DISSERTATION

# ADDRESSING NITROGEN AND WATER AVAILABILITY CHALLENGES IN SEMI-ARID MAIZE CROPPING SYSTEMS

Submitted by

Tyler Donovan

Department of Soil and Crop Sciences

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Doctoral Committee:

Advisor: Meagan Schipanski
Co-Advisor: Louise Comas

Francesca Cotrufo
Richard Conant

# ABSTRACT

## ADDRESSING NITROGEN AND WATER AVAILABILITY CHALLENGES IN SEMI-ARID MAIZE CROPPING SYSTEMS

In many parts of the world, access to irrigation is threatened as competition for water resources increases and water availability decreases. This includes the Great Plains Region of the U.S. where roughly 25% of U.S. irrigated cropland is located. Loss of irrigation threatens global food security as irrigated lands are highly productive; accounting for just 20% of cropland but responsible for 40% of agricultural production. Thus, there is urgent need for achieving high yields with less water. Many studies have been conducted to increase crop productivity with limited water, but the interactive effect of nitrogen (N) and water availability on crop response has received limited attention with variable conclusions. Additionally, the effect of varying N availability under different water levels on soil N mineralization (Nmin), contribution of Nmin to crop N uptake, and the recovery and fate of N fertilizer has been largely unexplored. Soil Nmin is an important source of N for crops therefore, quantifying Nmin rates and contribution to crop N uptake is important for N management. Additionally, minimizing N losses is an important goal for agroecosystems as N losses come with an economic and environmental cost. The general aim of my dissertation was to explore the effects of N availability on soil N cycling and crop response within maize cropping systems, an important irrigated crop in the Great Plains Region, under contrasting water availability.

Examining field data from 2021 – 2023, I found that maize grain yield response to N was dependent on water. When water was limited, maize grain yield was maximized with ~ 200 kg N ha$^{-1}$, with excess N being detrimental for all three growing seasons. This was true even during 2023, which was an extremely wet year, and had reduced N fertilizer rates due to higher pre-plant soil residual N. Maize N uptake continued to increase with N availability beyond 200 kg N ha$^{-1}$, showing maize was not co-limited by N when water was limited water. Rather, excess N and subsequent N uptake had negative effects on root and shoot growth, potentially via effects on stomatal conductance and photosynthesis, leading to yield declines. Soil net Nmin surrounding peak maize N uptake exhibited an N × water interaction where increased N fertilization rate decreased net Nmin with full water but increased with N rate when water was limited. Soil N-acquiring enzyme activity, a proxy for gross Nmin, had a different response where it increased with N regardless of water. This could suggest N fertilizer increased plant available N through increased microbial mediated depolymerization of N containing compounds in the soil. The different responses were likely due to the exclusion of living maize plants and maize N uptake in the net Nmin incubation tubes. Across the entire season, both net Nmin and enzyme activity tended to be higher during maize vegetative stages than during early reproductive stages when N demands are the highest.

A $^{15}$N tracer study revealed that recovery was high, and losses low and unaffected by N and water treatments. This suggests that lower N rates should have lower N losses. Maize N uptake increased with N rate, but primarily from $^{15}$N fertilizer, rather than non-N fertilizer sources such as soil Nmin. This could be due to the asynchrony between soil N supply and maize N demand. Additionally, microbial biomass N at the end of the season suggests that immobilization occurred, but primarily for non-N fertilizer sources. Immobilization of non-N fertilizer sources later in the season when soil Nmin rates are low and maize N demands are high likely led to maize acquiring N from N fertilizer to meet its N requirements. The wet growing season during

2023 made the water treatments negligible. Future studies with treatment differences in water availability could reveal how water availability affects fate and recovery of N fertilizer, as well as contribution of non-N fertilizer sources, such as soil Nmin, to crop N uptake with different N fertilizer rates.

Overall, my findings show that water limited maize is not co-limited by N, and that excess N is detrimental to maize growth and yield. For limited water, reducing N fertilizer rate should reduce N losses while still maximizing yields and resource use efficiency. Reducing N fertilizer rate when water is fully available and maize N demands are high may be challenging. Higher N fertilizer rates appeared to increase bioavailable N through increased soil enzyme activity, however maize was not able to significantly increase uptake of N from sources other than the $^{15}$N fertilizer applied, such as soil Nmin, regardless of treatment. Maize was more reliant on N fertilizer rather than non-N fertilizer sources when supplied with high N fertilizer rates, while more reliant on non-N fertilizer sources when supplied with low N fertilizer rates. Management practices that increase internal N cycling, especially later in the season when maize N demands are greater, may help reduce the reliance on synthetic N fertilizer inputs thus reducing N losses without sacrificing productivity.

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# CHAPTER 1: INTRODUCTION

The two resources that have the greatest impact on crop productivity are water and nitrogen (Plett et al., 2020). Approximately 40% of global land surface is arid or semi-arid (Golla, 2021). Arid and semi-arid land is characterized by low precipitation where potential evapotranspiration is greater than precipitation making water the most limiting factor (Ahmed et al., 2022). Crop production in these regions is greatly enhanced when crop water demands are met via irrigation. Irrigated lands account for a relatively small percentage of arable land but are disproportionately productive relative to their rainfed and dryland counterparts. Globally, roughly 20% of arable land is irrigated, but this land accounts for approximately 40% of global food production (Mehta et al., 2024). Along with meeting crop water demands, nitrogen (N) additions greatly increase crop yields. Of the essential crop nutrients, N is needed in the highest quantity, yet it is often one of the most limiting for yield (Drinkwater et al., 2017; Karthika et al., 2018). Therefore, N additions can greatly increase yields. Additions of N fertilizer have largely been responsible for the yield increases observed in the last several decades, so much so that it is estimated that over half of the global population could not be sustained without it (Ladha et al., 2016; X. Zhang, Davidson, et al., 2015).

Loss of access to irrigation and the increased likelihood of water limitations threaten crop production in many regions of the world. This includes the Great Plains Region in the United States where roughly a quarter of irrigated crop production in the United States occurs (Derner et al., 2015). In the Great Plains Region, irrigated land is shifting to dryland production, and loss of irrigated land may continue largely due to depleted water level in the Ogallala Aquifer and increased competition for water resources (Evett et al., 2020). This loss of irrigation can be exacerbated by shifting precipitation patterns limiting water supply during key crop growth stages, increasing temperatures, and more frequent and severe drought that the region has and is projected to experience (Derner et al., 2015). Loss of irrigation and increased water stress can greatly reduce yields, economic viability of regions, and ecosystem services such as soil organic matter concentrations (Núñez & Schipanski, 2023; D. R. Rudnick, Irmak, Ray, et al., 2017). Therefore, improving crop performance with less water is necessary.

Much research has been dedicated to identifying management strategies (Nielsen et al., 2005; Schneekloth et al., 2020; H. Zhang et al., 2019), and crop traits for breeding programs (Evett et al., 2020; Gleason et al., 2022, 2024) that are beneficial for crop production with limited water. One area that is lacking clear understanding is the effects of N availability when water is limited. Crop yield and sink demand determines the potential N uptake, and water limitations reduce crop growth thus N demands and uptake (Ciampitti & Vyn, 2011; Lemaire et al., 1996; Zamora-Re et al., 2020). However, limited water also reduces transpiration, and transpiration drives mass flow which is the main mechanism by which crops accumulate N from the soil (Lambers & Oliveira, 2019). Additionally, soil N mineralization (Nmin), which is an important N source for crops, tends to decrease with limited water (Elrys et al., 2021; Z. Li et al., 2019). Therefore, reduced crop N uptake could be due to reduced soil N mineralization (Nmin) as well as reduced diffusion and mass flow (He & Dijkstra, 2014). Thus, crops may be co-limited by N during water limitations and additional N may be beneficial.

The effect of additional N on crop performance when water is limited is variable. Studies have found that increased N additions are beneficial to crop yield and water use efficiency when water is limited (Ashraf et al., 2016; O'Neill et al., 2004; Pan et al., 2024), while others have found thresholds where moderate N is beneficial, but excess N does not increase yield, and may even decrease yield (Y. Li et al., 2020; Pandey, Maranville, & Admou, 2000; D. R. Rudnick & Irmak, 2013). Along with potential yield loss, excess N fertilizer decreases soil organic matter accumulation, increases soil acidity, and reduces profits for producers (Poffenbarger et al., 2017; Shapiro et al., 2008; D. Tian & Niu, 2015). Further, N losses to the environment increase with increased N additions causing harm to the environment (Delgado et al., 2023; Ladha et al., 2016). Given the economic and environmental impact of N additions further exploration of the effects of N when water is limited are needed. This is especially true given that it is common for producers to purposefully add high amounts of N as "insurance" to ensure there is ample N for high yields (Flynn et al., 2023; Udvardi et al., 2021).

The interaction between N and water availability has been explored in different reviews (Cramer et al., 2009; Ding et al., 2018; Drobnitch et al., 2024), but none have explored soil Nmin. Indeed, studies exploring the interactive effect of N and water availability on soil Nmin rates at all are rare. This is an important knowledge gap worth considering as Nmin is often the largest N source for crops during the growing season (Yan et al., 2020). Both N and water can directly affect soil Nmin by meeting the metabolic needs of the soil microbial community that carryout Nmin (Mooshammer et al., 2014; Schimel, 2018). The effects can also be indirect as N and water alter the quality and quantity of plant inputs into the soil which in turn affects soil Nmin (Booth et al., 2005; Brown et al., 2014; Huo et al., 2017). Further, the breakdown of hydrological connectivity in water limited soils reduces microbial activity and Nmin (Deng et al., 2021). Understanding the effects of N and water on Nmin rates is important for managing N with the aim of synchronizing crop N needs with soil Nmin (Ma et al., 1999).

Besides quantifying Nmin rates, quantifying the contribution of Nmin to crop N uptake is important for improving N management and becoming less reliant on external N inputs. The use of $^{15}$N tracer studies allows for such quantification. Tracer studies can be used to partition the sources of N utilized by crops (N derived from $^{15}$N fertilizer applied, Ndff, or other sources of N, non-Ndff) along with quantifying the "fate" (crop uptake, remain in soil, unrecovered or lost) of the labeled N addition (Poffenbarger et al., 2018; Rimski-Korsakov et al., 2012; Y. Wang et al., 2017). Maximizing recovery of N inputs and promoting internal N cycling are important goals for agroecosystems in order to minimize the environmental harm of N additions (Breza et al., 2023; Grandy et al., 2022; Yan et al., 2020), but is another important knowledge gap with respect to the interaction of N and water availability.

The general objective of my dissertation was to explore the interactive effects of N and water availability on maize cropping systems in Eastern Colorado. Maize was used as the model crop for the experiment as it is an important crop for the region, especially where irrigation is available. Specifically, I aimed to answer the following questions:

1. What is the impact of N availability on maize grain yield when water is limited?
2. What are the mechanisms contributing to maize grain yield response?
3. What are the effects of N and water availability on soil Nmin rates surrounding peak maize N uptake and the entire season?
4. Which sources of N (Ndff vs non-Ndff) is maize utilizing with different N and water availabilities?
5. How is the fate of N fertilizer changed by N rate and water availability?

In 2021 a field experiment was established where maize was grown continuously with two water availabilities and six N fertilizer rates. In Chapter 2, I followed the experiment for three growing seasons (2021 – 2023) and took different measurements to inform the first two questions. Specifically, maize grain yield, aboveground biomass, belowground biomass, and aboveground maize N uptake were measured. In Chapter 3 I address the third question by measuring *in-situ* net Nmin and soil enzyme activity over the entire maize growing season during the 2021 and 2022 growing seasons. In Chapter 4, micro-plots for a $^{15}N$ tracer study were established during the 2023 growing season to answer the fourth and fifth questions. Using the labeled N fertilizer allowed me to differentiate between sources of N the maize was utilizing as well as determine the fate of the fertilizer applied.

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# CHAPTER 2: EXCESS NITROGEN DECREASES MAIZE GRAIN YIELD WHEN WATER IS LIMITED

## 2.1 INTRODUCTION

Water is often the most limiting resources for crop growth with increased water limitations potentially becoming more common in the Great Plains Region (Deines et al., 2020; Derner et al., 2015; Plett et al., 2020). This threatens the viability of rural economies that rely on irrigation as well as food security as irrigated lands are disproportionately productive; accounting for roughly 20% of arable land yet responsible for nearly 40% of calorie production (Garces-Restrepo et al., 2007). Given this, preserving water resources and improving water use efficiency is gaining attention as water limitations become more widespread and frequent across the globe (Nozari et al., 2024; Schipanski et al., 2023; Zou et al., 2021). As the global population grows, agricultural demand grows. Under *status quo* projections, the demand for agricultural products will increase by 75% by 2050 compared to 2005 levels (Godfray et al., 2010; Hunter et al., 2017; Tilman et al., 2011). Therefore, it is important to develop productive cropping systems adapted to limited water conditions while also protecting water quality and availability for future generations.

One area that needs more research is the effect of nitrogen (N) availability on crop performance under limited water. When water is limiting crops can be co-limited by N due to reduced soil N supply and mobility (Borken & Matzner, 2009; He & Dijkstra, 2014), hindering crop N uptake (Gonzalez-Dugo et al., 2010) and reducing grain yield. Adding additional N can help crops overcome this N co-limitation by increasing N availability and N uptake and therefore can increase yields and water use efficiency under water limitations (Cossani et al., 2012; Di Paolo & Rinaldi, 2008; Y. Li et al., 2019). These yield increases can be attributed to increased photoprotective mechanisms (Ahmadi et al., 2010; Eissa & Roshdy, 2019; Song et al., 2019), osmotic adjustment and cell membrane stability (Nematpour et al., 2019; Saneoka et al., 2004; L. X. Zhang et al., 2012) increased root growth, root length diameter, and water use (W. Liu et al., 2018; Ogola et al., 2002; L. Wang et al., 2018), increased sink potential and increased grain fill (A. Ullah et al., 2022), and stomatal conductance and photosynthetic rates (Dinh et al., 2017; X. Wang et al., 2016; Zhong et al., 2019).

However, there is conflicting evidence about the positive effects of high N with low water availability. Some studies have found that too much N availability can be detrimental to plant growth when water is limiting (Flynn et al., 2023; Mon et al., 2016). Additionally, water limitations can reduce crop N demand and uptake even when N availability is high (Gonzalez-Dugo et al., 2010; Pandey, Maranville, & Chetima, 2000; Peng et al., 2010). Along with potential yield reductions, excess N can hurt ecosystem services such as reducing soil carbon relative to "optimal N" (Poffenbarger et al., 2017; Singh, 2018), and cause soil acidification (D. Tian & Niu, 2015). Reduced plant N uptake can increase nitrate ($NO_3$) leaching by as much as 34% the following season due elevated soil N concentrations (Loecke et al., 2017). Losses of N to the environment are already high and increase rapidly when excess N is applied (Puntel et al., 2016). Agricultural N losses have contributed to nearly doubling the amount of reactive N in the biosphere in the last century (Battye et al., 2017; Gruber & Galloway, 2008), with use and demand for N expected to increase in the agricultural sector in the future (Vishwakarma et al., 2022; Zhang et al., 2015). Therefore, there is urgent need for a better understanding of how N and water availabilities affect crop growth to help evaluate the tradeoffs associated with further increasing N use.

Effectively managing N is challenging. Producing high cereal crop yields requires high total N uptake, but the supply of soil N needs to be in synchrony with plant N demands over the growing season (Ma et al., 1999a; Xu et al., 2012). Grain yields can be hard to predict making it difficult to know how much additional N fertilizer is needed to meet crop demands (Grandy et al., 2022). It is common for most, if not all, fertilizer to be added prior to the growing season based on optimal growing conditions and yield potential (Grandy et al., 2022; Udvardi et al., 2021), or fields are held near N saturation to ensure there is ample N for high grain yields (McSwiney et al., 2010; Udvardi et al., 2021). This is problematic due to variable precipitation and irrigation availability in season (D. R. Rudnick, Irmak, Ray, et al., 2017), and increasing unpredictability of water limitations (Deines et al., 2020; Derner et al., 2015).

The objective of this field study was to investigate maize response to different N and water availabilities and identify the underlying factors contributing to these responses. A better understanding of maize response can lead to improved N and water management thus improving yields and resource use efficiency. Specifically, we sought to: 1) examine maize grain yield and aboveground biomass in response to N and water availability; 2) quantify total aboveground maize N uptake and identify if maize yield was limited by N uptake or other processes; and 3) examine maize resource use efficiency (water productivity, WP, and nitrogen use efficiency, NUE) in response to N and water availability.

## 2.2 MATERIALS AND METHODS

### 2.2.1 SITE DESCRIPTION

The experiment was conducted at the USDA-ARS Great Plains Research Center in Akron, Colorado (40°09 N, 103°09 W, 1,383 m). The dominant soil type is a Weld silt loam (fine, smectitic, mesic Aridic Argiustoll). The field site is within a semiarid climate with average monthly temperatures ranging from 23 °C in the summer to – 5 °C in the winter, and average annual precipitation of 420 mm occurring primarily (69%) from May – September (Schneekloth et al., 2020). In 2019 and 2020, the field was managed as a fully irrigated maize system with limited N applications to reduce residual soil N levels for the start of this study. Prior to this, the field had been managed using no-till practices since before 1990 for multiple dryland or irrigated crops.

A field trial was established in 2021 with data collected during the 2021, 2022, and 2023 growing seasons. On May 6, 2021, a 104-day maturity maize hybrid (DKC 54-64) was planted at 79,000 seeds ha$^{-1}$ and on May 10, 2022, the same maize hybrid was planted at 84,000 seeds per ha$^{-1}$. On May 8, 2023 a 106-day maturity maize hybrid (DKC 56-65) was planted at 84,000 seeds ha$^{-1}$. The field remained in no-till except for 2023 which was strip-tilled prior to planting. Maize residue was not harvested, and remained on the field.

We utilized a split-plot experimental design with a total of 36 plots. Plots were 12 rows wide and approximately 37 m long with 0.76 m row spacing. There were 3 blocks with 2 water treatments randomly assigned within each block and 6 N availability treatments (sum of N fertilizer applied, N in irrigation water, and pre-planting soil inorganic N 0 – 90 cm) randomly assigned within each of those water treatments. Water treatments consisted of full water (100% ET), and limited water (70% ET), which is near average seasonal precipitation for the region (dryland conditions). During the 2021 and 2022 growing season the N treatments started at 22 kg N ha$^{-1}$ and increased in increments of 50 – 51 kg ha$^{-1}$ with the highest rate being 275 kg N ha$^{-1}$ capturing low to excessive N additions. Soil residual inorganic N was much higher at the start of 2023 so N application rates were reduced. When considering both residual N and applied N, N treatments were similar across the growing seasons. During the 2023 growing season the full water N rates were 22, 56, 106, 168, 212, and 247 kg N ha$^{-1}$. For limited water, N rates were 22, 56, 106, 123, 123, and 202 kg N ha$^{-1}$. At planting, liquid ammonium phosphate at a rate of 22 kg N ha$^{-1}$ and 67 kg P ha$^{-1}$ was added across all plots during the 2021 growing season. During the 2022 and 2023 growing seasons liquid ammonium phosphate at a rate of 22 kg N ha$^{-1}$ and 44 kg P ha$^{-1}$ was added at planting across all plots. Growth stages were documented with standard notation of stages for maize (Abendroth et al., 2011). Nitrogen was side dressed at V6-V7 (6th-7th leaf vegetative stage) as urea ammonium nitrate (UAN, 32-0-0) banded to the soil surface approximately 5 cm from the row to create the different nitrogen treatments. Side dressed fertilizer was immediately followed by a watering event of 13 mm to incorporate it into the soil.

Soil inorganic N was measured before planting each season in the spring. In 2021, 3 soil samples were taken from each block with a tractor mounted hydraulic probe (Giddings, Windsor, CO) to a depth of 90 cm. Inorganic N amounts ranged from 24 kg ha$^{-1}$ – 35 kg ha$^{-1}$. In 2022 and 2023, 2 samples were taken from each plot to a depth of 90 cm with the same hydraulic probe. Soil inorganic N ranged from 11 kg N ha$^{-1}$ – 24 kg N ha$^{-1}$ across the different treatments in 2022, and. from 29 – 124 kg N ha$^{-1}$ in 2023. Maize rows shifted to the north ~ 15 – 20 cm with each growing season so rows were not right on top of each other from one season to the next. The plot treatments were in the same location for each growing season, verified by GPS coordinates from the previous season. Spring residual inorganic N measurements were only used to adjust N application rates in 2023.

### 2.2.2 EVAPOTRANSPIRATION MEASUREMENT AND IRRIGATION MANAGEMENT

The method used for measuring evapotranspiration (ET) is described by (Schneekloth et al., 2020). Briefly, neutron probes (model 503DR, Concord, CA) were used to measure soil water content on a weekly basis (Bowman & King, 1965). Soil water measurements were done in 0.3 m increments to a depth of 1.8 m. Neutron probes were installed in the 6th row of each plot (the middle row) shortly after maize emergence and were removed after harvest.

A simplified water balance method was used to calculate ET (Bowman & King, 1965):

$$ET = PAV + IAV + \Delta MAV\ (+\ CAV + RAV + GAV),$$

where ET is evapotranspiration, PAV is total precipitation, IAV is net irrigation, CAV is total capillary rise from below rooting zone, ΔMAV is net change in soil water, RAV is net change in soil water due to surface flow, GAV is total percolation of water below the rooting depth. Drainage, capillary rise, and surface flow were assumed negligible. Using the water balance approach, we calculated the consumptive water use for the growing season.

Water was applied with a linear sprinkler system. The amount of water applied was based on growth stage and soil moisture content. The full water treatment avoided water stress, while the limited water treatment represented the long-term average annual precipitation amounts for dryland production in the region. This average corresponds to approximately 70% consumptive water use relative to the full water. The limited water treatment received more water during the reproductive stages, to reduce stress during this critical growth stage, compared to the vegetative stages, but still experienced some water stress during reproductive stages especially in 2022. The spring of 2021 was much wetter than the spring of 2022 allowing water applications to start later in 2021. The 2023 growing season had above average precipitation so the irrigation did not start until August, and the limited water treatment did not receive any supplemental irrigation.

### 2.2.3 GRAIN YIELD, ABOVEGROUND BIOMASS, AND PLANT NITROGEN ACCUMULATION

Aboveground maize nitrogen uptake was measured at silking (R1) and again at physiological maturity (R6). Maize N uptake was measured by collecting 5 whole consecutive plants from all the plots from one of the center 2 rows. In 2023 the data for R1 stalks was lost prior to analysis and therefore data is not available. In 2023 dry weights were not recorded for R6 leaves and stalks. Dry stalk weights were estimated using the ratio of fresh stalks to dry leaf from 2021 for all plots. Dry leaf weights were estimated using the ratio of dry leaf to fresh stalks from 2021 for all plots. Maize samples were separated by leaves, stalks, grain, and cobs, dried at 60° C and weighed. Plant samples were then ground, homogenized, and a subsample was analyzed for total N concentration using a dry combustion elemental analysis (LECO Tru-SPEC, St. Joseph, MI, USA). Total N uptake was calculated by multiplying the oven dry biomass of each plant component by its corresponding N concentration and summing all the aboveground parts. Maize N uptake between R1 and R6 was calculated by taking the difference between N uptake from R6 samples and R1 samples. Maize grain yield and aboveground biomass were calculated at R6 using the same samples for N uptake. The grain was separated from the ears, weighed, measured for moisture content, and adjusted to 15.5% moisture. Maize N uptake, yield, and biomass data was converted to a per hectare basis using the harvested area.

### 2.2.4 BELOWGROUND BIOMASS

Maize root biomass was only measured during the 2021 growing season after maize reached physiological maturity and had been harvested. Two soil cores per plot were taken with a 3.8 cm diameter tractor mounted hydraulic probe (Giddings, Windsor, CO): one in-row core, and one inter-row core. Soil cores were taken to a depth of 180 cm and were split into 7 depths (0 – 15, 15 – 30, 30 – 60, 60 – 90, 90 – 120, 120 – 150, and 150 – 180 cm). Visible roots were removed from each soil depth increment by hand-picking from samples. Soil cores were passed through a 2 mm sieve and roots were washed to remove soil and rocks from the sample. Root samples were then oven-dried at 60°C, weighed, and sub-replicated averaged for each plot on a ground area basis. Root to shoot ratio (R:S) was calculated by dividing total root biomass from the entire soil profile by total aboveground biomass at R6.

### 2.2.5 WATER PRODUCTIVITY AND NITROGEN USE EFFICIENCY

Grain yield and maize N uptake were used to calculate water productivity (WP) and nitrogen use efficiency (NUE) metrics. Water Productivity was calculated using Equation 1 where higher values indicate more grain yield per total evapotranspiration (ET) used during the crop growing season.

Equation 1: $\frac{Grain\ Yield\ (kg\ grain\ ha^{-1})}{Evapotranspiration\ (mm)}$

Partial N Balance grain ($PBN_{grain}$) was calculated using Equation 2 where values > 1 indicate soil N mining, or more nitrogen being removed than added and values < 1 indicate potential over application.

Equation 2: $\frac{Maize\ Nitrogen\ Uptake\ grain\ (kg\ N\ ha^{-1})}{Nitrogen\ fertilizer\ rate\ (kg\ N\ ha^{-1})}$

N Utilization Efficiency (NUtE) was calculated using Equation 3 where higher values indicate more grain yield per unit nitrogen accumulated by the plant.

Equation 3: $\frac{Maize\ Grain\ Yield\ (kg\ ha^{-1})}{Maize\ Nitrogen\ Uptake\ (kg\ N\ ha^{-1})}$

### 2.2.6 STATISTICAL ANALYSIS

A linear mixed effects model was used for all analyses to determine how the response variables (yield, aboveground biomass, belowground biomass, N uptake, grain C:N ratio, WP, and NUE) were affected by water treatment, nitrogen availability (N fertilizer rate, soil residual N 0 – 90 cm, and N in applied water), year, and their interactions. Model selection was guided by Akaike Information Criterion (AIC) scores and visual inspection of model fit (i.e. graphed residuals and fitted values and quantile-quantile plots). The split plot design and repeated measures analyses were integrated into the mixed model structure through the inclusion of block and its interactions with irrigation and N rate as random factors. Water availability and year were treated as categorical and N availability was treated as continuous. Response variables and N availability were checked for normality, equal variance, and homogeneity and were transformed to meet model assumptions. Grain yield, total aboveground biomass at R1 and R6, total N uptake, N uptake from germination to R1, and N uptake from R1 – R6 had a quadratic relationship with N availability, which was modeled using a quadratic polynomial term. Maize WP and NUtE were log transformed to meet model assumptions and N availability was modeled using a quadratic polynomial term. Maize NUE $PNB_{grain}$ was log transformed to meet model assumptions and N availability was modeled using a quadratic polynomial term. Maize belowground biomass was log transformed for both the entire soil profile and the top 0 – 60 cm to meet model assumptions. An ANOVA with the "Kenward-Roger" degrees of freedom adjustment was used to assess the significance of the fixed effects and their interactions within the context of this mixed-effects model. Analyses were performed in R version 4.2.2 using the lme4, lmeTest, and emmeans packages (R Core Team, 2022).

## 2.3 RESULTS

### 2.3.1 PRECIPITATION, IRRIGATION, AND NITROGEN AMOUNTS

The 10-yr average annual precipitation for the site is 420 mm with 313 mm occurring between May – October (Schneekloth et al., 2020). Total annual precipitation (January – December) for 2021 was near average with 421 mm of precipitation. However, 76% of the precipitation was concentrated between March – May. Total precipitation in 2022 was 14% below average with 359 mm of precipitation. In 2022, precipitation was more evenly distributed across the growing season with 56% of precipitation occurring between May – August but this was still below average during this time frame. For 2023 precipitation was much higher than average with 577 mm of precipitation occurring. Precipitation was above average or near average from January – August (except for April) and 75% of the precipitation occurred between May – August.

For full water availability, 288, 470, and 115 mm of irrigation water were added during the 2021, 2022, and 2023 growing seasons respectively to meet crop ET demands. For the limited water availability treatment, 89, 199, and 0 mm of irrigation water were added during the 2021, 2022, and 2023 growing seasons respectively (Fig. 2.1, S. Table 2.6). Total average ET in the limited water treatment was 76% (396 mm), 71% (556 mm), and 92% (582 mm) of the full water treatment in the 2021, 2022, and 2023 growing seasons, respectively.

The applied water had 8 ppm $NO3^{-1}$ which was equivalent to adding 2 kg N $ha^{-1}$ per 25 mm of water applied. This was equivalent to adding 23 and 7 kg N $ha^{-1}$ for the full and limited water, respectively in 2021. In 2022 an additional 38 and 16 kg N $ha^{-1}$ was added from the applied water for the full and limited water, respectively. In 2023 an additional 9 kg N $ha^{-1}$ was added to the full water treatment from water applied. The 2021 growing season total N availability (pre-planting soil residual N 0 – 90 cm, N fertilizer added, and N in applied water) ranged from 74 – 327 kg N $ha^{-1}$ for full water, and 58 – 311 kg N $ha^{-1}$ for limited water. In 2022 N availability ranged from 79 – 326 kg N $ha^{-1}$ and 50 – 312 kg N $ha^{-1}$ for full and limited water, respectively. In 2023 N availability ranged from 67 – 310 kg N $ha^{-1}$ and 59 – 302 kg N $ha^{-1}$ for full and limited water, respectively (Table 2.1).

### 2.3.2 GRAIN YIELD AND ABOVEGROUND BIOMASS

Maize grain yields increased curvilinearly with N availability but were dependent on water availability resulting in an N × water interaction (Fig. 2.2a, $P < 0.01$). With full water availability in all 3 growing seasons, rates of yield increases diminished with higher N availability but did not decrease or plateau. Across all 3 growing seasons with limited water, grain yields increased curvilinearly with N availability until the 4th highest N availability (~ 200 kg N ha$^{-1}$). As N availability increased beyond 200 kg N ha$^{-1}$, yields declined. Average grain yields were nearly identical with full water availability across the three growing seasons. However, with limited water availability average grain yields in 2023 were 46% and 6% greater than 2022 and 2021, respectively, leading to water × year interaction ($P < 0.01$). Maximum yields with limited water were achieved with 25% (411 mm), 27% (563 mm), and 7% (600 mm) less ET compared to full water for 2021, 2022, and 2023, respectively. When water was limited, N availability (a sum of N fertilizer applied, soil residual N, and N from irrigation water) could be reduced by an average of 37% (36, 37, and 38% for the 2021, 2022, and 2023 growing seasons respectively) relative to full water to achieve maximum yields.

Aboveground biomass at R6 followed a similar trend as grain yield where biomass increased curvilinearly with N rate and was dependent on water availability as well as year, resulting in an N × water and N × Year interaction (Fig. 2.2b, $P < 0.01$ & $P < 0.01$, respectively). For 2021 maximum biomass was achieved with the 5th highest N availabilities (~ 270 kg N ha$^{-1}$) for both full and limited water. In 2022 and 2023 the maximum biomass was achieved with the highest N availability (~ 320 kg N ha$^{-1}$) for full water and the 4th highest N availability (~ 200 kg N ha$^{-1}$) for limited water. Biomass at R1 increased curvilinearly with N availability and tended to be greatest with ~ 215 kg N ha$^{-1}$ (S. Fig. 2.1, $P < 0.01$). The effect of water availability was dependent on growing season resulting in a water × Year interaction (S. Fig. 2.1, $P = 0.011$).

Maize grain yield and aboveground biomass exhibited very strong positive relationships with total aboveground maize N uptake at the R6 for the lower N availabilities (N treatment 1 – 4).

The relationships were positive, but weaker at the higher N availabilities (N treatment 4 – 6) (S. Table 2.4 & 2.5). When water was limited strong, positive relationships were observed, though not as strong as full water for N treatments 1 – 4. For N treatments 4 – 6 negative relations were observed in the 2022 for both yield and biomass, and 2023 for yield (S. Table 2.4 & 2.5).

### 2.3.3 BELOWGROUND BIOMASS

For the entire soil profile (0 – 180 cm) there was no water nor N effect (S. Fig. 2.2, $P = 0.3$ & 0.75 respectively). There was a depth effect where on average root biomass decreased with soil depth, except for 150 – 180 cm which was greater than biomass at 120 – 150 cm (S. Fig. 2.2, $P < 0.01$). There was a 3-way interaction between N × water × depth for the top 0 – 60 cm depths (Fig. 2.3, $P = 0.07$). For limited water maximum root biomass was achieved at the 4th N availability (210 kg N $ha^{-1}$) for all depths. The highest N availability (311 kg N $ha^{-1}$) had the lowest root biomass in the top 0 – 15 cm depth, while the 3rd N availability (159 kg N $ha^{-1}$) had the lowest biomass for the 15 – 30 cm and 30 – 60 cm depths. For full water maximum root biomass was achieved at the 3rd N availability (175 kg N $ha^{-1}$) for the surface 0 – 15 cm and 15 – 30 cm depths, while the 6th N availability (327 kg N $ha^{-1}$) had the highest root biomass for the 30 – 60 cm depth. Lowest root biomass was found at the lowest N availability (74 kg N $ha^{-1}$), 4th N availability (226 kg N $ha^{-1}$, and 2nd N availability (125 kg N $ha^{-1}$) for the 0 – 15, 15 – 30, and 30 – 60 cm depths, respectively. Maize R:S ratio was not affected by water, nitrogen, nor and N × water interaction ($P = 0.15$, 0.36, 0.21 respectively, S. Fig. 2.3).

### 2.3.4 MAIZE NITROGEN UPTAKE

Maize N uptake for the entire season (from germination – physiological maturity (R6)) increased curvilinearly with N availability, especially when water was fully available, and was greatest during the 2023 growing seasons resulting in an N × water × year interaction (Fig. 2.4, $P = 0.04$).

Maximum N uptake was achieved at the highest N availability and was 47%, 25%, and 55% higher with full water during the 2021, 2022, and 2023 growing season respectively.

Maize N uptake was also examined from germination to silking (R1) and from R1 to R6 in 2021 and 2022. Germination to R1 followed a similar trend as the entire season and exhibited an N × water interaction where N availability increased N uptake especially with full water (Fig. 2.5a & 2.5c, $P = 0.06$). This N × water interaction was more pronounced during the 2021 growing season. There was a water × year effect ($P = 0.01$) where N uptake with full water was 31% greater than limited water for the 2021 season, but only 5% greater in the 2022 season. Maize N uptake increased with N availability, and increased from one season to the next, especially in 2023 except for the lowest N availability leading to an N × year effect ($P = 0.04$). From R1 – R6, maize N uptake increased curvilinearly with N availability and was higher with full water availability (Fig. 2.5b & 2.5d, $P < 0.01$ & 0.09). From R1 – R6 maize N uptake was 65% higher in the full water treatment. No N × water interaction was observed during these later growth stages ($P = 0.87$). Maize N uptake between R1 – R6 was 30% greater in 2022 than 2021 ($P = 0.02$). The change in maize N uptake and growth in response to the treatments also changes maize grain C:N ratio. An N × water interaction was observed where the C:N ratio decreased with N availability, especially for limited water (S. Fig. 2.3, $P = 0.01$). The 2022 growing season had the lowest grain C:N ratio ($P = 0.06$).

### 2.3.5 WATER PRODUCTIVITY

Maize WP exhibited a three-way interaction between N and water availability and growing season resulting in an N × water × Year interaction (Fig. 2.6, $P = 0.08$). With full water availability maize WP increased curvilinearly with N availability and was greatest in the 2021 growing season. With limited water, excess N availability was not beneficial to WP and WP decreased beyond 3rd N availability (159 kg N $ha^{-1}$) in 2021, and beyond the 4th N availability (~ 200 kg N $ha^{-1}$) for the 2022 and 2023 growing seasons.

### 2.3.6 NITROGEN USE EFFICIENCY

Maize NUE $PNB_{grain}$ was greater with full water availability, and the effect of N rate was dependent on growing season resulting in an N × year interaction (Fig. 2.7a, $P = 0.01$ & $P < 0.01$). For the 2021 and 2022 growing season maize NUE $PNB_{grain}$ was balanced ($PNB_{grain} = 1$) between the two lowest N rates (22 and 73 kg N $ha^{-1}$) for both water treatments. For the 2023 season with full water NUE $PNB_{grain}$ was above 1 even at the highest N rate (247 kg N $ha^{-1}$, $PNB_{grain} = 1.05$) because a substantial portion of the available N was residual in the soil from the previous year. With limited water NUE $PNB_{grain}$ was balanced between the two highest N rates (123 and 202 kg N $ha^{-1}$).

Maize NUtE was greater with full water availability and decreased with N rate (Fig. 2.7b, $P < 0.01$ & $< 0.01$). Maize NUtE was 17% greater with full water availability compared to limited. Maize NUtE decreased sharply from the lowest to 2nd lowest N rate, and progressively less declines as N increased 2nd lowest rate. Maize NUtE varied across growing seasons and was 24% and 21% greater in the 2021 growing compared to the 2022 and 2023 growing season respectively ($P < 0.01$).

## 2. 4 DISCUSSION

Past research has suggested that water limitations can lead to N co-limitations due to reduced N availability and hindered plant N uptake. Thus, adding additional N can increase crop performance when water is limited (A. Ullah et al., 2022; H. Ullah et al., 2019; Zulfiqar et al., 2017). We did not find evidence for this claim. In contrast we found that high N application and N availability reduced maize grain yield and aboveground biomass when water was limited.

Maize N uptake increased with N availability and was greater with full water availability. This was true both pre- and post-silking but did not translate to yield gains when water was limited, showing maize was not co-limited by N. With limited water availability root biomass in the top 0 – 60 cm depths was maximized at the 4th N availability, and was greatly reduced at the highest N availability relative to the 4th. The hormetic effect of N, where initially beneficial but eventually negative (Schirrmacher, 2021), on grain yield when water is limited is likely explained by reduced root biomass and excessive N uptake. Improving resource use efficiency (WP and NUE) is an important goal for agroecosystems and has motivated local and regional initiatives and research aimed at improving water and N management (D. Rudnick et al., 2020; Sawadgo et al., 2021; Wardle et al., 2015). In order to achieve maximum yields and resource use efficiency N fertilizer needs to be adjusted based on water availability and yield potential. The amount N should be reduced by, and the reduction in yield will depend on many factors especially soil N availability and water stress timing and severity.

### 2.4.1 MAIZE GRAIN YIELD, ABOVEGROUND BIOMASS, BELOWGROUND BIOMASS AND N UPTAKE

With full water availability, maize aboveground biomass and grain yield increased with N availability while excess N decreased biomass and yield when water was limited leading to an N × water interaction. The hormetic effect of N with limited water has been found in several other studies (Al-Kaisi & Yin, 2003; Flynn et al., 2023; Pandey, Maranville, & Admou, 2000). In some crops, such as triticum, N fertilizer can increase vegetative biomass and water use leading to water limitations during reproductive stages thus yields decline (i.e. Y. Liu et al., 2021). For the current study, aboveground biomass at R1 and R6 show that N fertilizer did not increase vegetative growth and consequently vegetative water use leading to water limitations during grain fill. Several studies have found that N does not increase maize vegetative leaf area or water use (Mansouri-Far et al., 2010; Ogola et al., 2002; D. R. Rudnick, Irmak, Djaman, et al., 2017). Alternatively, the yield and biomass suppression with limited water at the higher N availability in the current study could be due to high N concentrations in the root zone and leaves eliciting a concentration dependent stomatal closure thus limiting photosynthesis, carbohydrate production, and water use (Cramer et al., 2009; Song et al., 2019). Additionally, root biomass in the top 0 – 60 cm decreased as N availability increased beyond 210 kg N $ha^{-1}$ for limited water. The highest N availability greatly reduced root biomass relative to the 4th N availability and yielded the lowest biomass in the surface 0 – 15 cm depth. In the current study, there was no evidence that resource allocation changed as R:S was unaffected by the treatments. Excessive N has been shown to be detrimental to root growth and function and could exacerbate water limitations leading to yield loss (Y. Chen et al., 2015; Ordóñez et al., 2021). Root growth at depths with water availability is important for tolerating water limitations, and deep roots for deep water acquisition can be advantageous when water is limited (Comas et al., 2013). Root growth at shallower depths is important for plant water acquisition as well. Additions of irrigation water were often less than 25 mm per event. The same was true for in-season precipitation events, especially later in the growing season when maize is more sensitive to water stress. These irrigation and precipitation events may not have penetrated the soil profile very deeply. Reduced root biomass in the top 0 – 60 cm with limited water therefore may have reduced maize water acquisition and use and subsequently negatively impacted grain yield and aboveground biomass.

The results show that N fertilizer rates need to be reduced, and residual soil N needs to be accounted for when water is limited as yields declined at the upper N availability. Across all 3 growing seasons maximum yields with limited water were achieved with 37% less N relative to full water. This optimal level of N under ~ 20% less water (25%, 27%, and 6% in 2021, 2022, 2023 respectively), resulted in 36%, 58%, and 29% less grain yield compared with the fully fertilized and full water maize treatment for the 2021, 2022, and 2023 growing season respectively. Optimal N availability and the yield when water is limiting relative to full water will depend on a variety of factors. Plant N demand will decrease with yield potential but yield potential will be dependent on the timing of water stress (Comas et al., 2019; Peng et al., 2010; Zou et al., 2021). Management practices and soil properties affect soil water available for crop use (Oldfield et al., 2019; Schneekloth et al., 2020) along with indigenous soil N supply and optimal N rate (Carpenter-Boggs et al., 2000; Coulter & Nafziger, 2008; Elrys et al., 2021). Accounting for soil residual N may be especially important in limited water environments. In 2023 soil residual N was much higher than the previous two growing seasons leading to reductions in N fertilizer applied. Even though the 4th N fertilizer rate was 29% lower for limited water in 2023 compared to 2021 and 2022 (123 and 174 kg N ha$^{-1}$ respectively) maximum yield was 17% and 68% greater in 2023 compared to limited water 2021 and 2022 maximum yields. Additionally, a yield decline was still observed beyond the 4th N rate in 2023 showing the importance of residual soil N concentrations. In some cases, the highest grain yield may not be the most profitable (Poffenbarger et al., 2017). Therefore, producers may choose to maximize profits rather than grain yields.

Total aboveground maize N uptake increased with N availability, especially with full water availability resulting in an N × water interaction. The same interaction was observed for pre-silking N uptake while post-silking N uptake increased with N and water availability, but no interaction was observed. It is well documented that N uptake increases with N (Barbieri et al., 2008a; Ciampitti et al., 2013; Ma et al., 1999b) and increased water individually (Djaman et al., 2013; Eissa & Negim, 2019; He & Dijkstra, 2014) including pre- and post-silking (Ciampitti et al., 2013; Djaman et al., 2013; Guo et al., 2022). Maize N uptake in response to N × water availability is variable in the literature. High N availability can suppress maize N uptake in water limited environments (i.e. Flynn et al., 2023). Other studies have found that N uptake increases with N and is greater will full water but no interaction is observed Click or tap here to enter text.(Al-Kaisi & Yin, 2003; Pandey et al., 2000; Zamora-Re et al., 2020). Finally, several studies have found N × water interactions where N availability greatly increased N uptake when water is not limited (Bennett et al., 1989; Hammad et al., 2017; Rimski-Korsakov et al. 2009).

Maize N uptake is primarily driven by plant N demand, mass flow, and soil N availability (Ciampitti & Vyn, 2011; Gonzalez-Dugo et al., 2010; Lambers & Oliveira, 2019). Limited water availability reduces N uptake as N demand and mass flow are reduced. However, N uptake can persist, and be high when N is available, as seen in the current study. Additionally, reduced water increases diffusion of N from the soil to the root zone increases allowing for N uptake to continue (Gonzalez-Dugo et al., 2010). Finally, Buljovcic & Engels (2001) found that maize N uptake rates can recover after re-wetting the soil meaning N uptake can increase after precipitation or irrigation events.

Maize N uptake both pre- and post-silking increases leaf chlorophyll content, leaf longevity, photo-assimilate production, kernel weight and grain yield (Ciampitti et al., 2013; Ciampitti & Vyn, 2011; Subedi & Ma, 2005). Accumulation of N both pre- and post-silking is also important for meeting the N demands of grain during the grain fill period (Masclaux-Daubresse et al., 2010; Nasielski et al., 2019). In the current study, N uptake pre-silking increased with N availability, especially with full water. This N × W interaction may have increased the amount of N available for remobilization thus slightly decreasing the demand for N uptake at later stages. Therefore, no N × water interaction was observed for N uptake post-silking.

Maize N uptake and grain yield are generally positively related (Ciampitti & Vyn, 2011; Cossani et al., 2012; Hammad et al., 2017), and we found that to be the case when water was fully available. With limited water we found a positive, yet weaker relationship with grain yield and biomass for the lower N availability and no relationship if not negative a correlation at the higher N availability. With limited water availability maize was still able to accumulate N, but it was not beneficial for yield after a certain point. A recent review exploring N and water effects on plant performance concluded that moderate N supply may be beneficial while high N can be detrimental when water is limited in line with our findings (Drobnitch et al., 2024).

Maize grain C:N ratio decreased with N availability, especially with limited water. In grain, N is stored primarily as proteins which are important for future seed germination, and lower C:N ratios indicate higher protein content (Masclaux-Daubresse et al., 2010; Xu et al., 2012). Higher protein content is associated with increased grain quality, and other studies have found that increased N availability and uptake increase the quality of grain (Biswas & Ma, 2016; Wasaya et al., 2018). It is important to balance high yields, sustainability, and nutrient density when managing agroecosystems (Henry et al., 2012).

### 2.4.2 WATER PRODUCTIVITY

The effect of N availability on water productivity in water limited systems is somewhat unclear in the literature. Studies have found that WP increases with N (Mansouri-Far et al., 2010; Ogola et al., 2002; Pandey, Maranville, & Chetima, 2000), while others have reported WP decreasing with N (i.e. Y. Wang et al., 2017). Still, the findings of the current study where moderate N supply is beneficial, but excess is detrimental is in line with other studies in the literature (Al-Kaisi & Yin, 2003; Flynn et al., 2023; D. R. Rudnick & Irmak, 2013).

The variable responses in the literature are likely due to both environmental and management factors changing WP through different mechanisms. Ultimately, the change in WP will depend on the magnitude of change in ET components and grain yield (Hatfield & Dold, 2019; Ullah et al., 2019). Additions of N fertilizer can increase transpiration, thus increasing grain yield and WP (D. R. Rudnick, Irmak, Djaman, et al., 2017). However, too much N can inhibit stomatal conductance leading to reduced transpiration, crop growth and yield (Ding et al., 2018). Reduced yields will reduce WP, unless ET is reduced. For smaller crop canopies ET is dominated by soil evaporation, and frequent partial wetting of the soil can lead to high evaporative losses especially when the canopy is sparse (Lenka et al., 2009; Ogola et al., 2002; D. R. Rudnick, Irmak, Djaman, et al., 2017). Therefore, seasons with high soil evaporation can have high ET without necessarily having high crop transpiration. Thus, ET remains high without increasing yields thereby reducing WP.

Management practices such as retaining crop residue can decrease soil evaporation thereby improving WP in semi-arid cropping systems (Schneekloth et al., 2020). In irrigated systems, matching water supply with crop water demands will increase WP through reduced soil evaporation but increased crop transpiration and yields (Comas et al., 2019; Hatfield & Dold, 2019; Quemada & Gabriel, 2016). Zou et al., (2021) found that deficit irrigation can increase WP relative to the fully watered control treatment so long as there was minimal water stress during late vegetative and reproductive stages. This was due to similar yields, but lower ET compared to the fully watered treatments. Maximizing WP is an important goal in water limited regions and requires management strategies that minimize soil evaporation while maintaining or increasing transpiration and crop yields.

### 2.4.3 NITROGEN USE EFFICIENCY

The NUE metrics measured varied by growing season, were greater with full water availability, and declined as N rate increased. Across the 3 growing seasons NUE was similar when comparing NUE at the N rate that achieved maximum grain yield for the two water availabilities. Maize $PNB_{grain}$ was 0.76 and 0.7 kg N kg $N^{-1}$, and NUtE was 61 and 63 kg grain kg $N^{-1}$ for limited and full water respectively. Maize NUE metrics can vary by environment, management and crop rotation, but our findings were in line with values reported in the literature (Ciampitti & Vyn, 2012; Grassini & Cassman, 2012; Wortmann et al., 2011). When comparing NUE metrics of the same N rate across water availability, limited water always had lower efficiency further highlighting the need to adjust N rates based on water availability. Additionally, NUE $PNB_{grain}$ and yield per unit N applied were greater in 2023 when N fertilizer rates were reduced due to high pre-planting soil residual N. Accounting for soil N will allow for less N fertilizer to be applied thus increasing NUE.

Improving N uptake and utilization efficiency should increase NUE metrics and yields while decreasing N requirements and environmental harm (Perchlik & Tegeder, 2017). Increased root growth early in the season and maintaining growth during reproductive stages can increase N uptake thus limiting N losses (Asibi et al., 2019; Garnett et al., 2009). Additionally, management aimed at synchrony between plant N demand and N availability will increase NUE (Loecke et al., 2012; Quemada & Gabriel, 2016; Udvardi et al., 2021). When N losses are reduced, maize yield per unit N applied increases (Davies et al., 2020). The inherently high mobility of mineral N in soil will always make synchrony challenging and has motivated research focused on managing N availability from more stable soil N pools (Breza et al., 2023; Grandy et al., 2022). Improving sink capacity, N partitioning, and photosynthetic machinery can increase utilization efficiency (Noor, 2017; Perchlik & Tegeder, 2017; Udvardi et al., 2021). Increasing utilization efficiency when water is limited may become especially important as water availability becomes less certain. Water stress decreases nitrate reductase enzyme activity leading to reduced N assimilation and remobilization and therefore NUtE (Dinh et al., 2017; Xing et al., 2019). Reduced N remobilization is especially problematic during grain fill as remobilizing N from vegetative tissue to the grain supports kernel formation and prevents pollinated kernels from being aborted (Nasielski et al., 2019). Water stress negatively affected NUtE, particularly in the drier 2022 season, when plant N uptake was nearly 2-fold greater under limited water, high N fertilizer but still yielded less than the full water, lowest N treatment.

## 2.5 CONCLUSION

In summary, the effect of increasing N availability on maize yield and biomass was dependent on water availability. This led to an N × water interaction where grain yields increased with N availability when water was fully available, but excess N was detrimental when water was limited. Water limited maize was not co-limited by N as the highest N uptake did not lead to highest grain yields. In order to maximize grain yields and resource use efficiency while reducing N losses it is necessary to adjust N inputs based on water availability and yield potential. Accounting for residual soil N and in-season N mineralization may become increasingly important to ensure excess N is not available in water limited regions.

# CHAPTER 2 TABLES AND FIGURES

**Table 2.1**: Total nitrogen (N) availability (kg N ha$^{-1}$) and the sources for all treatments and growing seasons. Soil residual N corresponds to pre-plant soil inorganic N ($NH_4$ and $NO_3$) 0 – 90 cm, N fertilizer rate corresponds to the amount of N fertilizer added, Irrigation water N corresponds the amount of N added due to irrigation water having some $NO_3$ in it, and Total N is the sum of all N sources.

**Nitrogen (N) Availability (kg N ha$^{-1}$)**

| | | Full water 2021 | | | | Limited water 2021 | | |
|---|---|---|---|---|---|---|---|---|
| **N treatment** | **Soil residual N** | **N fertilizer rate** | **Irrigation water N** | **Total N** | **Soil residual N** | **N fertilizer rate** | **Irrigation water N** | **Total N** |
| 1 | 29 | 22 | 23 | 74 | 29 | 22 | 7 | 58 |
| 2 | 29 | 73 | 23 | 125 | 29 | 73 | 7 | 109 |
| 3 | 29 | 123 | 23 | 175 | 29 | 123 | 7 | 159 |
| 4 | 29 | 174 | 23 | 226 | 29 | 174 | 7 | 210 |
| 5 | 29 | 224 | 23 | 276 | 29 | 224 | 7 | 260 |
| 6 | 29 | 275 | 23 | 327 | 29 | 275 | 7 | 311 |

| | | Full water 2022 | | | | Limited water 2022 | | |
|---|---|---|---|---|---|---|---|---|
| **N treatment** | **Soil residual N** | **N fertilizer rate** | **Irrigation water N** | **Total N** | **Soil residual N** | **N fertilizer rate** | **Irrigation water N** | **Total N** |
| 1 | 19 | 22 | 38 | 79 | 12 | 22 | 16 | 50 |
| 2 | 17 | 73 | 38 | 128 | 12 | 73 | 16 | 101 |
| 3 | 15 | 123 | 38 | 176 | 14 | 123 | 16 | 153 |
| 4 | 18 | 174 | 38 | 230 | 16 | 174 | 16 | 206 |
| 5 | 14 | 224 | 38 | 276 | 24 | 224 | 16 | 264 |
| 6 | 13 | 275 | 38 | 326 | 21 | 275 | 16 | 312 |

| | | Full water 2023 | | | | Limited water 2023 | | |
|---|---|---|---|---|---|---|---|---|
| **N treatment** | **Soil residual nitrogen** | **N fertilizer rate** | **Irrigation water N** | **Total N** | **Soil residual N** | **N fertilizer rate** | **Irrigation water N** | **Total N** |
| 1 | 36 | 22 | 9 | 67 | 37 | 22 | 0 | 59 |
| 2 | 38 | 56 | 9 | 103 | 35 | 56 | 0 | 91 |
| 3 | 38 | 106 | 9 | 153 | 43 | 106 | 0 | 149 |
| 4 | 30 | 168 | 9 | 207 | 68 | 123 | 0 | 191 |
| 5 | 34 | 212 | 9 | 255 | 124 | 123 | 0 | 247 |
| 6 | 54 | 247 | 9 | 310 | 100 | 202 | 0 | 302 |

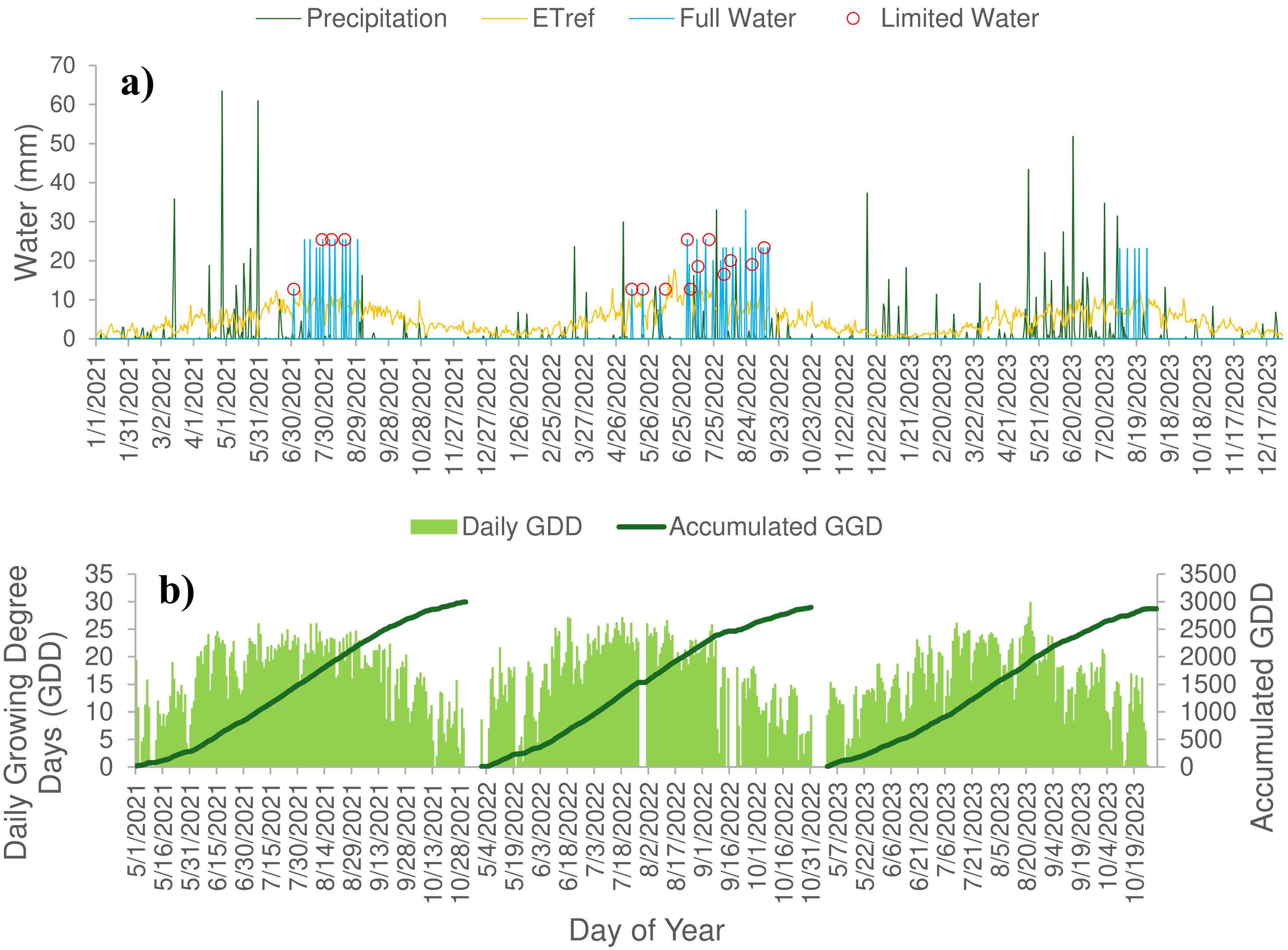

**Figure 2.1**: a) Daily precipitation, irrigation additions, and reference evapotranspiration (ET) for the three growing seasons of the experiment. b) Daily and accumulated growing degree days (GDD) during the three growing seasons of the experiment.

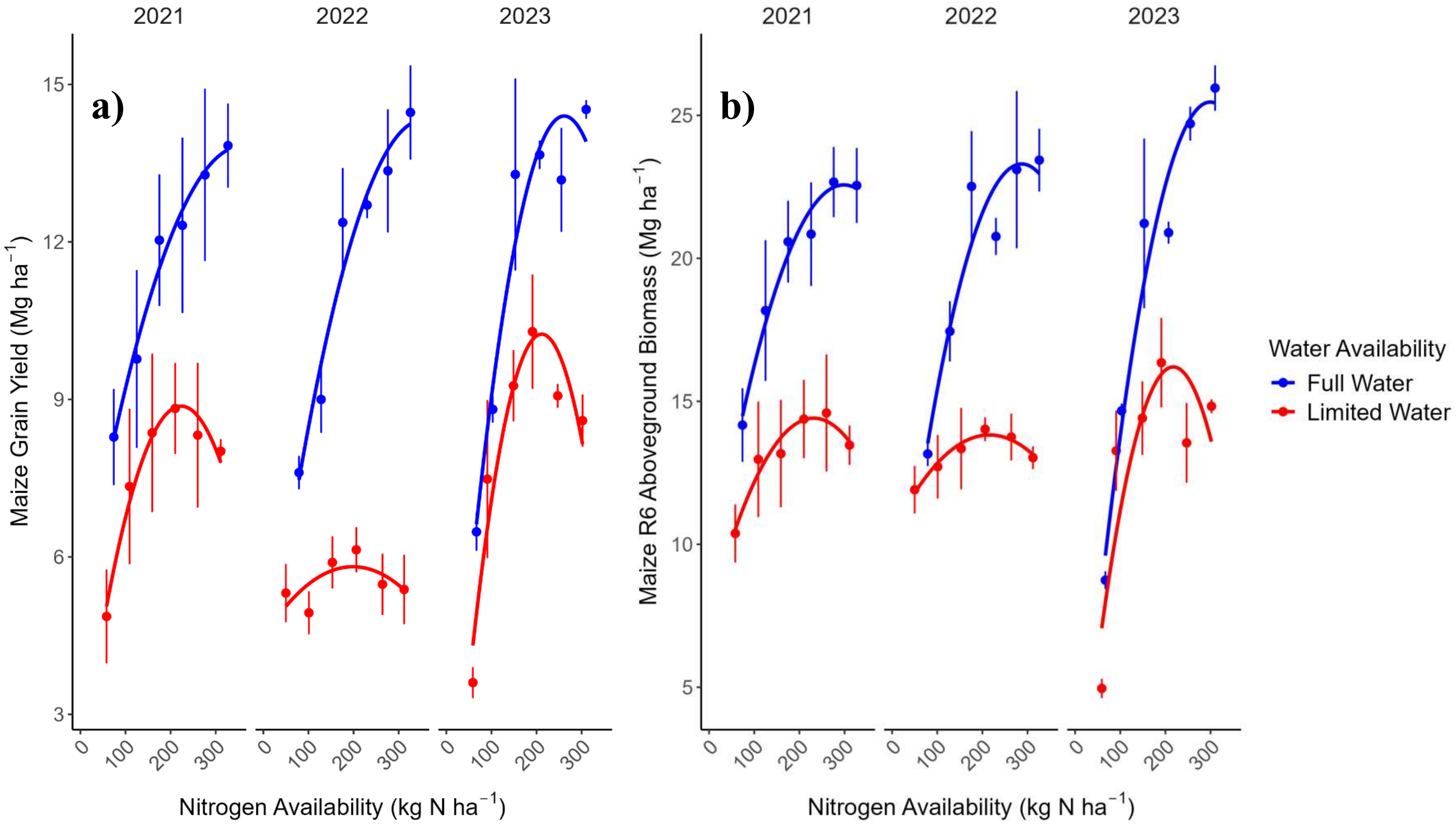

**Figure 2.2**: a) Maize grain yield by growing season, water availability, and nitrogen availability. Maize grain yield was adjusted to 15.5% moisture. b) Total maize aboveground biomass by growing season, water availability, and nitrogen availability. "R6" corresponds to physiological maturity which is when samples were collected. Error bars depict ± SE.

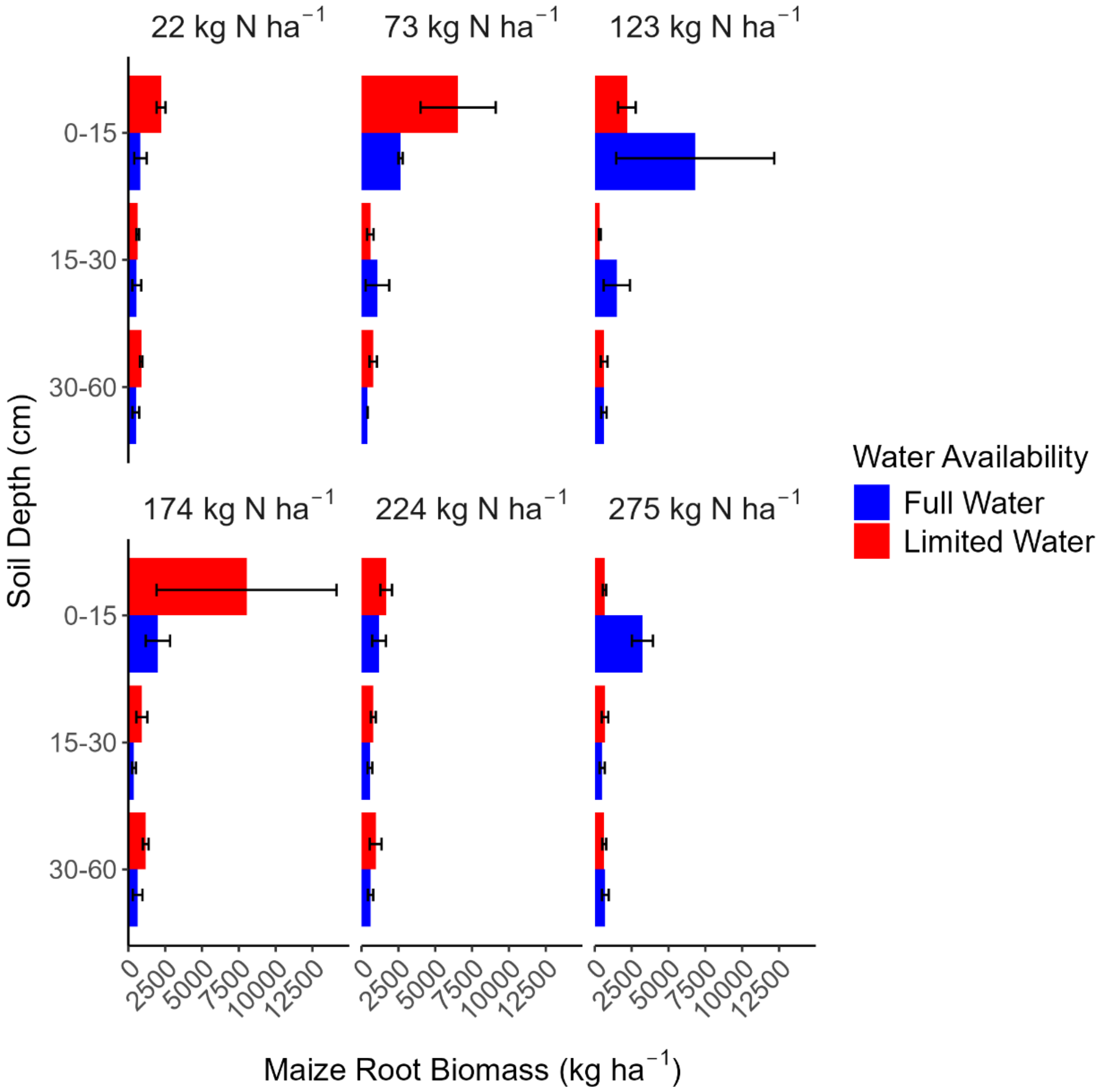

**Figure 2.3:** Maize root biomass for the 2021 growing season by water availability, soil depth increment, and nitrogen (N) fertilizer rate. The N fertilizer rates ranged from 22 – 275 kg N ha$^{-1}$ and the total N availability (sum of N fertilizer rate, pre-plant soil N, and N in irrigation water applied) are defined in Table 1. Root samples were in the fall after maize reached physiological maturity and was harvested. Nitrogen fertilizer rate rather than availability (sum of fertilizer rate, pre-plant soil N, and N in irrigation water) to be consistent for both water treatments. Error bars depict ± SE.

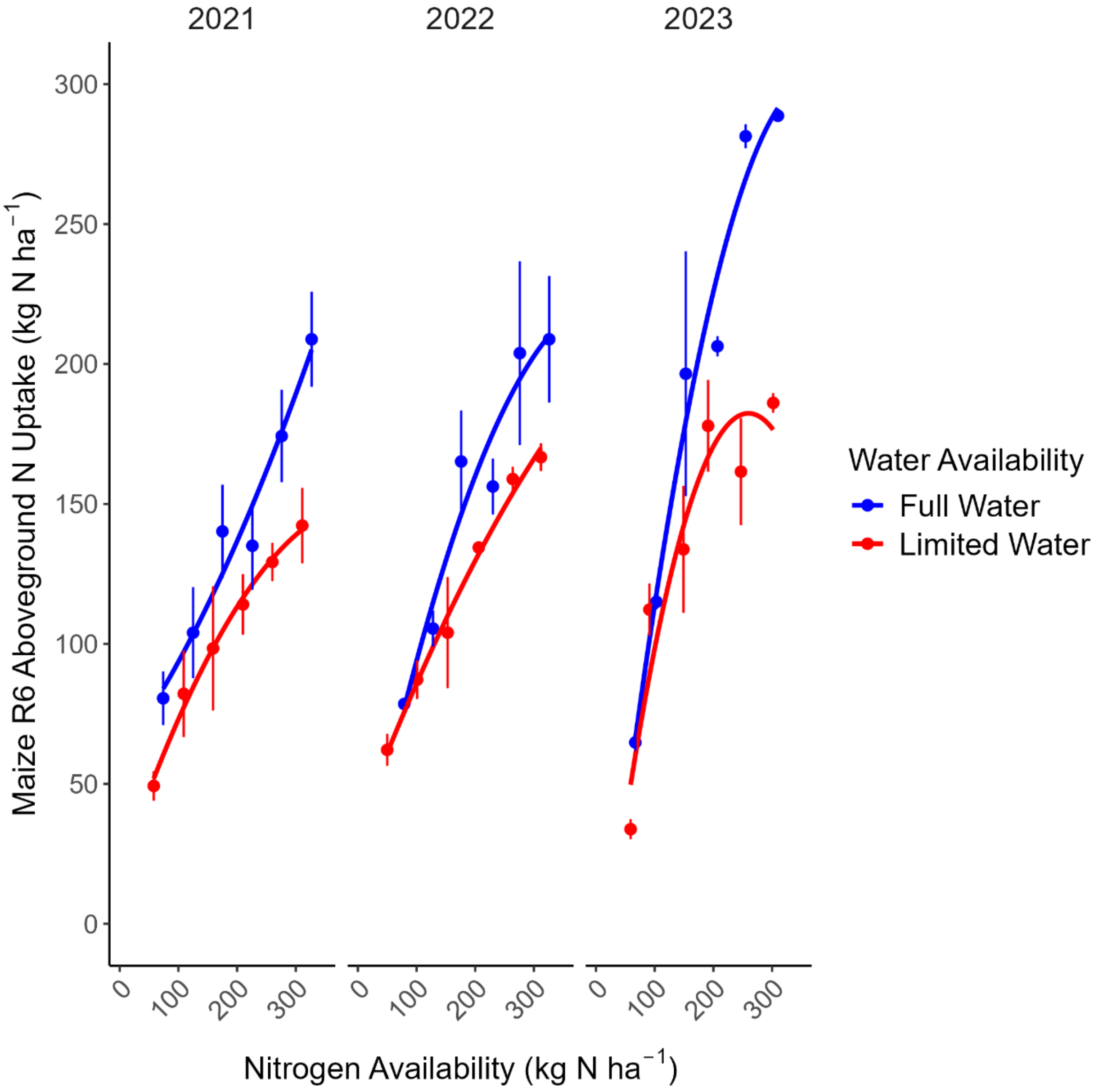

**Figure 2.4:** Maize nitrogen (N) uptake over entire growing season (germination – physiological maturity) by growing season, water availability, and nitrogen availability. Error bars depict ± SE.

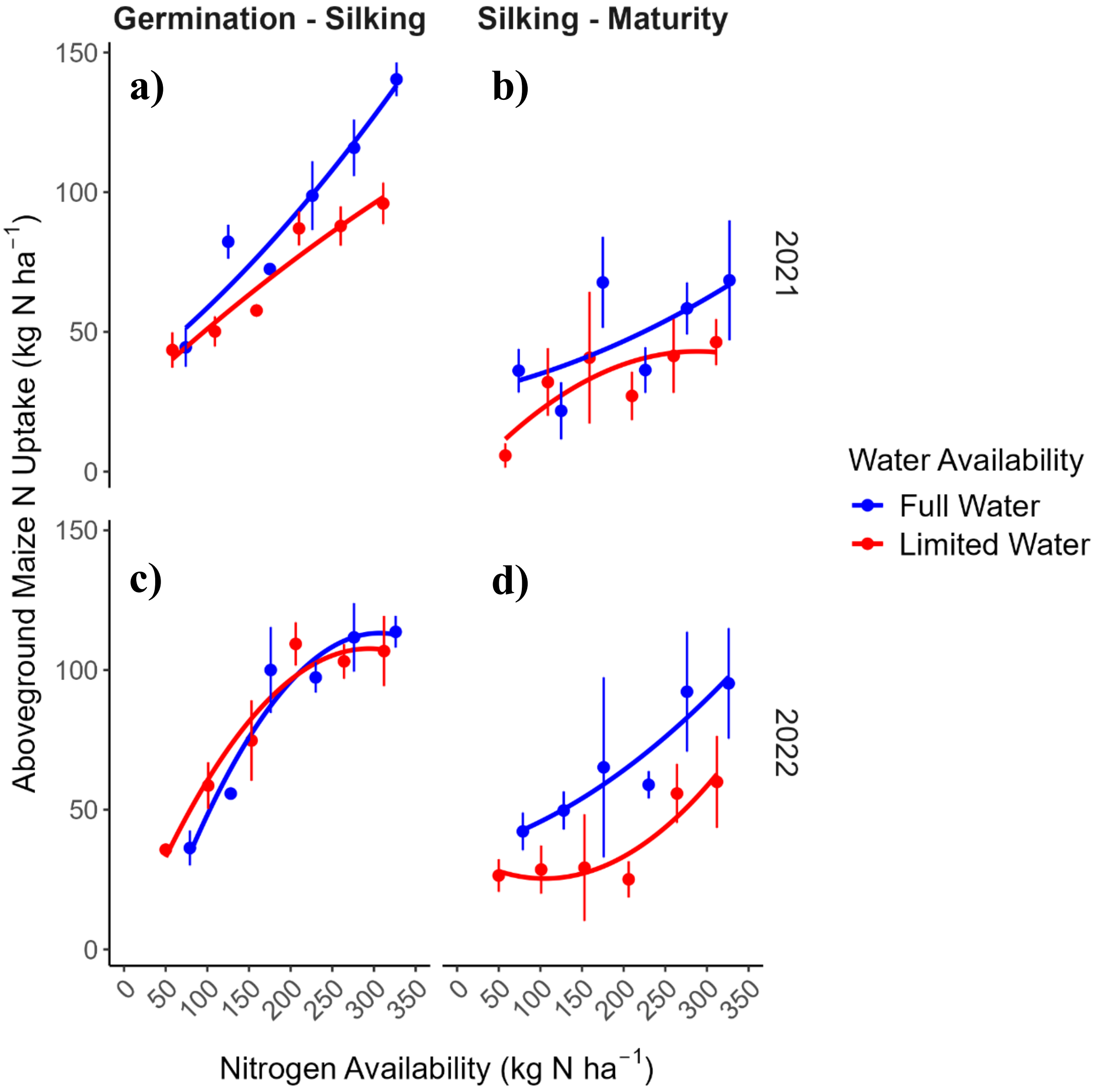

**Figure 2.5**: a) Aboveground maize nitrogen (N) uptake from germination – silking (R1) by water and nitrogen availability for the 2021 growing season. b) Aboveground maize nitrogen (N) uptake from R1 – physiological maturity (R6) by water and nitrogen availability for the 2021 growing season. c) Aboveground maize nitrogen (N) uptake from germination – R1 by water and nitrogen availability for the 2022 growing season. d) Aboveground maize nitrogen (N) uptake from R1 – R6 by water and nitrogen availability for the 2022 growing season. Error bars depict ± SE.

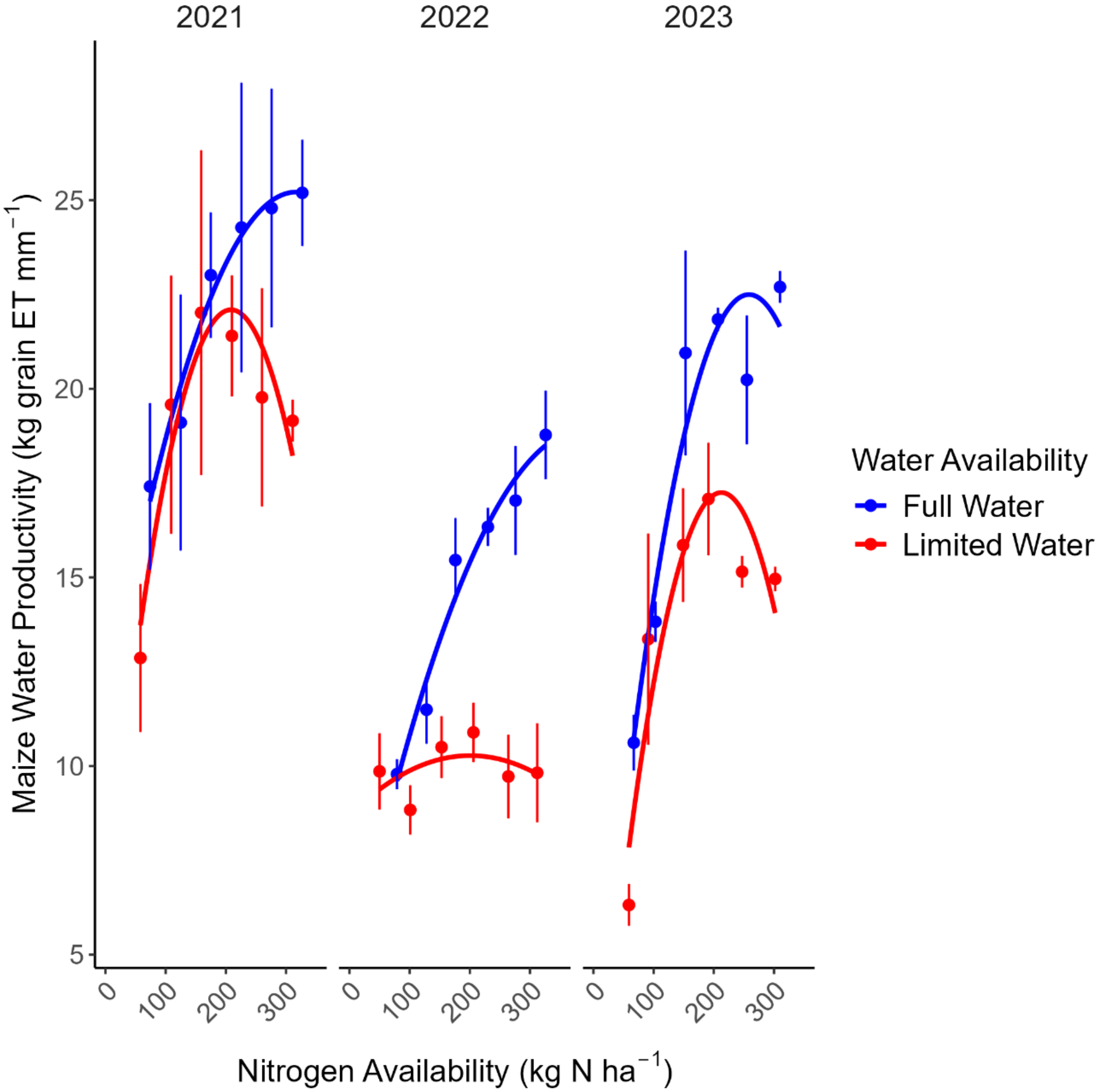

**Figure 2.6**: Maize water productivity by growing season, water availability, and nitrogen availability. Water productivity was calculated as grain yield (kg ha$^{-1}$) divided by crop ET (mm) over the entire season. Error bars depict ± SE.

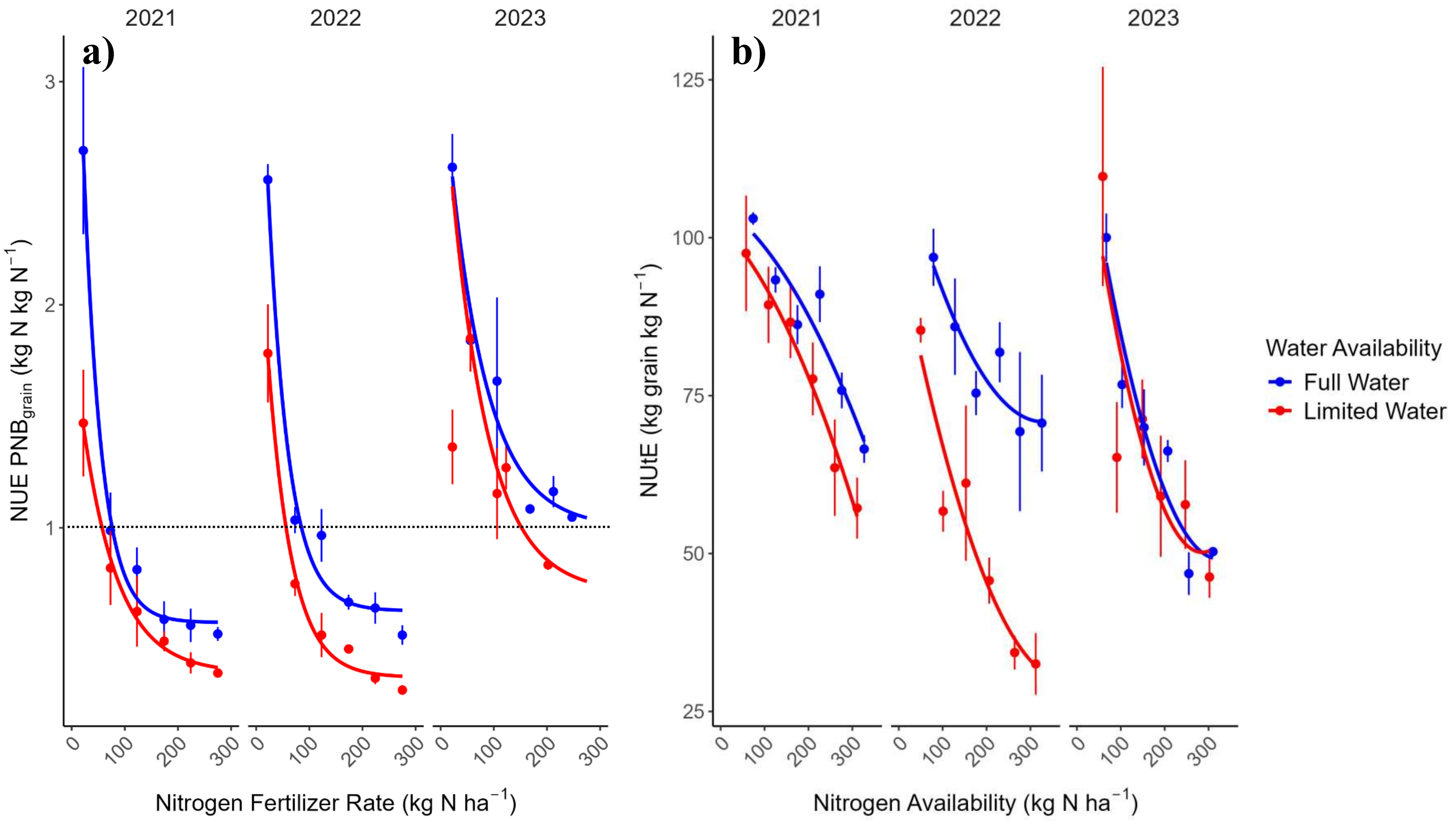

**Figure 2.7**: a) Maize nitrogen use efficiency (NUE) partial nitrogen (N) budget grain (PNB$_{grain}$) by growing season, water availability, and N fertilizer rate. Maize NUE PNB$_{grain}$ was calculated by dividing maize N uptake in the grain only (kg N ha$^{-1}$) at physiological maturity (R6) by N fertilizer applied (kg N ha$^{-1}$). Values above the dotted black line show that more N is accumulated in the grain and removed at harvest relative to N applied as fertilizer. Values below 1 show that more N fertilizer was added relative to N removed.

Values equal to 1 show a balanced N fertilizer budget (amount of N removed at harvest is equal to N added as fertilizer). Maize NUE $PNB_{grain}$ uses N fertilizer instead of N availability (sum of N fertilizer, pre-plant residual N, and N in irrigation water) on the x-axis because it is specifically a "fertilizer budget". Nitrogen fertilizer rates are defined in Table 1 and 2023 had lower N rates due to higher residual soil N before planting. b) Maize nitrogen utilization effiency (NUtE) by growing season, water availability, and nitrogen availability. Maize NUtE is calculated as grain yield (kg $ha^{-1}$) divided by total aboveground maize N uptake (kg N $ha^{-1}$) at R6. Higher values indicate greater yield per unit N uptake. Error bars depict ± SE.

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# CHAPTER 3: NITROGEN AND WATER AVAILABILITY AFFECT SOIL NITROGEN MINERALIZATION AND MAIZE NITROGEN UPTAKE DYNAMICS

## 3.1 INTRODUCTION

Turnover of soil organic nitrogen (N) is an important source of N for crops, capable of supplying over 50% of the N crops need in a growing season even under high levels of inorganic N fertilizer applications (Gardner & Drinkwater, 2009; Poffenbarger et al., 2018). However, plants must compete for inorganic N with other sinks, namely microbial uptake and sorption, *i.e.* chemical associations on soil surfaces (Daly et al., 2021). Inorganic N that is not taken up by plants or stabilized in one of these sinks can be lost to the environment, sometimes "cascading" through different environments. Agricultural production systems are the primary source of reactive N globally, contributing to soil acidification, surface water eutrophication, and increased atmospheric concentrations of nitrous oxide ($N_2O$) (Galloway et al., 2003; Robertson et al., 2013). Given the environmental, economic, and soil fertility implications, proper management of soil N is important for sustainable crop production.

While extensive research has focused on improving N fertilizer management, less attention has been given to how management influences the turnover of soil organic N. Soil N mineralization (Nmin) is the complex, microbially-mediated conversion of organic N to inorganic N. Microbial extracellular enzymes cleave N containing monomers making them bioavailable for microbial use and, in some cases, plant use (Schimel & Bennett, 2004). Microbes can use these monomers, or substrates, to meet their carbon and N needs. Excess N in the form of ammonium ($NH_4$) is released when microbial N needs are met and through microbial predation along the soil food web (Mooshammer et al., 2014; Whalen et al., 2013). Once in the soil, $NH_4$ is often rapidly oxidized by nitrifiers to nitrite ($NO_2$) and then further oxidized to nitrate ($NO_3$) (Kuypers et al., 2018), with $NH_4$ and $NO_3$ being the main forms of N that plants utilize (Asibi et al., 2019).

Soil Nmin rates have been extremely challenging to quantify because they are governed by multiple dynamic factors including the climate, litter quality, microbial access to substrates, and soil physical, chemical, and biological properties (Colman & Schimel, 2013; Dungait et al., 2012; Z. Li et al., 2019; Y. Liu et al., 2017; Mooshammer et al., 2014). Rates vary across the growing season and are primarily controlled by soil temperature and moisture (Gonçalves & Carlyle, 1994; Ma et al., 1999a). Estimating Nmin rates in agroecosystems can be further complicated as management factors including crop rotation, N additions, and tillage also heavily influence Nmin rates (Carpenter-Boggs et al., 2000; Mahal et al., 2018; Silgram & Shepherd, 1999). Soil Nmin rates can be quantified as gross Nmin, which is the total production of $NH_4$, or net Nmin, which is the difference between gross Nmin and gross immobilization (Hart et al., 1994). While gross Nmin may better reflect soil N available for plant uptake, it can be challenging to quantify, typically requiring the use of expensive stable isotope methods. Therefore, more studies rely on net Nmin assays as an indicator of soil organic N turnover.

Both N and water availability affect Nmin with potentially interacting consequences but are rarely explored together. Water limitations and irrigation retirement are expected to become more frequent in the Great Plains Region of the United States (Deines et al., 2020). It is common for high N rates, even to the point of soil N saturation, to be added to soils to ensure there is ample N for high yields (McSwiney et al., 2010; Udvardi et al., 2021). This N is often added early in the growing season in advance of current season weather impacts (Grandy et al., 2022). As water availability becomes less certain, there is an increased need for exploring the interactive effects of N and water availability on soil N cycling processes.

Fertilizer N additions can directly affect Nmin by meeting the microbial community's N demands and changing microbial activity. The stoichiometric decomposition theory predicts that if the microbial community is limited by N then adding N fertilizer will alleviate microbial N limitation, thus, increasing microbial activity and Nmin (R. Chen et al., 2014). On the other hand, the N mining theory suggests that if the microbial community is N limited then adding N fertilizer will suppress microbial activity and Nmin. This suppression occurs by meeting microbial N demand, thereby eliminating the need for microbes to decompose soil organic matter (Moorhead & Sinsabaugh, 2006). However, microbial communities in agricultural systems can vary in their relative degree of C- or N-limitation under different fertilizer and irrigation regimes (Lundquist et al., 1999; Ye et al., 2022), leading to inconsistent support for either theory.

Water is another important resource that directly affects the soil microbial community and thus Nmin. As soils dry, microbes experience water stress as their water potential declines leading to loss of cell turgor and eventually loss of cellular and metabolic function or death, contributing to reduced microbial activity and Nmin rates (Schimel, 2018). Conversely, at the other end of the moisture spectrum, saturated soils have low oxygen levels, which also leads to decreased microbial activity and mineralization rates. Optimal soil moisture (close to field capacity) thus, is likely to support higher rates of N min than either water limitation or excess (Barakat et al., 2016).

Additionally, water and N availability indirectly affect Nmin by altering soil organic matter quantity, accessibility, and quality. Water and N availability affect both above and belowground biomass production (Flynn et al., 2021; Ordóñez et al., 2021; Poffenbarger et al., 2017), and the quality of the biomass (C:N ratio) (Brown et al., 2014; He & Dijkstra, 2014; Ning et al., 2018), which in turn affects subsequent soil organic matter accumulation (Núñez & Schipanski, 2023;

Sherrod et al., 2003). Increased plant inputs to the soil and soil organic matter quantity and quality should enhance Nmin if the microbial community is not limited by other factors (Y. Liu et al., 2017; Mooshammer et al., 2014).

Soil water also indirectly affects Nmin by affecting diffusion as the soil goes through dry and wet cycles. As the soil dries, diffusion of substrates and extracellular enzymes that break down these substrates can be disrupted as water films are disconnected. These diffusive limitations along with osmotic regulation are two main reasons Nmin is reduced under drier soils (Borken & Matzner, 2009). However, Nmin is carried out by a broad range of soil organisms, allowing it to be drought tolerant in some instances (Homyak et al., 2017). Moreover, rewetting after a dry period can cause a “flash” of Nmin known as the “Birch Effect” (Borken & Matzner, 2009). After rewetting, both bacterial necromass and osmolytes becomes available. These N rich substrates can stimulate an increase in Nmin (Schimel, 2018).

Given the importance of soil Nmin for crop nutrition (Yan et al., 2020), the frequent overuse and subsequent N loss of N fertilizers (Battye et al., 2017; Zhang, Davidson, et al., 2015), and the increased likelihood of water limitations in the Great Plains Region (Derner et al., 2015; Nilahyane et al., 2023), we conducted a field experiment to better understand how different N and water availabilities alter soil N dynamics and plant responses. We hypothesized that N and water additions would each increase Nmin rates, including later in the season when maize N uptake is high.

## 3.2 METHODS

### 3.2.1 SITE DESCRIPTION

The experiment was conducted at the same field site described in Chapter 2, though measurements were only collected during the 2021 and 2022 growing season. The field experimental design and management practices were the same as described in Chapter 2.

### 3.2.2 SOIL SAMPLING

Soil samples were taken from both water treatments in the low, optimal, and excess N (N1: 22 kg N ha$^{-1}$: N5, 224 kg N ha$^{-1}$, & N6: 275 kg N ha$^{-1}$) plots approximately every two weeks from germination to approximately milk stage (R3) and then again at physiological maturity as we expected the soil N pool to be less dynamic during the later reproductive stages. A final soil sampling event occurred at physiological maturity (R6). At each sampling, four soil samples were taken per plot with a 1.75 cm diameter hand probe to a depth of 15 cm. Two samples were taken approximately 6 – 8 cm away from each side of one of the two middle rows of the plot. Soil samples were composited in the field and stored in a cooler with ice packs before being transported to the lab for analysis. Once back in the lab duplicate subsamples were taken for gravimetric water content, two more subsamples were taken for mineral N extractions (T0 for N mineralization estimates), and two more were taken for enzyme activity assays.

### 3.2.3 NITROGEN MINERALIZATION METHODS

To measure net N mineralization, undisturbed soil cores were incubated in-situ using PVC tubes (DiStefano & Gholz, 1986) with ion exchange resin (IER) lysimeters (Susfalk & Johnson, 2002) attached at the bottom (Fig. 3.2). Briefly, 6 cm outer diameter PVC tubes were cut into 25 cm long pieces for incubating soil cores, and 7.5 cm long sections for the resin lysimeters. Each lysimeter contained two sandbags with an IER bag in the middle. The sandbags and IER bags were held in place with cheese cloth and secured with zip ties. To prevent the sand and IER bags from falling out of the bottom of the lysimeter, a 1 cm tall section of 5 cm outer diameter PVC wrapped in cheese cloth was inserted and glued to the bottom of the lysimeter PVC. The sandbags had 25 g of sand each and the resin bags had 10 g of IER beads. Due to supply chain issues, Lewatit® NM 60 (Thermo Fisher Scientific) IER was used in 2021, and AmberLite® MB20 (Simga-Aldrich) IER was used in 2022. The bags were triple rinsed with DI water and stored at 4 °C until being brought to the field in a cooler with ice for installation. A lathe machine was used to reduce the thickness of the bottom of the incubation PVC tube and the top of the PVC lysimeter. Reducing the thickness of the PVC allowed the two pieces to slide over each other and be joined together with a screw.

Two incubation + lysimeter PVC tubes were installed in low, optimal, and excess N plots in both water treatments on the same day that soil sampling occurred. The PVC tubes straddled one of the middle rows of maize in the plots and were approximately 6 – 8 cm off the row. Cylinders were installed using a drop hammer to hammer the PVC 15 cm into the soil. The cylinders containing the top 15 cm of soil were carefully removed to avoid spilling and soil and the resin lysimeter bottoms were attached. A longer piece of PVC was then hammered into the hole to remove soil between 15 – 22.5 cm so that when the soil core plus lysimeter were returned to the hole for the incubation period the top surface of the soil inside the core was flush with the soil surface. The open-top soil cores and resin bags incubated in the field for approximately 2 weeks, except for the final incubation period which lasted about 1 month. After the incubation the tubes were removed from the field and transported back to the lab in a cooler with ice, the resin bags were removed and frozen until analysis. The soil was removed from each PVC tube, homogenized, and two subsamples were taken from each PVC tube: one for gravimetric water content, and one for mineral N extractions.

Gravimetric water content was measured for both the initial soil samples and incubated soils by oven-drying a 12 g subsample of soil at 105 °C for 48 hours and then reweighing it. Mineral N extractions were performed on the initial soil samples and incubated soils immediately after they got to the lab by shaking approximately 12 g of field-moist soil in 100 mL of 2M KCl for 1 hour on a reciprocal shaker. After shaking, the samples were filtered through Whatman filter paper No. 1, and stored at – 20 °C until analysis. Extractions were also performed on the previously frozen resin bags following the same procedure (using resin bag instead of soil).

During the 2021 growing season soil samples were taken and PVC incubation tubes were installed to the west of the neutron probe, and to the east of the neutron probe in 2022. In both growing seasons, each subsequent soil sample and PVC incubation moved to the east or west of the previous sampling event by ~ 1 m. Additionally, in both growing seasons sections of maize row that had skips, double plants, or were non-representative of the plot were avoided when soil samples were collected and incubation tubes installed.

### 3.2.4 CHEMICAL ANALYSIS FOR INORGANIC NITROGEN

Mineral N KCl extracts (both initial and incubated soil as well as IER) were analyzed colorimetrically on a microplate reader (Cytation 5, BioTek Instruments, Winooski, VT) to determine nitrate ($NO_3$-N) and ammonium ($NH_4$-N) concentrations. The Griess method using vanadium (III) chloride ($VCl_3$) was used to determine $NO_3$-N concentrations in the extracts (Doane & Horwáth, 2003), and $NH_4$-N was determined via the Berthelot method using salicylate-hypochlorite and citrate (Sims et al., 1995). All inorganic N values are reported as the sum of $NO_3$-N + $NH_4$-N.

Net nitrogen mineralization was calculated using Equation 1:

Equation 1: $Net\ N_{min} = ((IN_t + IN_r) - IN_0)\ /D$

Where Net $N_{min}$ refers to net nitrogen mineralization (kg N $ha^{-1}$ $day^{-1}$ ), $IN_t$ refers to inorganic N from the incubated soil samples, $IN_r$ refers to inorganic N from the IER, $IN_0$ refers to inorganic N from the initial soil sample, and D refers to the number of days of the incubation. Soil inorganic N was converted to kg $ha^{-1}$ based on the extractable N, the weight of the dry soil sample, the depth of the soil sample, and the bulk density of the soil. Bulk density data from a previous experiment at this field was used for this calculation. Briefly, 2 replicate 5.1 cm soil cores were taken from each block to a depth of 0 – 15 cm. Soil cores were passed through a 2 mm sieve and roots were removed. The soil cores were oven dried at 105 °C for 48 hours and the dry weight and volume was used to determine bulk density. Inorganic N from the IER was converted to kg $ha^{-1}$ using the same calculations as for the soil core and the assumption that the N in the IER was leached from that same volume of soil.

### 3.2.5 SOIL ENZYME ACTIVITY

Potential soil enzyme activity was measured for two enzymes related to nitrogen acquisition; namely NAG (*β*-1,4-N-acetyl-glucosaminidase), which degrades chitin, and LAP (L-leucine amino peptidase), which degrades proteins. We analyzed two lab soil replicates at field moisture conditions the day after sampling following the protocol outlined in (Saiya-Cork et al., 2002). In 2021, soil slurries were made by homogenizing 1 g of each sample in approximately 60 ml of 50 mM, pH 8.1, sodium acetate buffer. In 2022, the same protocol was used, but a tris buffer was used instead because we determined it was more appropriate for our alkaline soil conditions. After homogenizing, the slurry was pipetted into black, 96-well microplates and mixed with substrate. Slurries were also mixed with buffer only or with standards (10 mM 4 methylumbelliferone, or 7-amino-4methyl coumarin) as negative quenching controls. Samples were incubated for 4 h at 25 °C in the dark, and the fluorescence was read on a microplate reader

(Cytation 5, BioTek Instruments, Vermont, USA) at 365 nm excitation and 450 nm emission wavelengths.

### 3.2.6 GRAIN YIELD AND ABOVEGROUND NITROGEN ACCUMULATION

Along with the soil nitrogen measurements, aboveground maize N uptake rate, end of season aboveground N accumulation, and end of season grain yield were measured. Maize N uptake rate was determined by collecting shoots of 5 consecutive plants from all low, optimal, and excess N plots from the middle 2 rows. In 2021 samples were collected at vegetative stage 3, 5, and 11 (V3, V5, V11), at silking (R1) and physiological maturity (R6). In 2022 samples were collected at V5, V12, R1, and R6. The samples from R6 were used to determine end of season total N uptake. Data was unavailable for V11 in 2021 and V5 in 2022 due to samples being lost prior to analysis. Plants were separated into leaves, stalks, grain, and cobs, dried at 60 °C and weighed. Plant samples were then ground, homogenized, and a subsample was analyzed for total N concentration using a dry combustion elemental analyzer (LECO Tru-SPEC, St. Joseph, MI, USA). Maize N uptake at each growth stage was calculated by multiplying the oven dry biomass of each plant component by its corresponding N concentration and summing all the aboveground parts. Maize N uptake rate at each stage was calculated as the change in maize N content from the previous growth stage divided by the number of days between growth stages. Due to missing samples from both growing seasons, we combined data from both years by using the measured values from V3, V5, and V12, and the average of R1 and R6 across both years, where data from both years were available, to estimate maize N uptake at different growth stages. Grain yield was determined at R6 each year with the 5 plants collected for N uptake. The grain was separated from the ears, weighed, and adjusted to 15.5% moisture. Maize N uptake rates, total N uptake at the end of the season, and grain yields were converted to a per hectare basis using the harvested area.

### 3.2.7 STATISTICAL ANALYSIS

A linear mixed effects model was used for all analyses to determine how the response variables (extractable soil inorganic N, net Nmin, enzyme activity, maize N uptake (total and rate), grain yield) were affected by irrigation treatment (water availability), nitrogen fertilizer rate, year, maize growth stage (where applicable), and their interactions. During the 2022 growing season 2 soil samples were lost at the V6 growth stage for enzyme activity. Soil samples from block 3 of the full water optimal and excess N were misplaced and not analyzed for enzyme activity. The split plot design and repeated measures analyses were integrated into the mixed model structure through the inclusion of block and its interactions with irrigation and N rate as random factors. The predictors were treated as categorical variables with fixed effects. Response variables were checked for normality, equal variance, and homogeneity of variance. Inorganic soil N and enzyme activity were log-transformed to meet model assumptions. An ANOVA with the "Kenward-Roger" degrees of freedom adjustment was used to assess the significance of the fixed effects and their interactions within the context of this mixed-effects model. Post hoc means comparisons (Tukey) were conducted to assess treatment differences when the ANOVA revealed significant treatment effects. Analyses were performed in R version 4.2.2 using the lme4, lmeTest, and emmeans packages (R Core Team, 2022).

When looking at soil N dynamics we wanted to understand dynamics over the entire season as well as those spanning peak maize N uptake (R1 or blister, R2) (Ma et al., 1999a; Osterholz et al., 2017). Therefore, we have 2 separate analyses, one which includes the entire growing season, and the other with only the last 3 sampling events (3 incubations and 4 soil samples) that occurred after the treatments were applied, and cover before, during, and after peak maize N uptake. Both analyses used the same statistical approach as described above.

## 3.3 RESULTS

### 3.3.1 PRECIPITATION AND IRRIGATION AMOUNTS

Average annual precipitation (January – December) for the site is 420 mm (Schneekloth et al., 2020). Total 2021 precipitation (January – December) was similar to this average with 421 mm of precipitation occurring. However, 76% of this precipitation was concentrated between March – May. Total precipitation in 2022 was 14% below average with 359 mm of precipitation. In 2022, precipitation was more evenly distributed across the growing season with 56% of precipitation occurring between May – August. For the full water availability treatment 288 and 470 mm of irrigation water was added during 2021 and 2022 respectively to meet expected crop ET demands. For the limited water treatment 89 and 199 mm of irrigation water was added during 2021 and 2022 respectively.

### 3.3.2 N × WATER EFFECTS ON INORGANIC N AVAILABILITY AND NMIN

Over the course of the entire season extractable inorganic nitrogen (EIN) EIN was affected by a N fertilizer × water (N × W) interaction where EIN values were nearly identical at the low N treatment for both water treatments but were much higher in the limited water for the optimal and excess N rates *(P* = 0.02; S. Fig. 3.1). We found a similar trend when looking at vegetative stage 16 – physiological maturity (V16 – R6). The EIN was 27%, 141%, and 80% greater in the limited water treatment compared to the full water treatment for the low, optimal, and excess N rates, respectively (Fig. 3.3a, *P* = 0.07).

When focusing on net Nmin from VT – R6, we found an N x W interaction (Fig. 3.3b, *P* = 0.03). Under full water availability, net Nmin was highest in the low N treatment (0.52 = kg N ha$^{-1}$ day$^{-}$

$^{1}$) and declined by 67% and 85% as the N fertilizer rate increased to optimal N and excess N, respectively. Under limited water availability, net Nmin was close to zero in the low N and optimal N, while 0.34 kg N ha$^{-1}$ day$^{-1}$ accumulated in the excess N treatment (Fig. 3.3b).

### 3.3.3 N X WATER EFFECTS AND SEASONAL DYNAMICS OF SOIL ENZYME ACTIVITY

Both LAP and NAG enzyme activity increased with N rate regardless of water availability (Fig. 3.4, $P = 0.02$ & 0.01, respectively). Nitrogen fertilizer increased LAP activity by 39% and 22% in the optimal N and excess N treatments compared to the low N treatment, respectively. Soil NAG activity increased by 43% for both the optimal and excess N treatments compared to the low N treatment. Enzyme activity varied across and within growing seasons and changed with water availability leading to a W × Growth stage × Year interaction for both LAP and NAG (S. Fig. 3.3, $P < 0.01$ & $P = 0.01$ respectively). For LAP, activity tended to be lowest around R1/R2 especially for limited water during the 2022 growing season. For NAG, activity was lowest at R6 during the 2021 growing season and was lowest at R1/R2 during the 2022 growing season for limited water. Finally, LAP activity near the end of the season and peak maize N uptake (plot averages between R1/R2 and R3/R4) increased with end of season maize N uptake (r = 0.43, $P = 0.01$, Fig. 3.5a). Although there was a similar trend, there was no significant correlation between NAG and maize N uptake (r = 0.26, $P = 0.13$, Fig. 3.5b).

### 3.3.4 N X WATER EFFECTS ON CUMULATIVE MAIZE N UPTAKE AND YIELDS

Total maize N uptake tended to be higher in the 2022 growing season compared to the 2021 growing season ($P = 0.08$) with average N uptake being 131 kg ha$^{-1}$ and 147 kg ha$^{-1}$ for the 2021 and 2022 growing seasons respectively. Across both years, maize N uptake increased independently with increases in both N fertilizer and water availability ($P < 0.01$ & 0.04). Increased N fertilizer application increased maize N uptake by 146% and 169% when comparing the low N treatment to the optimal and excess N treatments, respectively. Full water availability increased N uptake by 26% relative to the limited water availability (Fig. 3.6a). There was no N × W interaction affecting N uptake *(P* = 0.35).

The effect of N fertilizer addition on grain yield was dependent on water availability resulting in an N × W interaction (Fig. 3.6b, *P* = 0.03). Under full water availability, grain yields increased by 68% and 78% for the optimal and excess N treatments relative to the low N treatment, respectively. Under limited water availability, grain yields increased by only 36% and 32% as N fertilizer rates increased from the lowest N level applied (Fig. 3.6b). There was no difference between grain yields across the two growing seasons *(P* = 0.12).

### 3.3.5 SYNCHRONY BETWEEN NET NMIN AND MAIZE N UPTAKE

For the entire growing season, net Nmin rates varied within and across the two growing seasons and changed with N fertilizer rate, resulting in a N fertilizer × Growth Stage × Year interaction (Fig. 3.7a, *P* = 0.01). Biweekly Net Nmin rates ranged from strong net immobilization to strong net mineralization with the highest rates of mineralization typically occurring earlier in the growing season before the main application of N fertilizer and when maize N demand was lower (Fig. 3.7a & b, S. Fig. 3.2).

Uptake rates were initially similar across plots but increased with N availability after N fertilizer treatments were applied, resulting in a Growth stage × N fertilizer interaction. Maize N uptake rates increased substantially from V3 and V5 and peaked from V12 to R1 with optimal and excess N under both water availabilities. With low N, maize N uptake rates were similar between V5 – R1 (Fig 3.7b). Maize N uptake rates also varied by water availability later in the growing season resulting in a Growing season × Water interaction (Fig. 3.7b, *P* = < 0.01). Maize N uptake rates were 32% higher in the limited water than in the full water treatment at V12. However, the full water treatment had greater maize N uptake rates during reproduction stages than the limited water treatment across all N fertilizer treatments (79% and 49% higher at R1 and R6, respectively).

Extractable inorganic N (EIN) varied across the season from germination through R6 and tended to be lower as R6 approached, especially for full water availability. Limited water availability had greater EIN especially in 2022 which had nearly double the EIN as 2021 (S. Fig. 3.1).

## 3.4 DISCUSSION

While there has been a large body of work investigating crop and soil N cycling responses to N fertilizer and water independently, few studies have examined the interactive effects of N fertilizer additions and varying water availability on soil N processes (e.g. Flynn et al., 2021; Y. Wang, Janz et al., 2017). We found that *in situ* net Nmin rates during peak maize N uptake were governed by an N × water interaction in incubation tubes that excluded plant uptake. Net Nmin rate decreased with increasing N application under full water availability, but net Nmin rate was highest at the highest N fertilizer application when water was limited. Outside of the incubation tubes, soil enzyme activity, which is closely correlated with gross Nmin (Booth et al., 2005), had a different response, increasing with N fertilizer regardless of water availability. The exclusion of living roots in the incubation net Nmin tubes may have contributed to contrasting results seen between net Nmin and soil enzyme activity via two mechanisms, excluding plant uptake and excluding active C inputs from root exudation (Booth et al., 2005; Huo et al., 2017; Kumar et al., 2017).

The results suggest distinct temporal patterns between net Nmin, gross Nmin, and plant N uptake. When measuring net Nmin over the entire growing season, we saw that Nmin peaked well before the peak in maize N uptake rates regardless of treatment. Net Nmin reflects N availability after microbial N demands are met, suggesting that there was a surplus of mineralized N early in the growing season. However, once maize N uptake increased, net Nmin rates likely diverged from gross N min rates as maize can effectively compete with microbes for N over the course of the growing season (Osterholz et al., 2017). Although net Nmin rates were lower during peak maize N uptake, Nmin often still represents a majority of N taken up by maize (Gardner & Drinkwater, 2009; Yan et al., 2020).

### 3.4.1 POST FERTILIZER SOIL N DYNAMICS

Net Nmin is the difference between two opposite and simultaneous microbial processes; namely gross mineralization and gross immobilization, and the net N left over from these processes is available for plant use (Y. Liu et al., 2017). Incubating soil cores in the field is a common method for measuring net Nmin and provides some advantages over lab incubation methods and other field methods (Hart et al., 1994). However, there are some limitations associated with this method. First, the incubation tubes did not include living plant roots which influence soil Nmin via rhizodeposition as well as N uptake (Meier et al., 2017; Zhu et al., 2016). Additionally, net Nmin is not well correlated with gross mineralization, especially as the duration of the incubation increases, as most soil inorganic N pools have a turnover time of ~ 1 day (Booth et al., 2005). While gross mineralization (total production of $NH_4$) measurements can be costly, time consuming, and complicated relative to other methods, it may be a better measure of soil N supplying capabilities especially since plants can compete effectively against soil microbes for inorganic N and do not need to wait for “leftover net N” (Elrys et al., 2021; Osterholz et al., 2017). Despite some of these limitations, net Nmin is still commonly used and is considered a good “index” of plant available N (Hart et al., 1994; Li et al., 2019; Schimel & Bennett, 2004).

Further, in reality the amount of inorganic N that is available to plants is somewhere between gross and net Nmin rates (Booth et al., 2005).

To better understand how temporal dynamics in soil N cycling aligned with varying maize N demands across growth stages, we analyzed the effects of N fertilizer and water availability on soil N dynamics before, during, and after peak N uptake. The results showed an N x W interactive effect on net Nmin rates. When looking at the individual effects of N fertilizer and water availability on Nmin alone, we see different responses in the literature among studies. Additions of N fertilizer have increased Nmin rates relative to zero N (Biau et al., 2012; Ma et al., 1999b; Mikha et al., 2006), though the highest N fertilizer rates do not always yield the highest Nmin rates (Al-kaisi et al., 2008; Fujita et al., 2018; Ouyang & Norton, 2020). Nitrogen fertilizer additions can also suppress Nmin (Carpenter-Boggs et al., 2000; Mahal et al., 2019), “destabilize” Nmin where N fertilizer has both the highest and lowest Nmin rates (Studt et al., 2021), and yield different responses based on the cropping system (Breza et al., 2023b). When considering water alone, Nmin rates tend to be highest when there is adequate soil moisture (Barakat et al., 2016; Neve & Hofman, 2002). or in regions with higher precipitation (Colman & Schimel, 2013; Elrys et al., 2021b; Z. Li et al., 2019b), and irrigation tends to increase Nmin rates (Valé et al., 2007). Nmin can be fairly drought tolerant due to the broad range of microbes that mediate Nmin processes and pulses of Nmin after rewetting dry soils are common  (Homyak et al., 2017; Y. Wang, Jensen, et al., 2017). However, the flashes cannot always compensate for the reduction in mineralization that occurred during the dry period (Mikha et al., 2005a). Additionally, high magnitude perturbations of soil moisture can reduce Nmin (Barakat et al., 2016).

Nitrogen × water experiments that report Nmin rates, especially in agronomic field settings, are rare. However, the finding of an N × water interaction in the present study is consistent with Y. Wang, Janz et al., (2017), which is one of the few studies reporting Nmin with different N and water availabilities. Placing the results of the current study within the context of previous studies of just N or water treatments, suggests that this interactive effect may be the result of multiple, potentially interacting, mechanisms. In the current study, an accumulation of inorganic N (from N fertilizer and mineralization) under full water availability, in the absence of plant N uptake in the incubation tubes, may have suppressed further Nmin (Booth et al., 2005). Under limited water availability, N fertilizer may have helped microbes synthesize nitrogenous osmolytes needed to maintain their water potential during water stress (Kakumanu et al., 2013). These osmolytes are released, causing a "flash" of Nmin when soils are rewetted (Schimel, 2018). Additionally, the N × W interaction could have been due to the differing effects N and water have on root growth and function (Flynn et al., 2021; Ordóñez et al., 2021; Zhu et al., 2016). While the incubation tubes did not include living roots, freshly severed maize roots would be present from installation of the tube and treatment effects on root inputs could create treatment "legacy effects" in the tubes. For example, increased fine root growth in the limited water treatment with more N addition, would contribute to differences in C availability in incubation tubes as fine roots quickly turnover, stimulating Nmin rates (B. Chen et al., 2014; Jilling et al., 2018).

Depolymerization of N containing polymers is a rate-limiting step for Nmin as polymers are not immediately bioavailable due to their size (Geisseler et al., 2010; Mooshammer et al., 2014b; Schimel & Bennett, 2004). Extracellular enzymes LAP and NAG are excreted by soil microorganisms to cleave N containing substrates, specifically proteins and chitin, releasing bioavailable monomers (Jian et al., 2016). Depolymerization of proteins is especially important for Nmin as 60% of the organic N in plant and microbial cells (Chen et al., 2018). Both soil N cycling enzyme activities assayed in this study (LAP and NAG) increased with N fertilizer application across both water availabilities, which is in line with several other studies (Chen et al., 2018; Uwituze et al., 2022; Zhang, Dong, et al., 2015). These results suggest that N fertilizer increased gross Nmin via increased depolymerization of N containing compounds, which was not reflected by the net Nmin measurement.

The effect of N additions on the direction and magnitude of LAP and NAG is variable in the literature with positive (Chen et al., 2018; Fujita et al., 2018; Saiya-Cork et al., 2002), neutral (Jian et al., 2016), and time × N fertilizer interactions being reported (Grandy et al., 2013). Microbial biomass and composition can elicit different responses to N additions (Geisseler & Scow, 2014; Z. Guo et al., 2019; Treseder, 2008) and depend on the ecosystem, N fertilizer rates, and duration of the study (Jia et al., 2020). Additions of N can directly increase enzyme activity by alleviating N limitations and increasing microbial biomass and activity (Chen et al., 2018; R. Chen et al., 2014), as well as through plant mediated mechanisms (Huo et al., 2017; X. Liu et al., 2017). The microbial activation hypothesis suggests that exudates from plant roots increase microbial activity as a priming effect on soil N mineralization and soil N-acquiring enzyme activity has been shown to increase in response to plant growth and labile root exudates (Cheng & Kuzyakov, 2005; Kumar et al., 2018). This would support the results in the current study of LAP activity typically being higher during vegetative stages when root exudation is greater. Soil N-acquiring enzyme activity also increases with plant growth due to increased competition for N between soil microbes and plants. In order to fulfill the additional nutrient needs, enzymes are produced to mine soil organic matter for N (Kumar et al., 2017). This may support the finding in the present study where LAP activity increased with maize N uptake. This can lead to a positive feedback loop where N fertilizer increases crop N uptake, which increases microbial mineralization of organic matter further increasing N availability and crop N uptake. Future N × water studies that quantify gross Nmin and the contribution of soil Nmin to crop N use can contribute to strategies aimed at improving N management (Osterholz et al., 2017; Yan et al., 2020). Additionally, measuring root exudation, including across different hybrids and crops with varying exudation capacities, may help reveal the extent to which root exudation shapes soil N cycling.

Water deficits and retirement of irrigated lands have shown to decrease microbial biomass and enzyme activity (Flynn et al., 2021; Núñez et al., 2022). However, some soil microbial communities can also be quite drought tolerant (Fierer et al., 2003; Homyak et al., 2017). Additionally, the historic climate and dryland management practices used could have provided a "home-field advantage" for the microbial community (i.e. soil microbes were accustomed to drier soil conditions) thus muting the effect of water availability (Ayres et al., 2009; Mahal et al., 2019). This may have been the reason there was no main effect of water availability on enzyme activity in the current study.

### 3.4.2 MAIZE N UPTAKE AND GRAIN YIELD

In the current study, maize was not co-limited by N when water was limited as the excess N fertilizer rate led to greater N uptake, but did not increase yields compared to the optimal N rate. Plant response to water and N together is greater than its response to each resource in isolation, and maximum plant growth is achieved when both resources are non-limiting (Quemada & Gabriel, 2016b).

It is important to properly manage resources such as N fertilizer to optimize economic return and avoid environmental consequences (Xin Zhang et al., 2015). Here we found that excess N was not beneficial to yield with limited water and N needs to be adjusted based on water availability and yield potential. Finding the optimal N rate is challenging (McDaniel et al., 2020; Puntel et al., 2016), especially when water availability is highly variable as it is in the Great Plains (Derner et al., 2015; Rudnick, et al., 2017; Schlegel et al., 2019). Given the high variability of water in the Great Plains and the increasing likelihood of water scarcity, applying N fertilizer in multiple split applications during the season—rather than applying most of the nitrogen at the beginning—based on water availability and plant N needs may become more important for improving economic returns and resource use efficiency (Quemada & Gabriel, 2016). Additionally, accounting for contributions of soil Nmin to N availability can help prevent over-applying N fertilizer.

### 3.4.3 NET NMIN AND MAIZE N UPTAKE RATES OVER THE ENTIRE SEASON

When considering the entire growing season (germination – R6), net Nmin rates were not affected by water availability or N rate individually, did not exhibit an N × water interaction, and peaked relatively early in the growing season. Although this decline in Nmin after the initiation of N fertilizer could suggest that fertilizer addition suppressed Nmin, we also found a decline in the low N treatment, suggesting there was a background, temporal pattern in net Nmin that was independent of the treatments.

While Nmin rates often vary within and across growing seasons, (Loecke et al., 2012; Studt et al., 2021) the findings of Nmin rates being higher in the earlier vegetative stages than the latter is in line with other studies (Ma et al., 1999b; Mahal et al., 2019). Variations in Nmin rates across and within growing seasons are often attributed to variability in precipitation and temperature, soil inorganic pool size, plant C inputs, and microbial community structure and activity (Gonçalves & Carlyle, 1994; Mahal et al., 2019; Studt et al., 2021).

Maize N uptake rates increased with N fertilizer and peaked at R1 which is in line with the literature (Guo et al., 2022; Ma et al., 1999a; Osterholz et al., 2017). Maize N uptake rates were greater with full water availability except at V12. This may have been due to increased investment in belowground growth in response to water stress. Increased belowground growth can increase N uptake but often comes at the expense of aboveground growth (Flynn et al., 2021). Greater N uptake rates were only temporary as full water increased aboveground growth (S. Table 3.4) and therefore N demand (Noor, 2017b; Peng et al., 2010). Although Nmin rates peaked early in the growing season, there was still Nmin occurring when plant N uptake was highest. Previous studies have shown that Nmin and its subsequent support of plant N uptake during grain fill can be beneficial as leaf N content and photosynthetic rates are maintained (Osterholz et al., 2018; Subedi & Ma, 2005).

Repeated measurements of EIN provides a robust indicator of the temporal trends in plant available N throughout the growing season. We found a decline in EIN as the growing season progressed and plant N uptake increased, which was consistent with that observed by others (Mahal et al., 2019). Soil EIN represents the balance between inputs of mineral N via fertilizer and/or mineralization and plant N uptake. We found increased EIN with water deficits during later growth stages likely due to reduced plant N uptake (He & Dijkstra, 2014). In addition, we found higher EIN in the 2022 growing season compared to the 2021 growing. This was likely due to the legacy effect of the repeated fertilizer additions, since treatments were repeated in the same plots in the second year. This is in agreement with other studies with repeated fertilizer additions over multiple seasons (Brown et al., 2014b; Dhillon et al., 2018) (Fujita et al., 2018; Grandy et al., 2013).

## 3.5 CONCLUSION

In conclusion, the different responses in net Nmin, soil enzyme activity and maize N uptake to N and water treatments highlight the complex plant-soil-microbial interactions governing soil N cycling. Further research quantifying gross Nmin and identifying the contribution of different N sources to maize N use will help improve soil N and fertilizer management. Properly managing N and ensuring excess N is not available will help ensure high yields are realized while N losses are reduced and will become more important as water becomes more limited.

# CHAPTER 3 TABLES AND FIGURES

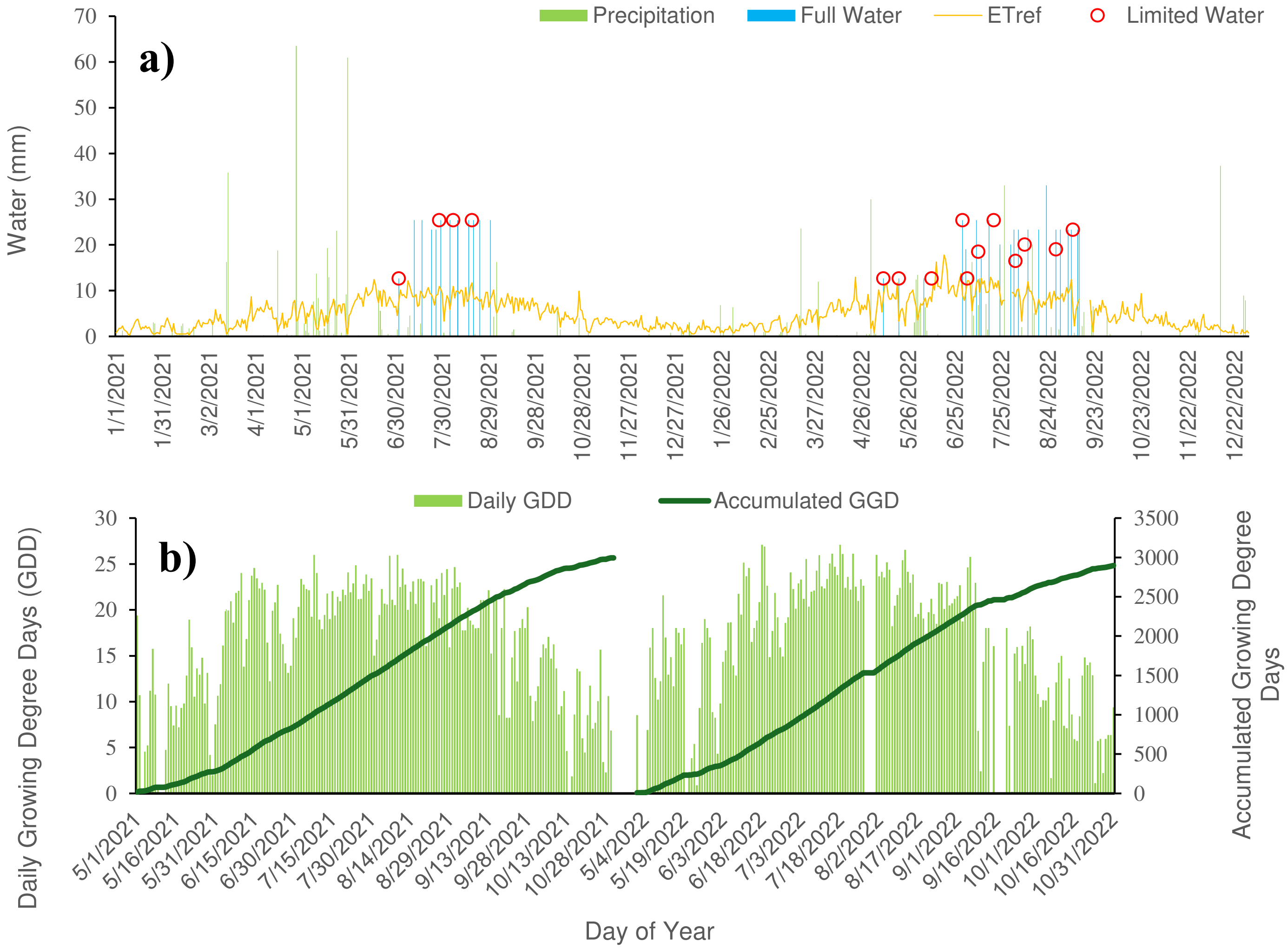

**Figure 3.1:** a) Daily precipitation, irrigation applied, and reference evapotranspiration (ET) (mm) during the two growing seasons of the experiment. Irrigation events with a red circle identify days when the limited water treatment was irrigated; b) Daily and accumulated growing degree days (GDD) during the two growing seasons of the experiment.

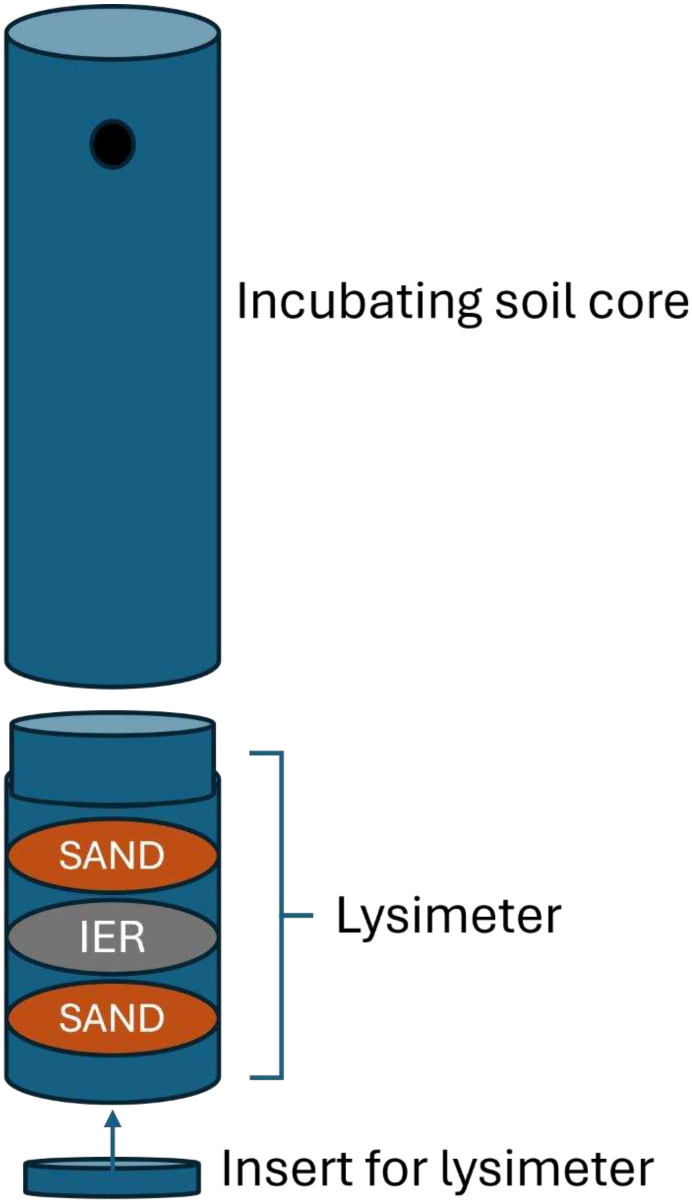

**Figure 3.2**: Schematic showing PVC set up for in-situ undisturbed soil core incubations for net nitrogen mineralization measurements. The top PVC piece contain undisturbed soil core from 0 – 15 cm. The lysimeter was attached to the top PVC piece and had two sandbags and one ion exchange resin bag which captured inorganic N as it moved through the core. The insert for the lysimeter kept the bags in place.

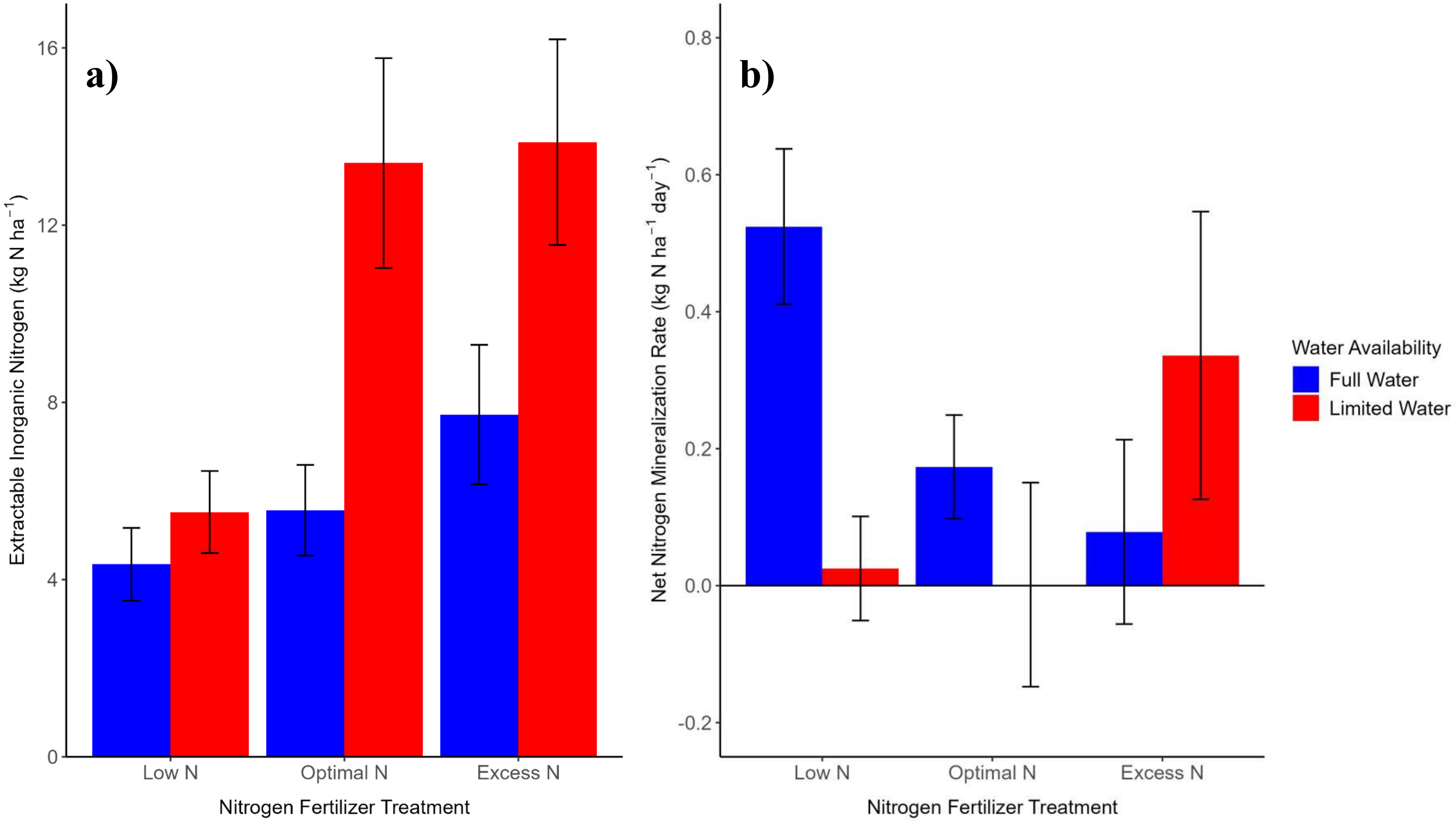

**Figure 3.3:** a) Soil extractable inorganic nitrogen (EIN) in the top 0 – 15 cm by nitrogen fertilizer and water availability averaged across the two growing seasons from approximately vegetative stage 16 – physiological maturity (V16 – R6). b) Net nitrogen mineralization (Nmin) rates in top 0 – 15 cm by nitrogen fertilizer and water availability averaged across the two growing seasons from approximately V16 – R6. Both EIN and Nmin include NH4-N + NO3-N. Error bars depict ± SE.

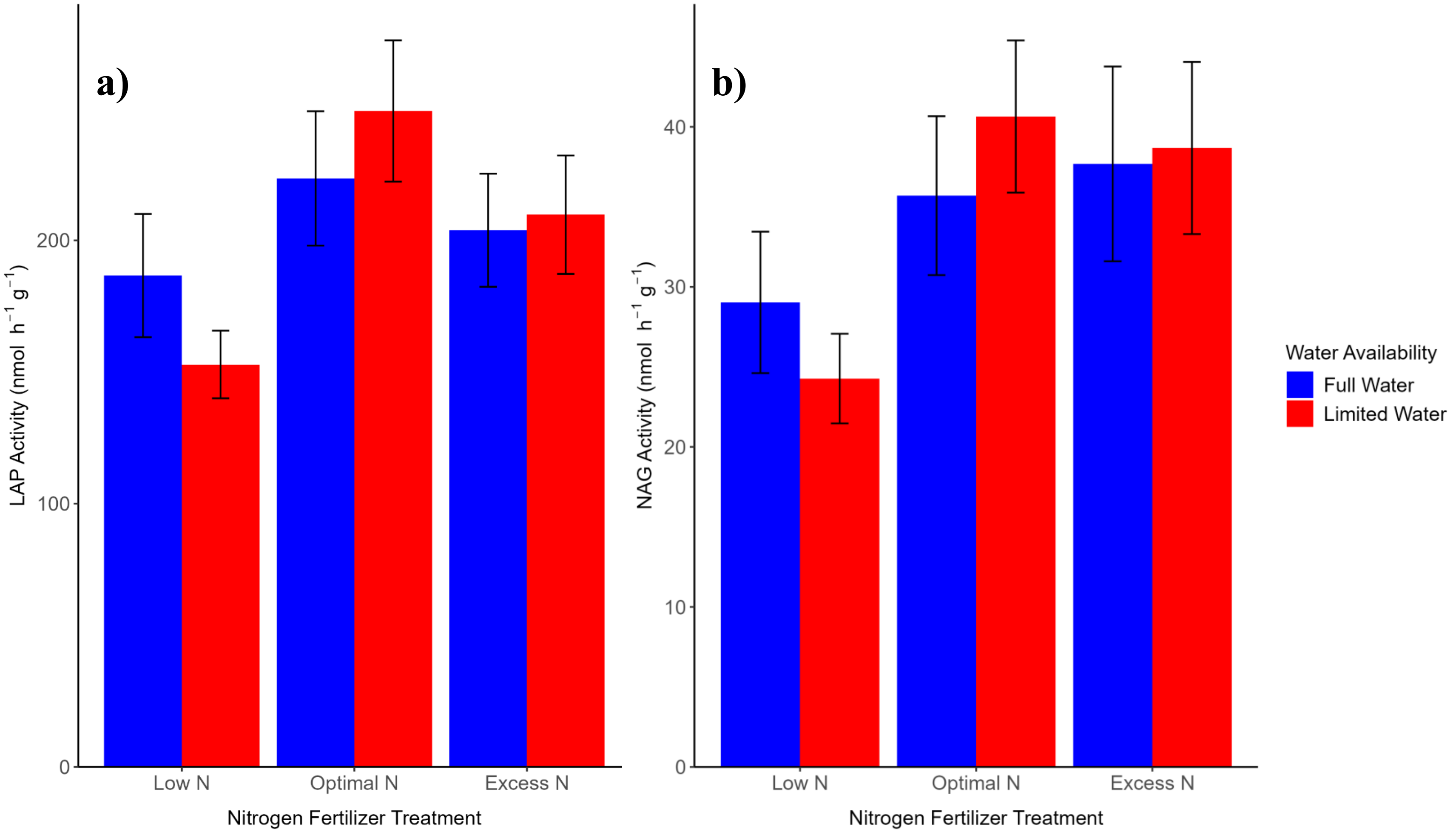

**Figure 3.4:** Soil enzyme activity corresponding to a) L-leucine amino peptidase (LAP) activity and b) *β*-1,4-N-acetyl-glucosaminidase (NAG). Both show enzyme activity in the top 0 – 15 cm by nitrogen fertilizer and water availability averaged across the two growing seasons from approximately vegetative stage 16 – physiological maturity (V16 – R6). Error bars depict ± SE

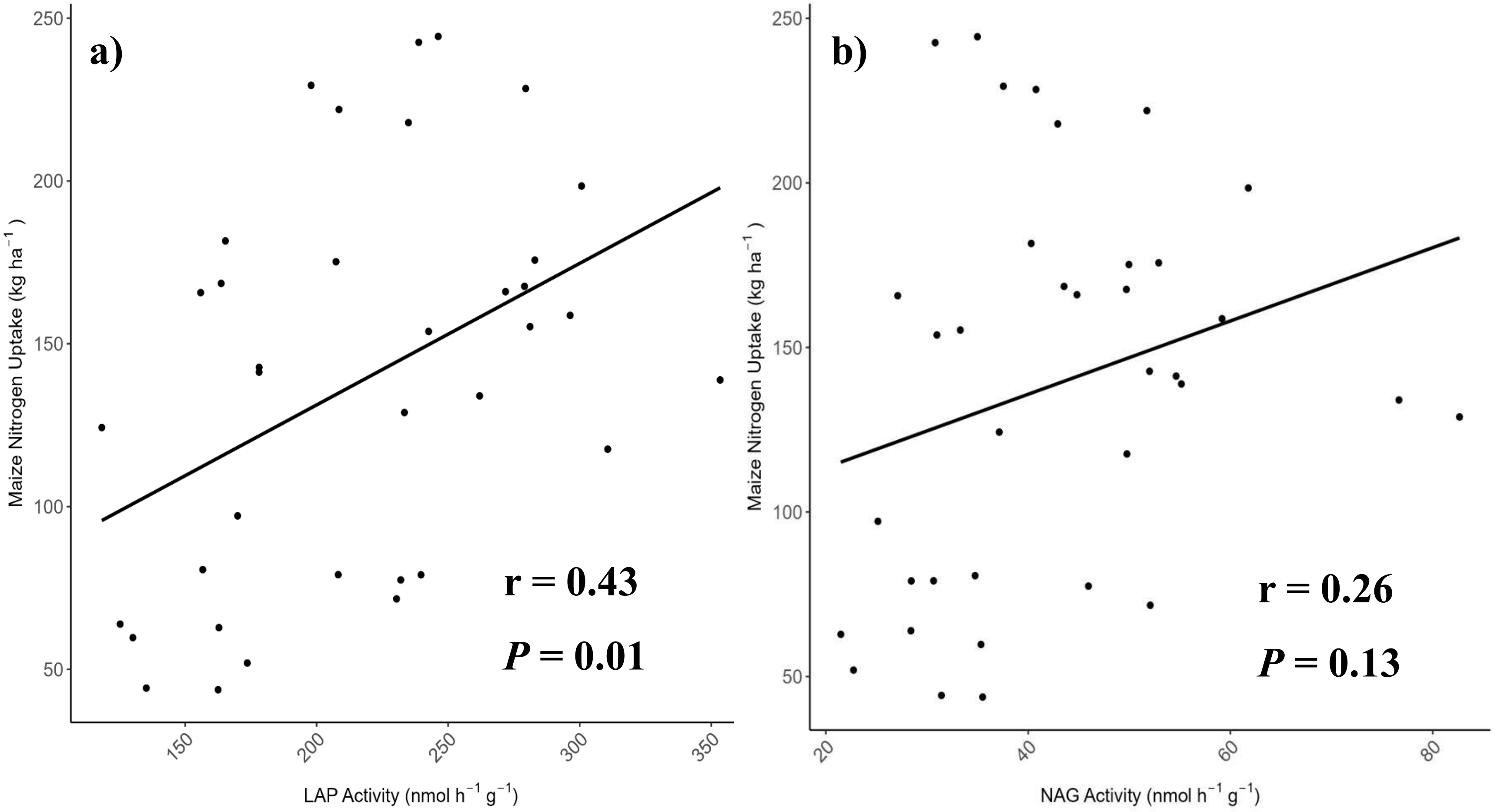

**Figure 3.5:** Pearson's correlation scatterplot between soil enzyme activity between approximately R1 and R3 and end of season maize nitrogen uptake for a) L-leucine amino peptidase (LAP) activity and b) *β*-1,4-N-acetyl-glucosaminidase (NAG). Data shown is averaged across the two growing seasons and treatments.

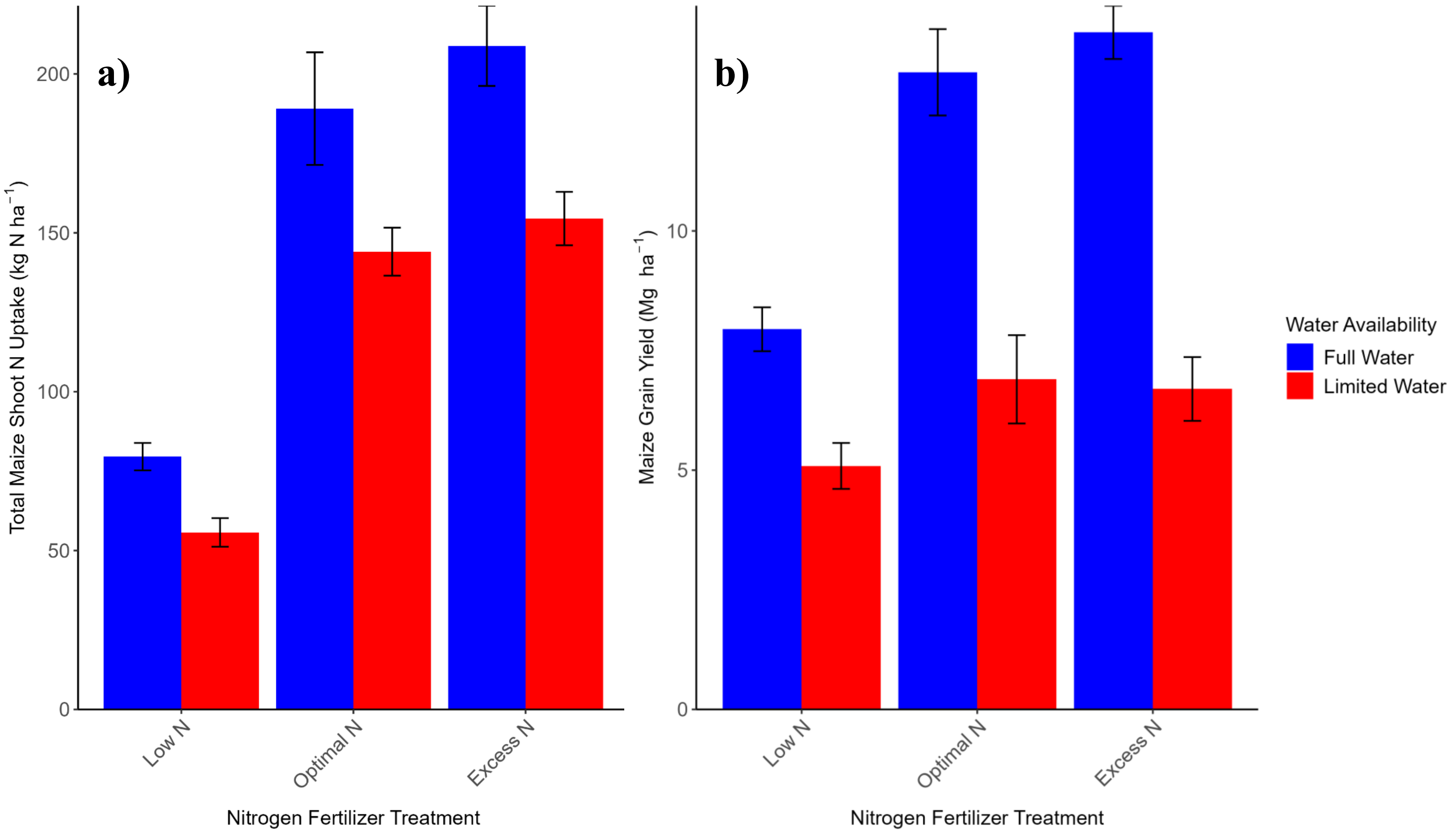

**Figure 3.6:** a) Total aboveground maize nitrogen uptake at physiological maturity by nitrogen fertilizer and water availability across the two growing seasons. b) Maize Grain Yield at physiological maturity by nitrogen fertilizer and water availability averaged across the two growing seasons. Grain yields were adjusted to 15.5% moisture content. Error bars depict ± SE.

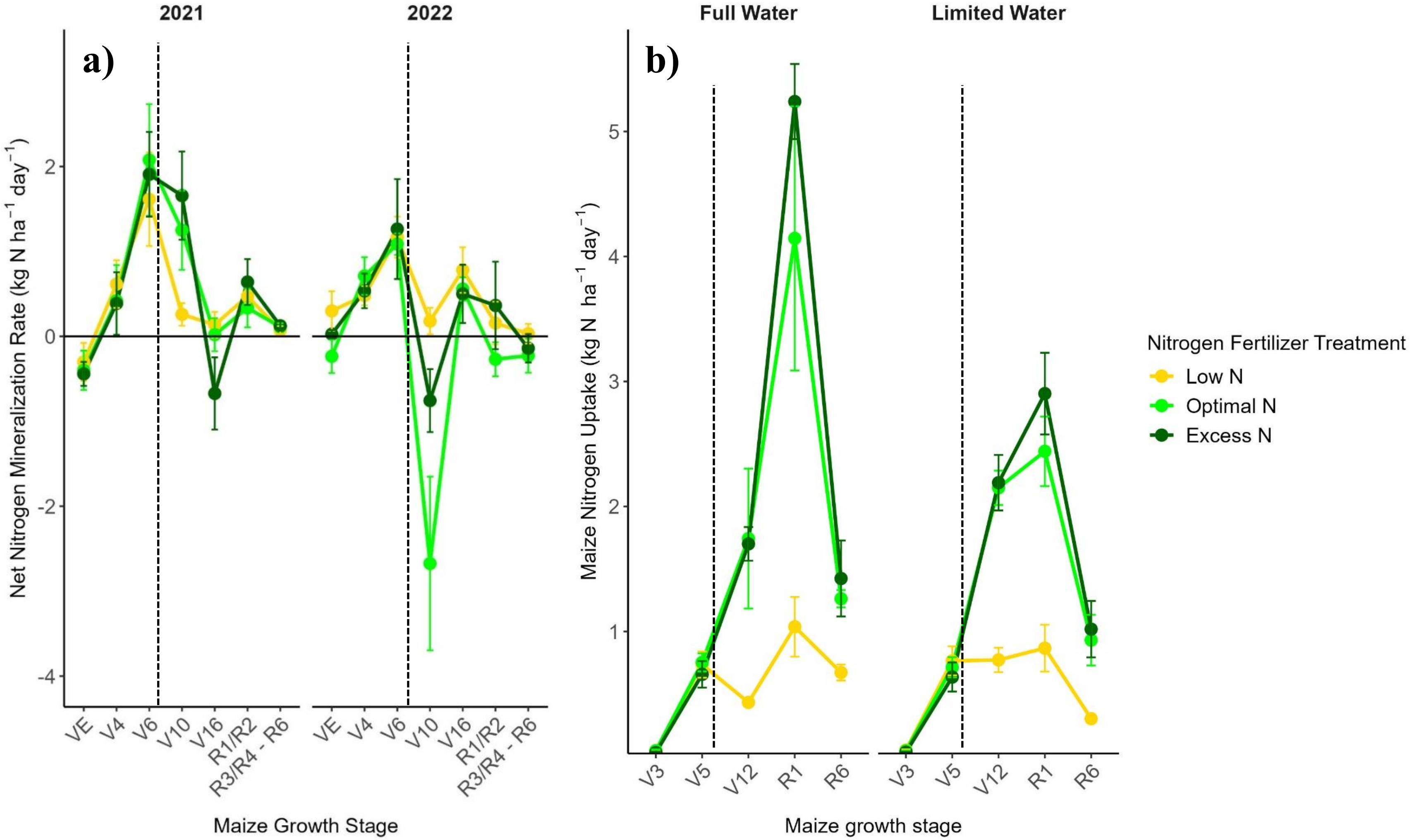

**Figure 3.7:** a) Net nitrogen mineralization (Nmin) rates (kg N ha$^{-1}$day$^{-1}$) during each incubation period in the top 0 – 15 cm over the entire growing season by nitrogen fertilizer treatment and growth stage. Nmin rates include NH4-N + NO3-N. The growth stage is labeled by the approximate maize growth stage when soil core incubations began. Incubations were done consecutively where the next incubation would start as soon as the previous incubation ended with the final incubation lasting from approximately R3/R4 – R6. b). Maize nitrogen uptake rate (kg N ha$^{-1}$day$^{-1}$) by maize growth stage, nitrogen fertilizer treatment, and water treatment. Maize growth

stage corresponds to when plant samples were collected, and plant N content and uptake rates were determined. “V” corresponds to vegetative stage and the number corresponds to how many collard leaves are present. “R” corresponds to reproductive stage and the number corresponds to which stage with R1, R2, R3 and R4 corresponding to silking, blister, milk, and dough stage respectively. “R6” corresponds to physiological maturity which is when the final sample of the growing season was taken. Error bars depict ± SE. Nitrogen fertilizer application was applied in two doses, one at planting and one at V7 which is indicated by the dotted black line.

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# CHAPTER 4: FATE OF NITROGEN FERTILIZER AND ITS CONTRIBUTION TO MAIZE NITROGEN UPTAKE

## 4.1 INTRODUCTION

Inorganic nitrogen (N) fertilizer is the largest source of N additions to crop lands (Battye et al., 2017), however recovery by agronomic crops is low, often lower than 50% (Gardner & Drinkwater, 2009; Yu et al., 2022). Fertilizer N that is not utilized by the current season crop can be used by future crops if it stays near the root zone, or if it is immobilized and subsequently mineralized (Tian et al., 2023). Therefore, long-term fertilizer N recovery is greater than initial recovery (Guo et al., 2021; Vonk et al., 2022; Yan et al., 2020). While improved genetics and agronomy have increased grain yields while minimizing N losses (Dohleman et al., 2024), approximately 41% of N added to crops in the U.S. is considered “surplus” (i.e. not used by the crop) (J. Zhang et al., 2021). Surplus N can be lost to the environment and global scale estimates of N losses range from 23 – 70% (Coskun et al., 2017; Ladha et al., 2016; Vonk et al., 2022). Fertilizer N recovery efficiency (NRE) can be defined as the proportion of N fertilizer that is taken up by the crop ($NRE_{crop}$) or is retained in the soil ($NRE_{soil}$). Fertilizer that is not recovered in these sinks is considered unrecovered or “lost” and contributes to the amount of reactive N in the environment (Cassman et al., 2002). Widespread use and subsequent loss of N fertilizer to the surrounding environment has nearly doubled the amount of reactive N in the biosphere (Battye et al., 2017; Gruber & Galloway, 2008), which can have adverse effects on soil, water, and air quality at the local to global level (Galloway et al., 2003).

Achieving high grain yields while minimizing N fertilizer losses is an important goal of agroecosystems. Multiple factors affect NRE of different pools including crop N uptake dynamics, N fertilizer management, water availability, residue management, and climate and soil properties (Asibi et al., 2019; Quan et al., 2021; Yu et al., 2022). Therefore, NRE can vary widely over space and time (Huggins & Pan, 2003; Ottman & Pope, 2000; Poffenbarger et al., 2018). In semi-arid regions, water and N availability are especially relevant for NRE. Water limitations reduce plant N uptake leaving more in the soil where it is susceptible to being lost (Cassman et al., 2002; Hauck & Bremner, 1976). Irrigation or above average precipitation can increase plant N uptake (Al-Kaisi & Yin, 2003; Djaman et al., 2013; Guo et al., 2022) and reduce ammonia volatilization (Holcomb et al., 2011), but can also increase leaching especially in sandy soils (Bundy & Andraski, 2005; Quemada et al., 2013). Leaching can be especially high when soil inorganic N concentrations are elevated such as after a drought year or when excessive N fertilizer is added (Loecke et al., 2017). Increasing N fertilizer applications tends to increase total plant N uptake, but typically also reduces $\mathrm{NRE}_{\mathrm{crop}}$ relative to lower N fertilizer rates (Asibi et al., 2019; Hammad et al., 2017; Wortmann et al., 2011). As water limitations become more prevalent in semi-arid regions (Deines et al., 2020; Derner et al., 2015), understanding the effects of N and water on NRE and the fraction of N lost will become increasing important in order to sustainably manage N for high yields and minimal losses.

Crops have access to different sources of N during the growing season with soil N mineralization and current season N fertilizer being the two main sources (Yan et al., 2020). Management practices and water and N availability can alter the relative reliance of crops on these difference N sources (Bundy & Andraski, 2005; X.-J. Liu et al., 2017), including changing N availability by changing soil N mineralization rates (Booth et al., 2005; Carpenter-Boggs et al., 2000; Mahal et al., 2018). The inherent mobility and potential for N losses associated with inorganic N fertilizers has motivated research focused on the ability of more stable soil organic matter pools to supply crop N needs to reduce reliance on inorganic N inputs (Grandy et al., 2022; Liebman et al., 2008;

Osterholz et al., 2017). Understanding how N and water availability alter the pools of N that crops utilize—specifically, nitrogen derived from $^{15}$N fertilizer (Ndff) and nitrogen derived from non-$^{15}$N fertilizer sources (non-Ndff)—can inform strategies to reduce crop reliance on extern N inputs thereby reducing losses (Breza et al., 2023).

There are two methods used for directly quantifying NRE: the “N-difference” method and the direct method using $^{15}$N fertilizer. Both methods have their weaknesses due to the confounding effects of adding N fertilizer on root growth, N uptake, and soil N mineralization rates as well as “pool-substitution” for $^{15}$N specifically (Cassman et al., 2002; Hauck & Bremner, 1976). Therefore, one method may be preferrable to the other depending on the research goal. The use of $^{15}$N fertilizer allows for positive identification of the labeled N in the system which can be useful for quantifying the fate of N as well as partitioning N pools used by crops (Hauck & Bremner, 1976; Quan et al., 2021; Rimski-Korsakov et al., 2009).

Maize (*Zea mays*) is an important crop in eastern Colorado, especially where irrigation is available (Derner et al., 2015), and it requires large amounts of N fertilizer to achieve high yields (Bauder et al., 2020). To better understand the effects of N and water availability on NRE and the fraction of N fertilizer lost, as well as quantify the source of maize N , we conducted a field experiment with two water availabilities and three $^{15}$N fertilizer rates. The objective of this study was to calculate nitrogen recovery efficiency (NRE) for different pools (maize and soil) and the fraction of N lost, as well as to quantify which pools—$^{15}$N fertilizer (Ndff) or non-$^{15}$N fertilizer (non-Ndff)—maize utilizes in order to inform improved N fertilizer management in the future. We expected higher N rates to have higher N losses, and the full water treatment to increase $NRE_{Maize}$.

## 4.2 METHODS:

### 4.2.1 SITE AND MAIN PLOT DESCRIPTION

The field experiment was conducted at the same field site described in Chapter 2, and used the same management practices.

### 4.2.2 MICROPLOT $^{15}$N STUDY

During the 2023 growing season, paired microplots were established in all of the N1, N2, and N3 plots, which had received 22 kg N ha$^{-1}$, 73 kg N ha$^{-1}$, 123 kg N ha$^{-1}$ respectively during both the 2021 and 2022 growing seasons. These plots were selected to represent a range of N rates and also due to their similar initial residual soil inorganic N from 0 – 90 cm that ranged from 36 – 43 kg N ha$^{-1}$. The microplots were 2.286 m wide (3 rows) and 2.286 m long and they received the same fertilizer treatment as the main plots at planting where banded liquid ammonium phosphate with 22 kg N ha$^{-1}$ and 44 kg P ha$^{-1}$ were applied as starter fertilizer. On June 27, 2023, which was V6, the treatments were applied to the main plots and microplot pairs. The main plots received the N fertilizer rates described in Chapter 2 where urea ammonium nitrate (UAN 32-0-0) was banded to the soil surface approximately 5 cm from the row to create the different nitrogen treatments. The next day the N treatments were established for the microplot pairs where 1 microplot (control) received no additional N fertilizer and the other received $^{15}$N-enriched $^{15}NH_4{}^{15}NO_3$ (5 atom %). The microplots that received $^{15}$N-enriched fertilizer received 38 kg N ha$^{-1}$, 95 kg N ha$^{-1}$, and 169 kg N ha$^{-1}$ in the N1, N2, and N3 plots respectively representing Low, Medium, and Optimal N. The side dressed enriched N fertilizer amounts were based on yield data from 2021 and 2022, and residual N fertilizer in the soil profile. Enriched fertilizer was added using a handheld backpack sprayer (6L per subplot) in a band ~ 5 cm from the maize row, mimicking the banded fertilizer that was applied to the main plots the day before.

### 4.2.3 PLANT SAMPLING

In the main plots, aboveground maize samples were collected at vegetative stage 4, 12 (V4, V12), and reproductive stage 1 (silking, R1) and 6 (physiological maturity, R6). Maize N uptake was determined by collecting shoots of 5 consecutive plants from all low, optimal, and excess N plots from the middle 2 rows. Plants were separated into leaves, stalks, grain, and cobs, dried at 60 °C and weighed. Plant samples were then ground, homogenized, and a subsample was analyzed for total N concentration using a dry combustion elemental analyzer (LECO Tru-SPEC, St. Joseph, MI, USA). Maize N uptake at each growth stage was calculated by multiplying the oven dry biomass of each plant component by its corresponding N concentration and summing all the aboveground parts. Maize N uptake at V4 from the main N1, N2, and N3 plots was used to determine N uptake of non-Ndff sources for the microplots before the $^{15}$N fertilizer was applied.

In the microplots, maize was sampled for grain yield and N uptake at R6 on September 27, 2023. Five plants were harvested from the center row of each microplot and plants were separated into grain, cob, leaf, and stalk. The plant parts were dried at 60 °C, weighed, and ground. Grain yield was determined by separating the grain from the ear and adjusting it to 15.5% moisture.

### 4.2.4 SOIL SAMPLING AND ANALYSIS

Soil profile sampling occurred post maize harvest on November 13, 2023. A tractor mounted hydraulic probe was used to collect 3 soil cores from each plot to a depth of 120 cm. The cores were split at increments of 0 – 15 cm, 15 – 30 cm, 30 – 60 cm, and 60 – 120 cm and composited in the field. Soil cores were taken in or near the middle row of the microplot with 1 sample being directly in the row and the other 2 straddling the middle row by approximately 10 cm. Soil cores were stored in a cooler on ice while being transported to the lab and were stored at – 20 °C before processing. Soil sample processing involved passing soil through a 2mm sieve, oven drying a 10 g subsample for 48 h at 105 °C to quantify gravimetric water content, and an additional subsample of sieved soil was dried and roller ground for later analyses.

### 4.2.5 MICROBIAL BIOMASS ANALYSIS

Microbial biomass N was measured in the surface soil depth, 0 – 15cm, using a chloroform fumigation-extraction method (Fierer & Schimel, 2003). Thawed, fresh soil samples (20 g) were sieved to 2 mm and shaken in 50 mL of 0.05M $K_2SO_4$ for 4 h at 150 rpm on a reciprocal shaker with or without 1 ml chloroform, centrifuged, and filtered through a 0.45 μm filter. All extracts were frozen at – 80 °C and then lyophilized (freeze dried) for later analysis.

### 4.2.5 NITROGEN CONCENTRATION AND $^{15}N$ ABUNDANCE

Subsamples from the microplots of dried, ground, homogenized maize (all organs: grain, cob, leaves, stalk), soil (all depths: 0 – 15, 15 – 30, 30 – 60, and 60 – 120 cm), and $K_2SO_4$ extracts were analyzed to determine N concentration and $^{15}N$ abundance using EA/IRMS (elemental analyzer coupled with Delta V Advantage isotope ratio mass spectrometer; Costech Analytical Technologies, Inc., Valencia, CA and Thermo Fisher Scientific Inc., Waltham, MA, respectively).

Maize yield and N uptake were converted to a per hectare basis using harvest area. Soil N concentrations were scaled to kg per hectare basis using the dry weight of the soil, depth of soil sample, and bulk density of the soil. Bulk density data from a previous experiment at this field was used for this calculation. Briefly, 2 replicate 5.1 cm soil cores were taken from each block to a depth of 0 – 75 cm and processes as described in Chapter 3.

### 4.2.8 FERTILIZER RECOVERIES AND SOURCE OF MAIZE N UPTAKE:

Fertilizer N recovery for maize (grain, cob, leaves, stalk), soil (0 – 15 cm, 15 – 30 cm, 30 – 60 cm, and 60 – 120 cm), and microbial biomass were calculated using the following equation:

1. $Fertilizer\ N\ fraction = \frac{Atom\%^{15}N_{sample} - Atom\%^{15}N_{zero}}{Atom\%^{15}N_{fertilizer} - Atom\%^{15}N_{zero}}$
2. $Recovered\ N\ derived\ from\ ^{15}N\ fertilizer\ (Ndff) = Fertilizer\ N\ fraction\ \times\ N\ content_{sample}$
3. $Fertilizer\ nitrogen\ recovery\ efficiency\ (NRE) = \frac{Recovered\ Ndff}{Fertilizer\ applied} \times 100$

where Atom%$^{15}$N$_{sample}$ is the atom % $^{15}$N values of maize , soil, or microbial biomass samples, Atom%$^{15}$N$_{zero}$ is the atom % $^{15}$N value for the paired zero N control subplot, and Atom%$^{15}$N$_{fertilizer}$ is the atom % $^{15}$N value for the fertilizer applied the subplots. The N content$_{sample}$ is the N content of the maize organs, soil depths, or microbial biomass samples. Total recovered Ndff fertilizer for maize was calculated by summing recovered Ndff for all organs (grain, cob, leaves, stalks), and total recovered Ndff for soil was calculated by summing all depths (0 – 15 cm, 15 – 30 cm, 30 – 60 cm, and 60 – 120 cm). Likewise, NRE$_{Maize}$ for maize was calculated by summing NRE for all maize organs, and NRE$_{Soil}$ for soil was calculated by summing all soil depths. Unrecovered or “Lost” Ndff was calculated by taking the difference of $^{15}$N applied and $^{15}$N fertilizer recovered in maize and soil. The fraction of fertilizer that was lost was calculated by subtracting NRE$_{Maize}$ and NRE$_{Soil}$ from 100.

Maize N uptake from non-Ndff sources and the percent of N uptake from the two sources – $^{15}$N fertilizer (Ndff) or non-$^{15}$N fertilizer (non-Ndff)– was calculated by difference using the following equations:

4. $Maize\ uptake\ non^{15}N\ fertilizer = Total\ N\ uptake - uptake\ from\ Ndff$
5. $Maize\ N\ uptake\ source\ percent = \frac{uptake\ from\ Ndff\ or\ non{-}Ndff}{Total\ N\ uptake} \times 100$

Maize N uptake from non-Ndff and percent of maize N uptake from the two sources was calculated at the individual organ level and total was calculated by summing all organs.

### 4.2.9 STATISTICAL ANALYSIS

A linear mixed effects model was used for all analyses to determine how the response variables (NRE, lost, Ndff recovered, non-Ndff uptake, grain yield, and maize N uptake source percent) were affected by water availability (irrigation treatment), nitrogen rate, maize organ and soil depth where applicable, and their interactions. The split plot design and repeated measures (where applicable i.e. $NRE_{Soil}$ by soil depth) analyses were integrated into the mixed model structure through the inclusion of block and its interactions with irrigation and N rate as random factors. All predictors were treated as categorical. Response variables and N availability were checked for normality, equal variance, and homogeneity and were transformed to meet model assumptions. To meet model assumptions Total $NRE_{Soil}$, $NRE_{MB}$ , recovered Ndff in MB, and the percentage of maize N uptake from non-Ndff were log transformed. Maize models that included maize organs, and soil models that included soil depth as predictors also needed log transformations to meet model assumptions. An ANOVA with the "Kenward-Roger" degrees of freedom adjustment was used to assess the significance of the fixed effects and their interactions within the context of this mixed-effects model. Paired t-tests were used to determine whether nitrogen uptake differed between paired microplots (control plots and plots that received enriched fertilizer). Analyses were performed in R version 4.2.2 using the lme4, lmeTest, and emmeans packages (R Core Team, 2022).

## 4.3 RESULTS

### 4.3.1 WATER AND NITROGEN AVAILABILITY

Annual precipitation (January – December) was 577 mm for which was well above average for the site (420 mm) (Schneekloth et al., 2020). Precipitation was above average especially May – July which accounted for 65% of the precipitation observed during 2023. To meet the ET demands for full water availability a total 116 mm of irrigation water was added. Irrigation was added weekly starting August 3rd and it ended August 28th. Limited water received 0 mm of irrigation water due to the high precipitation during the growing season (Fig. 4.1)

Total crop N availability was calculated as the sum of residual soil N before planting, starter N fertilizer, N in irrigation water, and enriched side-dressed $^{15}N$ fertilizer. The N treatments ranged from 105 – 238 kg N $ha^{-1}$ and 97 – 234 kg N $ha^{-1}$ for full and limited water, respectively (Table 4.1).

### 4.3.2 FATE AND RECOVERY OF NDFF

Maize recovery of N derived from the side-dressed $^{15}N$ fertilizer (Ndff) increased with N fertilizer rate and ranged from 18 – 108 kg N $ha^{-1}$ (Fig. 4.2, $P > 0.01$). Recovery of Ndff in soils increased with N rate, and tended to be greater in limited water and trended towards an N × water interaction (Fig. 4.2, $P = 0.09$). With full water recovery ranging from 11 – 35 kg N $ha^{-1}$, and limited water ranging from 12 – 56 kg N $ha^{-1}$. With full water, the medium N rate had the highest amount of Ndff in soil, with 220% and 19% more than the low and optimal N rates, respectively. When water was limited, the optimal N rate had 350% and 122% more Ndff than the low N rate and medium N rate respectively. Unrecovered, or lost Ndff, tended to be higher with more N fertilizer but was highly variable. Ultimately, the amount of N fertilizer lost was not affected by water, N, or an interaction (Fig. 4.2, $P = 0.70$, 0.59 & 0.23 respectively) and on average, 13 kg N $ha^{-1}$ was lost. The coefficient of variance (CV) for lost Ndff was roughly double that of recovered Ndff by maize or soil (CV = 139, 65, and 69 respectively).

Nitrogen recovery efficiency (NRE) was unaffected by water, N, or an interaction for all pools. Average $NRE_{Maize}$ was 56% making it the largest pool of recovered Ndff, but no water, N, or an interaction effects were observed (Fig. 4.3, $P = 0.84$, 0.16, & 0.69 respectively). Average $NRE_{Soil}$ was 29% but no treatment effects were observed (Fig. 4.3, $P = 0.39$, 0.61, & 0.32 respectively). Average Lost, or unrecovered, was 15% making it the smallest pool of Ndff, but no treatment effects were observed (Fig. 4.3, $P = 0.81$, 0.67, & 0.31 respectively).

### 4.3.3 MAIZE YIELD AND N UPTAKE

Maize grain yield increased with N fertilizer and tended to be higher with full water availability (Fig. 4.4, $P = 0.01$ & 0.08 respectively). Optimal N fertilizer increased maize yield by 13% and 51% compared to medium and low N respectively. Full water increased yields by 28% compared to limited water.

Total maize N uptake—uptake of N derived from $^{15}N$ fertilizer (Ndff), and uptake from non-$^{15}N$ fertilizer sources (non-Ndff)—increased with N fertilizer *($P > 0.01$)* and was largely due to increased uptake of Ndff. Maize N uptake of Ndff increased with N rate and ranged from 18 – 108 kg N $ha^{-1}$ (Fig. 4.5a, $P > 0.01$). Over the entire season, average N uptake of non-Ndff sources was 79 kg N $ha^{-1}$ (Fig. 4.5a). Optimal N led to a slight increase in uptake of non-Ndff sources compared to medium and low N, though this increase was not significant ($P = 0.47$). The slight N uptake with optimal N came between V4 – R6 (after the treatments were applied). Before the $^{15}N$ was applied, average N uptake at V4 ranged from 17 – 20 kg N $ha^{-1}$ across N rates. Average N uptake of non-Ndff sources post V4 ranged from 54 – 69 kg N $ha^{-1}$ with optimal N being the highest (S. Fig. 4.1). However, there was no N effect on maize N uptake of non-Ndff sources at V4, or between V4 – R6 therefore increases were not significant ($P = 0.72$ & 0.58). Full water increased maize N uptake from Ndff and non-Ndff sources by 5% and 18% respectively, but this effect was not significant ($P = 0.62$ & 0.38 respectively). Additionally, no N × water interaction for uptake from Ndff and non-Ndff ($P = 0.78$, 0.90, respectively). Average maize N uptake in the zero N control plots was 86 kg N $ha^{-1}$ and no water, N, no N × water interaction was observed (S. Fig 4.2, $P = 0.30$, 0.82, 0.97 respectively).

The relative source of maize N varied with N rate (Fig. 4.5b, $P > 0.01$). Maize acquired 20 – 56% of its N from Ndff, and Ndff was the main source of N with optimal N. Maize acquired 46 – 80% of its N from non-Ndff sources and was more reliant on non-Ndff sources at lower N rates. There was no water effect on the percentage of maize N uptake from the two sources (Ndff or non-Ndff) ($P = 0.38$ & 0.44 respectively).

Within the maize plant, there was an N × organ interaction on N uptake from Ndff or non-Ndff sources (Fig. 4.6a, $P = 0.06$ & 0.04 respectively). Increasing N fertilizer rate increased N uptake of Ndff for all maize organs, especially in the grain. For non-Ndff a different pattern was observed. Grain, cob, and stalk uptake of non-Ndff was relatively constant across the N treatments with average N uptake of non-Ndff sources being 53, 5, 8 kg N $ha^{-1}$ respectively. However, leaf N uptake of non-Ndff sources ranged from 9 – 16 kg N $ha^{-1}$ and was greatest at the optimal N rate. Maize leaves acquired a majority of their N from non-Ndff regardless of N fertilizer rate. All other maize organs (grain, cob, stalks) accumulated a majority of their N from Ndff at the optimal N rate, and a majority of their N from non-Ndff at the low and medium N rate. For the control plots there was only an organ effect where grain accumulated the most N, followed by leaves, then stalk and cobs (59, 12, 8, 6 kg N $ha^{-1}$, respectively) (S. Fig. 4.2, $P >$

0.01). Maize N uptake of non-Ndff in the leaves was greater in the optimal N plots compared to the control plot (*P* = 0.06).

Within the maize plant, $NRE_{Maize}$ exhibited an N × organ interaction where $NRE_{Maize}$ increased with N rate for all organs except for leaves (S. Fig. 4.3, P > 0.06). Grain had the greatest NRE within the plant and accounted for 69 – 70% of $NRE_{Maize}$. Grain $NRE_{Maize}$ increased by 12 and 33% from low N compared to medium and optimal respectively. Leaves had the second highest $NRE_{Maize}$, but $NRE_{Maize}$ was lowest at optimal N. Stalk and Cob $NRE_{Maize}$ was greatest with optimal N, and stalk NRE was greater than cob.

### 4.3.4 SOIL RECOVERY OF NDFF

When looking at soil Ndff by depth results show that Ndff is greater near the surface and increases with N rate (Fig. 4.7a, *P* > 0.01 & *P* = 0.03). Soil Ndff ranged from 3 – 17 kg N $ha^{-1}$ for the 4 depths measured. The top 0 – 15 cm had the greatest amount of Ndff and had 292% more Ndff than the next closest depth of 60 – 120 cm. The bottom depth, 60 – 120 cm, had 45% and 21% more Ndff than the 15 – 30 cm and 30 – 60 cm depth respectively. Across all depths soil Ndff increased with N rate. The medium N rate had 157% more Ndff than the low N rate, and the optimal N rate had 42% more Ndff than medium N. The $NRE_{Soil}$ varied by soil depth (Fig. 4.7b, *P* > 0.01). Of the 29% $NRE_{Soil}$, 60% was in the top 0 – 15 cm, followed by 11%, 13%, and 16% in the 15 – 30 cm, 30 – 60 cm, and 60 – 120 cm respectively.

Within the top 0 – 15 cm Ndff in microbial biomass N (MBN) increased with N rate (Fig. 4.8a, *P* = 0.04). The optimal N rate had 270% and 9% more Ndff in MBN compared to low and medium N respectively. There was no effect of water, nor was there an N × water interaction (*P* = 0.40 & 0.77). There was no effect of water, N, nor an N × water interaction on $NRE_{MBN}$ (Fig. 4.8b, *P* = 0.40, 0.48, 0.77). While recovered Ndff did increase with N rate, it only constituted 4 – 5% of

Ndff recovered in the top 0 – 15 cm of the soil. There was no water, N, nor N × water effect on non-Ndff sources in MBN (Fig. 4.8a, $P = 0.38$, 0.25, & 0.32 respectively). In MBN non-Ndff sources were made up the majority of N for all treatments ranging from 86 – 97%. Optimal N led to the lowest reliance on non-Ndff in MBN, though it was still very high (86%).

## 4.4 DISCUSSION

Quantifying the fate of N fertilizer and pools of N crops are utilizing in response to N and water availability is becoming more important as water availability becomes less certain. Overall, maize nitrogen fertilizer recovery efficiency was consistently high (56%), and was almost double that of soil (29%). On average, the fraction of N fertilizer that was lost was relatively low (15%) which suggests that higher N fertilizer rates will lose more kg N $ha^{-1}$. Maize N uptake from N fertilizer increased with increasing N rate. Maize N uptake from non-Ndff sources did not exhibit any treatment effects, indicating soil N supply capacity and crop access non-Ndff sources did not significantly differ by treatments. Additionally, non-Ndff sources were more important N sources for lower N fertilizer rates than the optimal N rate.

The results suggest that reducing N inputs while still meeting crop N demands will help maintain crop productivity while mitigating N losses. Higher yielding maize crops with higher N demands are more reliant on Ndff than non-Ndff, such as soil N mineralization, to meet their N needs. Therefore, reducing N inputs may be challenging, but management practices aimed at increasing soil N mineralization rates and the synchrony of soil N supply with crop N demand can help reduce crop reliance on external N inputs. In the current study, there was no difference in water availability until reproductive stages due to well above average precipitation in 2023. Future studies with more extreme differences in water availability will be valuable to better understand N × water interactions.

### 4.4.1 FATE AND RECOVERY OF NDFF:

In the current study, $NRE_{Maize}$ was 56% which is on the higher end of $NRE_{Maize}$ values reported in meta-analyses and reviews which often range from33 – 54% (Cassman et al., 2002; Quan et al., 2021; Yan et al., 2020). However, in the current study $^{15}N$ fertilizer was added around V6, not at planting. This may have helped increase $NRE_{Maize}$ as maize N demand starts to increase around V6 (Ma et al., 1999a). Other studies have found that $NRE_{Maize}$ of side-dressed N is greater than N applied pre-planting (Guo et al., 2021; Roberts et al., 2016). Additionally, retaining crop residue, as this field has been historically managed, can lead to immobilization of N due to high C:N ratios of residues thereby temporarily preventing N from being lost (McSwiney et al., 2010). Over the growing season this immobilized N can be released and made available to the crop thus increasing $NRE_{Maize}$ (Quan et al., 2021). On the other hand, high levels of immobilization increases pool substitution of $^{15}N$ which could decrease $NRE_{Maize}$ as microbes immobilize $^{15}N$ but release non-enriched N which is subsequently accumulated by the crop (Cassman et al., 2002; Hauck & Bremner, 1976). In the current study, $NRE_{MBN}$ was constant across treatments suggesting that pool substitution was not affected by the treatments and likely did not affect $NRE_{Maize}$. Maize root N uptake was not measured in the current study but including it would have slightly increased $NRE_{Maize}$ (Flynn et al., 2023; Rimski-Korsakov et al., 2009).

Generally, $NRE_{Maize}$ decreases as N rate increases (Asibi et al., 2019) though that was not the case in the current study. Matching N supply with N demand, increasing crop N uptake, and increasing availability of other resources (e.g. water) that increase biomass growth can increase $NRE_{Maize}$ (Poffenbarger et al., 2018; Quemada & Gabriel, 2016; Ullah et al., 2019). In the current study, above average precipitation provided favorable conditions for biomass production and crop N uptake thus there was no water effect. Maize N demand is driven by growth potential and soil N availability (Gonzalez-Dugo et al., 2010; Peng et al., 2010). In the current study, low N had the lowest N uptake and yield due to it being N limited, while optimal N had greater N availability thus greater N demand and uptake, contributing to similar $NRE_{Maize}$ across treatments. Roberts et al., (2016) found that $NRE_{Maize}$ was the same when 135 or 185 kg N $ha^{-1}$ was added to irrigated maize. Similarly, Rimski-Korsakov et al., (2012) found that $NRE_{Maize}$ was the same when 70 or 140 kg N $ha^{-}$ was added and $NRE_{Maize}$ only decreased when 250 kg N $ha^{-1}$ was applied. When N rates do not greatly exceed maize N demands, and when N losses are reduced, $NRE_{Maize}$ is not reduced.

The $NRE_{Soil}$ for the current study was 29% which is similar to values reported in the literature 28 – 36% (Quan et al., 2021; Yan et al., 2020). $NRE_{Soil}$ varies by location, management practices, and soil type (Poffenbarger et al., 2018; Vonk et al., 2022; C. Zhang et al., 2021). While high precipitation or irrigation can increase N losses via nitrate ($NO_3^-$) leaching, thus reducing $NRE_{Soil}$, this is primarily true for sandy soils that have high water permeability and low N retention (Quemada et al., 2013). Additionally, leaching can be reduced with reduced tillage (Khan et al., 2017), and will decrease when crop N uptake is high. The results show that 60% of Ndff recovered was in the top 0 – 15 cm, and that 16% was recovered in the bottom 60 – 120 cm. Thus, some N did move down the soil profile suggesting some leaching did occur. Of the gas losses, ammonium volatilization was likely not the dominant pathway due to N being applied as a solution and a precipitation event shortly after application. Rather, above average precipitation could have created conditions for high denitrification (Holcomb et al., 2011; Meisinger & Randall, 1991). Overall, the fraction of $^{15}N$ fertilizer that was lost was relatively low, and unaffected by the treatments. Gaseous losses of $N_2O$ are often 1% of N applied, and losses of N via leaching are proportional to N applied but tend to increase rapidly with excess N applied

(Puntel et al., 2016; Venterea et al., 2012). This should suggest that more N would be lost at higher N rates, though the percentage lost would be constant until excess N is applied. In the current study, higher N rates tended to more Ndff lost relative to the low N treatment, however results were highly variable, and no treatment effect was observed. Management practices that allow for lower N rates while maintaining high yields could help mitigate N losses without sacrificing productivity.

### 4.4.2 MAIZE YIELD AND N UPTAKE

Maize grain yield was more sensitive to water than plant N uptake. Full water increased grain yields relative to limited water, but there was no significant water effect on N uptake. Maize N demand increases with N fertilizer so long as other factors are not limiting growth (de Wit, 1992), and above average precipitation during the growing season provided ample water for high N uptake for both water treatments. The full water treatment did receive an additional 116 mm of water during the reproductive stages, and maize N uptake can increase with water availability during reproductive stages; however, a majority of maize N is accumulated during vegetative and early reproductive stages and uptake ceases during later reproductive stages (Donovan et al., in prep; Guo et al., 2022; Ma et al., 1999a). Therefore, additional water during this time, especially during the later stages, did not affect N uptake. Grain yields on the other hand are very sensitive to water availability during all reproductive stages until maturity, thus additional water during this time increases yields (Aslam et al., 2015).

Maize accumulates N from both Ndff and non-Ndff sources. Non-Ndff sources include soil N mineralization, residual soil N from previous seasons, N fixation, N deposition, and organic inputs with soil N mineralization often being the largest source (Ladha et al., 2016; Udvardi et al., 2021; Yan et al., 2020). Starter N fertilizer applied at planting would also contribute to non-

Ndff sources in the present study as starter fertilizer was not enriched. Although rates were low (22 kg N ha$^{-1}$), so it would not constitute a large fraction of non-Ndff. In the current study, maize acquired a majority of its N from Ndff when optimal N was applied and was more reliant on non-Ndff sources at lower N rates. Other studies have found that uptake of enriched fertilizer increases with rate, but non-Ndff sources are still the primary source of N for maize (Rimski-Korsakov et al., 2012; Roberts et al., 2016; Stevens et al., 2005).

The present study included a control plot that received no fertilizer beyond what was applied as starter fertilizer. Using a control plot with no N fertilizer is common in the literature (Poffenbarger et al., 2018; Rimski-Korsakov et al., 2012; Stevens et al., 2005), including studies who’s control plots only received N fertilizer at plant such as the present study (Roberts et al., 2016). Using a 0N control plot allows quantifying how N uptake of non-Ndff sources changes when N fertilizer is added. In the current study, maize N uptake of non-Ndff sources between control plots and plots that received enriched fertilizer was similar, suggesting a minimal effect of N fertilizer on N uptake from non-Ndff sources. However, in the enriched plots maize N uptake of non-Ndff was slightly, though not significantly, higher with optimal N relative to lower N rates. Fertilizer N additions can increase plant N uptake from non-fertilizer sources due to “priming” effects where N additions increase soil N mineralization (Stevens et al., 2005), as well as plant mediated responses such as increased root growth and exudation increasing volume of soil explored by roots and priming the rhizosphere leading to increased soil N mineralization (X.-J. Liu et al., 2017). Additionally, Donovan et al., (in review) found that N fertilizer increased soil enzyme activity and that enzyme activity was greater during maize vegetative stages when maize N uptake is lower. This suggests N fertilizer increases bioavailable N via microbial depolymerization of N containing compounds; a rate limiting step for soil N mineralization

(Daly et al., 2021). However, there was asynchrony between soil N supply and crop N demand which has been found in several other studies (Fernández et al., 2017; Ma et al., 1999b; Mahal et al., 2019).

The tendency to have higher N uptake of non-Ndff sources with optimal N could be due to a priming or plant mediated effect. Although the asynchrony between soil N supply and crop N demand hindered the crop's ability to uptake more non-Ndff. Looking at maize N uptake of the different sources by maize organ seems to support this. Higher N fertilizer rates increased N uptake of non-Ndff sources and leaves always acquired a majority of their N from non-Ndff. Additionally, leaf N uptake of non-Ndff sources was higher with optimal N relative to leaf N uptake of non-Ndff sources in the control plot. Leaves are a major N sink early in the season, supporting photosynthesis as well as being stored for translocation to new sinks later in the season (Tegeder & Masclaux-Daubresse, 2018). As soil N mineralization is often greater during vegetative stages of maize, leaves could access soil N mineralization to meet their N demands. Grain N, the dominant N sink later in the season, is supported by N uptake during reproductive stages as well as translocation (Masclaux-Daubresse et al., 2010; Tegeder & Masclaux-Daubresse, 2018). Maize N uptake peaks during early reproductive stages (Osterholz et al., 2017), and in the current study maize accumulation of non-Ndff is constant across the treatments, while uptake of Ndff increased. This suggests that uptake and translocation of Ndff, and not non-Ndff was primarily supporting grain N demands. Favorable growing conditions may have led to high N uptake throughout the growing season thereby increasing leaf stored N and reducing the need to remobilizing leaf photosynthetic N to grain during reproductive stages (Ciampitti et al., 2013; Masclaux-Daubresse et al., 2010; Tegeder & Masclaux-Daubresse, 2018). Therefore, non-Ndff acquired by the leaves early in the growing season could be retained in the leaf supporting photosynthesis, while Ndff acquired later in the season could be transported to grain during the reproductive stages.

Additionally, increased crop growth increases competition for N between soil microbes and the crop thus soil microbes produce enzymes to mine soil organic matter for N to meet their own N needs (Kumar et al., 2017). Competition should be greatest around peak maize N uptake, which typically has lower soil N mineralization rates (Donovan et al., in review; Mahal et al., 2019). Soil microbes can effectively outcompete crops for N in the short term (Osterholz et al., 2017), and may have outcompeted maize for N uptake during growth stages with peak uptake rates. Indeed, the data from the present study suggests that immobilization was occurring, and that a majority of MBN was from non-Ndff sources. This would lead to maize crops being more reliant on Ndff rather than the newly mineralized N microbes are using for their own N demands. Future studies with biomass samples collected throughout the growing season, as opposed to just end-of-season sampling, will reveal the temporal and partitioning patterns of Ndff and non-Ndff sources of maize N uptake throughout the season. Additionally, quantifying gross N mineralization and immobilization at different growth stages will reveal soil N supply capacity across the season.

The increased reliance on N fertilizer rather than soil N mineralization and other non-Ndff sources of the higher yielding maize could make it challenging to reduce N inputs. Long term N additions increase soil capacity to supply N to crops (Poffenbarger et al., 2018b; Tian et al., 2023b), therefore reducing N inputs too much for too long may result in soil N mining and a reduction in soil N supply capacity. Adding N fertilizer in smaller, but more frequent doses, to match crop N needs presents logistical challenges, but can help increase N recovery and may allow for lower N rates to be used as losses are reduced (Quemada & Gabriel, 2016; Roberts et al., 2016; Udvardi et al., 2021). There are several management approaches that can support building soil N while still supporting crop growth via N mineralization. For example, complex crop rotations increase soil N mineralization relative to two-crop rotations or monocultures (Breza et al., 2023; Grandy et al., 2022), and the use of leguminous cover crops can help offset N fertilizer needs via biological N fixation and mineralization of cover crop residue (Schipanski et al., 2014; Schipanski & Drinkwater, 2011). Additionally, grazing cover crops with livestock can help make them more profitable in water-limited systems (Kelly et al., 2021), and livestock manure has improved synchrony between N release and crop N demand compared to synthetic N fertilizer (Loecke et al., 2012; Ma et al., 1999b).

### 4.4.3 SOIL AND MICROBIAL BIOMASS NDFF

For the entire soil profile, recovered Ndff increased with N rate, and tended to be higher with limited water. This may have been due to full water slightly increasing maize N uptake but also N losses, though not significantly in either case. Of the Ndff recovered in the soil, a majority was in the top 0 – 15 cm. Retention of N in the soil is controlled by inherent soil properties such as silt and clay content as well as soil organic matter content which can change through management practices (Barrett & Burke, 2002; Núñez & Schipanski, 2023; Poffenbarger et al., 2017). Retaining N in the soil prevents it from entering the surrounding environment and can be utilized by crops in following growing seasons, though it is often not a major source of N for crops (Guo et al., 2021; Stevens et al., 2005; Vonk et al., 2022). Once in the soil, N flows between bioavailable N and MBN, and soil organic matter fractions of mineral associated or particulate organic matter (Cotrufo et al., 2013; Daly et al., 2021). Mineral associated organic matter (MAOM) N is typically considered to be more slowly available due to the silt and clay mineral surface associations. Therefore, soil minerology can influence a soil's capacity to build and retain high MAOM concentrations (Castellano et al., 2012, 2015; Poffenbarger et al., 2018). Particulate organic matter (POM) on the other hand is not bound to soil mineral surfaces and soils do not become POM saturated (Castellano et al., 2015). Semi-arid environments have low soil organic matter content (Cotrufo & Lavallee, 2022; Núñez & Schipanski, 2023; Oldfield et al., 2019), therefore the soils in the current study were likely far from saturation, allowing for increased Ndff retention.

The increase of Ndff in MBN as N rate increase suggests microbial immobilization of N was occurring. End of season immobilization of N is likely more important for temporary Ndff retention than immobilization during the growing season as turnover of MB is typically less than 1 year (Li et al., 2019). Additionally, microbial activity decreases with temperatures, so immobilization later in the season should help stabilize N as turnover of organic N is reduced (Y. Liu et al., 2017; Miller & Geisseler, 2018). The increase of Ndff in MBN does not necessarily mean soil N availability was greatly decreased. Soil N mineralization and immobilization occur simultaneously as N is incorporated into MB or released depending on metabolic needs (Elrys et al., 2021; Mooshammer et al., 2014). Crops can effectively compete with soil microbes for N over the course of the growing season, and over half of mineralizable N is derived from MBN (Drinkwater et al., 2017; Li et al., 2019). Further, immobilization rates can change over time as microbial N needs are satisfied (Aoyama & Nozawa, 1993; Mikha et al., 2005). Taking maize N uptake and MBN data together suggests that mineralization was the more dominant pathway earlier in the growing season, while immobilization was more dominant later in the season. This would explain why optimal N only slightly, and not significantly, increased maize N uptake of non-Ndff sources. Depolymerizing soil enzyme activity increased with N fertilizer rate and was greater during vegetative stages relative to early reproductive stages when maize N uptake was greatest (Donovan et al., in review). Therefore, in the current study optimal N could have increased soil N mineralization and maize N uptake during growth stages prior to maximum N uptake. However, during peak N uptake, soil N immobilization of primarily non-Ndff sources was occurring. Therefore, in order to meet the high N demands, maize utilized Ndff instead of non-Ndff.

## 4.5 CONCLUSION

Understanding the fate of N fertilizer and the pools of N crops are utilizing is important for efficient N management. We found that the Ndff recovered (kg N $ha^{-1}$) increased with N rate in maize and soil but the proportion recovered (NRE) did not. The fraction of $^{15}N$ fertilizer that was lost was consistent across the treatments suggesting that higher N fertilizer rates would lose more N. Reducing N inputs in order to reduce N losses may be challenging as the higher yielding, higher N demanding maize with optimal N was more reliant on Ndff than non-Ndff sources. This was likely due to seasonal soil N mineralization patterns, with lower rates occurring at peak maize N growth stages. Above average precipitation during the growing season made the water treatments negligible, and future studies with more extreme water differences will provide insights into how water affects NRE, and pools of N used by maize with different levels of N. Management practices aimed at increasing soil N mineralization rates and synchrony of soil N supply with crop N demand can help ensure the high N needs of high yielding maize are met without being over reliant on synthetic N fertilizer. Future studies with sampling throughout the season, rather than just physiological maturity, can reveal the temporal dynamics of NRE and pools of N utilized.

# CHAPTER 4 TABLES AND FIGURES

**Table 4.1**: Nitrogen availability (kg N ha$^{-1}$) for all treatments. Soil residual nitrogen was determined pre-planting 0 – 90 cm, all plots received the same starter N fertilizer at planting, side-dressed $^{15}$N are the treatment amounts, nitrogen from irrigation water was determined based on ppm NO$_3^-$ in irrigation water and amount added, and total N availability is the sum of all the columns listed.

| | **Full Water** | | | | | **Limited Water** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Nitrogen treatment** | **Soil residual nitrogen** | **Starter Nitrogen fertilizer rate** | **Side-dressed $^{15}$N** | **Nitrogen from irrigation water** | **Total Nitrogen Availability** | **Soil residual nitrogen** | **Starter Nitrogen fertilizer rate** | **Side-dressed $^{15}$N** | **Irrigation water nitrogen** | **Total Nitrogen availability** |
| Low | 36 | 22 | 38 | 9 | 105 | 37 | 22 | 38 | 0 | 97 |
| Medium | 38 | 22 | 95 | 9 | 164 | 35 | 22 | 95 | 0 | 152 |
| Optimum | 38 | 22 | 169 | 9 | 238 | 43 | 22 | 169 | 0 | 234 |

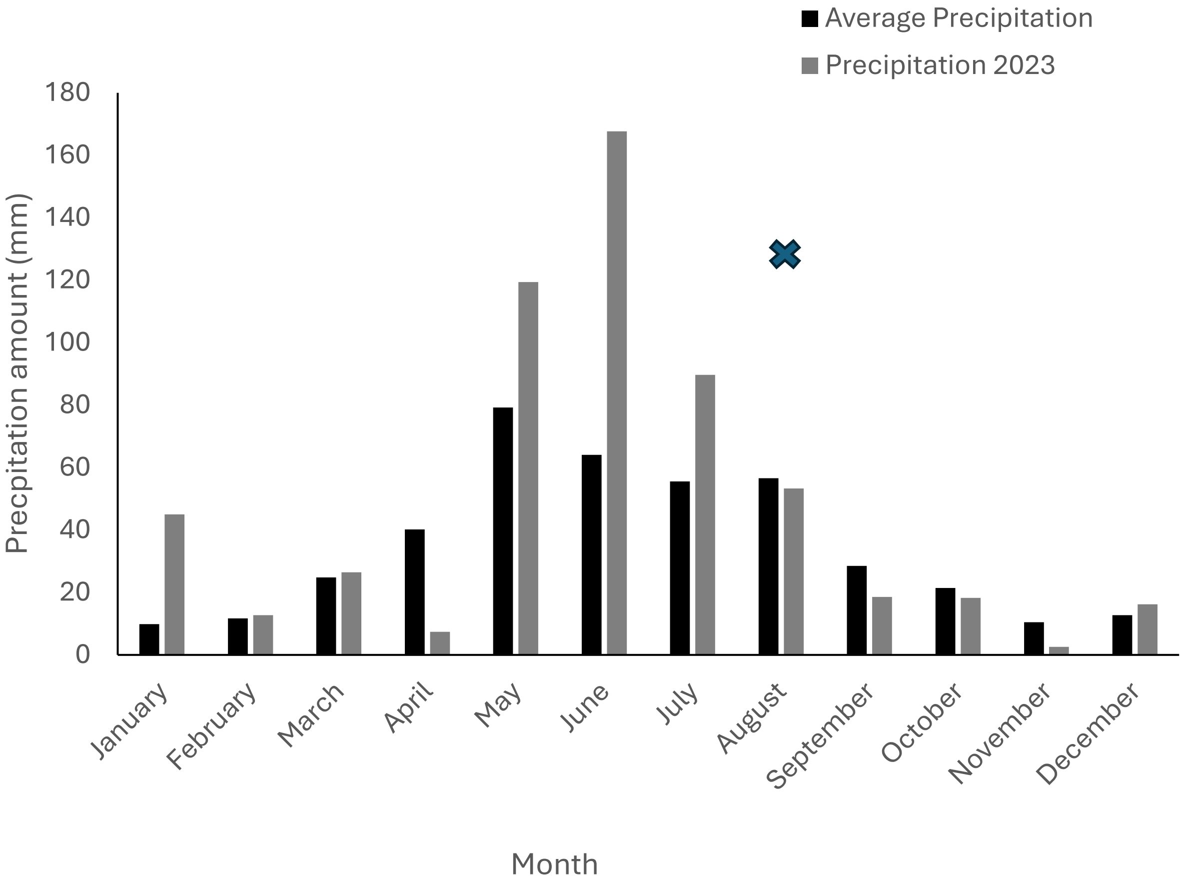

**Figure 4.1:** Long term average monthly precipitation and monthly precipitation for the 2023 growing season. The full water treatment had an additional 116 mm of irrigation water during the month of August indicated by the “x”. The limited water treatment did not receive any supplemental irrigation water.

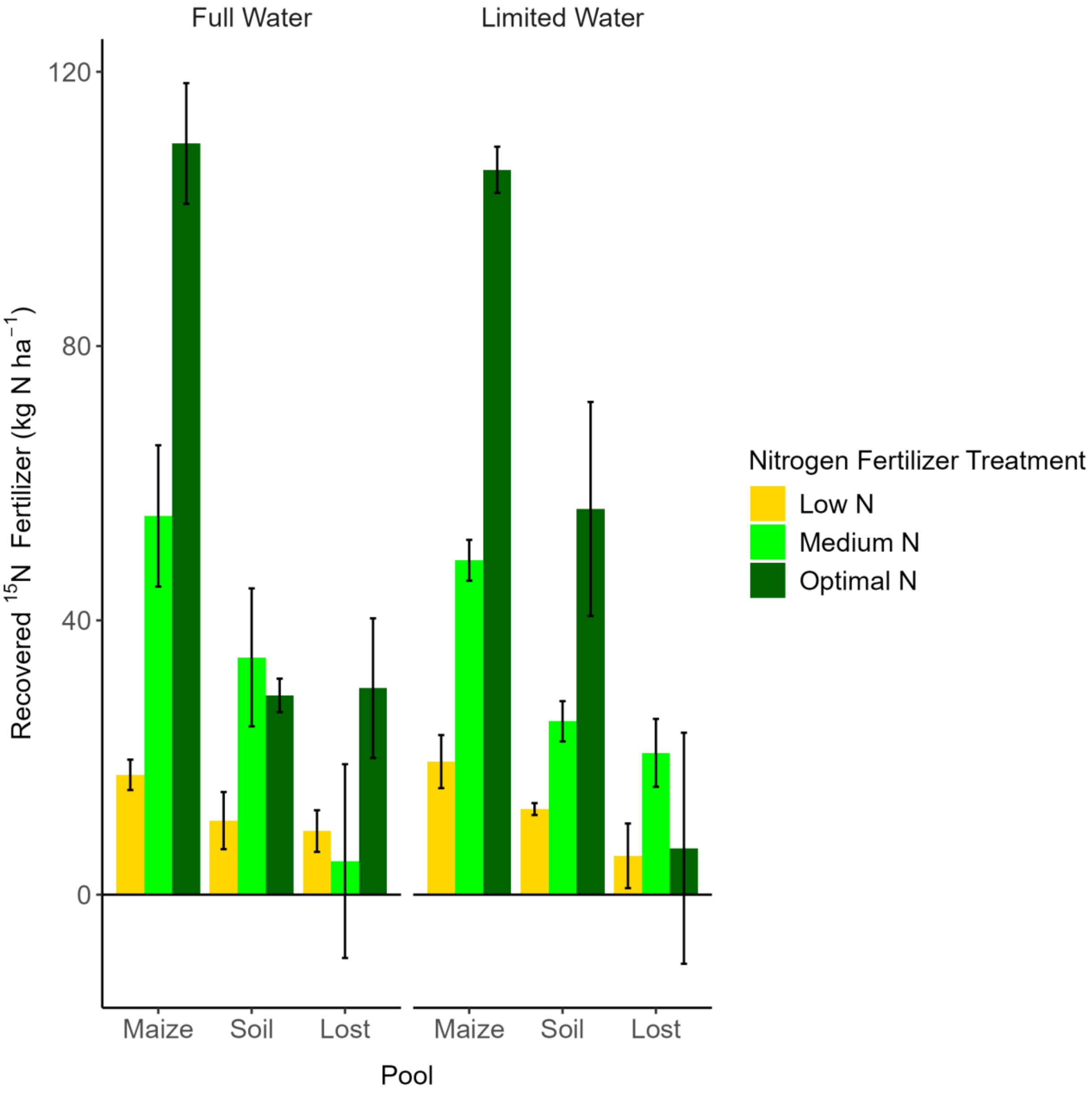

**Figure 4.2:** Recovered nitrogen (N) derived from side-dressed $^{15}$N fertilizer (Ndff) by N fertilizer treatment and water availability. Maize corresponds to all aboveground maize organs (grain, cob, leaves, and stalk) at physiological maturity. Soil corresponds to the entire soil profile 0 – 120 cm after grain harvest. Lost corresponds to Ndff that was not recovered by maize or the soil. Error bars indicate ± SE.

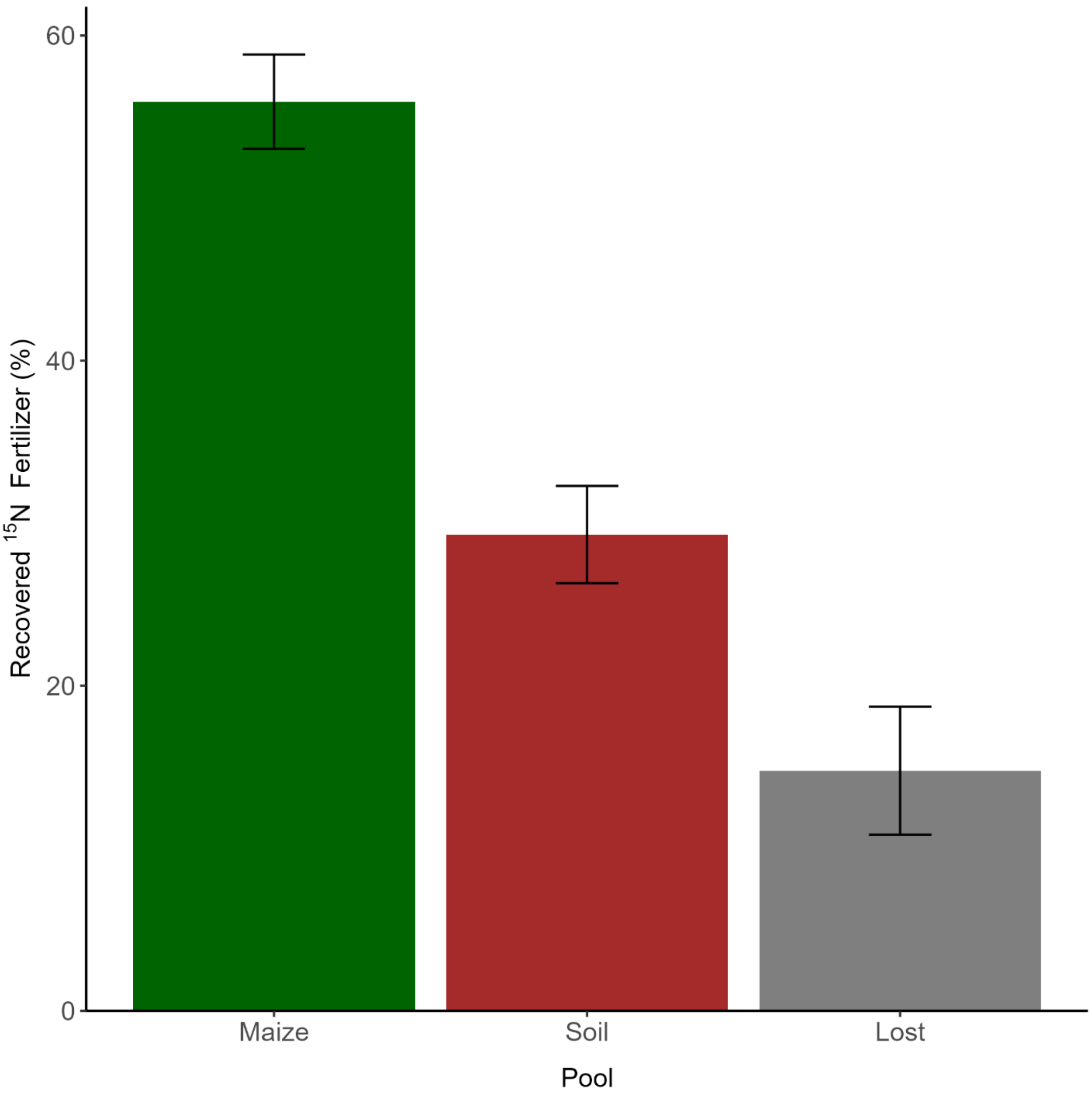

**Figure 4.3:** Nitrogen (N) fertilizer recovery efficiency (percent of $^{15}$N fertilizer recovered, NRE) averaged over N fertilizer treatment and water availability. Maize corresponds to all aboveground maize organs (grain, cob, leaves, and stalk) at physiological maturity. Soil corresponds to the entire soil profile 0 – 120 cm after grain harvest. Lost corresponds to $^{15}$N fertilizer that was not recovered by maize or the soil. Error bars indicate ± SE.

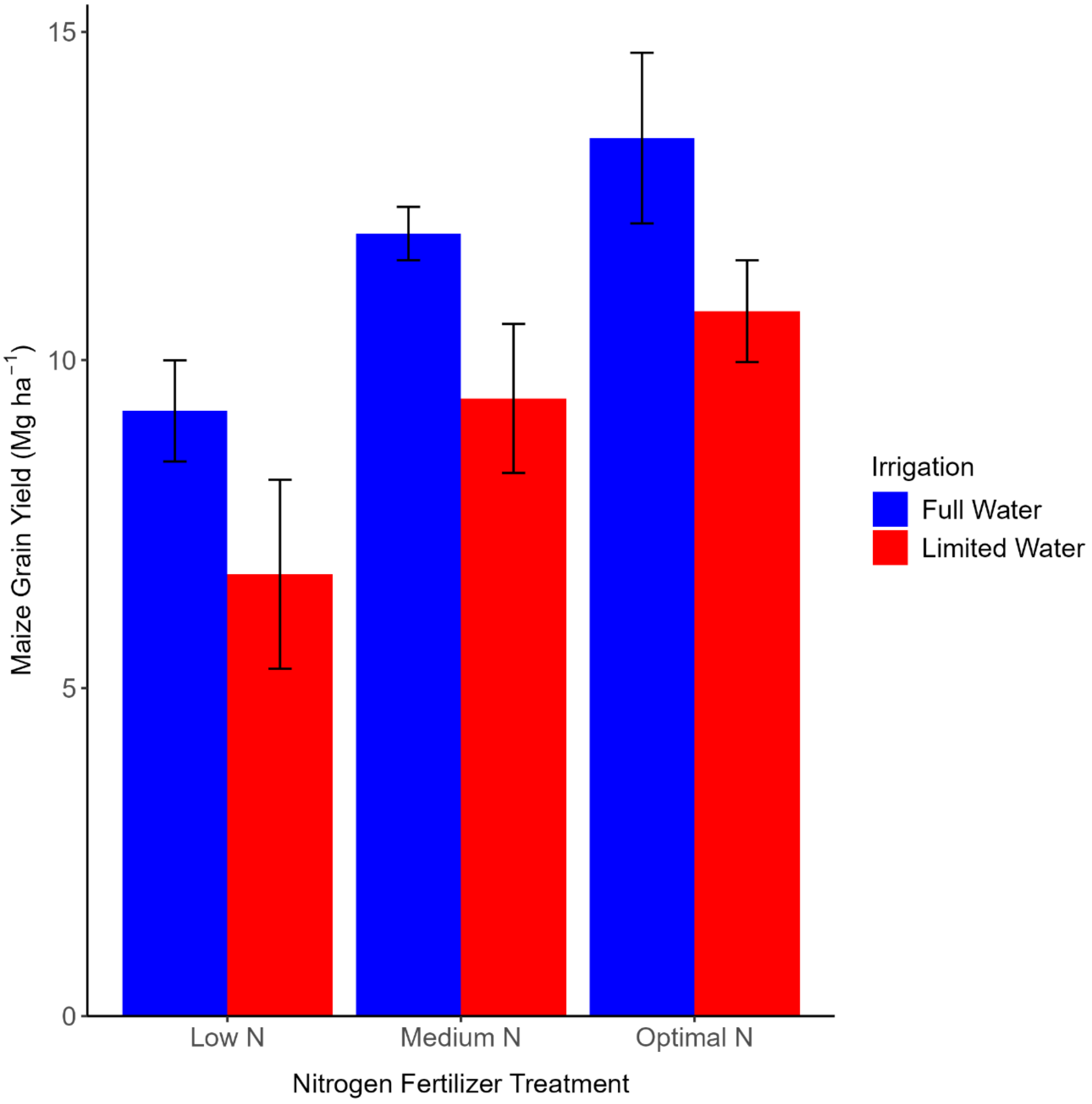

**Figure 4.4:** Maize grain yield by nitrogen fertilizer treatment and water availability. Maize grain yield was adjusted to 15.5% moisture. Error bars indicate ± SE.

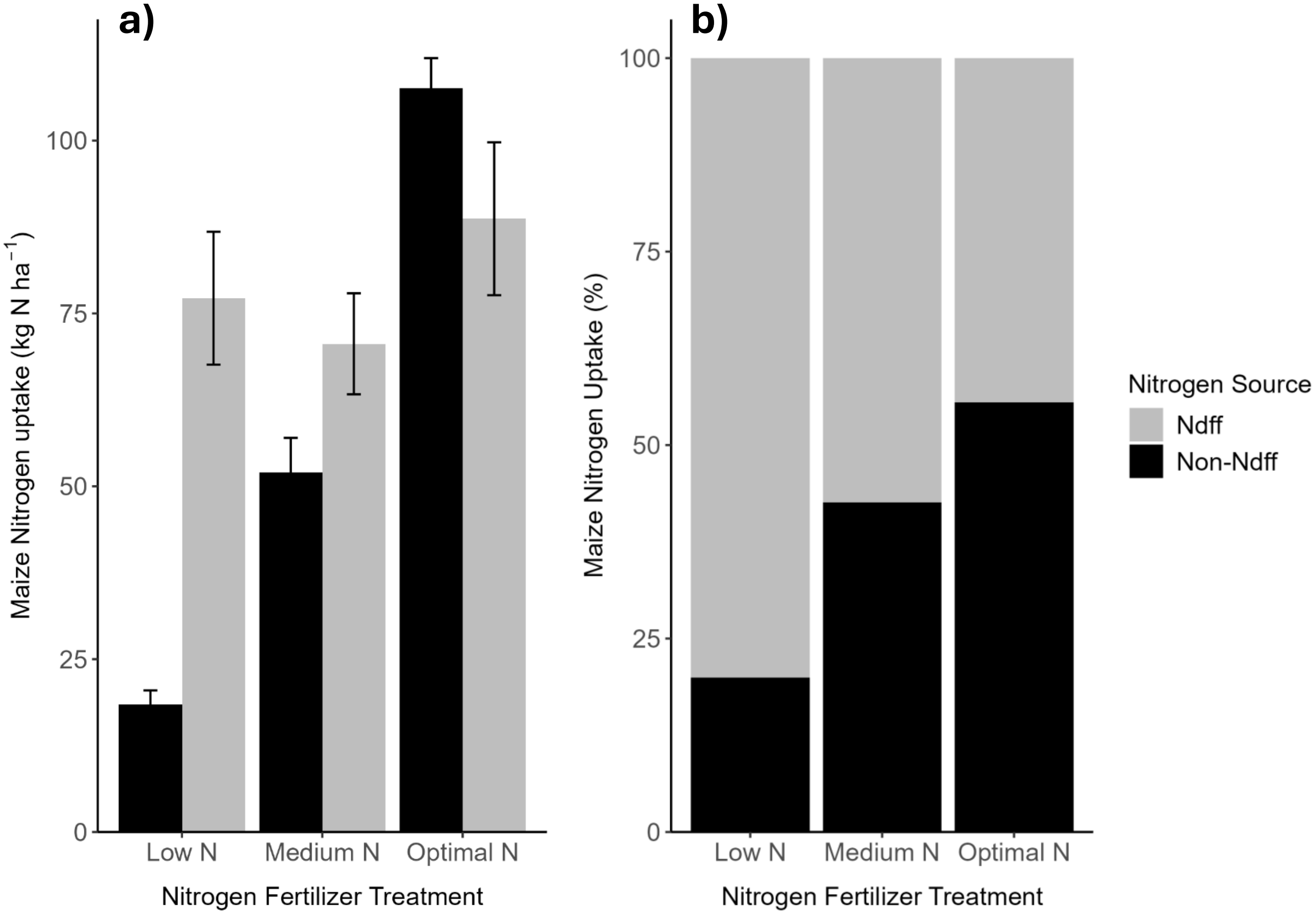

**Figure 4.5:** a) Maize nitrogen (N) uptake (kg N ha$^{-1}$) from N derived from $^{15}$N fertilizer (Ndff) or non-$^{15}$N fertilizer sources (non-Ndff) by N fertilizer treatment. b) Percent of maize N uptake that came from Ndff or non-Ndff sources by nitrogen fertilizer treatment averaged over water availability. N uptake did not vary by water availability and data shown is averaged across water treatments. Error bars indicate ± SE.

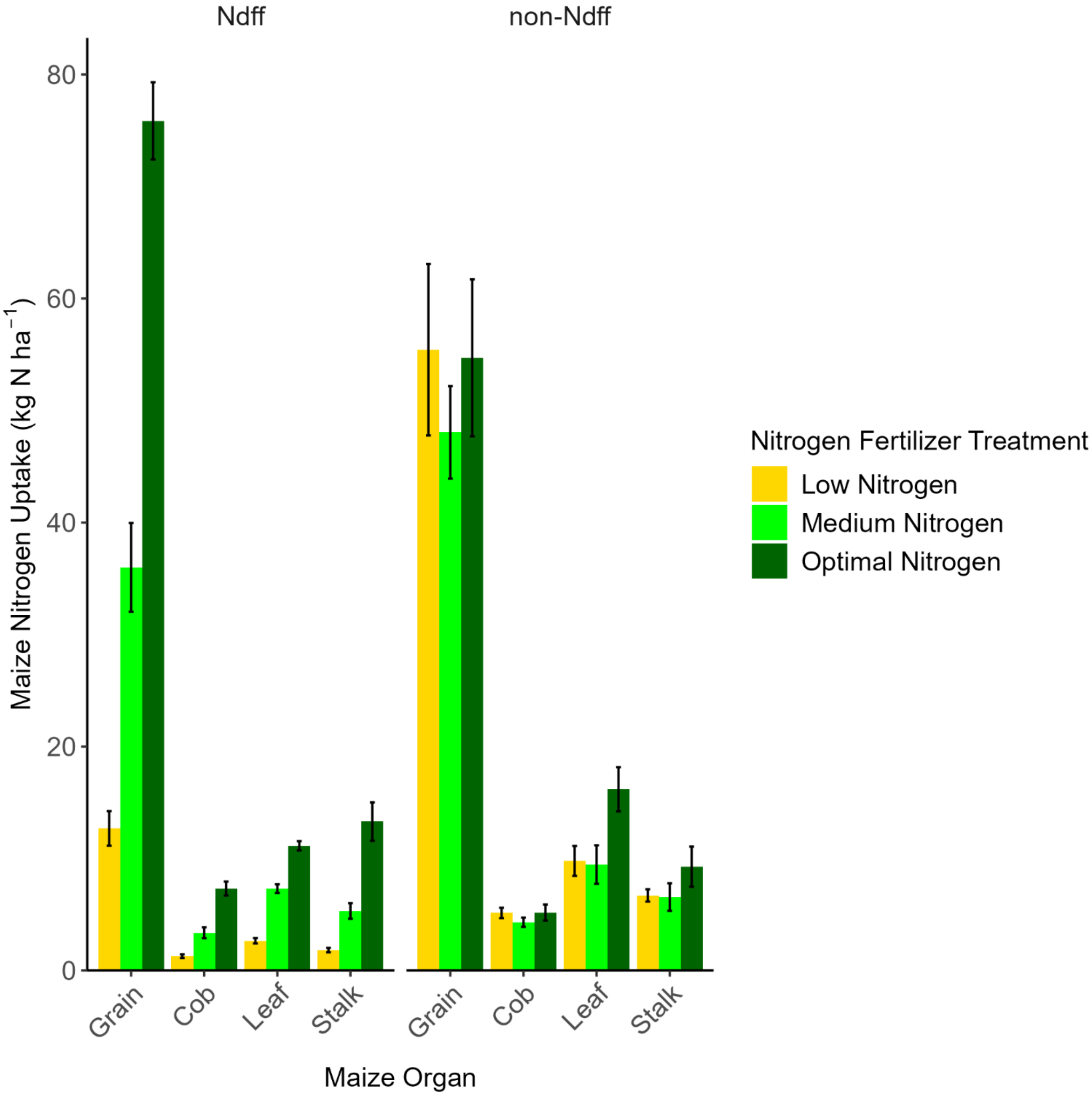

**Figure 4.6**: Maize nitrogen (N) uptake from N derived from $^{15}$N fertilizer (Ndff) or non-Ndff (non-$^{15}$N fertilizer) sources by N fertilizer treatment and maize organ across the two water treatments. Error bars indicate ± SE.

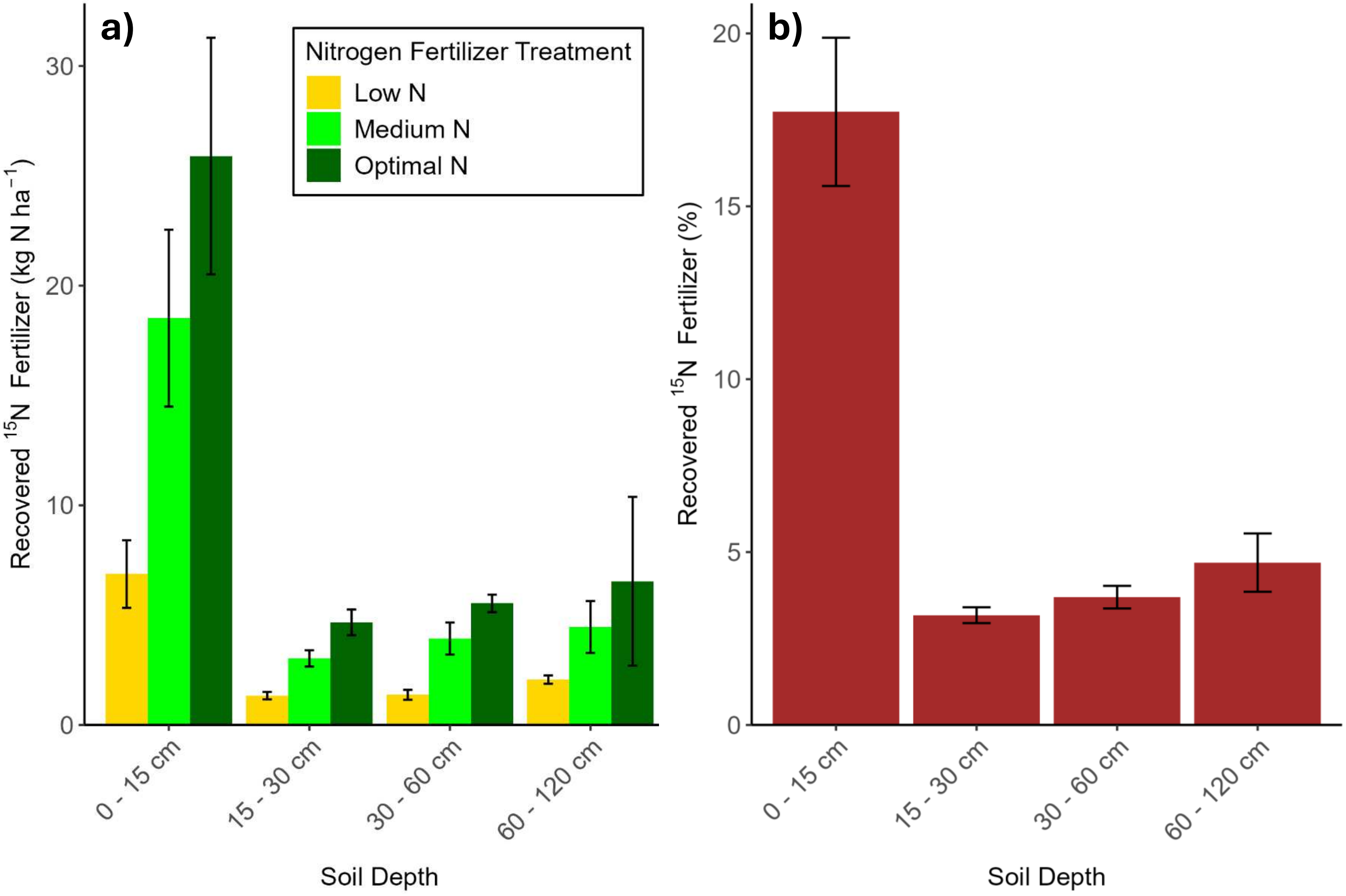

**Figure 4.7:** a) Nitrogen (N) derived from $^{15}$N fertilizer (Ndff) recovered in soil by depth and N fertilizer treatment. Data is averaged across water availability. b) Soil N recovery efficiency (percent of $^{15}$N applied that was recovered) by soil depth. Data is averaged across N fertilizer treatment and water availability. Error bars indicate ± SE.

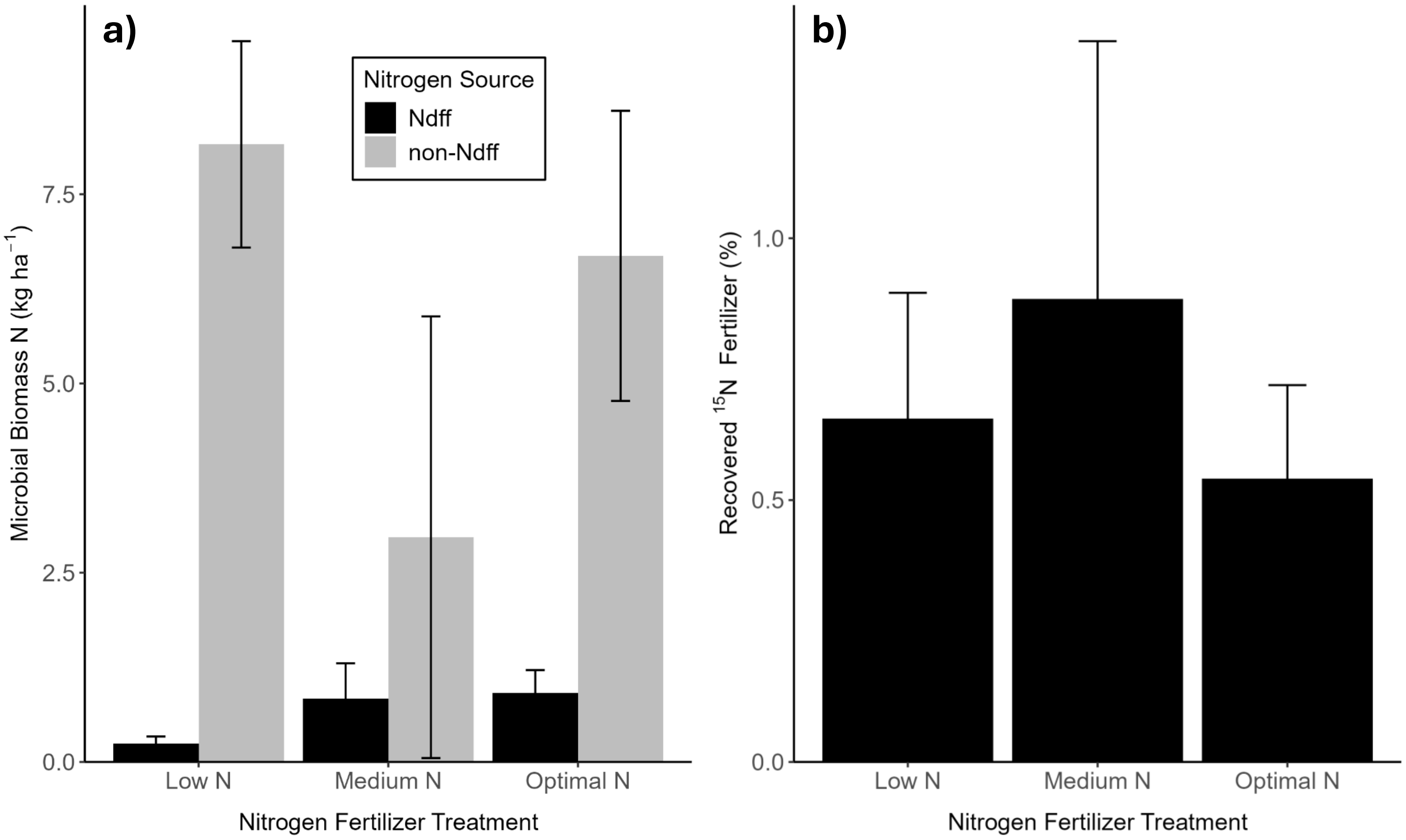

**Figure 4.8:** a) Microbial biomass nitrogen (N) from nitrogen derived from $^{15}$N fertilizer (Ndff) or non-Ndff (non-$^{15}$N fertilizer) sources by N N treatment. Data is averaged across water availability. b) N fertilizer recovery efficiency (percent of $^{15}$N applied that was recovered, NRE) in microbial biomass by N fertilizer treatment averaged across water availability. Microbial biomass was only measured in the top 0 – 15 cm. Error bars indicate ± SE.

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# CHAPTER 5: SUMMARY

Access to irrigation has greatly increased crop production in the Great Plains Region, but access to irrigation is threatened as competition for water resources increases, and ground water storage in the Ogallala Aquifer decreases (Hrozencik, 2021; Norwood, 1995). The need for achieving high yields with limited water will only increase as the global population and demand for agricultural production grows (Hunter et al., 2017). The general objective of my dissertation was to explore how nitrogen (N) and water availability affect semi-arid maize cropping systems in the Great Plains Region. The effects of N availability when water is limited remain unclear with respect to crop performance, and relatively unexplored with respect to soil N mineralization (Nmin) rates, contribution of Nmin to crop N uptake, and the recovery and fate of N fertilizer. I used a field experiment to understand the effects of N and water availability on maize cropping systems. Specifically, I investigated how N and water availability affect maize grain yield, soil Nmin rates and their contribution to maize N uptake, and the recovery of N fertilizer. Taken together, the results of my dissertation show that excess N is detrimental to maize grain yields when water is limited. Adjusting N fertilizer rates based on water availability and yield potential will not only lead to higher yields and resource use efficiency but will also reduce N losses from the system. I found that ~ 200 kg N $ha^{-1}$ maximized grain yields and resource use efficiency when water was limited, while maximum yield was achieved with ~ 320 kg N $ha^{-1}$ with full water. Optimal N amount will vary with management practices and especially water stress timing and severity. Soil Nmin rates and soil enzyme activity tended to be higher before peak maize N uptake resulting in asynchrony between soil N supply and maize N demand for all treatments. Increased N fertilizer rate increased microbial enzyme activity related to depolymerization of N containing compounds suggesting an increase in plant available N. Maize leaves, the major N sink early in the season, did increase N uptake of non-$^{15}$N fertilizer sources as N fertilizer rate increased. This potentially suggests that plant available N did in fact increase due to increased enzyme activity and depolymerization. However, for the entire plant, there appeared to be a limited capacity for maize to acquire this N as uptake from non-$^{15}$N fertilizer sources was relatively constant across treatments. Overall, higher N fertilizer rates increased N uptake, but the increased N uptake came primarily from $^{15}$N fertilizer while uptake from other sources was relatively constant. One explanation for this may be asynchrony between soil N supply and crop N demand. Later in the season when maize N demands are greatest, soil Nmin is low limiting its contribution to maize N uptake. Additionally, microbial biomass data at the end of the season suggests that immobilization was occurring, and a majority of the N acquired by the microbes was non-$^{15}$N fertilizer. Therefore, maize utilized N from the fertilizer applied rather than internal N cycling to meet its N demands. Recovery of $^{15}$N fertilizer was high, and the fraction lost was constant across treatments.

Maize response to N is dependent on water availability, and limited water maize needed 37% less N than full water in the current study to maximize yields. Excess N led to yield declines relative to moderate N supply with limited water. Accounting for soil residual N and in-season soil Nmin may become increasingly important for ensuring excess N is not available as water availability becomes more limited. Management strategies that improve the synchrony between soil Nmin and maize N uptake will help decrease reliance on external N inputs and decrease N losses while still achieving high yields.

# REFERENCES CHAPTER 5

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# APPENDEX: SUPPLEMENTARY TABLES AND FIGURES

**S. Table 2.1**: Analysis of variance summary statistics for maize yield (Fig. 2.2a), maize aboveground (AG) biomass (BM) at physiological maturity (R6) (Fig. 2.2b), total maize aboveground nitrogen uptake for entire season (Fig. 2.4), maize nitrogen uptake from germination (G) to silking (R1) (Fig. 2.5a & c), maize nitrogen uptake from R1 – R6 (Fig. 2.5b & d), maize water productivity (WP) (Fig. 2.6), maize partial nitrogen balance grain ($PNB_{grain}$) (Fig. 2.7a), maize nitrogen utilization efficiency (NUtE) (Fig. 2.7b), maize aboveground biomass at R1 (S. Fig. 2.2), root to shoot ratio (R:S) (S. Fig. 2.3), and maize grain C:N ratio (S. Fig. 2.4). Water refers to the water treatment (full water 100% ET, or limited water 70% ET). Nitrogen refers to nitrogen availability (sum of pre-plant soil residual nitrogen 0 – 90 cm, nitrogen fertilizer rate, and nitrogen in irrigation water) for all analysis except for partial nitrogen balance grain in which case it refers to just nitrogen fertilizer rate. Year refers to the growing season. Bold values indicate significance at $a < 0.05$.

| **Effect** | **Grain Yield** | **Maize AG BM (R6)** | **Total nitrogen uptake** | **Nitrogen uptake G – R1** | **Nitrogen uptake R1 – R6** | **WP** | **$PNB_{grain}$** | **NUtE** | **Maize AG BM (R1)** | **Maize Grain C:N Ratio** |
|---|---|---|---|---|---|---|---|---|---|---|
| Water (W) | **< 0.02** | **0.01** | **0.03** | 0.18 | 0.09 | **0.05** | **0.01** | **0.02** | 0.14 | 0.91 |
| Nitrogen (N) | **< 0.01** | **< 0.01** | **< 0.01** | **< 0.01** | **< 0.01** | **< 0.01** | **< 0.01** | **< 0.01** | **< 0.01** | **< 0.01** |
| Year (Y) | **< 0.01** | 0.99 | **< 0.01** | 0.48 | **0.02** | **< 0.01** | **< 0.01** | **< 0.01** | **< 0.01** | 0.06 |
| N x W | **< 0.01** | **< 0.01** | **0.02** | 0.06 | 0.87 | **0.001** | 0.16 | 0.1 | 0.1 | **0.014** |
| W x Y | **< 0.01** | 0.96 | **< 0.01** | **0.01** | 0.21 | **0.04** | 0.16 | **< 0.01** | **0.01** | 0.90 |
| N x Y | **0.01** | **< 0.01** | **< 0.01** | **0.04** | 0.33 | **< 0.01** | **< 0.01** | **0.01** | 0.96 | 0.84 |
| N x W x Y | 0.22 | 0.33 | **0.04** | 0.8 | 0.65 | 0.08 | 0.15 | 0.11 | 0.98 | 0.29 |

**S. Table 2.2:** Analysis of variance summary statistics for maize root biomass for the entire soil profile (0 – 180 cm) (S. Fig. 2.1), and maize root biomass from the top 0 – 60 cm of the soil profile (Fig. 2.3). Water refers to the water treatment (full water 100% ET, or limited water 70% ET). Nitrogen refers to nitrogen availability (soil residual nitrogen 0 – 90 cm, nitrogen fertilizer rate, and nitrogen in irrigation water). Depth refers to the soil depth biomass was sampled from. Bold values indicate significance at *a* < 0.05.

| **Effect** | **Root Biomass Full profile** | **Root Biomass 0 – 60 cm** |
|---|---|---|
| Water (W) | 0.3 | 0.08 |
| Nitrogen (N) | 0.75 | 0.95 |
| Depth (Y) | **< 0.01** | **< 0.01** |
| N x W | 0.53 | 0.17 |
| W x D | 0.58 | 0.1 |
| N x D | 0.33 | 0.45 |
| N x W x D | 0.5 | 0.07 |

**S. Table 2.3**: Analysis of variance summary statistics for maize root to shoot ratio (R:S) (S. Fig. 2.3). Water refers to the water treatment (full water 100% ET, or limited water 70% ET). Nitrogen refers to nitrogen availability (soil residual nitrogen 0 – 90 cm, nitrogen fertilizer rate, and nitrogen in irrigation water). Bold values indicate significance at *a* < 0.05.

| **Effect** | Maize R:S ratio |
|---|---|
| Water (W) | 0.15 |
| Nitrogen (N) | 0.36 |
| N × W | 0.21 |

**S. Table 2.4:** Pearson's correlation between maize grain yield and total aboveground maize nitrogen uptake at physiological maturity (R6). Correlations are shown by growing season, water availability, and nitrogen treatment. Nitrogen is grouped by lower nitrogen treatments (1 – 4) and higher nitrogen treatments (4 – 6). Bold values indicate significance at *a* < 0.05.

| **Growing season** | **Water availability** | **Nitrogen treatment** | **Correlation (r)** | **$R^2$** | ***P* – value** | **Lower 95% CI** | **Upper 95%** |
|---|---|---|---|---|---|---|---|
| 2021 | Full Water | 1 – 4 | 0.96 | 0.92 | **< 0.01** | 0.86 | 0.99 |
| 2022 | Full Water | 1 – 4 | 0.94 | 0.89 | **< 0.01** | 0.80 | 0.98 |
| 2023 | Full Water | 1 – 4 | 0.98 | 0.96 | **< 0.01** | 0.93 | 0.99 |
| 2021 | Full Water | 4 – 6 | 0.76 | 0.57 | **0.02** | 0.19 | 0.95 |
| 2022 | Full Water | 4 – 6 | 0.42 | 0.18 | 0.26 | -0.34 | 0.85 |
| 2023 | Full Water | 4 – 6 | 0.16 | 0.03 | 0.68 | -0.56 | 0.74 |
| 2021 | Limited Water | 1 – 4 | 0.94 | 0.88 | **< 0.01** | 0.79 | 0.98 |
| 2022 | Limited Water | 1 – 4 | 0.57 | 0.32 | **0.05** | -0.01 | 0.86 |
| 2023 | Limited Water | 1 – 4 | 0.88 | 0.77 | **< 0.01** | 0.61 | 0.97 |
| 2021 | Limited Water | 4 – 6 | 0.27 | 0.08 | 0.47 | -0.48 | 0.79 |
| 2022 | Limited Water | 4 – 6 | -0.40 | 0.16 | 0.29 | -0.84 | 0.36 |
| 2023 | Limited Water | 4 – 6 | -0.07 | 0.00 | 0.86 | -0.70 | 0.63 |

**S. Table 2.5**: Pearson’s correlation between total aboveground maize biomass and total aboveground maize nitrogen uptake at physiological maturity (R6). Correlations are shown by growing season, water availability, and nitrogen treatment. Nitrogen is grouped by lower nitrogen treatments (1 – 4) and higher nitrogen treatments (4 – 6). Bold values indicate significance at $a < 0.05$.

| **Growing season** | **Water availability** | **Nitrogen treatment** | **Correlation (r)** | **$R^2$** | ***P* – value** | **Lower 95% CI** | **Upper 95%** |
|---|---|---|---|---|---|---|---|
| 2021 | Full Water | 1 – 4 | 0.96 | 0.92 | **< 0.01** | 0.85 | 0.99 |
| 2022 | Full Water | 1 – 4 | 0.98 | 0.95 | **< 0.01** | 0.92 | 0.99 |
| 2023 | Full Water | 1 – 4 | 0.99 | 0.97 | **< 0.01** | 0.95 | 1.00 |
| 2021 | Full Water | 4 – 6 | 0.77 | 0.60 | **0.01** | 0.23 | 0.95 |
| 2022 | Full Water | 4 – 6 | 0.95 | 0.91 | **< 0.01** | 0.79 | 0.99 |
| 2023 | Full Water | 4 – 6 | 0.93 | 0.87 | **< 0.01** | 0.70 | 0.99 |
| 2021 | Limited Water | 1 – 4 | 0.90 | 0.81 | **< 0.01** | 0.68 | 0.97 |
| 2022 | Limited Water | 1 – 4 | 0.72 | 0.51 | **0.01** | 0.24 | 0.91 |
| 2023 | Limited Water | 1 – 4 | 0.95 | 0.89 | **< 0.01** | 0.81 | 0.98 |
| 2021 | Limited Water | 4 – 6 | 0.47 | 0.22 | 0.20 | -0.28 | 0.86 |
| 2022 | Limited Water | 4 – 6 | -0.22 | 0.05 | 0.57 | -0.77 | 0.52 |
| 2023 | Limited Water | 4 – 6 | 0.80 | 0.64 | **0.01** | 0.29 | 0.96 |

**S. Table 2.6**: a) Irrigation water added (mm) by month for all three growing seasons. b) Precipitation amount (mm) by month for all three growing seasons. Total refers to precipitation for each month (January – December) for all three years, crop year refers to precipitation that occurred between October of the previous year through September of the current year, and June – August refers to precipitation that occurred between those months of the current year.

a) **Irrigation Added (mm)**

| | ***2021*** | | ***2022*** | | **2023** | |
|---|---|---|---|---|---|---|
| **Month** | **Full Water** | **Limited Water** | **Full Water** | **Limited Water** | **Full Water** | **Limited Water** |
| May | x | x | 25 | 25 | x | x |
| June | x | x | 38 | 38 | x | x |
| July | 136 | 38 | 140 | 57 | x | x |
| August | 152 | 51 | 150 | 56 | 115 | x |
| September | x | x | 117 | 23 | x | x |
| **Total** | **288** | **89** | **470** | **199** | **115** | **0** |

b) **Precipitation (mm)**

**Total (mm)**

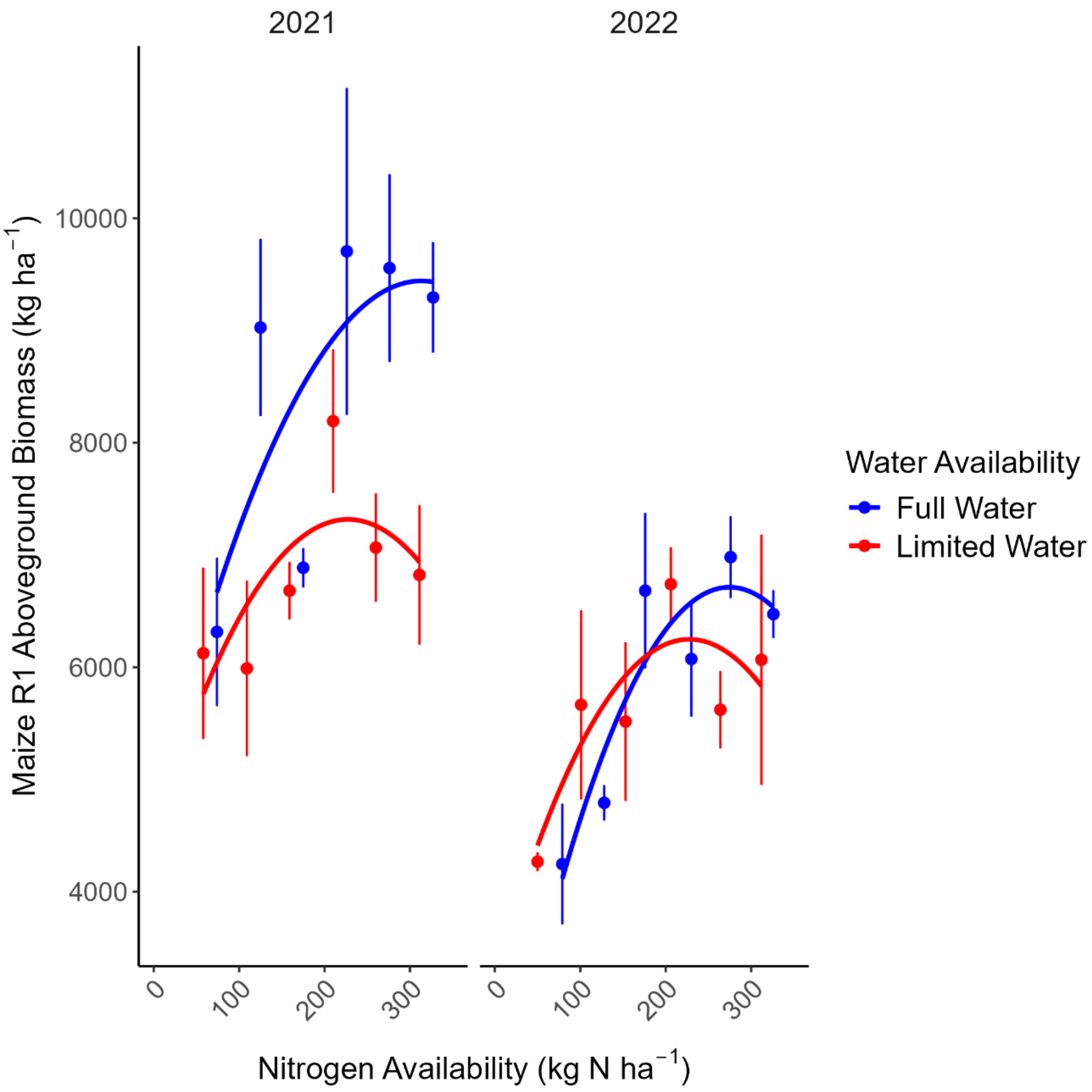
| Month | *2021* | *2022* | 2023 |
|---|---|---|---|
| January | 8 | 12 | 45 |
| February | 11 | 10 | 13 |
| March | 56 | 47 | 26 |
| April | 87 | 2 | 7 |
| May | 176 | 49 | 119 |
| June | 18 | 34 | 168 |
| July | 12 | 89 | 90 |
| August | 14 | 31 | 53 |
| September | 23 | 21 | 19 |
| October | 11 | 7 | 18 |
| November | 1 | 3 | 3 |
| December | 3 | 55 | 16 |

**Crop Year (mm)**

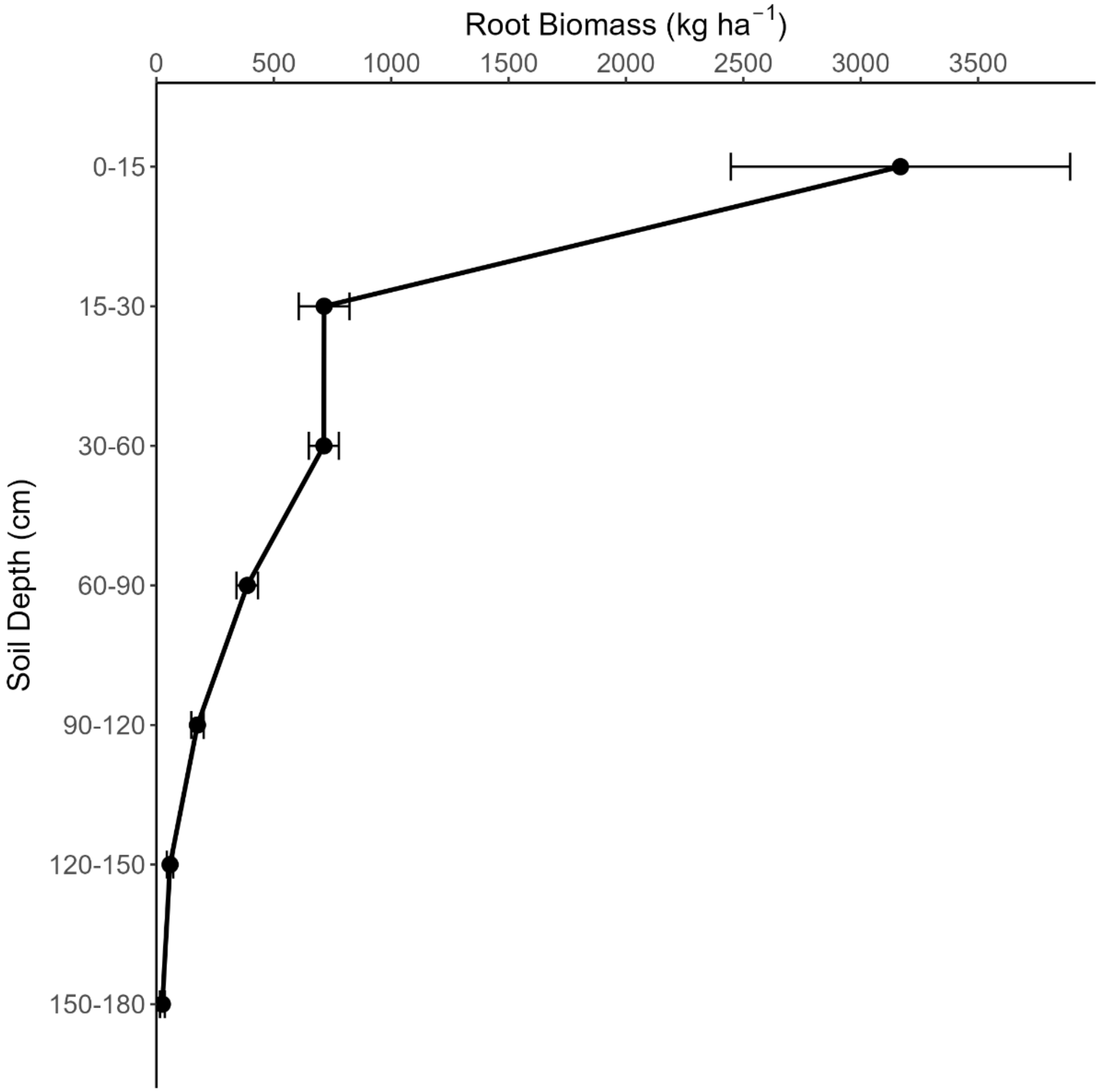
| *2021* | *2022* | *2023* |
|---|---|---|
| 432 | 310 | 605 |

**June - August (mm)**

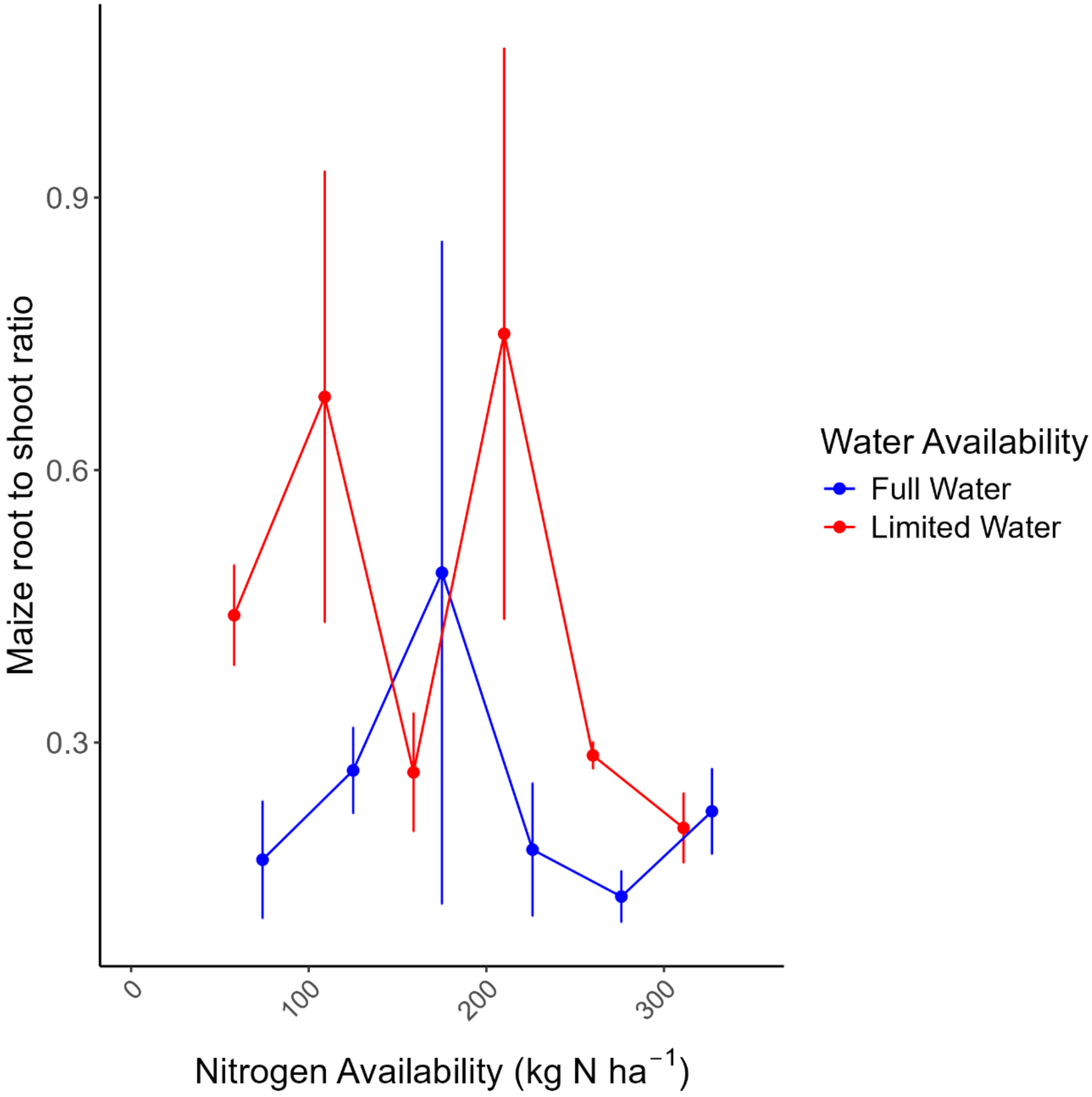
| *2021* | *2022* | 2023 |
|---|---|---|
| 44 | 154 | 311 |



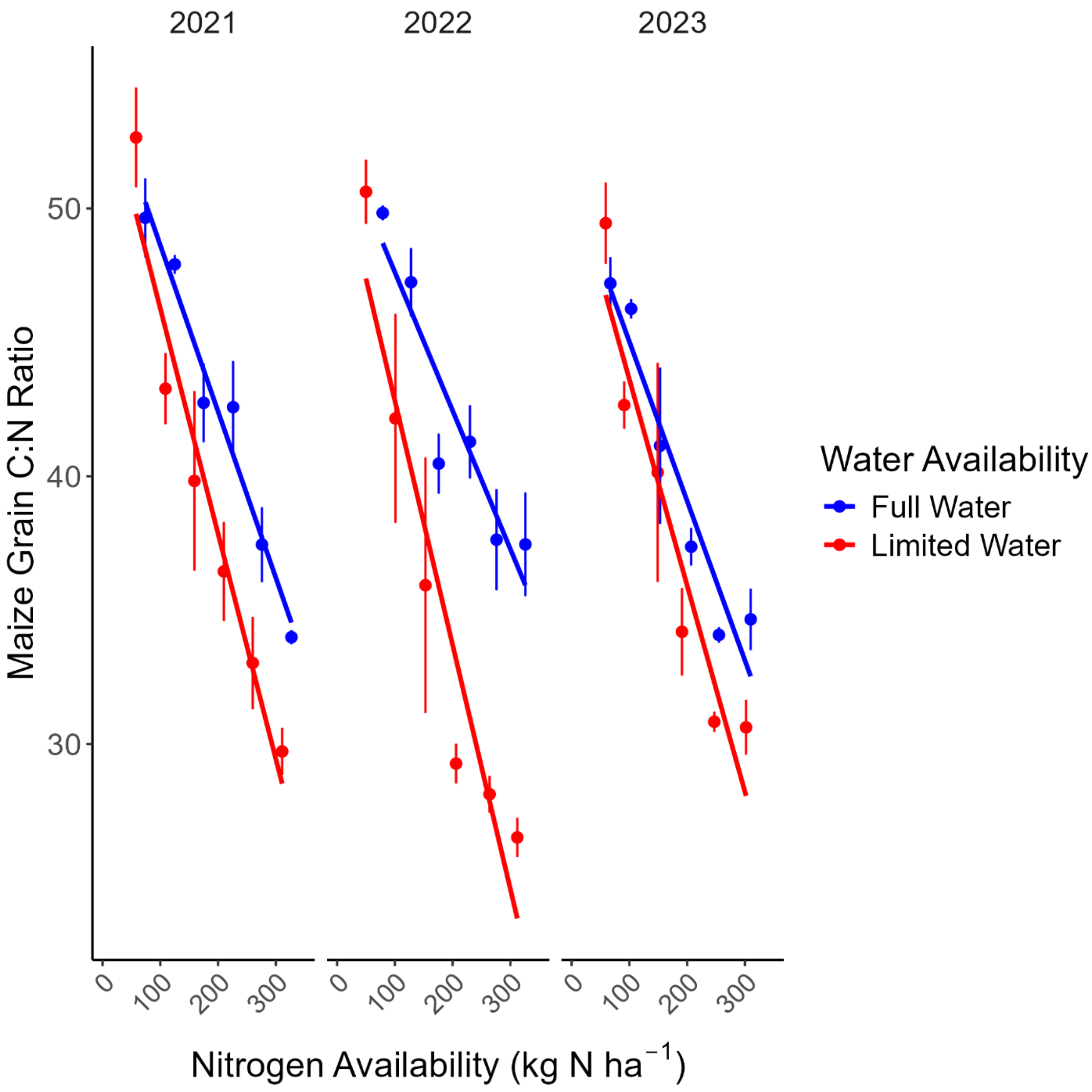
**S. Figure 2.1**: Maize aboveground biomass at silking (R1) by growing season, water availability, and nitrogen availability. Error bars depict ± SE.



**S. Figure 2.2:** Maize root biomass by soil depth increment averaged across water and nitrogen availabilities. Maize root biomass was only collected in the fall of 2021 after maize reached physiological maturity and was harvested. Error bars depict ± SE.



**S. Figure 2.3:** Maize root to shoot ratio (R:S) by water and nitrogen availability. Maize shoots correspond to the dry weight of all aboveground biomass at physiological maturity (R6). Maize roots correspond to the dry weigh of root biomass 0 – 180 cm. Error bars depict ± SE.



**S. Figure 2.4:** Maize grain carbon (C) to nitrogen (N) ratio by water and nitrogen availability and growing season. Error bars depict ± SE.

**S. Table 3.1**. Analysis of variance summary statistics for soil extractable inorganic nitrogen (EIN) for the entire season (S. Fig. 3.1), net nitrogen mineralization (Nmin) rates over the entire season (Fig. 3.7a), EIN from approximately vegetative stage 16 (V16) – physiological maturity (R6) (Fig. 3.3a), Net Nmin rates from approximately V16 – R6 (Fig. 3.3b), soil N-acquiring enzyme activity leucine amino peptidase (LAP) from approximately V16 – R6 (Fig. 3.4a), b-1,4-N-acetyl-glucosaminidase (NAG) from approximately V16 – R6 (Fig. 3.4b), LAP for the entire season (S. Fig. 3.3a), and NAG for the entire season (S. Fig. 3.3b) . Bold values indicate significance at $a < 0.05$.

| **Effect** | **Full Season Extractable Inorganic N** | **Full Season Net Nmin** | **Soil Extractable N (V16 – R6)** | **Net N Mineralization (V16 - R6)** | **Lecumine Amino Peptidase (LAP)** | **b-1,4-N-acetyl-glucosaminidase (NAG)** | **Full Season LAP** | **Full Season NAG** |
|---|---|---|---|---|---|---|---|---|
| Water Availability (W) | 0.11 | 0.16 | 0.06 | 0.29 | 0.94 | 0.93 | 0.54 | 0.47 |
| N Fertilizer (N) | **< 0.01** | 0.2 | **< 0.01** | 0.32 | **0.02** | 0.01 | **0.01** | **0.01** |
| Growth Stage (GS) | **< 0.01** | **< 0.01** | **< 0.01** | 0.02 | **< 0.01** | < 0.01 | **< 0.01** | **< 0.01** |
| Year (Y) | **< 0.01** | **< 0.01** | **< 0.01** | 0.92 | **< 0.01** | < 0.01 | **< 0.01** | **< 0.01** |
| W x N | **0.02** | 0.15 | 0.07 | **0.03** | 0.39 | 0.25 | 0.21 | 0.12 |
| W x GS | 0.15 | 0.44 | 1.00 | 0.09 | **< 0.01** | 0.01 | **0.01** | **0.02** |
| N x GS | 0.07 | 0.16 | 0.12 | 0.23 | 0.78 | 0.61 | 0.46 | 0.75 |
| W x Y | 0.79 | 0.88 | 0.08 | 0.51 | **0.02** | 0.87 | **0.01** | 0.94 |
| N x Y | 0.2 | **0.01** | 0.723 | 0.56 | 0.82 | 0.16 | 0.63 | 0.04 |
| GS x Y | **< 0.01** | **< 0.01** | **< 0.01** | **< 0.01** | **< 0.01** | **< 0.01** | **< 0.01** | **< 0.01** |
| W x N x GS | 0.83 | 0.61 | 0.25 | 0.2 | 0.4 | 0.34 | 0.48 | 0.25 |
| W x N x Y | 0.23 | 0.08 | 0.58 | 0.38 | 0.84 | 0.65 | 0.41 | 0.55 |
| W x GS x Y | **0.03** | 0.360 | 0.093 | 0.20 | **< 0.01** | **< 0.01** | **< 0.01** | **0.01** |
| N x GS x Y | 0.33 | **0.01** | 0.793 | 0.98 | 0.603 | 0.516 | 0.667 | 0.641 |
| W x N x GS x Y | 0.81 | 0.070 | 0.946 | 0.08 | 0.140 | 0.354 | 0.325 | 0.77 |

**S. Table 3.2:** Analysis of variance summary statistics for total maize shoot N uptake at end of season (Fig. 3.6a) and end of season grain yield adjusted to 15.5% moisture content (Fig. 3.7b). Bold values indicate significance at *a* < 0.05.

| **Effect** | **Maize N uptake** | **Maize Grain Yield** |
|---|---|---|
| Water Availability (W) | **0.04** | **0.01** |
| N Fertilizer (N) | **<0.01** | **<0.01** |
| Year (Y) | 0.08 | 0.12 |
| W x N | 0.35 | **0.03** |
| W x Y | 0.44 | 0.11 |
| N x Y | 0.48 | 0.57 |
| W x N x Y | 0.83 | 0.17 |

**S. Table 3.3:** Analysis of variance summary statistics for maize nitrogen uptake rates across the growing season (Fig. 7b). Bold values indicate significance at *a* < 0.05.

| **Effect** | **Maize N uptake Rate** |
|---|---|
| Water Availability (W) | 0.11 |
| N fertilizer (N) | **< 0.01** |
| Growth Stage (GS) | **< 0.01** |
| W x N | 0.3 |
| W x GS | **< 0.01** |
| N x GS | **< 0.01** |
| W x N x GS | 0.17 |

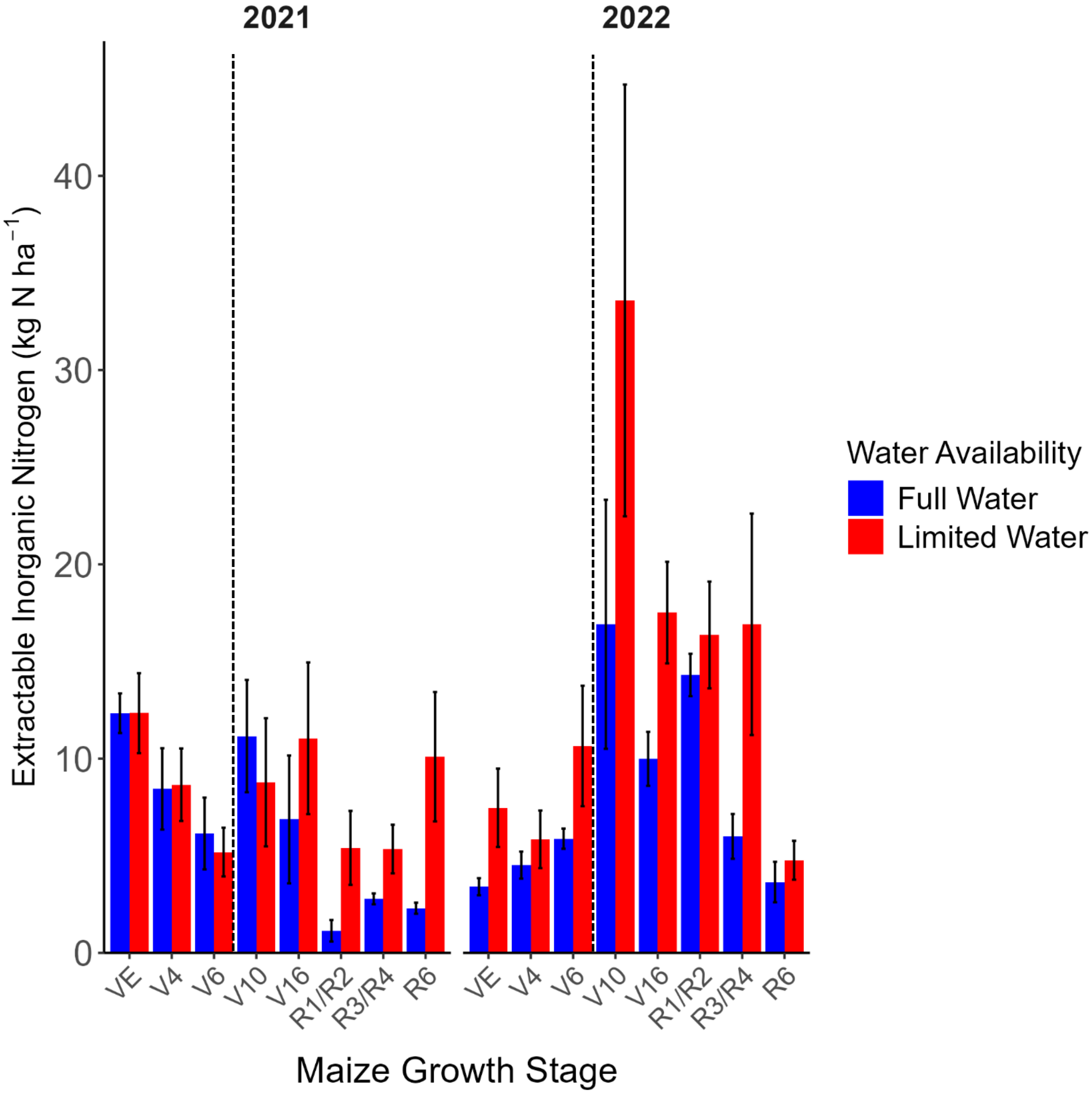
**S. Table 3.4:** A) Total aboveground biomass (kg ha$^{-1}$), N uptake (kg N ha$^{-1}$), and C:N by growth stage, water availability, and N fertilizer treatments. B) Aboveground biomass (kg ha$^{-1}$), N uptake (kg N ha$^{-1}$), and C:N by water availability and N fertilizer treatments.

**A)** ***Aboveground Biomass, maize N uptake, and C:N***

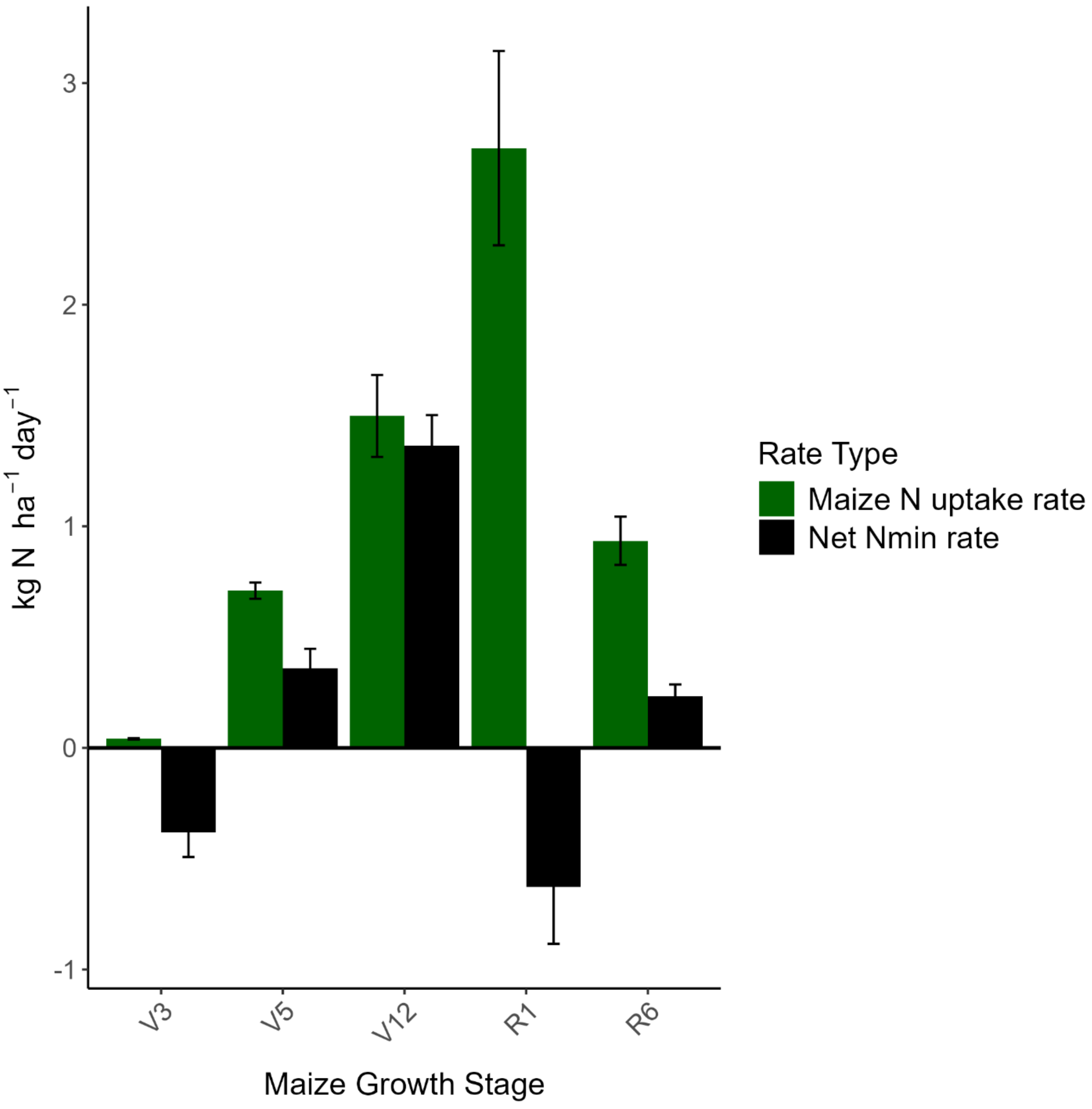
| Growth Stage | Water Availability | N treatment | Biomass (kg ha$^{-1}$) | kg N ha$^{-1}$ | C:N |
|---|---|---|---|---|---|
| V3 | Full Water | Low | 23 | 0.7 | 13.4 |
| | | Optimal | 28 | 0.9 | 13.2 |
| | | Excess | 20 | 0.6 | 14.8 |
| | Limited Water | Low | 31 | 1.0 | 13.4 |
| | | Optimal | 28 | 0.9 | 13.5 |
| | | Excess | 20 | 0.7 | 13.6 |
| V5 | Full Water | Low | 696 | 16.2 | 18.5 |
| | | Optimal | 718 | 16.7 | 18.4 |
| | | Excess | 618 | 14.4 | 18.7 |
| | Limited Water | Low | 718 | 17.1 | 18.3 |
| | | Optimal | 660 | 15.9 | 18.1 |
| | | Excess | 598 | 14.0 | 18.4 |
| V12 | Full Water | Low | 1796 | 32.4 | 24.0 |
| | | Optimal | 2522 | 51.6 | 19.8 |
| | | Excess | 2803 | 48.4 | 23.3 |
| | Limited Water | Low | 2635 | 46.2 | 25.5 |
| | | Optimal | 2448 | 58.9 | 16.4 |
| | | Excess | 2400 | 57.8 | 16.8 |
| R1 | Full Water | Low | 5280 | 40.4 | 55.4 |
| | | Optimal | 8269 | 113.8 | 31.3 |
| | | Excess | 7884 | 127.0 | 26.4 |
| | Limited Water | Low | 5196 | 39.6 | 55.4 |
| | | Optimal | 6343 | 95.5 | 29.2 |
| | | Excess | 6444 | 101.4 | 27.4 |
| R6 | Full Water | Low | 13665 | 79.5 | 73.1 |
| | | Optimal | 22885 | 189.1 | 53.4 |
| | | Excess | 22990 | 208.8 | 48.1 |
| | Limited Water | Low | 11144 | 55.7 | 86.0 |
| | | Optimal | 14172 | 144.1 | 42.6 |
| | | Excess | 13249 | 154.5 | 37.4 |

**B)** ***Aboveground Biomass, maize N uptake, and C:N at physiological maturity by organ***

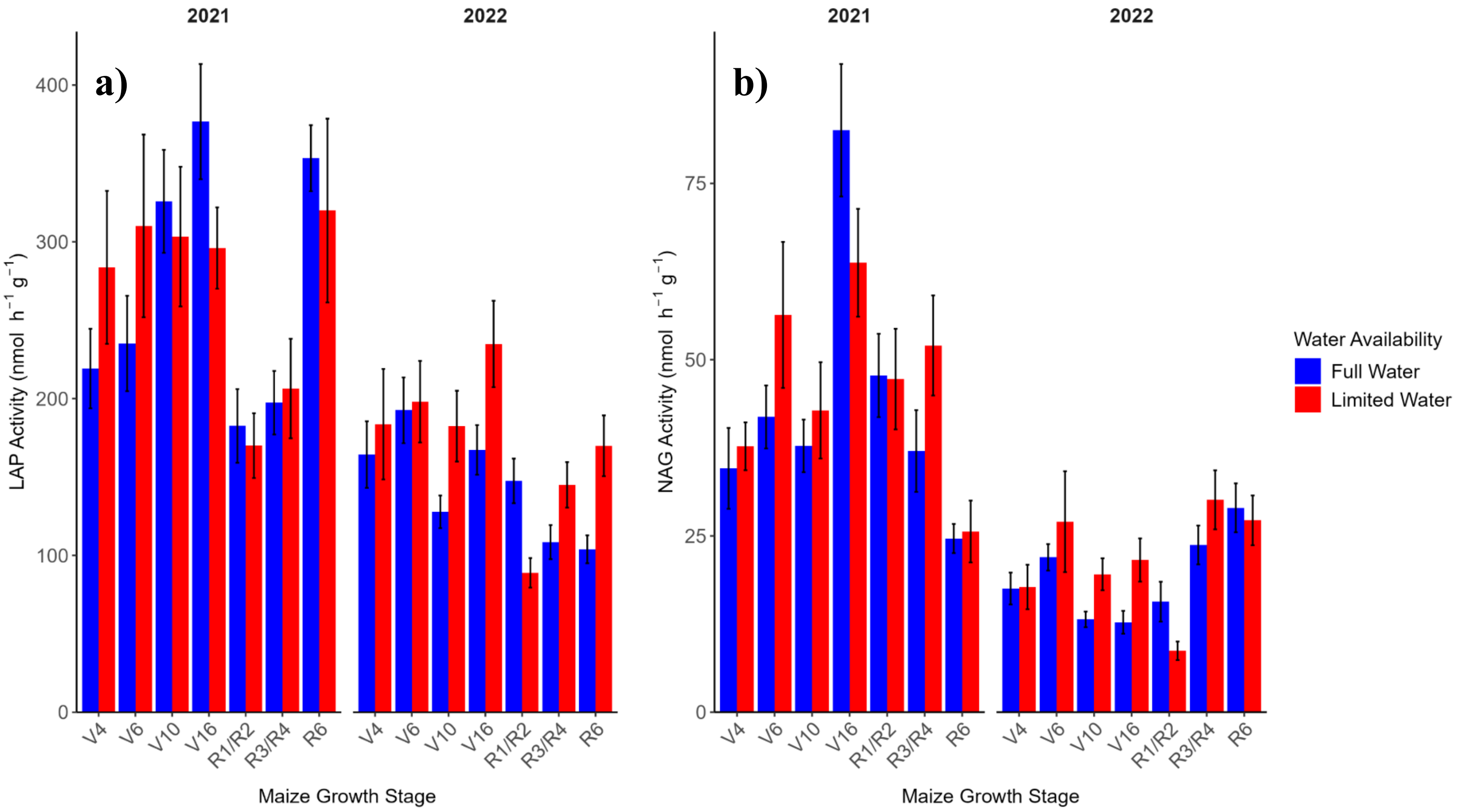
| Organ | Water Availability | N treatment | Biomass (kg ha$^{-1}$) | kg N ha$^{-1}$ | C:N |
|---|---|---|---|---|---|
| Leaf | Full Water | Low | 1847 | 9.7 | 75.2 |
| | | Optimal | 2574 | 25.6 | 44.6 |
| | | Excess | 2730 | 33.4 | 35.4 |
| | Limited Water | Low | 2110 | 8.9 | 97.1 |
| | | Optimal | 2582 | 24.9 | 47.1 |
| | | Excess | 2464 | 24.4 | 44.9 |
| Stem | Full Water | Low | 3340 | 6.8 | 210.0 |
| | | Optimal | 5615 | 17.4 | 149.0 |
| | | Excess | 5280 | 19.8 | 124.5 |
| | Limited Water | Low | 3261 | 6.3 | 226.4 |
| | | Optimal | 3885 | 26.5 | 77.5 |
| | | Excess | 3412 | 33.9 | 50.8 |
| Cob | Full Water | Low | 1770 | 5.3 | 157.7 |
| | | Optimal | 2991 | 11.3 | 127.9 |
| | | Excess | 3020 | 12.1 | 116.4 |
| | Limited Water | Low | 1465 | 4.7 | 146.7 |
| | | Optimal | 1850 | 12.0 | 76.5 |
| | | Excess | 1722 | 10.7 | 79.8 |
| Grain | Full Water | Low | 6708 | 57.8 | 49.7 |
| | | Optimal | 11705 | 134.8 | 37.5 |
| | | Excess | 11961 | 143.5 | 35.7 |
| | Limited Water | Low | 4308 | 35.8 | 51.6 |
| | | Optimal | 5855 | 80.7 | 30.6 |
| | | Excess | 5651 | 85.5 | 28.1 |



**S. Figure 3.1:** Soil extractable inorganic nitrogen (EIN) in the top 0 – 15 cm across the entire growing season by water availability and growing season. EIN includes both NH4-N + NO3-N. Growth stage corresponds to the approximate growth stage of maize when the soil sample was collected. “V” corresponds to vegetative stages with VE representing emergence, V4 – V16 representing the amount of collard leaves present. “R” corresponds to reproductive stages with R1, R2, R3 and R4 corresponding to silking, blister, milk, and dough stage respectively. “R6” corresponds to physiological maturity which is when the final sample of the growing season was taken. Error bars depict ± SE. Nitrogen fertilizer application was applied in two doses, one at planting and one at V7 which is indicated by the dotted black line.



**S. Figure 3.2:** Average plant nitrogen uptake and net nitrogen mineralization (Nmin) rates at different maize growth stages across both growing years and treatments. See methods for determining Nmin and N uptake rates. Growth stage corresponds to the approximate growth stage of maize when the soil sample was collected. “V” corresponds to vegetative stages with V4 – V16 representing the amount of collard leaves present. “R” corresponds to reproductive stages with R1 and R6 corresponding to silking physiological maturity respectively. Error bars depict ± SE. Error bars depict ± SE.



**S. Figure S3:** Soil enzyme activity panel a) corresponds to L-leucine amino peptidase (LAP) activity and panel b) corresponds to activity *β*-1,4-N-acetyl-glucosaminidase (NAG) activity. Both panel a and b show enzyme activity in in top 0 – 15 cm by maize growth stage, growing season and water availability averaged over nitrogen fertilizer treatments. Growth stage corresponds to the approximate growth stage of maize when the soil sample was collected. “V” corresponds to vegetative stages with V4 – V16 representing the amount of collard leaves present. “R” corresponds to reproductive stages with R1, R2, R3 and R4 corresponding to silking, blister, milk, and dough stage respectively. “R6” corresponds to physiological maturity which is when the final sample of the growing season was taken. Error bars depict ± SE. Error bars depict ± SE.

**S. Table 4.1:** Analysis of variance summary statistics for nitrogen (N) derived from fertilizer ($^{15}$N, Ndff) recovered in maize (Fig. 2, 5a), nitrogen recovery efficiency (NRE) for maize (Fig. 3), maize grain yield (Fig. 4), maize N uptake from non-Ndff sources (Fig. 5a), percent of maize N uptake from Ndff (Fig. 5b), percent of maize N uptake from non-Ndff (Fig. 5b), maize N uptake at vegetative stage 4 (V4) (S. Fig. 1), maize N uptake between V4 and physiological maturity (S. Fig. 1), maize N uptake in the zero N control plots (S. Fig. 2), and total maize N uptake (uptake of Ndff and non-Ndff). Water refers to water availability treatment (full water 100% ET, or limited water 70% ET). Nitrogen refers to nitrogen fertilizer rate (low, medium, or optimal). Bold values indicate significance at *a* < 0.05.

| **Effect** | **Maize N uptake of Ndff** | **NRE Maize** | **Maize Grain Yield** | **Maize N uptake non-Ndff** | **Maize N percent Ndff** | **Maize N percent non-Ndff** | **Maize N uptake V4** | **Maize N uptake post V4** | **Control Maize N uptake** | **Total Maize N uptake** |
|---|---|---|---|---|---|---|---|---|---|---|
| Water (W) | 0.62 | 0.84 | 0.09 | 0.38 | 0.38 | 0.44 | 0.65 | 0.44 | 0.30 | 0.404 |
| Nitrogen (N) | > **0.01** | 0.16 | **0.01** | 0.47 | > **0.01** | > **0.01** | 0.72 | 0.58 | 0.82 | > **0.01** |
| N x W | 0.78 | 0.69 | 1.00 | 0.90 | 0.75 | 0.88 | 0.89 | 0.98 | 0.97 | 0.97 |

**S. Table 4.2**: Analysis of variance summary statistics for nitrogen (N) derived from fertilizer ($^{15}$N, Ndff) recovered in the entire soil profile (Fig. 4.2), Ndff that was unrecovered, or lost, (Fig. 4.2), nitrogen recovery effecincy (NRE) for the entire soil profile (Fig. 4.3), the percentage of Ndff that was unrecovered or lost (Fig. 4.3), recovered Ndff in microbial biomass N (MBN) (Fig. 4.8a), MBN that is non-Ndff (Fig. 4.8a), and NRE of microbial biomass (MB) (Fig. 4.8b). Water refers to water availability treatment (full water 100% ET, or limited water 70% ET). Nitrogen refers to nitrogen fertilizer rate (low, medium, or optimal). Bold values indicate significance at *a* < 0.05.

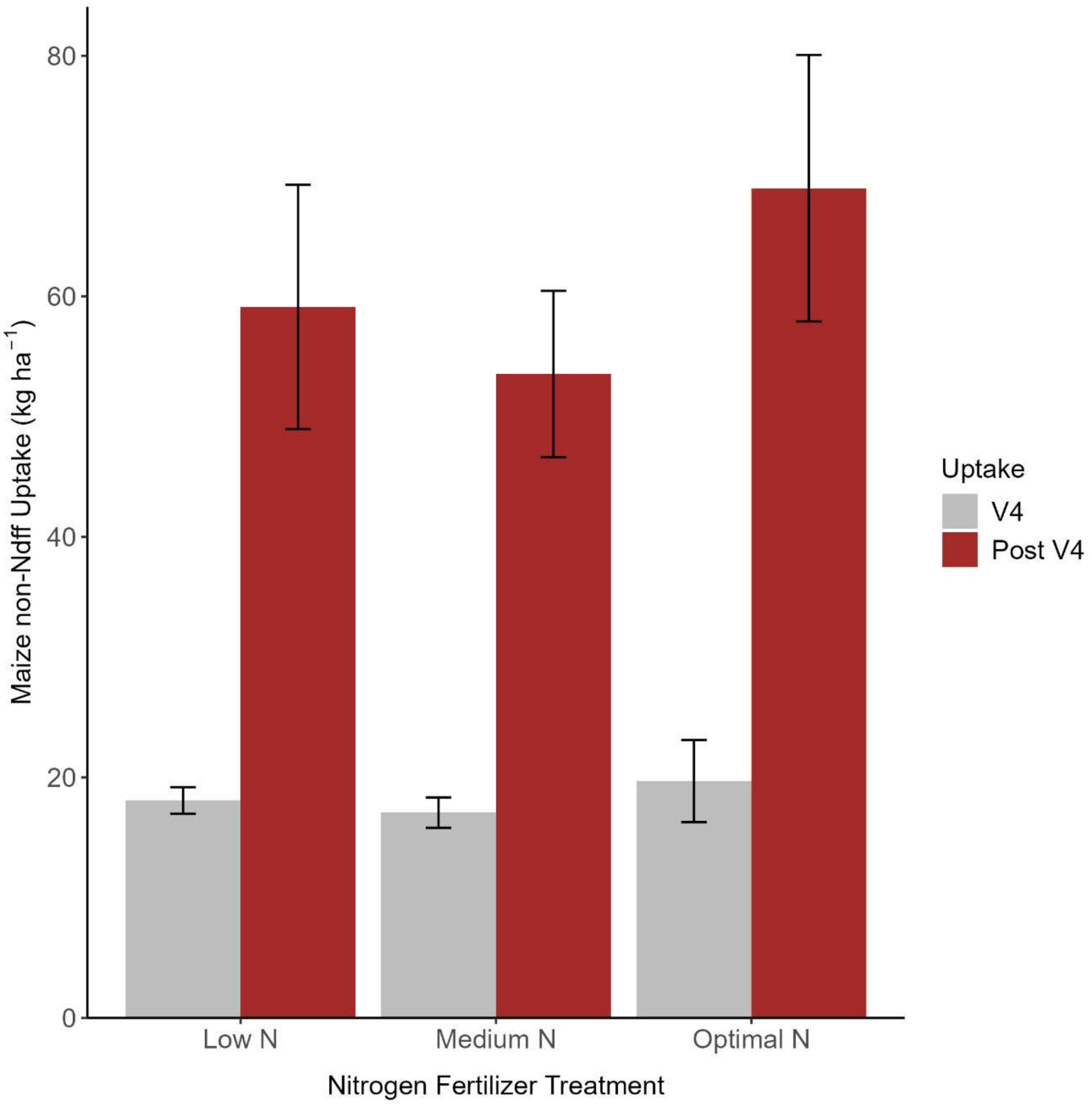
| **Effect** | **Recovered Ndff Soil** | **Unrecovered Ndff (Lost)** | **NRE Soil** | **% Lost** | **Recovered Ndff MB** | **MBN Non-Ndff** | **NRE MB** |
|---|---|---|---|---|---|---|---|
| Water (W) | 0.38 | 0.70 | 0.39 | 0.81 | 0.4 | 0.38 | 0.40 |
| Nitrogen (N) | **0.01** | 0.59 | 0.61 | 0.67 | **0.04** | 0.26 | 0.47 |
| N x W | 0.09 | 0.23 | 0.32 | 0.31 | 0.77 | 0.32 | 0.77 |

**S. Table 4.3:** Analysis of variance summary statistics for maize nitrogen (N) uptake that was nitrogen derived from fertilizer ($^{15}N$, Ndff) (Fig. 4.6), maize N uptake that was non-Ndff (Fig. 4.6), and nitrogen recovery efficiency (NRE) for maize (S. Fig. 4.2). Water refers to water availability treatment (full water 100% ET, or limited water 70% ET). Nitrogen refers to nitrogen fertilizer rate (low, medium, or optimal). Organ refers to maize organ (grain, cob, leaf, stalk). Bold values indicate significance at *a* < 0.05.

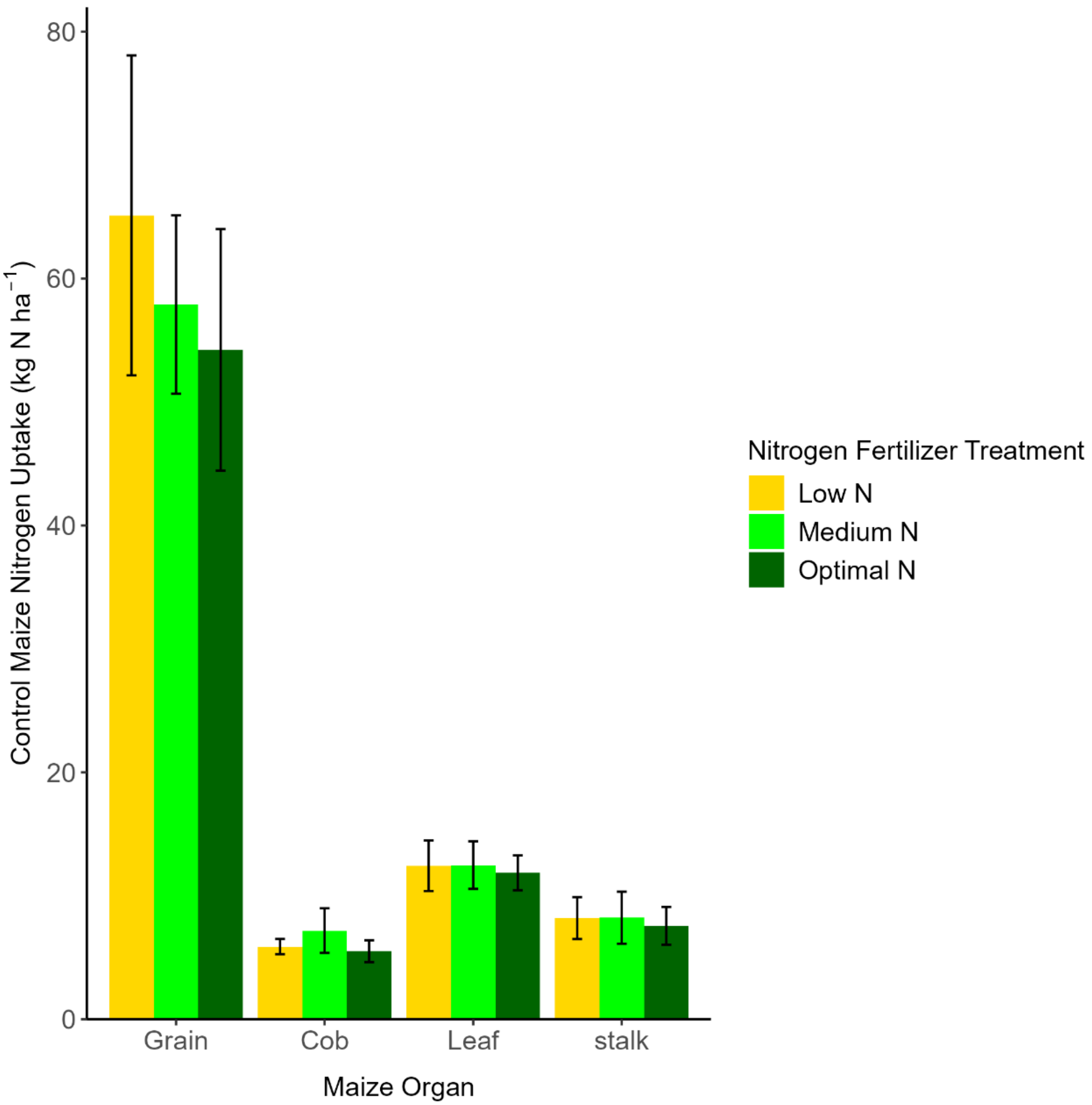
| **Effect** | **Maize N uptake Ndff** | **Maize N uptake non-Ndff** | **NRE Maize** | **Control Maize N uptake** |
|---|---|---|---|---|
| Water (W) | 0.95 | 0.41 | 0.95 | 0.21 |
| Nitrogen (N) | **> 0.01** | 0.27 | 0.18 | 0.89 |
| Organ (O) | **> 0.01** | **> 0.01** | **> 0.01** | **>0.01** |
| N × W | 0.81 | 0.79 | 0.81 | 1.00 |
| W × O | 0.37 | 0.07 | 0.37 | 0.54 |
| N × O | 0.06 | **0.04** | 0.06 | 0.99 |
| N × W × O | 0.87 | 0.65 | 0.87 | 0.87 |

**Table S4:** Analysis of variance summary statistics for nitrogen derived from fertilizer ($^{15}N$, Ndff) in the soil (Fig. 4.7a), and nitrogen recovery efficiency (NRE) in the soil (Fig. 4.7b). Water refers to water availability treatment (full water 100% ET, or limited water 70% ET). Nitrogen refers to nitrogen fertilizer rate (low, medium, or optimal). Depth refers to soil depth (0 – 15 cm, 15 – 30 cm, 30 – 60 cm, 60 – 120 cm). Bold values indicate significance at *a* < 0.05.

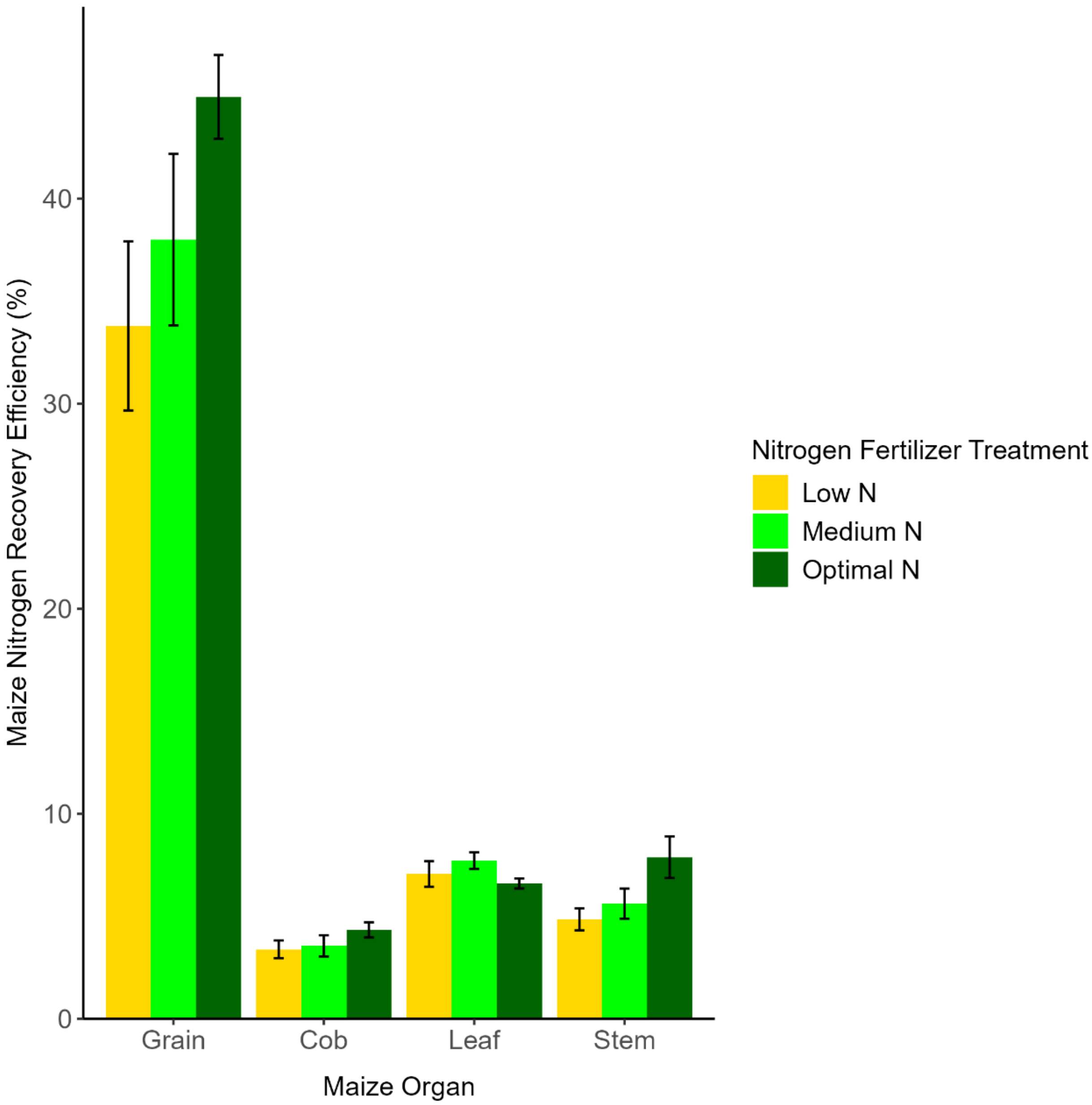
| **Effect** | **Soil recovered Ndff** | **NRE Soil** |
|---|---|---|
| Water (W) | 0.33 | 0.33 |
| Nitrogen (N) | **0.01** | 0.58 |
| Depth (D) | **> 0.01** | **> 0.01** |
| N × W | 0.57 | 0.57 |
| W × D | 0.72 | 0.72 |
| N × D | 0.35 | 0.35 |
| N × W × D | 0.17 | 0.17 |



**S. Figure 4.1:** Maize nitrogen uptake of non-nitrogen derived from fertilizer (non-Ndff, non- $^{15}$N labeled fertilizer). Data is shown as N uptake between germination and vegetative stage 4 (V4), and again from V4 – physiological maturity. Fertilizer was side-dressed ~ vegetative stage 6. Data is shown by nitrogen fertilizer treatment and averaged across water availability. Error bars indicate ± SE.



**S. Figure 4.2:** Maize nitrogen (N) uptake in the control plots (plots that did not receive any $^{15}$N fertilizer) by N fertilizer rate and maize organ. Data is averaged across the two water treatments. Error bars indicate ± SE.



**S. Figure 4.3**: Maize nitrogen (N) recovery efficiency (percent of $^{15}$N fertilizer that was recovered in the plant) by maize organ and N fertilizer rate. Data shown is averaged across the two water treatments. Error bars indicate ± SE.