applied, was similar between irrigation treatments but was significantly improved with lower N rates. Nitrogen fertilizer applied above 150 kg ha⁻¹ under DI and 210 kg ha⁻¹ under FI treatments appeared to have no additional benefit for yield. Therefore, this study did not support the suggestion that high N benefits maize yield under water stress. Our results indicate that N rate should be reduced if irrigation water supplies cannot meet the full water demand of the crop. Despite having N

treatments that spanned more than 100 kg N ha⁻¹, N rate had little effect on yield, in contrast to other studies that have found increasing N fertilizer to benefit maize yield under water stress (Al-Kaisi & Yin, 2003; Eissa & Roshdy, 2019). It could be that our N rates (146-291 kg N ha⁻¹) were too high to show the benefit of N under water stress. Additionally, N fertilizer treatments can also be muted non-fertilizer N sources. Indeed, non-fertilizer N sources (e.g., from mineralization of soil organic matter) can be quite substantial and need to be considered when deciding N application rate (Yan et al., 2020). For example, Teixeira et al. (2014) found that unfertilized maize under dryland and irrigation was able to take up 50 kg N ha⁻¹ and 150 kg N ha⁻¹, respectively. Without knowing the relative contribution of different N sources (fertilizer, mineralized organic matter, residual inorganic N from prior years) used by the crop, we may still conclude that N rates were high since yield did not appear to be N limited even in the low N treatment.

Nitrogen uptake

As reflected by reduced yield and overall biomass production, DI decreased N uptake compared to FI in this study, confirming our hypothesis that reduced growth under DI would reduce crop N demand and lower N removal from the soil. The difference in plant N uptake was due to both N uptake in vegetative biomass and grain. It has been shown that maize grown under water-limited conditions typically requires less N to achieve maximum grain yield compared to well-watered maize (Moser et al., 2006), likely because smaller plants need less N. Similar to reduced N uptake, the ratio of N uptake: N applied (a proxy for N recovery efficiency) was reduced under DI compared to FI, suggesting greater potential for fertilizer N loss. Our ability to speculate on this, however, is limited because our N recovery metric does not distinguish between fertilizer N vs. that taken up from other soil N sources (e.g., mineralized from soil

organic matter or previous fertilizer applications). Conducting a similar experiment with isotope tracing of the various N sources in plants would allow for a more precise understanding of how DI impacts the transformation and fate of soil N.

While plant N uptake is largely determined by plant growth and N demand, soil N availability and root uptake capacity also play critical roles in plant N uptake (Wang et al., 2017). Nitrogen uptake depends on water availability (Ercoli et al., 2008), but without increasing soil moisture (i.e., irrigation water shortages), increasing the concentration of N in the soil and making it more accessible to roots could enhance N uptake. This idea, however, was not supported in this study, where increasing N application under DI by 145 kg N ha⁻¹ (from the low to high treatment) reduced N uptake. Under FI, where soil moisture was more available, increasing N by 118 kg ha⁻¹ (from the low to high N treatment) resulted in an additional 22 kg ha⁻¹ of N uptake by the plant. However, this was only a small increase in N uptake compared to the large amount applied.

Maize root biomass and root mass fraction were not significantly impacted by irrigation and N treatment, so it is difficult to speculate what impact root biomass, or root length by proxy, had on N uptake. The lack of difference in root growth was surprising, as previous work at this field site has shown DI to increase maize root growth at depth (Comas et al., 2013; Flynn et al., 2021). The deficit imposed in this study was milder than in Flynn et al. (2021) and we suspect that this may explain the lack of an effect.

In summary, our results indicate that plant demand was a key driver of N uptake and that increasing soil N availability also increased plant N uptake to some extent for the FI treatment but not for DI, and we could not conclude that increased root growth under DI was able to increase N uptake.

Residual nitrogen and N₂O emissions

In line with the lower plant N uptake under DI, we observed a corresponding buildup of NO₃⁻ in the deeper soil layers (30-60 and 60 – 90 cm) compared to FI after one year of treatment implementation. Others have also found end-of-season soil NO₃⁻ to be higher under DI than FI in maize-based systems (Gheysari et al., 2009; Kirda et al., 2005; Teixeira et al., 2014). As was used in this study, drip irrigated systems typically have lower fertilizer N leaching losses because water and nutrients are delivered directly to the rooting zone, resulting in overall lower soil moisture and increased synchrony with plant demand. In contrast, flood irrigated systems often have excess water being applied, especially at the top of the field, and thus more significant fertilizer leaching losses below the root zone (Gärdenäs et al., 2005; Hanson et al., 2006). Nitrogen remaining at the end of the season is vulnerable to multiple loss pathways, including via leaching and gaseous fluxes (Barakat et al., 2016), or can be immobilized and rereleased via mineralization for take up by plants in subsequent seasons (Hart et al. 1993). While we did not follow the fate of the residual NO₃⁻ after the growing season, we know that the amount of N leached from soil tends to increase with the amount of excess N applied (Goulding, 2000). Residual N at the end of the growing season is vulnerable to leaching even in semi-arid environments because even small amounts of water (e.g., less than 3 cm) can move soil NO₃⁻ down 15-20 cm in a loamy sand soil (Endelman et al., 1974) and large spring rains frequently occur (Klocke et al., 1999). The end-of-season residual NO₃⁻, therefore, represents a concern for environmental degradation but also an inefficient use of an expensive input that could impact the farmer's net profits.

Irrigation and N treatment had no significant impact on cumulative N₂O emissions in this study. This was surprising, as reduced water application and soil moisture under DI has been

shown to decrease N₂O emissions (Ning et al., 2019; Flynn et al., 2022) because both nitrification and denitrification are influenced by soil moisture (Baggs et al., 2000). At the same time, reducing N fertilizer inputs should reduce N substrates available for soil N₂O producing processes, thus lowering N₂O emissions (Abalos et al., 2014; Signor & Cerri, 2013), so it was surprising that our N treatments had little effect. The lack of treatment impacts suggests that our lowest irrigation and N levels did not meaningfully limit N₂O producing processes. Applying lower N rates than were used in this study could introduce competition from plants for soil N that would limit microbial N₂O producing processes. Flynn et al. (2022) showed that relatively extreme DI (40% ET during the late vegetative stage) may be needed to reduce GHG emissions. It is also important to note that we only measured in-season N₂O emissions. It is possible that residual soil N under DI could lead to greater off-season emissions compared to FI. Other factors controlling N₂O emissions could also have suppressed our treatment effect, including compaction, temperature, pH, organic matter, and texture (Bremner, 1997; Snyder et al., 2009). Soil organic matter, which is <1% at this field site, may have been especially limiting. Soil carbon availability can limit N₂O emissions because carbon is a key substrate and source of energy for microbial activity and associated soil N transformations (Cameron et al., 2013). Despite having no treatment differences, the EFs were notably low in this study compared to other irrigated maize systems utilizing sprinkler irrigation because drip-irrigated systems are already efficient at mitigating GHG emissions (Adviento-Borbe et al., 2007; Guardia et al., 2017; Halvorson et al., 2016; Sanz-Cobena et al., 2017). This resulted in an EF much lower than the IPCC empirical estimate of 1%, which assumes a linear relationship between fertilizer N inputs and N₂O emissions. While DI and N levels had little impact on N₂O emissions, future

research could reach broader conclusions about the effects of reduced irrigation and N rates by studying DI in more conventional irrigation systems such as sprinkler or flood irrigation.

4.5 Conclusion

Optimizing water and N use are critical for sustaining crop production with limited water resources and mitigating agricultural impact due to fertilizer use on the environment. In this experiment, we found that DI reduced maize yields and overall plant N uptake. Our data show that less N is needed to optimize yield under DI compared to FI and that when less N is taken up by the crop, more residual soil NO_3^- was left at the end of the growing season under DI at depth (30-60 and 60-90 cm). This excess N can be lost via groundwater leaching or N_2O emissions. Our findings illustrate the importance of measuring N dynamics under alternative irrigation strategies such as DI because of the potential to alter the fate of N in agricultural systems. We suggest that the N fertilizer rate should be reduced in proportion to the amount of irrigation water available for crop production because we found no evidence that a high rate of N fertilizer benefited crop growth under water stress, and doing so will reduce N fertilizer costs and reduce deleterious N losses.

TABLES AND FIGURES

Table 4.1 Precipitation, irrigation, evapotranspiration (ET), and N application data for two irrigation treatments (full irrigation, FI; deficit irrigation, DI) with three nitrogen levels (high, H; medium, M; and low, L) within one season of corn production near Greeley, Colorado, USA.

Irr. Trt.	N Trt.	Precipitation (mm)	Irrigation (mm)	Water applied (mm)	ET (mm)	Fertilizer (kg ha ⁻¹)	N in irrigation water (kg ha ⁻¹)	Total N applied (kg ha ⁻¹)
FI								
	H	104	497	601	581	132	149	281
	M	104	497	601	581	53	149	202
	L	104	497	601	581	14	149	163
DI								
	H	104	441	544	531	159	132	291
	M	104	441	544	531	81	132	213
	L	104	441	544	531	14	132	146

Table 4.2 Mean water productivity (WP), N productivity (NP), N recovery (measured as N uptake: N applied), leaf area index (LAI), N₂O emissions, and emissions factor (EF) under two irrigation levels [full (F) and deficit (D)] and three N nitrogen fertilizer levels [high (H), medium (M) and low (L)]. Values in parentheses show standard error. Different letters indicate significant differences between N treatments within irrigation level. ANOVA p-values for each main effect are presented at the bottom of the table with significant effects ($P < 0.05$) in bold.

Irr. Trt.	N Trt.	WP (kg mm ⁻¹ ET)	NP (kg grain kg ⁻¹ N applied)	N uptake: N applied	LAI	N ₂ O (g N ₂ O-N ha ⁻¹)	EF %
FI							
	H	23.1 (1.4)	47 (3) a	1.01 (0.07) a	4.7 (0.3)	477 (76)	0.17 (0.03)
	M	17.06 (0.6)	73 (2) b	1.47 (0.08) b	4.8 (0.1)	629 (152)	0.31 (0.08)
	L	22.9 (1.2)	81 (4) c	1.61 (0.10) c	4.6 (0.1)	400 (40)	0.25 (0.020)
DI							
	H	21.2 (0.9)	38 (2) a	0.81 (0.04) a	3.9 (0.1)	525 (71)	0.18 (0.02)
	M	23.4 (1.0)	58 (3) b	1.18 (0.03) b	4.3 (0.2)	373 (75)	0.17 (0.04)
	L	24.0 (1.4)	87 (5) c	1.80 (0.04) c	4.3 (0.2)	392 (59)	0.27 (0.04)
ANOVA							
	Irr	0.48	0.08	0.04	<0.01	0.33	0.34
	N	0.69	<0.01	<0.01	0.21	0.28	0.05
	Irr x N	0.53	0.44	0.03	0.30	0.63	0.74

Table 4.3 Mean root biomass (kg ha⁻¹) under two irrigation levels [full (F) and deficit (D)] and three N nitrogen input levels [high (H), medium (M), and low (L)]. Values in parentheses show standard error. Different letters indicate significant differences between N treatments. ANOVA p-values for each main effect are presented at the bottom of the table with significant effects ($P < 0.05$) in bold.

Irr. Trt.	N Trt.	Root biomass (kg ha ⁻¹) by soil depth (cm)				Total Root Biomass (kg ha ⁻¹)
		0-15	15-30	30-60	60-90	
FI						
	H	1251 (320)	603 (129)	575 (121)	393 (95) a	2824 (525)
	M	1552 (86)	351 (59)	614 (100)	160 (43) ab	2678 (35)
	L	1160 (398)	449 (126)	391 (85)	126 (44) b	2125 (565)
DI						
	H	797 (148)	555 (39)	573 (138)	73 (34) a	1998 (302)
	M	866 (305)	470 (11)	409 (43)	181 (24) b	1927 (354)
	L	1460 (411)	440 (81)	488 (36)	289 (54) b	2679 (419)
ANOVA						
Irr		0.27	0.75	0.65	0.27	0.30
N		0.20	0.08	0.25	0.62	0.67
Irr x N		0.36	0.54	0.67	<0.01	0.13

Table 4.4 Mean N concentration (%) of leaves and stems, roots and grain, at R6, under two irrigation levels [full (F) and deficit (D)] and three N input levels [high (H), medium (M), and low (L)]. Values in parentheses show standard error. ANOVA p-values for each main effect are presented at the bottom of the table with significant effects ($P < 0.05$) in bold.

Irr. Trt.	N Trt.	Leaf	Stalk	Root	Grain
FI					
	H	1.43 (0.14)	0.61 (0.07)	1.35 (0.06)	1.26 (0.03)
	M	1.70 (0.04)	0.61 (0.05)	1.10 (0.08)	1.21 (0.02)
	L	1.63 (0.05)	0.65 (0.01)	1.24 (0.08)	1.19 (0.02)
DI					
	H	1.53 (0.08)	0.76 (0.02)	1.69 (0.06)	1.23 (0.01)
	M	1.53 (0.10)	0.80 (0.04)	1.53 (0.12)	1.24 (0.01)
	L	1.47 (0.06)	0.71 (0.04)	1.35 (0.09)	1.21 (0.01)
ANOVA					
Irr		0.28	<0.01	<0.01	0.60
N		0.53	0.37	<0.01	0.02
Irr x N		0.07	0.96	0.45	0.08

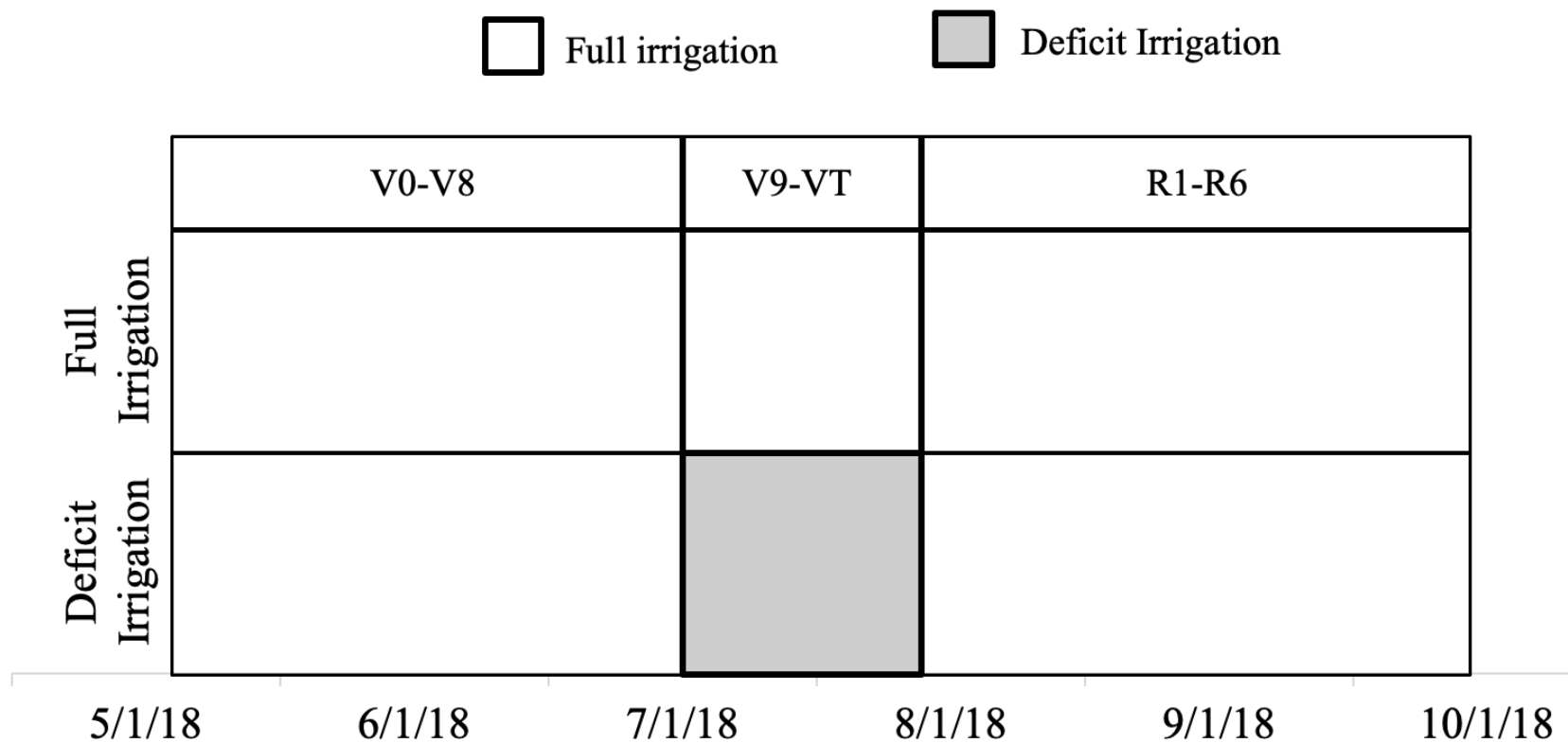


Figure 4.1 For the deficit irrigation treatment, deficit irrigation (75% of full ET) occurred from July 3, 2018, to July 31, 2018, during the V9-VT growth phases and full irrigation (100% of full ET) occurred before and after. For the full irrigation treatment, full irrigation occurred during the entire growing season. Research on maize took place at the limited irrigation research farm in Greeley, Colorado.

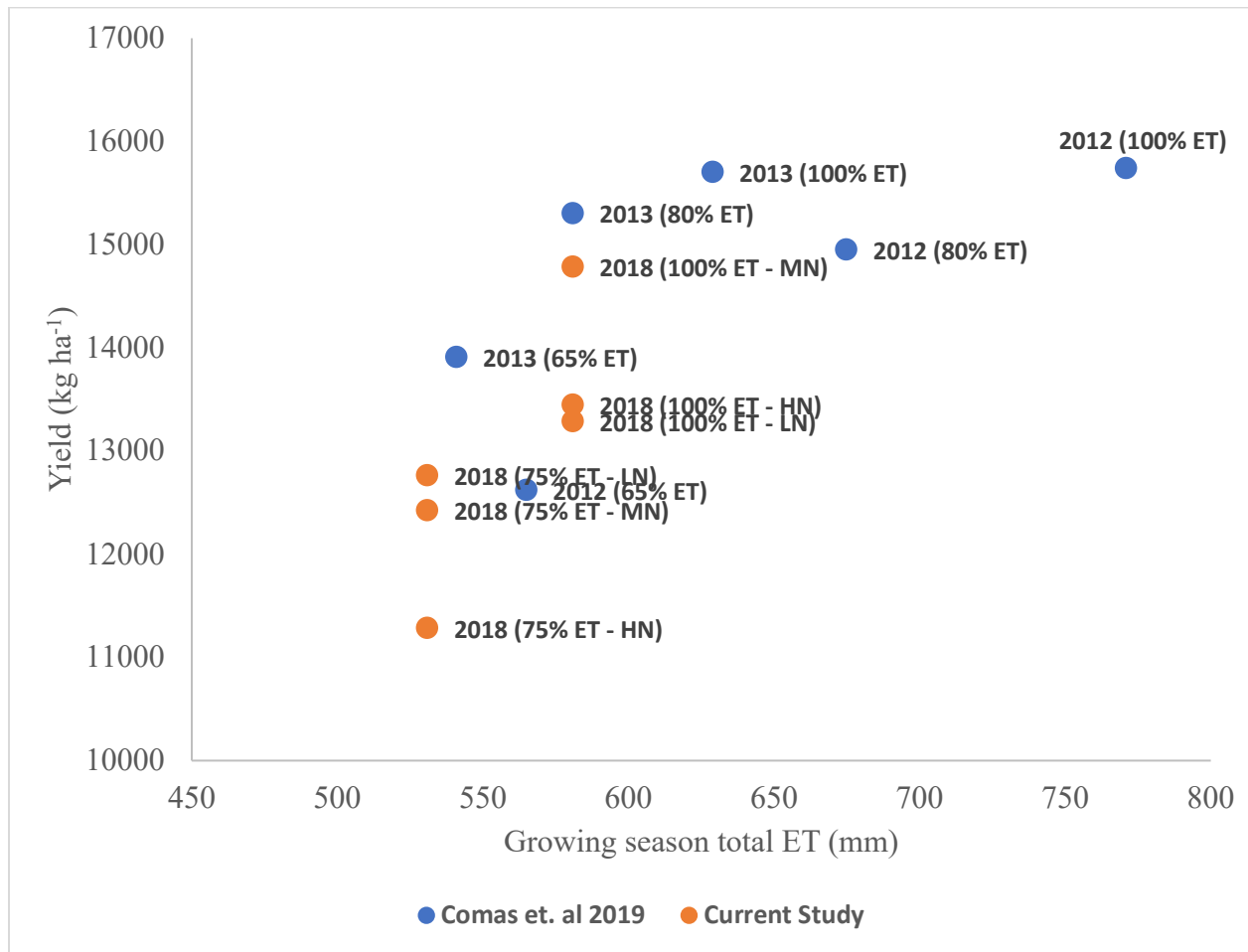


Figure 4.2 Comparison of the mean yield response to the total ET used by the maize to produce grain between this study (2018) and previous deficit irrigation (DI) study (2012 and 2013) by Comas et al. (2019). Data were collected at the limited irrigation research farm in Greeley, Colorado. For the 2012 and 2013 data points, data labels refer to the target ET level during the late vegetative and maturation growth stages. For the current study (2018) data labels refer to the target ET level during the late vegetative stage and no DI was used in the maturation growth stage.

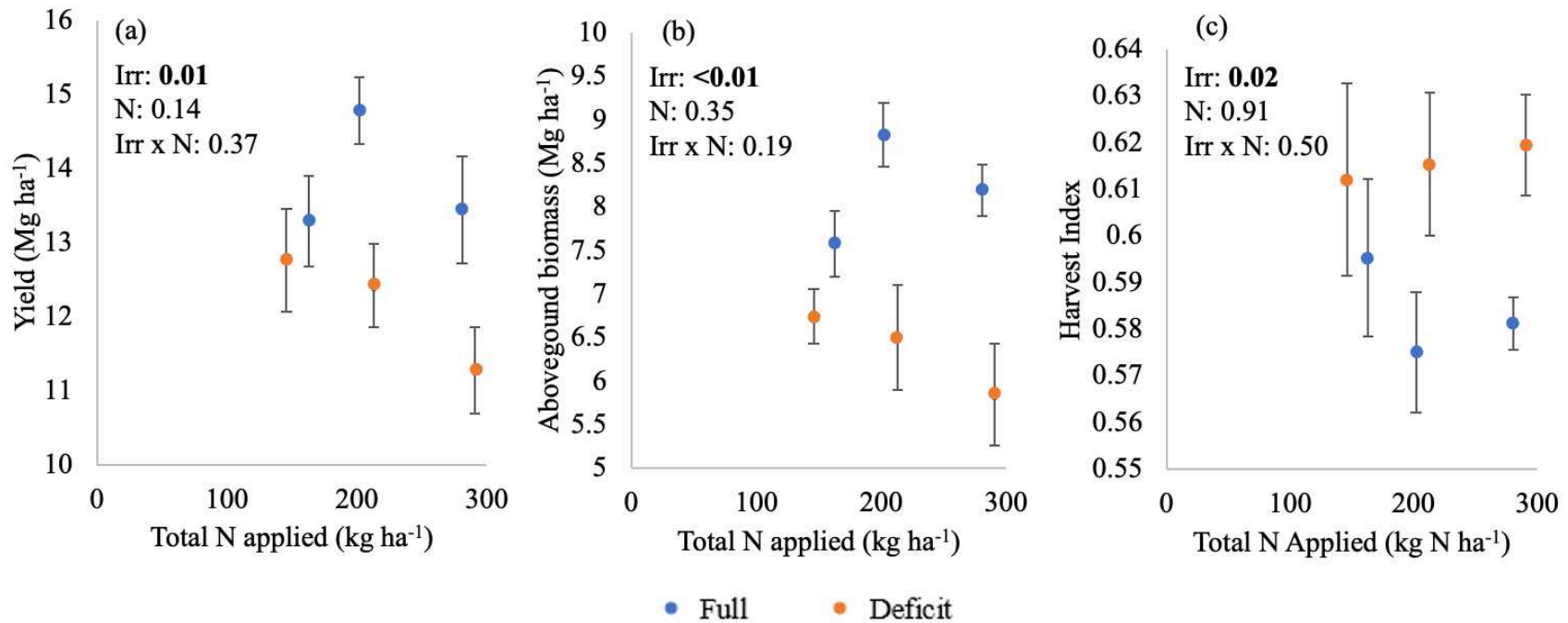


Figure 4.3 Average a) yield, b) aboveground biomass, and c) harvest index under two irrigation levels [full (F) and deficit (D)] and three N nitrogen application levels [high (H), medium (M), and low (L)]. Dots represent individual irrigation and N combinations, while error bars represent standard error. ANOVA p-values for each plot the main factors irrigation (Irr) and N rate (N) as well as their interaction (Irr x N) are provided in the top left corner of each plot.

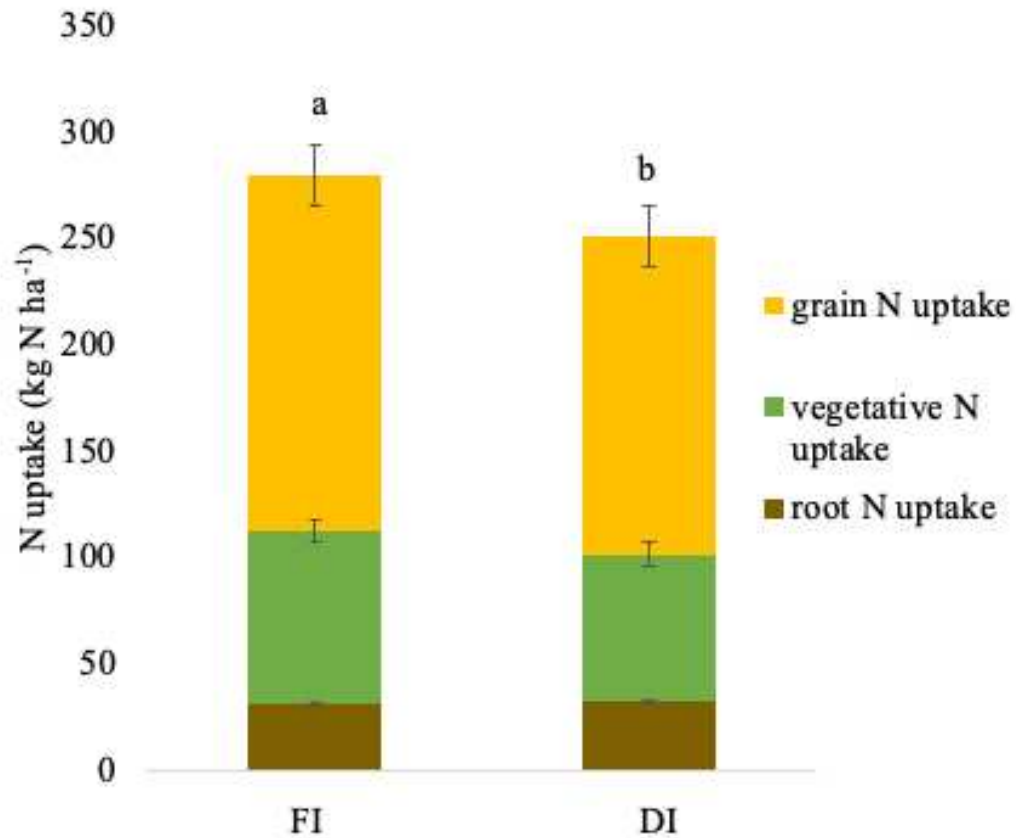


Figure 4.4 Mean N uptake in the roots, vegetative biomass, and grain under full irrigation (FI) and deficit irrigation (DI). Different letters indicate a significant difference in total recovery (the sum of root, vegetative, and grain N uptake, $P < 0.05$). Error bars represent standard error.

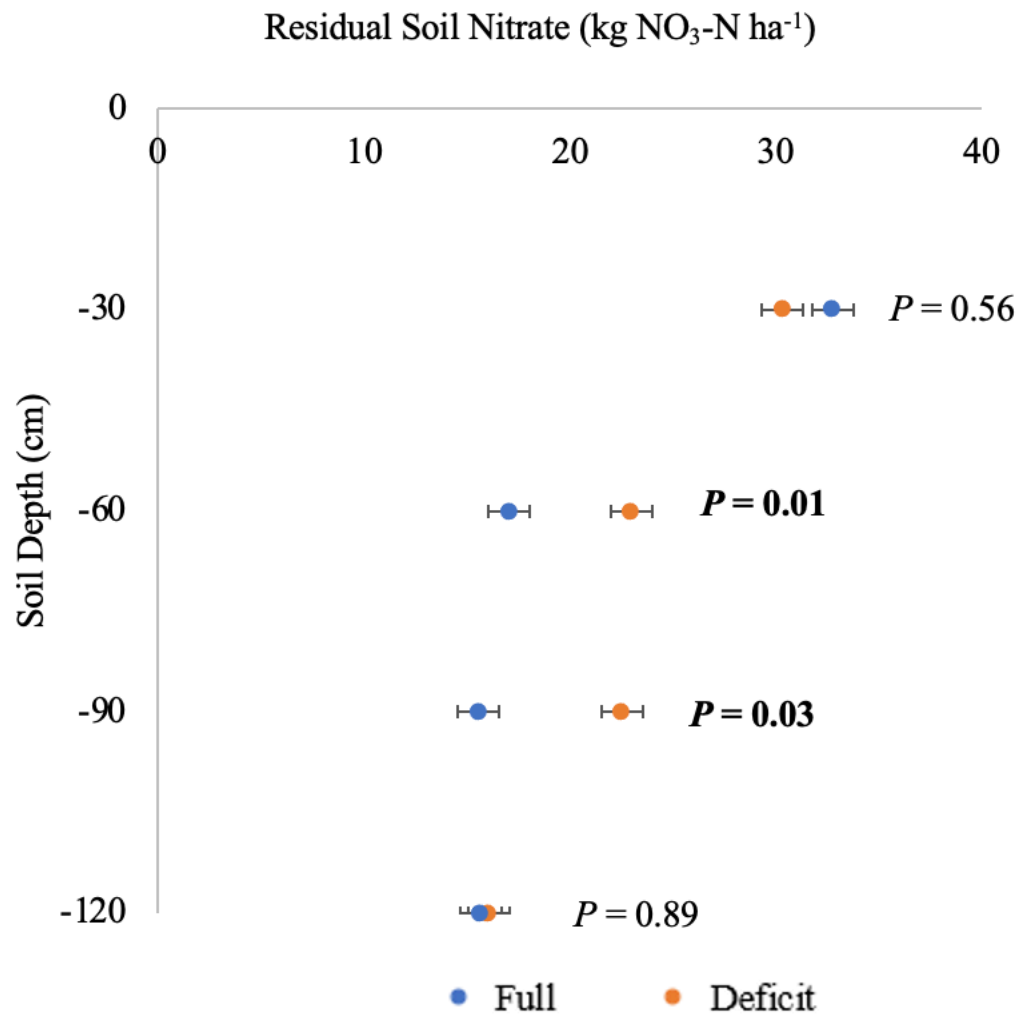


Figure 4.5 End-of-season residual soil NO₃- measured in terms of NO₃-N (kg ha⁻¹) at four depths (0-30, 31-60, 61-90, 91-120 cm) in a maize field averaged across two irrigation levels [full (F) and deficit (D)]. Error bars represent standard error. ANOVA p-values for each depth are presented and bold represents a significant effect ($P < 0.05$)

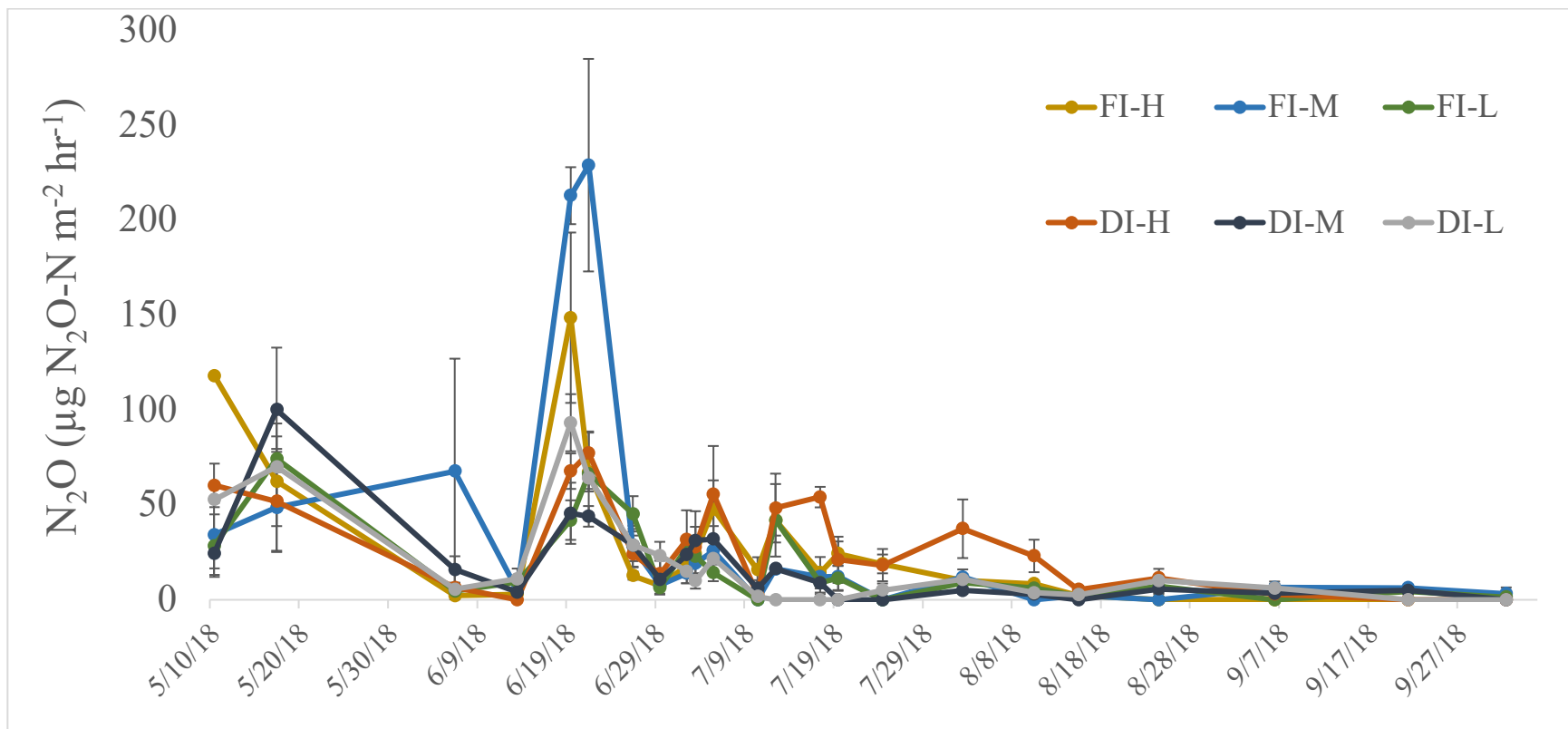


Figure 4.6 N₂O emissions ($\mu\text{g N}_2\text{O-N m}^{-2} \text{hr}^{-1}$) during the 2018 maize growing season at the Limited Irrigation Research Farm near Greeley, Colorado. Treatments are designated by the level of irrigation (full irrigation, FI; deficit irrigation, DI) and N fertilizer level (high, H; medium, M; low, L) during the late vegetative growth stages (V9-VT).

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CHAPTER 5: SORGHUM BIOMASS ALLOCATION SHIFTS TO ROOTS UNDER DEFICIT IRRIGATION AND NITROGEN LIMITATION

5.1 Introduction

Sorghum is a globally important food staple and is increasingly planted worldwide because of its productivity in water-limited environments (Borrell et al., 2014; Mutava et al., 2011; Visarada, 2018). Sorghum can be grown for vegetative biomass to produce animal feed or biofuel but is also grown for grain. Grain yield is a complex and composite trait affected by whole plant strategies for reaching reproductive maturity. Sorghum grain yield is usually proportional to shoot biomass, but if drought stress is severe enough to reduce total shoot biomass by more than 50%, harvest index may also be reduced (Craufurd and Peacock, 1993). Crop breeding and management efforts have therefore focused on maximizing aboveground biomass and yield, which can have consequences for root biomass and morphology (Fess et al., 2011; Waines and Ehdaie, 2007; Zhu et al., 2001).

From natural systems, we know that plants shift biomass allocation among organs to acquire the resources that most limit growth (Bloom et al., 1985). For example, plants increase root production in response to water deficits. Most research on sorghum has focused more on shoot growth and yield under drought conditions, but has largely ignored belowground responses (Bell et al., 2018; Borrell et al., 2014; Mutava et al., 2011). Another drought tolerance strategy for grain sorghum is reducing the canopy size during water stress to reduce pre-flowering water demand, increasing water availability during grain filling and leading to greater yield (Borrell et al., 2014). Less is known about how sorghum responds to different levels of N availability. In general, sorghum can take up more N than maize under dry conditions (Lemaire et al., 1996). However, sufficient soil moisture is still needed for sorghum to uptake and incorporate N into

plant tissues. N deficiency in sorghum can cause reduced leaf area, rate of photosynthesis, and lower dry matter accumulation (Sigua et al., 2018). The interaction of water and N availability on plant growth has received relatively little attention and merits further research, as limitation of these resources is often co-occurring and may become more common with global trends in water use and the efforts to reduce reliance on inorganic N fertilizer inputs.

Excess N fertilizer is often applied in agricultural settings to maximize yield, but this represents a significant cost to farmers and contributes to environmental degradation. Uncertainty exists with regards to how to adjust fertilizer rates in water-limited agricultural systems (Chilundo et al., 2017). In some cases, high N has been shown to ameliorate the impacts of drought stress on crop production (Randhawa et al., 2017; Saneoka et al., 2004; Shangguan et al., 2000; Song et al., 2019). This has been attributed to the critical role of N as a building block in plant biomass and photosynthetic structures (Song et al., 2019). However, in some cases, additional N applied in water-limited conditions goes unused and increases the likelihood of undesirable N losses to the environment (Cassman et al., 2002; Galloway et al., 2003). Too much N can also be a detriment to crop yield. For example, high N can stimulate high leaf area which increases transpiration, making the plant more vulnerable to water stress. Deficit irrigation (DI) can promote root growth during the vegetative growth stage and this could ultimately aid in both water and N uptake (Comas et al., 2013). For this reason, as well as the beneficial impacts of roots on soil N dynamics and soil health, roots are of great interest for improving agricultural sustainability.

Our objective was to evaluate the impact of DI and N level on sorghum shoot and root growth during the vegetative stage of development. We tested a range of sorghum genotypes to draw broad conclusions about sorghum response to water and N limitation. We hypothesized

that: 1) DI would increase the relative allocation to roots, and that 2) under DI, high N would increase N uptake, net photosynthesis (A_n), instantaneous nitrogen use efficiency (NUE_i), and shoot biomass.

5.2 Methods

Genotype selection and Experimental design

This study was conducted in a greenhouse at the USDA-ARS Crops Research Laboratory in Fort Collins, CO. Five diploid lines of grain *Sorghum bicolor* were selected to span a broad range of variation in WUE and grain yield response under drought based on the dataset of Turner et al. (2016; Table 5.1). The five genotypes were seeded in the dark at 25 °C in Petri dishes on filter paper with fungicide solution (Maxim XL, Syngenta). Seeds were germinated for one week, or until they had at least 2 cm each of shoot and root tissue, before transplanting into 7.57 L black polypropylene pots filled with fritted clay (Profile® Greens Grade™ in Emerald, PROFILE Products, LLC., Buffalo Grove, IL, USA). Fritted clay is a non-nutritive substrate with high water storage capacity that washes away easily from roots when dried, making it well-suited for studies of root biomass and morphology.

Experimental groups were established containing one pot each of each sorghum genotype. Genotypes were randomized spatially within groups. Pots were irrigated by polypropylene irrigation lines that delivered fertigated water via an inline Dosatron (model D14, Dosatron Inc., Clearwater, FL) to 3.7 liter minute⁻¹ emitters (1 emitter per pot). Plants were grown in well-watered and nutrient-rich conditions in the initial growth stages. Pots were irrigated to water holding capacity each afternoon at 1600 h and simultaneously fertigated at 80 ppm N. During natural daytime, sunlight increased ambient irradiance to a maximum of 1000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Plants were grown in a supplemental 14:10 hr daylight regime using banks

of LED lights providing $300 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. The greenhouse was maintained on a day/night cycle of 28/17°C.

Grow More fertilizer concentrate (20-20-20 blend) was mixed into the irrigation water to deliver 80 ppm of N (3.90 % Ammoniacal N, 5.90 % nitrate N, 10.20% urea N) to all treatments. After eight weeks, groups of plants were randomly assigned to three different treatments. Five groups were assigned to a full irrigation-high N (FI-HN) treatment that continued to receive 100% replenishment of daily evapotranspiration (ET) and 80 ppm N via manual watering (FI-HN). Three of the FI-HN plant groups were randomly weighed each day to track mean daily ET to calculate how much water to add to create deficit treatments. Eight groups were assigned to a deficit irrigation-high N treatment (DI-HN), with 30% of control ET and 80 ppm N, while five groups were given 30% of control ET and just 30 ppm N to create a deficit irrigation-low N (DI-LN) treatment. Phosphorous, potassium, and micronutrients were kept constant for all treatments using a modified Hoagland's solution. These experimental treatments were implemented for two weeks, after which destructive measurements occurred when plants had reached the five or six-leaf stage (V5/V6).

Non-destructive measurements

During week 9 of the experiment, gas exchange measurements were taken from a subset of the replicates in each treatment: three replicates for FI-HN, four for DI-HN groups, and four for DI-LN. Measurements were made between 0800 and 1100 h, the period when we found leaves to be most photosynthetically active. The lamina of 3rd collared leaf from the top was placed from each plant into a fluorescence/gas exchange cuvette (LI-6400-40, LI-COR Biosciences, Lincoln, Nebraska, USA), avoiding the midrib. Leaves were permitted to acclimate to conditions in the cuvette (1200 PAR, 25°C T_{leaf} , 400 ppm CO₂) for 10 min or more until the

rate of photosynthesis remained constant. At that point, instantaneous measurement of net photosynthetic rate (A_n) was made. Temperature and vapor pressure deficit were kept between 24.6 – 30.2 °C and 1.36 – 2.45 kPa, respectively, for measurements.

Destructive measurements and root scanning

After two weeks of treatment implementation (10 total weeks of plant growth), shoot biomass was cut from the plant base. Leaves were scanned with an LI-3100C leaf area meter (LI-COR Biosciences, Lincoln, Nebraska, USA). Roots were removed from pots, thoroughly rinsed, and collected through a 2 mm sieve. All plant material was dried at 60°C and weighed. Root systems were rehydrated for 24h and separated into coarse (>0.15 mm in diameter) and fine roots. A subsection of roots from each class was scanned and analyzed in WinRHIZO (Regent Instruments, Inc., Canada), dried, and reweighed to quantify specific root length.

N content measurements

Shoot material was ground and passed through a 2 mm sieve to analyze shoot tissue N concentration via combustion (Ward Laboratories, Inc., Kearney, NE, USA). Total N uptake was calculated by multiplying the mass of the plant material by its corresponding N concentration.

Calculations

Shoot mass fraction (SMF) was calculated as the total aboveground biomass divided by the total plant biomass. Similarly, root mass fraction (RMF) was calculated as the total root biomass divided by the total plant biomass. Specific leaf area (SLA, $\text{cm}^2 \text{g}^{-1}$) was calculated as the total leaf area divided by the total leaf mass. Specific root length (SRL, cm g^{-1}) was calculated as total root length divided by the total root mass. Instantaneous N use efficiency (NUE_i) was calculated as A_n divided by N concentration of the leaf used for photosynthesis

measurement. Shoot biomass NUE was calculated as shoot biomass divided by N uptake in the shoot.

Statistical analysis

The effect of irrigation and N treatment (FI-HN, DI-HN, DI-LN) and sorghum genotype and the interaction between them was assessed using a two-way ANOVA with treatment (3 levels) and genotype (5 levels) as a split-plot design. Response variables (biomass, shoot, and root morphology, N uptake, and N efficiency metrics) were checked for normality and homogeneity of variance. Where normality was not met, log transformations were used. Post hoc treatment comparisons were performed using the `emmeans()` package and `pairs()` function. Analyses were performed using R version 4.0.3.

5.3 Results

Sorghum biomass allocation was significantly affected by both treatment and genotype, but there were no significant interactions between these factors (Table 5.2). All sorghum genotypes responded similarly to the three water and N treatment combinations in the shoot, root, and total biomass, SMF, and RMF. Under FI-HN, Btx642 had the highest total biomass.. Under DI-HN, Btx623 had the highest biomass. Under DI-LN, Btx642 and BTx623 had the highest and same biomass. Shoot biomass was reduced on average by 50% for the DI-HN and DI-LN treatments compared to FI-HN (Fig. 5.1a). Root biomass increased by an average of 18% under DI-LN compared to DI-HN (Fig. 5.1a). Total plant biomass across genotypes was reduced 41% and 37% by DI-HN and DI-LN, respectively, compared to FI-HN (Table 5.2). Compared to FI-HN, sorghum SMF decreased 14% and 21% under DI-HN and DI-LN, respectively (Fig. 5.1b), while RMF increased 38% and 58% under DI-HN and DI-LN, respectively (Fig. 5.1b).

Averaged among genotypes, leaf area was reduced at least 50% by DI (HN and LN) compared to FI, but the effect strength differed by genotype (Table 5.3). For example, Btx642 and Btx623 had the first and second greatest leaf area under FI-HN, but leaf area of Btx642 was more reduced by DI than that of Btx623, leading to a significant interaction. Leaves were similar in size (data not shown, there were no treatment differences) under DI as FI, but the number of leaves per tiller was significantly reduced under DI compared to FI (Table 5.3). Specific leaf area followed a similar trend to leaf area and was reduced 51% by DI compared to FI. There was no difference in leaf area or SLA between N levels under DI (Table 5.3). Net photosynthesis (A_n) was reduced 48% and 63% by DI-HN and DI-LN, respectively, compared to FI-HN but with significant interacting genotype responses to DI (Table 5.3). Specifically, Btx642 and Btx623 had the highest A_n under FI-HN conditions but had the lowest A_n under DI-HN. Finally, NUE_i was not affected by treatment or genotype (Table 5.3).

Total root length and fine root length tended to be greater under DI-LN, although not significantly different from DI-HN and FI-HN (Table 5.4). Total root length and fine root length varied significantly among genotypes, with Btx642 having the greatest root length under DI-LN. Coarse root length was shorter under DI-HN than DI-LN and FI-HN with a significant genotype effect (Table 5.4). Under FI-HN, coarse root length was highest for Btx623. Under DI-HN, coarse root length was highest for Btx642. Under DI-LN, coarse root length was the highest for Btx623. The ratio of fine to coarse roots was highest under DI-HN but not significantly different from DI-LN or FI-HN (Table 5.4). Under FI-HN, the ratio of fine: coarse roots was greatest for Btx642. Under DI-HN, the ratio of fine: coarse roots was greatest for Tx7000. Under DI-LN, the ratio of fine: coarse roots was greatest for Btx642. The SRL of coarse roots was, on average, greatest under FI-HN, followed by DI-LN and then DI-HN. Under FI-HN, the SRL of coarse

roots was greatest for Tx7000. The SRL of fine roots was, on average, highest under DI-LN, followed by FI-HN and then DI-HN. Under FI-HN, the SRL of fine roots was greatest for Btx642. The ratio of root length to leaf area increased significantly from FI-HN to DI-HN and increased again from DI-HN to DI-LN but not significantly so (Table 5.4).

Averaged among genotypes, shoot tissue N concentration and N uptake were reduced by 35% and 67%, respectively, under DI-HN compared to full FI-HN (Table 5.5). DI-LN decreased N concentration by another 53% compared to DI-HN and resulted in, on average less N uptake, though not significantly different from DI-HN (Table 5.5). Under FI-HN, the greatest biomass N concentration was found in Tx7000. A similar pattern among genotypes was found in shoot tissue N concentration in the other treatments. Shoot biomass NUE differed significantly by treatment and genotype but without significant interaction (Table 5.5). All genotypes had reduced N concentration under DI-HN and more so under DI-LN compared to FI-HN. The ratio of N uptake to total root length was reduced by 63% and 79% by DI-HN and DI-LN, respectively, compared to FI-HN (Fig. 5.2a). On average, shoot biomass NUE increased 35% from FI-HN to DI-HN and increased another 20% from DI-HN to DI-LN (Fig. 5.2b).

5.4 Discussion

Impact of DI and N level on sorghum biomass allocation

Water and N are the greatest limiting factors in crop production globally. This study sought to understand the impacts of DI and N limitation on five sorghum genotypes in a greenhouse experiment. Deficit irrigation reduced shoot growth and shifted proportional biomass allocation to the roots for all the genotypes tested here. The combined of water and N further shifted sorghum biomass allocation belowground. This supports our first hypothesis and aligns with the results of a previous study, which found that sorghum root: shoot ratio increased under

drought conditions, similar to that observed for other species (Miller, 2018). The increase in RMF was due to maintenance and expansion of the root systems with a substantial decrease in shoot biomass in response to lower water availability. Cell expansion in shoots is generally more sensitive than in roots under water deficits (Hsiao and Xu, 2000). Deficit irrigation combined with N limitation further increased RMF compared to DI-HN without creating an additional reduction in shoot biomass production. The five genotypes used in this study responded similarly to the irrigation and N treatments, but there were significant shoot and root biomass differences between the genotypes. However, the magnitude of genotype responses was generally unrelated to previous drought tolerance designations (Turner et al., 2016).

The shift toward belowground biomass allocation is noteworthy because such investment usually comes at a cost to aboveground biomass and yield (Lynch, 2003). Root growth can be expensive, costing 50% of daily assimilated C (Lambers et al., 2002). However, root growth is a necessary tradeoff for maximizing overall growth, especially in systems with low or heterogeneous soil nutrient distribution (Hodge, 2004). Therefore, even though root investment is expensive, it provides a necessary soil exploration to access a greater volume of water and nutrients and ultimately enhance shoot growth (Jansen et al., 2006; Li et al., 2016).

Impact of DI and N level on sorghum morphology

Root morphology, not just root biomass, was impacted by irrigation and N treatments in this study. Plants generally respond to decreased water and nutrient availability by producing more fine roots, but a shift in morphology such as producing roots with greater SRL (thinner roots per biomass investment) further increases this response. Greater SRL may enable improved water uptake and has been associated with drought tolerance (Comas et al., 2013; Ostonen et al., 2007; Trubat et al., 2012; Zobel et al., 2007). We found relatively little response of root length or

SRL to DI but observed a much greater increase in these responses from HN to LN under DI for all the sorghum genotypes. It could be that low water conditions alone did not trigger root morphological changes here since sorghum is already a drought-tolerant crop (Fracasso et al., 2016; Mutava et al., 2011). Increased SRL can improve N uptake by crops in a field setting with heterogeneous distribution of nutrients by increasing the root surface area interacting with the soil and the volume of soil explored (Hodge, 2006).

Successful plant growth strategies involve balancing above- and belowground growth and optimizing the allocation to resource acquisition to maximize growth (Maire et al., 2009; Trubat et al., 2012). For example, the ratio of root length to leaf area is a proxy for comparing the balance of belowground soil foraging with aboveground light interception (Freschet et al., 2015; Ryser and Eek, 2000). This ratio significantly increased under DI-HN compared to FI-HN, with a tendency towards further increase under DI-LN compared to DI-HN, and the RMF was higher under DI-LN compared to DI-HN. Although plant investment in root length per unit of leaf area increased as water and N resources became more limited, the amount of N taken up per unit root length decreased significantly under DI and decreased further with LN. This demonstrates that increased root investment in terms of biomass and length, which improve soil exploration and surface area for absorption, does not necessarily compensate for the lack of moisture and the critical role that water plays in N uptake by moderating the movement of nutrients to the root and facilitating diffusion into the root itself (Kunrath et al., 2020; Lemaire et al., 1996).

Impact of DI and N level on sorghum N uptake

High N addition under DI led to increased N uptake per unit root length and greater N concentration in plant tissues than DI-LN, but the benefit to plant functioning was unclear. We hypothesized that DI-HN, compared to DI-LN, would enhance N uptake and help maintain

physiological function and improve plant growth under water stress as other studies have shown (Randhawa et al., 2017; Saneoka et al., 2004; Shangguan et al., 2000; Song et al., 2019). This hypothesis was partially supported in that applying more N appears to increase N uptake, but this did not improve A_n , NUE_i , or shoot biomass production. Nitrogen is needed in high amounts to build and maintain photosynthetic structures (Song et al., 2019). However, water stress is equally or more important in regulating A_n , and DI conditions appeared to have superseded the effect of the N level (Moussa, 2008; Song et al., 2020). Therefore, our study provides little to no evidence to support previous findings that higher N can relieve water stress and improve A_n and shoot growth in water-limited conditions (Cossani et al., 2012; Song et al., 2019; Wang et al., 2016; Xiong et al., 2015). It is possible that if this experiment continued beyond ten weeks, the difference in N uptake between DI-HN and DI-LN treatments might have widened and ultimately impacted A_n , shoot growth, and grain production. We note that even though the DI-HN and DI-LN treatments resulted in the same shoot biomass, the biomass under DI-LN had lower tissue N concentration. At the time of grain filling, N in the plant biomass is translocated to the grain. Nitrogen deficiency in sorghum plants at this stage would limit grain filling and yield (Holman et al., 2019; Sigua et al., 2018). A future study in which plants are grown to maturation could more fully elucidate the effect of higher N additions under DI on grain yield and quality.

The potential impact of increased root investment on soil N cycling and soil properties

There is growing interest in breeding or managing crop roots for enhancing soil-plant-microbe interactions that improve N dynamics and soil health (Junaidi et al., 2018; Moreau et al., 2019). The results of our study show partial merit to this idea as we observed similar flexibility among all five sorghum genotypes in terms of allocation to roots responsive to water and N

availability. Root growth can impact N cycling in the soil directly and indirectly. Directly, root proliferation and root biomass allocation are essential for nutrient uptake (Gersani and Sachs, 1992; Granato and Raper, 1989; Hodge, 2004). Indirectly, root carbon (C), in the form of exudates and biomass, can fuel mineralization of organic N forms that are plant-available (Bais et al., 2006; Herman et al., 2006). This mechanism is especially important in an agricultural field because even in fertilized cropping systems, 40-80% of plant N is likely derived from mineralization of organic matter (Drinkwater and Snapp, 2007). Additionally, a significant portion of fertilizer is lost from agricultural fields with negative impacts on the environment (Davidson, 2009; Davidson et al., 2014; Liu et al., 2010). Therefore, managing crops to shift from fertilizer dependence towards synergistic beneficial soil-plant-microbe associations is desirable. However, greater reliance on soil-plant-microbe associations often costs shoot growth and yield because a higher proportion of C resources is directed belowground (Richards et al., 2007). Our study provides evidence that this tradeoff may not be so straightforward. While we did not measure exudates, our study did show that under DI-LN, increased investment in root biomass came without a negative impact on shoot biomass.

Increasing root biomass is also desirable because of the positive effect roots have on soil structure, C storage, and benefits of root-derived organic matter on soil water holding capacity (Czarnes et al., 2000; Daynes et al., 2013; Gautam et al., 2020). Depending on the type of sorghum grown, grain, or forage, most of the aboveground biomass is often removed during harvest, which leaves roots as the primary sources of soil C. Deficit irrigation of maize has been shown to increase deep root growth, with potential implications for soil C stocks (Flynn et al., 2021). Root C is thought to be more stable than shoot C (Rasse et al., 2005) and is important in efforts to increase C sequestration in agricultural systems (Horton et al., 2021; Jarecki and Lal,

2003; Lal, 2016). Increasing soil C also has implications for improving water holding capacity, which is desirable in water-limited agroecosystems (Williams et al., 2016). Together, all these potential impacts of increasing root growth could help make agriculture more sustainable.

To meet present and future food demands, any management practices that aim for greater sustainability should weigh implications for yield (Power, 2010). In a best-case scenario, increased root growth would improve soil-plant-microbe associations, improve soil nutrient cycling, reduce dependence on fertilizers, and potentially increase yields. Our results suggest that when water is already limiting and DI is necessary, altering N management can change root growth without impacting shoot biomass. This could be especially important in heterogeneous field environments where the decomposition and mineralization of organic matter plays a vital role in supplying nutrients to the plant. This opens the door for further exploration of synergies and efficiencies that can be gained by leveraging plant responses in future research.

5.5 Conclusion

This study provides insight into the whole-plant response of sorghum to water and N limitation. Our main finding is that water and N limitation shifted biomass allocation to the roots. We also found that under DI, high N input led to higher N uptake but that A_n , leaf area, and biomass were not improved. Results here bring into question the idea that N additions can ameliorate the impacts of water stress. The flexibility we observed in root growth to water and N level could indicate an adaptive strategy to improve crop water and nutrient uptake capacity, but data from this greenhouse study did not fully support this idea. However, in a field environment with more heterogeneous nutrient distribution, the same root response flexibility could prove vital to improving soil N dynamics and root-derived soil health properties.

Table 5.1 Sorghum genotypes used in a greenhouse experiment to understand the impact of water and N limitation on crop growth and biomass allocation. For each genotype, information is provided on general drought tolerance characteristics, water use efficiency (WUE) ranking from Turner et al. (2016), genotype origin, and associated references.

Genotype	Characteristics	WUE	Origin	References
BTx623	Pre-flowering drought tolerant	1	Southern Africa	(Brown et al., 2006; Hart et al., 2001; Murray et al., 2008; Paterson et al., 2009; Rosenow et al., 1983)
RTx430	Pre-flowering drought tolerant	2	Sudan/Ethiopia	(Howe et al., 2006; Liu and Godwin, 2012; MacKinnon et al., 1987; Miller, 1984; Rosenow et al., 1983; Wu et al., 2014)
Tx7000	Pre-flowering drought tolerant	3	Sudan/Ethiopia/Egypt	(Evans et al., 2013; Kebede et al., 2001; Rosenow et al., 1983; Subudhi et al., 2000)
IS3620C	Drought susceptible	4	West Africa	(Brown et al., 2006; Burow et al., 2011; Kebede et al., 2001; Tuinstra et al., 1996)
BTx642	Pre-flowering drought susceptible	5	North-central Africa	(Evans et al., 2013; Subudhi et al., 2000)

Table 5.2 Mean and standard error of total, shoot, and root biomass, shoot mass fraction (SMF), and root mass fraction (RMF) for five sorghum genotypes under three treatments: Full irrigation and high nitrogen (FI-HN), deficit irrigation, and high nitrogen (DI-HN), and deficit irrigation with low nitrogen (DI-LN) grown in a greenhouse for 10 weeks. Error bars represent standard error. ANOVA p-values for each main effect are presented at the bottom of the table with significant effects ($P < 0.05$) in bold.

Treatment	Genotype	Total Biomass (g)	Shoot Biomass (g)	Root Biomass (g)	SMF	RMF
FI-HN	Btx623	92 (11)	70 (8)	17 (1)	0.72 (0.04)	0.21 (0.04)
	RTx430	90 (na)	75 (20)	11 (na)	0.81 (na)	0.13 (na)
	Tx7000	80 (14)	68 (7)	12 (1)	0.79 (0.01)	0.16 (0.02)
	IS3620C	80 (12)	61 (5)	10 (1)	0.79 (0.01)	0.14 (0.02)
	Btx642	111 (na)	78 (6)	17 (na)	0.75 (na)	0.17 (na)
DI-HN	Btx623	60 (4)	40 (2)	15 (2)	0.67 (0.01)	0.27 (0.02)
	RTx430	46 (2)	31 (1)	12 (1)	0.67 (0.01)	0.28 (0.01)
	Tx7000	57 (3)	38 (2)	15 (1)	0.67 (0.01)	0.28 (0.01)
	IS3620C	49 (5)	33 (4)	11 (2)	0.67 (0.02)	0.25 (0.01)
	Btx642	56 (3)	33 (1)	17 (2)	0.60 (0.01)	0.34 (0.02)
DI-LN	Btx623	64 (2)	38 (1)	20 (2)	0.60 (0.02)	0.35 (0.02)
	RTx430	54 (2)	33 (1)	16 (1)	0.61 (0.01)	0.33 (0.01)
	Tx7000	57 (2)	35 (1)	17 (1)	0.62 (0.01)	0.32 (0.01)
	IS3620C	50 (2)	32 (1)	11 (1)	0.64 (0.01)	0.26 (0.01)
	Btx642	64 (3)	36 (1)	20 (1)	0.57 (0.01)	0.36 (0.01)
Average	Treatment	<0.01	<0.01	0.03	<0.01	<0.01
	Genotype	<0.01	<0.01	<0.01	<0.01	<0.01
	Treatment x Genotype	0.49	0.90	0.32	0.17	0.07

Table 5.3 Mean and standard error of leaf area, specific leaf area (SLA), leaves per tiller, net photosynthesis (A_n) and instantaneous nitrogen use efficiency (NUEi) for five sorghum genotypes under three treatments: Full irrigation and high nitrogen (FI-HN), deficit irrigation, and high nitrogen (DI-HN), and deficit irrigation with low nitrogen (DI-LN) grown in a greenhouse for 10 weeks. Error bars represent standard error. ANOVA p-values for each main effect are presented at the bottom of the table with significant effects ($P < 0.05$) in bold.

Treatment	Genotype	Leaf Area (cm ²)	SLA (cm g ⁻¹)	Leaves per tiller	A_n (μmol C m ⁻² s ⁻¹)	NUEi
						(μmol C m ⁻² s ⁻¹ N g ⁻¹)
FI-HN	Btx623	1978 (101)	28 (3)	5.0 (0.2)	46 (5)	10.4 (0.9)
	RTx430	1688 (224)	22 (3)	5.4 (0.2)	37 (6)	8.3 (1.6)
	Tx7000	1316 (146)	18 (1)	4.0 (0.4)	38 (4)	8.0 (0.9)
	IS3620C	1169 (143)	18 (2)	4.5 (0.5)	32 (7)	7.7 (2.0)
	Btx642	2690 (551)	34 (5)	5.8 (0.3)	42 (6)	10.3 (1.3)
DI-HN	Btx623	675 (93)	17 (2)	1.8 (0.1)	16 (2)	5.7 (0.9)
	RTx430	206 (51)	7 (2)	1.7 (0.1)	21 (6)	7.1 (1.5)
	Tx7000	381 (78)	9 (2)	1.5 (0.1)	22 (2)	8.3 (0.8)
	IS3620C	411 (84)	11 (2)	1.3 (0.1)	20 (2)	7.8 (1.1)
	Btx642	397 (107)	12 (3)	1.9 (0.5)	15 (4)	5.8 (1.6)
DI-LN	Btx623	611 (81)	16 (3)	1.4 (0.2)	12 (4)	5.1 (1.6)
	RTx430	373 (84)	11 (2)	1.3 (0.1)	12 (4)	4.9 (1.5)
	Tx7000	331 (58)	9 (2)	1.0 (0.02)	13 (4)	5.5 (1.4)
	IS3620C	457 (103)	14 (3)	1.1 (0.1)	17 (3)	6.9 (1.1)
	Btx642	507 (61)	14 (2)	1.3 (0.1)	18 (5)	8.4 (1.1)
Average						
	Treatment	<0.01	<0.01	<0.01	<0.01	0.17
	Genotype	<0.01	<0.01	<0.01	0.86	0.73

Treatment x Genotype	<0.01	0.08	0.20	0.03	0.11
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Table 5.4 Mean and standard error of total root length, fine root length, coarse root length, ratio of fine: coarse root length, ratio of root length: leaf area, specific root length (SRL) of fine roots, SRL for coarse roots for five sorghum genotypes under three treatments: Full irrigation and high nitrogen (FI-HN), deficit irrigation, and high nitrogen (DI-HN), and deficit irrigation with low nitrogen (DI-LN) grown in a greenhouse for 10 weeks. Error bars represent standard error. ANOVA p-values for each main effect are presented at the bottom of the table with significant effects ($P < 0.05$) in bold.

Treatment	Genotype	Total Root length (m)	Fine root length (m)	Coarse root length (m)	Fine: coarse root length	SRL of fines (m g ⁻¹)	SRL of coarse (m g ⁻¹)	Root length: leaf area
FI-HN	Btx623	2151 (230)	2009 (244)	142 (21)	15 (4)	269 (50)	15 (2)	1.0 (0.02)
	RTx430	1089 (na)	989 (na)	99 (na)	10 (na)	299 (na)	13 (na)	0.6 (na)
	Tx7000	2068 (462)	1933 (482)	135 (53)	20 (9)	306 (38)	23 (8)	1.8 (0.2)
	IS3620C	1192 (25)	1115 (16)	77 (9)	15 (2)	369 (24)	11 (1)	1.2 (0.3)
	Btx642	3000 (na)	2886 (na)	114 (na)	25 (na)	370 (na)	12 (na)	0.9 (na)
DI-HN	Btx623	1897 (196)	1833 (193)	64 (6)	29 (3)	229 (20)	19 (1)	3.6 (0.9)
	RTx430	1098 (129)	1013 (128)	85 (13)	13 (3)	193 (27)	13 (2)	5.8 (1.3)
	Tx7000	2614 (378)	2526 (376)	88 (9)	30 (4)	280 (26)	14 (1)	8.6 (1.5)
	IS3620C	1323 (247)	1266 (238)	57 (10)	23 (2)	314 (35)	8 (1)	4.5 (1.1)
	Btx642	1979 (301)	1880 (298)	99 (14)	20 (3)	251 (28)	10 (1)	6.8 (2.2)
DI-LN	Btx623	2357 (162)	2161 (168)	196 (19)	12 (2)	270 (35)	17 (3)	4.2 (0.7)
	RTx430	1439 (313)	1330 (312)	109 (10)	14 (5)	211 (44)	12 (1)	4.8 (1.3)
	Tx7000	2862 (166)	2683 (137)	178 (29)	16 (2)	361 (35)	19 (3)	10.9 (3.3)

IS3620C	1793 (139)	1704 (131)	88 (13)	20 (2)	484 (22)	12 (2)	4.8 (1.0)
Btx642	3249 (524)	3122 (524)	127 (20)	27 (6)	357 (37)	11 (1)	6.5 (1.4)

Average

Treatment	0.21	0.27	<0.01	0.09	0.01	0.02	<0.01
Genotype	<0.01	<0.01	<0.01	0.09	<0.01	<0.01	0.07
Treatment x Genotype	0.66	0.64	0.01	0.05	0.31	0.26	0.90

Table 5.5 Mean and standard error of biomass N concentration (%), N uptake, N uptake per unit root length, and shoot biomass per unit N uptake for five sorghum genotypes under three treatments: Full irrigation and high nitrogen (FI-HN), deficit irrigation, and high nitrogen (DI-HN), and deficit irrigation with low nitrogen (DI-LN) grown in a greenhouse for 10 weeks. Error bars represent standard error. ANOVA p-values for each main effect are presented at the bottom of the table with significant effects ($P < 0.05$) in bold.

Treatment	Genotype	Biomass N Concentration (%)	N uptake (g)	kg N uptake per root length (kg m ⁻¹)	Shoot Biomass per N uptake (g g ⁻¹)
FI-HN	Btx623	4.3 (0.1)	3.0 (0.4)	1.4 (0.2)	23.2 (0.7)
	RTx430	4.8 (0.3)	3.6 (0.2)	2.9 (na)	20.9 (1.2)
	Tx7000	5.5 (0.05)	3.7 (0.4)	1.7 (0.2)	18.3 (0.2)
	IS3620C	4.8 (0.1)	2.9 (0.2)	2.5 (0.4)	20.9 (0.5)
	Btx642	4.4 (0.06)	3.4 (0.3)	1.3 (na)	22.7 (0.3)
DI-HN	Btx623	2.8 (0.3)	1.1 (0.04)	0.6 (0.1)	36.0 (1.2)
	RTx430	3.4 (0.3)	1.0 (0.04)	1.1 (0.1)	29.4 (1.1)
	Tx7000	3.3 (0.2)	1.3 (0.07)	0.5 (0.1)	30.0 (0.7)
	IS3620C	3.1 (0.3)	1.0 (0.1)	0.9 (0.1)	32.2 (1.1)
	Btx642	2.7 (0.3)	0.9 (0.03)	0.5 (0.1)	36.9 (1.5)
DI-LN	Btx623	2.0 (0.1)	0.7 (0.01)	0.3 (0.03)	49.7 (1.6)
	RTx430	2.4 (0.2)	0.8 (0.03)	0.6 (0.1)	41.9 (1.4)
	Tx7000	2.3 (0.1)	0.8 (0.03)	0.3 (0.03)	43.0 (0.3)
	IS3620C	2.3 (0.1)	0.7 (0.02)	0.4 (0.04)	42.8 (1.1)
	Btx642	2.0 (0.1)	0.7 (0.03)	0.3 (0.06)	49.3 (1.3)
Average					
	Treatment	<0.01	<0.01	<0.01	<0.01
	Genotype	<0.01	<0.01	<0.01	<0.01

Treatment
x Genotype

<0.01

0.15

<0.01

0.23

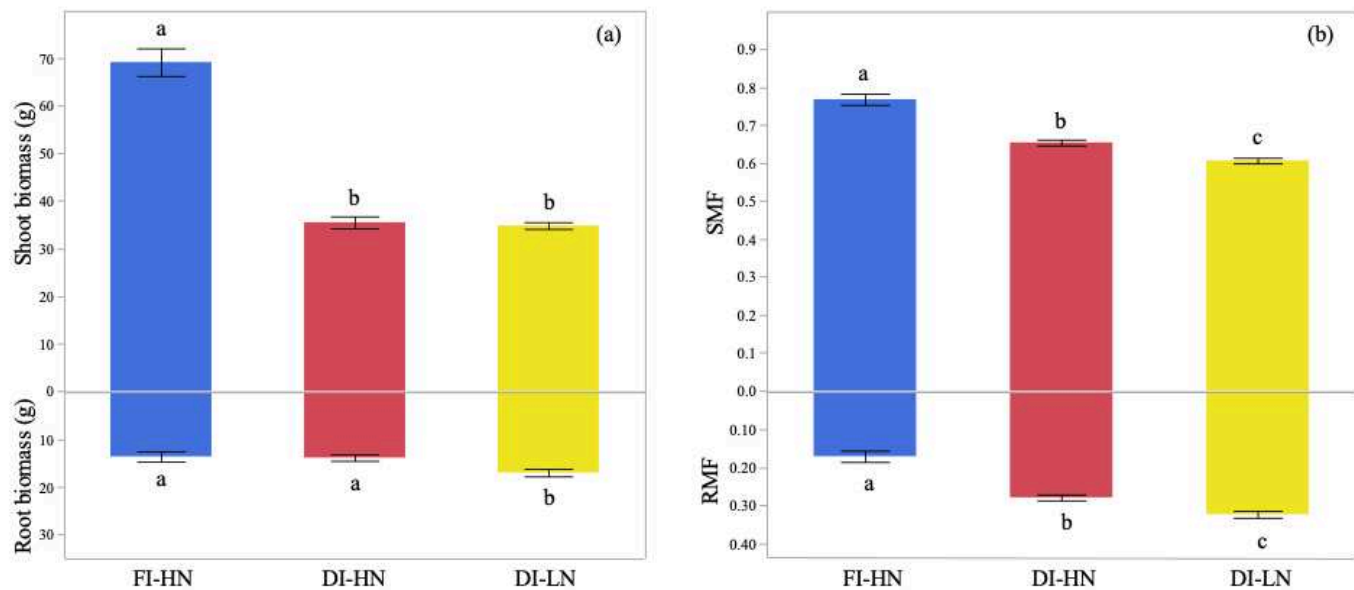


Figure 5.1 Mean a) sorghum shoot and root biomass and b) shoot mass fraction (SMF) and root mass fraction (RMF) for five sorghum genotypes under three treatments: Full irrigation and high nitrogen (FI-HN), deficit irrigation and high nitrogen (DI-HN), and deficit irrigation with low nitrogen (DI-LN) grown in a greenhouse for ten weeks. Error bars represent standard error. Different letters indicate a significant difference between treatments ($P < 0.05$).

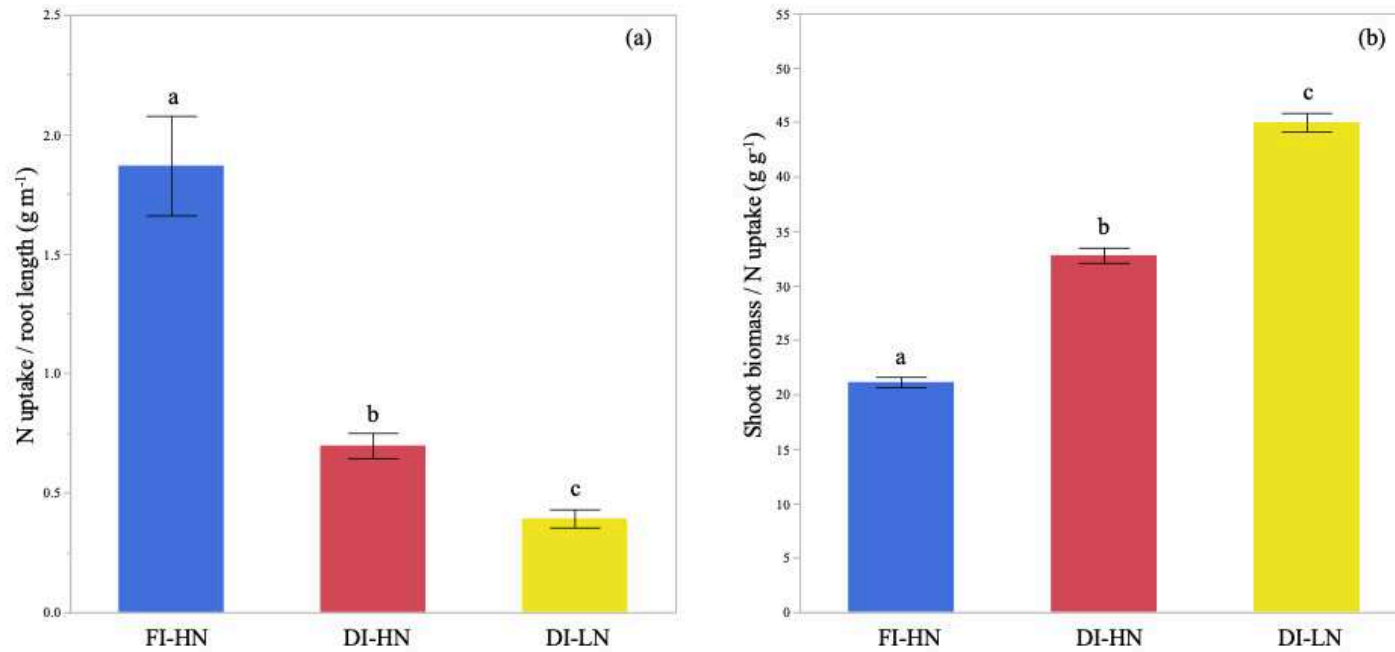


Figure 5.2 Mean a) N uptake per unit root length and b) shoot biomass per unit N uptake for five sorghum genotypes under three treatments: Full irrigation and high nitrogen (FI-HN), deficit irrigation and high nitrogen (DI-HN), and deficit irrigation with low nitrogen (DI-LN) grown in a greenhouse for ten weeks. Error bars represent standard error. Different letters indicate a significant difference between treatments ($P < 0.05$).

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