Advancing nitrogen (N) management in maize: yield response, N nutrition index, and in-season N diagnosis

by

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Abstract

Maize (*Zea mays* L.*)* is a vital global crop, supporting billions through food, feed, and industrial products, with nitrogen (N) as a major factor limiting its productivity. To meet the increasing food demand of a growing population, productivity must be balanced with environmental sustainability. In this context, research focused on advancing N management practices is essential to improve maize productivity, address environmental challenges, increase farmer profitability, and reduce the N footprint. This thesis is structured in four chapters (Chapter 1 – General introduction and Chapter 4 – Final remarks), outlining in-season N diagnostics, yield responses and ability of maize to recover from mild N stress. Chapter 2 examines a comprehensive database of ninety-four maize yield to fertilizer N response experiments across eight states in the US Midwest. The analysis focuses on identifying types of yield response to nitrogen nutrition index (NNI) and the key weather and soil factors that drive these patterns. Three distinct yield-NNI relationship types were identified: no response, linear response, and linear-plateau response. The study highlights pre-planting nitrate-N, the Shannon Diversity Index from the late vegetative stage to the end of the season, and cumulative precipitation from V9 to tasseling as critical factors influencing these relationships. Chapter 3 examines various in-season nitrogen (N) fertilization strategies and their effects on key crop response variables, such as crop growth, leaf area, and plant N status during the early stages of growth. It also assesses how these N scenarios affect yield and its components, focusing on the ability of maize to tolerate early season, temporary N deficiencies without yield penalties. The study highlights the potential of maize to recover from N stress with in-season applications as late as V14. These results suggest that a flexible management approach with strategic in-season N applications can mitigate early season N deficiencies and maintain yield, potentially reducing N rates through better

synchronization between crop N demand and N supply. The study also highlights the importance of maintaining adequate N levels during grain filling to optimize grain weight and achieve maximum yield. In summary, the key outcomes of this thesis include: 1) the identification of distinct yield-NNI relationship types and their dependence on key weather and soil factors, and 2) the potential of strategic in-season N applications to mitigate early-season N deficiencies, minimize the N footprint, and avoid yield penalties. These findings highlight the potential of timely N management in achieving a balance between optimizing maize grain yield and ensuring long-term environmental sustainability.

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Dedication

To my parents, whose unwavering support, love, and steadfast encouragement have been my foundation. Their personal and professional examples inspire me daily, shaping who I am and fueling my pursuit of excellence.

Chapter 1 - General Introduction

Maize (*Zea mays L.*) is one of the most important staple crops, providing food, feed, fiber, and industrial products to support the livelihoods of billions worldwide. As global food demand continues to rise due to the projected population growth, agriculture faces the dual challenge of increasing crop productivity and ensuring environmental sustainability (Fischer et al., 2014; Jägermeyr et al., 2021). Despite significant progress, recent trends indicate a slowdown in yield improvements for major crops, including maize, and there is an increasing to produce more food in a sustainable manner and to minimize environmental impacts (Gerber et al., 2024; Ray et al., 2013). In this context, improved management practices, in particular, those related to nutrient management, are essential to address these challenges, which could help to supply the right amount to the crop, improving its efficiency, while avoiding nutrient losses to the environment and increasing production costs (Tilman et al., 2011). Optimizing nutrient management and mitigating the effects of potential deficiencies are therefore essential strategies for achieving food security while promoting more sustainable agricultural farming systems.

Nitrogen (N) stands out as the most important limiting nutrient for maize production, and is known for its complex interactions with crop, soil and weather, making it difficult to manage effectively (Briat et al., 2020). Studies indicate that approximately 50% of the N fertilizer applied to crops is utilized by the plant, with the remaining N lost to the environment (Govindasamy et al., 2023; Ladha et al., 2005). This inefficiency can occur through processes such as volatilization, denitrification, and leaching (Halpern et al., 2022). The lost N not only reduces the effectiveness of fertilizer use but also contributes to ecological impacts such as water pollution and greenhouse gas emissions (Stewart and Roberts, 2012). These inefficiencies underscore the pressing need to enhance the effective use of N (increasing capture by the plant and enhancing its utilization) in maize systems to balance productivity and sustainability. Addressing this challenge requires advancing N status diagnostics and understanding N stress levels and timing to inform precise, timely, and effective N management decisions (Ciampitti and Lemaire, 2022).

Maize N uptake exhibits a temporal pattern aligned with crop developmental stages (Bender et al., 2013; Ciampitti et al., 2013). During the early growth stages, N uptake is considered to be relatively slow as plant N requirements are mainly directed to support root establishment and initial shoot development, then as maize enters the vegetative growth phase, N uptake accelerates significantly. This period of rapid uptake coincides with an exponential increase in biomass accumulation and leaf area expansion, processes that are critical for maximizing photosynthetic capacity and establishing the foundation for high yield potential. Research highlights that the peak N demand occurs just before flowering, underscoring the critical importance of ensuring sufficient N availability during this phase to avoid limiting growth and reproductive development (Hanway, 1962). However, recent studies also emphasized the importance of N uptake during the reproductive period to attain high yields (Ciampitti and Vyn, 2011; Fernandez et al., 2020)

Given the critical role of N status in maize growth and yield, it is essential to fully understand its dynamics throughout the growing season (Ciampitti and Lemaire, 2022). This includes investigating the main factors influencing N availability and uptake, such as weather conditions and soil properties, which play a key role in regulating crop growth and N supply (Correndo et al., 2021; Tremblay et al., 2012). Furthermore, it is essential to explore potential N management strategies aimed at synchronizing soil N availability with crop N demand (Ciampitti and Vyn, 2012). Such strategies should focus on optimizing the timing, and rate of N application to promote the effective use of N and minimize environmental losses. In order to assess in-season N status, determination of N nutrition index (NNI, actual to critical plant N concentration) is needed to obtain a more physiological-based and universal diagnosis tool for effectively (including profit and environment aspects) managing N for maize (Ciampitti and Lemaire, 2022). However, to date, there is a need to identify the main drivers of the relationship between maize grain yield and N status (e.g., NNI and using proximal sensors) and to explore the potential of maize to tolerate mild N deficiencies early in the season.

This thesis was designed to address two key aspects of maize N management: first, to investigate the primary drivers influencing yield response to in-season plant N status, and second, to assess the ability of maize to withstand temporary N deficiencies without incurring on yield penalties. The objectives of Chapter 2 were to: (i) study and identify different types of yield-NNI response curves and (ii) characterize the main drivers (soil and weather factors) underpinning the different responses. The objectives of Chapter 3 were to: (i) explore different in-season N fertilization strategies and their impact on crop growth, leaf area, and plant N status during the vegetative period, and (ii) evaluate the effect of those N scenarios on yield (and its components) to assess the ability to tolerate (without yield penalty) to early season and temporary N deficiencies.

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Chapter 2 - Nitrogen nutrition index as an in-season N diagnostic method for maize yield response to N fertilization

Under Review, Field Crops Research

Abstract

Assessing crop N status is essential for optimizing fertilizer N inputs for maize (*Zea mays* L.) crop and reducing the environmental footprint of this practice. The Nitrogen Nutrition Index (NNI) offers a promising method for improved in-season N diagnosis and management. However, there is a need to identify the different types of in-season responses for the relative yield (RY) to NNI (RY-NNI) relationship to develop better management tools and identify the main drivers (weather and soil factors) governing this process. This study aimed to identify the different RY-NNI relationships and characterize the main weather and soil drivers influencing these responses. We used ninety-four maize yield to fertilizer N response experiments collected using a standardized protocol from 2014 to 2016 growing seasons across the United States (US) Midwest (including eight US states). Bayesian modeling and conditional inference tree algorithm were employed to assess the different types of RY-NNI relationships and characterize key weather and soil drivers. Three distinct RY-NNI relationship were identified, 60% of the experiments exhibited a linear-plateau response (n = 56), 27% a linear response (n = 26), and the remaining 13% a no response (n = 12). Pre-planting nitrate-N (NO_3-N), the Shannon Diversity Index (SDI) from late vegetative (tasseling) to end of season (maturity), and the cumulative precipitation (CPP) from V9 to tasseling were key factors influencing RY-NNI responses. Together, these top three variables accounted for ~ 50% of the total relative variable importance. These findings enhance the use of NNI as an in-season N diagnostic tool by providing insights into types of RY-NNI relationships and their drivers.

Introduction

Nitrogen (N) is a critical nutrient for crop production due its role in crop growth and yield (Ciampitti and Vyn, 2014; Eickhout et al., 2006). However, accurate N management is challenging due to the complex interactions between soil, crop, and weather (Briat et al., 2020). Overapplication of N occurs frequently in maize (*Zea mays* L.) crops in the Midwest region of the United States, and farmers may not reduce N rates due to the perceived risk of yield loss (Houser, 2022). The relatively low cost of fertilizer N and lack of easy to use and pragmatic in-season N diagnostic methods are factors that discourage the adoption of precise N management which may reduce negative environmental externalities such as greenhouse gas emissions and NO3 leaching (Stulen et al., 1998; Sutton et al., 2021). In contrast, underapplication of N can lead to yield reduction (Ciampitti and Vyn, 2012), and therefore farmers overapply N as an "insurance" against potential maize yield losses (Houser, 2022).

The negative environmental externalities of current of N management highlight the need to implement accurate N recommendations to mitigate adverse effects while maintaining crop productivity (Zhang et al., 2015). The N nutrition index (NNI) is emerging as a promising approach towards developing a more universal and accurate N diagnosis method in maize (Ciampitti et al., 2022). This index is calculated as the ratio between the actual N concentration of the crop aboveground biomass (%Nact) and that required to maximize crop growth, the critical N concentration (%Nc) (Lemaire et al., 1984). Therefore, the NNI allows an in-season assessment of the crop N status by defining N-deficient ($NNI < 1$), N-sufficient ($NNI = 1$), and luxurious N consumption ($NNI > 1$) conditions for a given crop biomass. In maize, N deficiency around silking reduces plant growth rate, which leads to a reduction in grain number (Rodriguez et al., 2024a) and consequently grain yield (Uhart and Andrade, 1995). Therefore, a positive relationship between NNI around silking and grain yield is expected in maize (Ciampitti and Lemaire, 2022; Rodriguez et al., 2024a).

Crop N uptake is co-regulated by both soil N availability and crop growth (Gastal et al., 2015; Briat et al., 2020). In addition, uncertainties in defining the optimal N rate for maize challenge the use of pre-season methods since most of the variability (>75%) is driven by weather conditions around silking time for maize (Correndo et al., 2021a). Therefore, the complexity of developing in-season N diagnostic methods should not be overlooked and more dynamic approaches (based on plant N changes over time) can enable more precise N management. In this context, the use of NNI as an in-season N diagnostic tool can allow monitoring of changes and provide information for timely intervention to correct potential deficiencies. The yield-NNI relationship needs to be further evaluated to assess changes across site-years combinations (Ziadi et al., 2008a). Usually, yield is maximized under N sufficiency (when NNI is close to 1) (Plénet and Lemaire, 1999), but in other scenarios, yield is maximized at NNI > 1 (luxury N uptake) (Rodriguez et al., 2024a), or at NN I < 1 (N deficiency) (Maltese et al., 2020). However, the main drivers, mainly soil and weather variables, explaining differences in the yield-NNI relationship for maize have not been investigated using a large dataset across different environments.

Following this rationale, understanding the main drivers underpinning the relationship between yield and in-season NNI is crucial to provide new insights to improve N management strategies for maize systems with a focus on the US Midwest region. Therefore, this study aimed to: (i) examine and identify different types of yield-NNI response curves, and (ii) characterize the main drivers that govern these responses.

Material and Methods

Database description

Meta-data

The meta-data has been extracted from Ransom et al. (2021). Briefly, experiments on maize yield response to N fertilizer application were performed during three growing seasons, 2014, 2015, and 2016 in eight US Midwestern states (Illinois, Indiana, Iowa, Minnesota, Missouri, Nebraska, North Dakota, and Wisconsin) (Figure 2.1A). A standard protocol consisting of a randomized complete block design with four repetitions was followed in each state. The treatment structure was comprised by two N application timing. Each treatment comprises four replications of ammonium nitrate fertilizer rates ranging from 0 to 315 kg N ha^{-1}, with increments of 45 kg N ha^{-1}. These fertilizers were applied in either a single application at planting or a split application. In the split application, 45 kg N ha^{-1} was surface broadcast at planting, while the remaining fertilizer N was broadcast at the V9 corn developmental growth stage (Abendroth et al., 2011). Two additional treatments were included in the split scenario, 90 kg N ha^{-1} applied at planting, followed by either 90 or 180 kg N ha^{-1} at V9, resulting in a total of 16 treatments. For our analysis, the N application timing was considered as two different management treatments, resulting in ninety-four experiments (site-year-timing combinations) representing different weather conditions and soil characteristics (Figure 2.1B). Additional experiment information on the experiments can be found in Kitchen et al. (2017).

Soil and plant measurements

Soil

Soil data has been extracted from Ransom et al. (2021). Based on their study, briefly, soil samples were collected before planting to characterize the soil properties. A 120-cm deep soil core was obtained from each experimental block at every experiment site. The cores were divided by pedogenic diagnostic horizons to measure chemical and physical soil properties such as soil texture, soil organic matter (SOM), pH (1:1 soil to water ratio) and cation exchange capacity (CEC). These determinations were conducted according to the methods described in Kitchen et al. (2017). Weighted averages were calculated for the top 30 cm for those soil property variables, using the depth of each horizon within the first 30 cm. Preplant nitrate-N (NO_3-N) data for each plot at 0-30 cm depth was obtained from Kitchen et al. (2017).

Plant

Plant data has been extracted from Ransom et al. (2021). Briefly, plant samples were obtained from each treatment at the VT growth stage by collecting six whole plants at ground level. Aboveground biomass was dried in a forced air oven (60°C) until constant weight and dry matter was determined. Whole plant N concentration was measured on ground material (< 1-mm) using the Dumas combustion method (Bremner, 1996). Whole plant and yield were converted to mass per area (Mg ha^{-1}). For each experimental unit, the center rows were harvested to determine grain yield, and the weight was adjusted to 155 g H_2O kg^{-1}. More information regarding the plant measurement data used in this study is presented in Table 1. For each experiment, aboveground biomass and yield responses to N fertilization were shown. (Figures S2.1 and S2.2 in the supplementary material). In addition, for the experiments with available V9 and VT data, we have examined the relationship between RY-NNI for both stages, highlighting the VT as a more reliable moment. (Figure S2.3 in the supplementary material).

The relative yield (RY) for each experiment was determined by dividing the yield of each N rate treatment by the highest yield (Equation 1). The same calculation for RY was used in recent studies on RY vs NNI (Lacasa et al., 2024, Rodriguez et al., 2024a).

$$Relative\ yield\ (RY) = \frac{Observed\ yield}{Maximum\ observed\ yield\ within\ experiment} \quad (1)$$

Weather

On-site weather stations (HOBO U30 Automatic Weather Station; Onset Computer Corporation) were used to collect and record weather data. Hourly values were collected to calculate daily maximum and minimum temperature and total precipitation. These variables were used to calculate new features: Shannon diversity index (SDI) (Tremblay et al., 2012), extreme precipitation events (EPE) (Correndo et al., 2021b), and extreme temperature events (ETE) (Butler and Huybers, 2013), for four time periods throughout the growing season, 30 days before planting up to planting (1), planting up to V9 (time of the split application) (2), V9 up to VT (3), and VT up to R6 (4) (Table 2). All weather data has been derived from Ranson et al. (2021)

Nitrogen Status

The NNI was calculated as:

$$NNI = \frac{\%N_{act}}{\%Nc} \quad (2)$$

where $\%N_{act}$ is the actual N concentration (%) in aboveground biomass at the VT stage and $\%N_c$ is the critical N concentration calculated from the critical N dilution curve proposed by Ciampitti et al. (2021) ($\%N_c = 3.49\ biomass^{-038}$, Figure S.4 in the supplementary material).

This CNDC was derived from an extensive dataset comprising multiple maize hybrids grown in different environments in four countries, providing a robust estimate of critical N requirements under varying genotype, environment, and management (G x E x M) conditions. While some uncertainty in CNDC parameters has been noted, particularly at early growth stages and lower biomass levels, studies have shown that the overall pattern of N dilution in maize remains consistent (Plénet & Lemaire, 1999, Hermann and Taube, 2004, Makowski et al., 2020).

Statistical analysis

Model fitting

Three Bayesian models were fitted to the data to examine the relationship between relative yield (RY), and NNI. Their performance was evaluated, and each experiment was then classified according to the type of response it exhibited to NNI. The fitted models were:

$$y_i \sim Normal\ (\mu_i, \sigma^2) \tag{3}$$

$$\mu_i = \beta_0 \tag{4a}$$

$$\mu_i = \beta_0 + \beta_1 x_i \tag{4b}$$

$$\mu_i = \begin{cases} \beta_0 + \beta_1 x_i \ if \ x_i < bp, \\ \beta_0 + \beta_1 bp \ if \ x_i \geq bp \end{cases} \tag{4c}$$

$$\beta_0 \sim uniform\ (0, 1) \tag{5}$$

$$\beta_1 \sim uniform\ (0,\ 10) \tag{6}$$

$$bp \sim gamma\ (29.70,\ 27.25) \tag{7}$$

Where equation (3) determines the likelihood function for RY (y_i), with mean μ_i and constant variance σ^2. The underlying process of the mean response (i.e., expected value), μ_i, was modeled according to an intercept-only model (4a), a linear model (4b), and a linear-plateau model (4c). *Normal*, *uniform*, and *gamma* represent the normal, uniform, and gamma distributions used for priors, respectively (equations 3, 5-7). These models were fitted to the data in each experiment independently. The distributions for the intercept and slope were chosen to match the support of the parameters. The intercept must fall between 0 to 1 due to the RY range, and a positive slope is expected with increasing N, reflecting a positive response in RY (Correndo et al., 2021a; Ziadi et al., 2008b). The prior for the breakpoint (bp) was defined using a gamma distribution to ensure non-negativity and to match values reported in the literature (Figure S2.5 in the supplementary material). We used previously synthesized estimates from Rodriguez et al. (2024a) to parameterize the gamma distribution. The shape and rate parameters were derived using the moment-matching method, which allows the prior to incorporate biological expectations while remaining adaptable to the data.

The posterior distribution of the model parameters was obtained using the Hamiltonian Monte Carlo algorithm. Two parallel chains were run with 10,000 iterations and a burn-in period of 2,000. The convergence and mixing of the chains were assessed using the potential scale reductor factor (Gelman and Rubin, 1992). The model with the best performance was selected for each experiment based on the Watanabe-Akaike information criterion (WAIC), (Watanabe, 2010). The models were fitted using the *brms* package (Bürkner, 2017).

Conditional inference tree

To analyze the drivers of the relationship between RY response types and NNI, we employed a classification tree model using a conditional inference framework. This approach was chosen for its ability to handle nonlinear interactions between predictor variables, while reducing bias due to variable selection. The model predicts response type (no-response, linear, or linear-plateau) based on the soil and weather variables listed in Table 1. Models were fitted using the *rpart* package (Therneau and Atkinson, 1999). The *rpart* function iteratively splits the dataset to create subsets until a specified stopping criterion is reached. The Gini index (Breiman et al., 2017) was selected as the splitting criterion because it effectively handles moderate class imbalances. Similar methods have been applied in other agronomic studies, such Rodriguez et al. (2024b) and van Versendaal et al., (2025).

The relative importance of each weather and soil variable in the models was assessed by quantifying the significance of each variable based on its contribution to reducing node impurity (Gini index) using the *rpart* package in R. Higher importance scores are assigned to variables that contribute more significantly to the improvement of predictive accuracy of the model by effectively partitioning the tree nodes. All the statistical analyses were performed using the R software (R Core Team, 2024).

Results

Crop N status and relationship between RY and NNI

The RY-NNI relationship was determined for each experiment (94 in total, Figure S2.6 in the supplementary material). Of all the RY-NNI relationships, 13% of the experiments showed no response, while 27% depicted a linear response. The remaining 60% of the experiments exhibited a linear-plateau response over the range of NNI values (Figure 2.2).

The no-response experiments (Figure 2.2A) exhibited a greater median NNI of 1.13 ($IQR_{25-75} = 0.99 - 1.29$) relative to the responsive experiments (i.e., linear and linear-plateau responses). The responsive experiments had a median NNI of 1.11 for both linear ($IQR_{25-75} = 0.89 - 1.35$) and linear-plateau ($IQR_{25-75} = 0.91 - 1.29$), responses (Figure 2.2B, and 2.2C). The no-response experiments had a median absolute grain yield of 12.2 Mg ha^{-1} ($IQR_{25-75} = 10.2 - 14.5 \text{ Mg ha}^{-1}$), while the experiments with linear responses had a median of 11.1 Mg ha^{-1} ($IQR_{25-75} = 8 - 13.2 \text{ Mg ha}^{-1}$), and the experiments with linear-plateau responses had the highest median grain yield of 12.7 Mg ha^{-1} ($IQR_{25-75} = 10.4 - 14.3 \text{ Mg ha}^{-1}$).

For the model parameters, linear response experiments (Figure 2.2B) showed a median slope of 0.55 ($IQR_{25-75} = 0.49 - 0.79$), while the linear-plateau experiments (Figure 2.2C) had a median slope of 0.71 ($IQR_{25-75} = 0.52 - 0.88$), and median breakpoint ($bp,\ Equation\ 4c$) of 1.12 ($IQR_{25-75} = 1.03 - 1.51$). Lastly, there was a significant negative relationship between the median slope ($\beta_1,\ Equation\ 4b\ and\ 4c$) and median NNI for both the linear and linear-plateau response groups (Figure S2.7A, and B in the supplementary material).

Association between weather and soil variables with each response pattern

Pre-planting nitrate-N (NO3), the Shannon Diversity Index for the period from VT to maturity (SDI_4), and the cumulative precipitation from V9 to VT (CPP_3) were the most important variables accounting for the different types of RY and NNI relationships (Figure 2.3). The relative importance of the top 10 variables was obtained, with the first three (NO_3, SDI_4 and CPP_3) contributing ~ 50% of the total relative variable importance (Figure S2.8 in the supplementary material).

Pre-planting soil nitrate-N emerged as one of the soil variables strongly associated with yield response types (Figure 2.3). Greater soil nitrate-N levels (≥12 ppm, 30 cm depth) increased the likelihood of observing non-response RY-NNI patterns, regardless of changes in NNI status at the VT crop growth stage. Conversely, weather variables showed a strong relationship with response patterns, particularly the SDI for the period from tasseling to maturity and CPP for the period after sidedress application (V9) to tasseling stage (VT) (Figure 2.3).

Experiments with a more heterogeneous distribution of rainfall (SDI < 0.49) during tasseling up to maturity were more likely to show a linear response (Figure 2.3), with no saturation of crop N requirements. On the other hand, experiments characterized by an even rainfall distribution (SDI > 0.49) during the same period were more likely to show a linear-plateau RY-NNI response (Figure 2.3). Cases with the sum of all precipitation from V9 up to VT greater than 151 mm were associated with linear RY-NNI relationship (Figure 2.3). Conversely, experiments characterized with CPP below the threshold of 151 mm during this period were more likely to show a plateau response pattern (Figure 2.3).

Discussion

This study provided a new characterization of the most relevant yield-NNI patterns and the main weather/soil factors underlying these responses, providing a basis for in-season N management for maize crops. Understanding the relationship between yield and NNI is essential for tactical in-season N management, advancing towards robust crop N diagnostics and leading to improved N strategies (Ciampitti and Lemaire, 2022).

Current N management methods based on yield responses to external N inputs present a high uncertainty to define the optimal N requirements, with weather around silking strongly influencing this process (Correndo et al., 2021a). This high degree of uncertainty, and more static approaches, leads to low confidence among farmers and discourages them from using these methods to determine the optimal N rate (Correndo et al., 2021b; Morris et al., 2018). The NNI is a useful in-season diagnostic tool, for monitoring crop N changes over time and developing more effective (and dynamic) tactical N interventions (Ciampitti and Lemaire, 2022). This method can help to offset the current scenario with farmers tending to over-apply N to avoid yield losses due to stochastic weather and soil conditions (Babcock, 1992; Babcock and Hennessy, 1996; Rajsic et al., 2009). The development of an indirect indicator of crop NNI could be useful and could be related to the types of RY-NNI responses found in this study. These methods include sampling upper leaves to characterize their N concentration (Gastal et al., 2015) or expressing N content per unit of leaf dry matter (Ferruggia et al., 2004; Ziadi et al., 2009). At the same time, leaf greenness is a proxy for estimating the upper leaf N concentration (e.g. Matsunaka et al., 1997; Feibo et al., 1998; Ziadi et al., 2010), and also a relevant index of NNI, as a N deficiency diagnostic tool in wheat (Caviglia and Sadras, 2001). Therefore, promoting new phenotyping efforts for N sensing and developing new technologies (e.g. Atefi et al., 2021; Chen, 2021; Pinto et al., 2023) can provide the foundation for characterizing environments for responses to N via identification of the main factors driving this process.

The three patterns of RY and NNI explored (no-response, linear and linear-plateau responses) were mainly linked to soil and weather variables. Previous studies have already shown the importance of pre-planting nitrate in terms of N decisions for in-season N management (Bundy and Malone, 1988; Schmitt and Randall, 1994). Similarly to the influence of other factors on N management reported by Correndo et al. (2021a), soil organic matter and texture exhibited only minimal impact in this study. However, their role in N and water dynamics should not be overlooked. One possible explanation for limited influence is that their effects are highly dependent on precipitation patterns, which may overshadow their direct effects (Cassman et al., 2002). For instance, in sandy soils, high rainfall events can lead to substantial N losses through leaching, whereas in finer textured soils, water retention can mitigate these losses but also influence N availability through denitrification (Cameron et al., 2013, Tamagno et al., 2022). Environments with high nitrate conditions did not experience limitations of this nutrient (Binford et al., 1992), leading to less pronounced responses to fertilizer N application. Variables such as SDI and CPP were identified as key drivers of response patterns, highlighting the importance of weather, in our case, water distribution in the late vegetative period and critical period of maize (Correndo et al., 2021a; Tremblay et al., 2012). The relationship between water and N dynamics is well known, especially concerning N leaching due to high precipitation in a short period (Bowles et al., 2018). The interaction between water and N plays a crucial role in determining maize grain yield response to N status (Kunrath et al., 2020). High precipitation (extreme events) can lead to significant N losses via N_2O and N leaching, especially in poorly structured sandy soils (Cameron et al., 2013; Li et al., 2022; Tamagno et al., 2022).

Understanding these dynamics is essential for optimizing N management strategies and ensuring efficient N use in varying weather conditions (Tremblay et al., 2012).

We acknowledge the limitations of our study. Although it captured a wide range of environmental conditions, it was restricted to a single maize growth stage (VT). Future studies should extend NNI monitoring to earlier growth stages to allow timely N adjustments and minimize potential yield penalties. Promising approaches involved the use of proximal (e.g., sensor-based) and remote (e.g., planes, drones, satellites) approaches that provide non-destructive, real-time assessments of plant N status. The data derived from these approaches, obtained via canopy reflectance sensors and/or drones with multispectral or hyperspectral sensors, could help to establish an effective N diagnostic tool relating N status with yield response to N application. By integrating these technologies with crop growth models as a component of decision-support systems, farmers can optimize N applications throughout the growing season, improving the effective use of N while reducing the risk of yield loss due to N deficiency.

Conclusion

This study provides novel information on understanding the response of maize grain yield to NNI, with the additional component of assessing the main drivers underpinning different types of yield responses. Although the traditional linear-plateau was the most common, we also found no response and linear response groups. We have identified pre-planting nitrate-N, the Shannon Diversity Index in the grain filling period, and the cumulative precipitation in the late vegetative period (V9 up to VT) as the main drivers of these yield-NNI responses. Future efforts should focus on exploring the yield-NNI relationship at early vegetative stages and identifying non-destructive methods for rapid diagnostic of NNI during the crop growing season.

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Figures

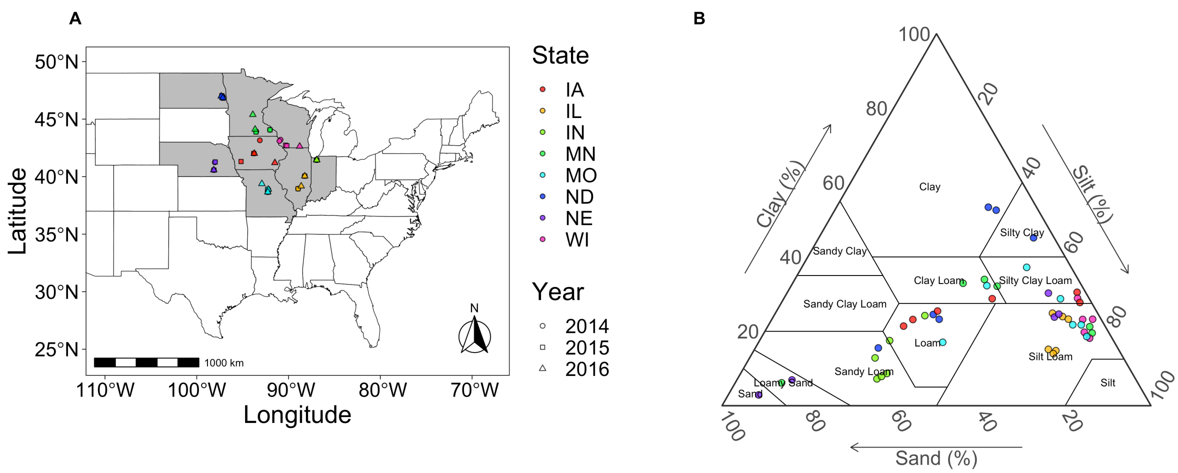

Figure 2.1. (A) Geographic distribution of field research experiments during the 2014, 2015, and 2016 growing seasons in the U.S. (B) Soil texture variability for each site in the database.

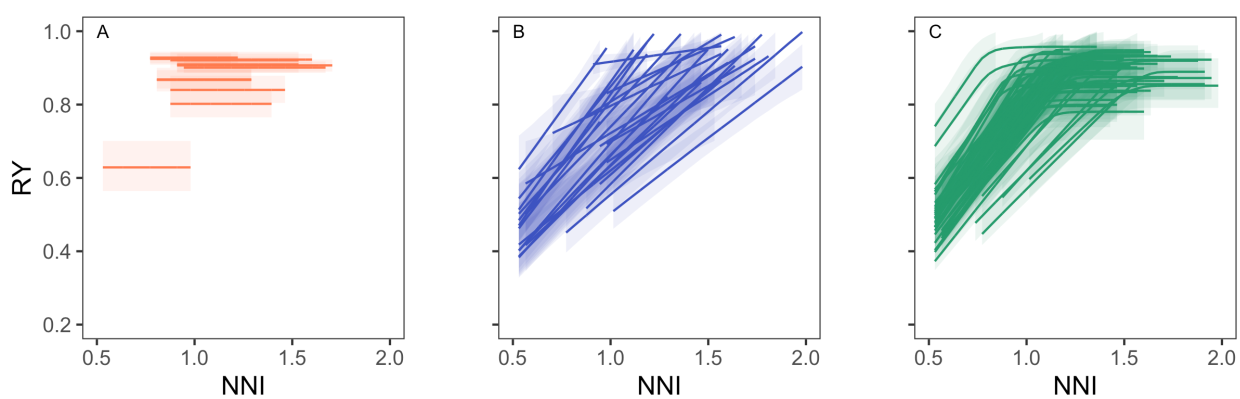

Figure 2.2. Relationship between relative grain yield (RY) and N nutrition index (NNI) of maize, A – Non-responsive experiments (n = 12), B – Linear response experiments (n = 26), C – Linear-plateau response experiments (n = 56). The shaded area represents the 95% credible interval of each model.

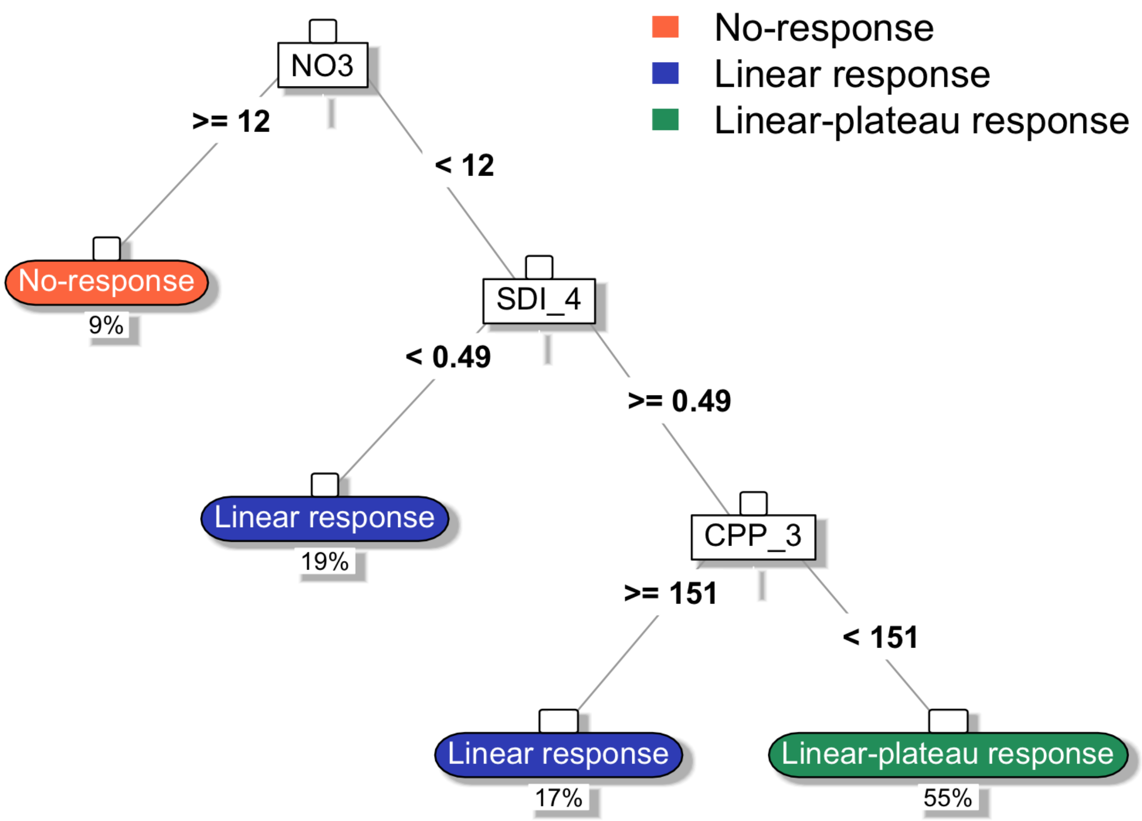

Figure 2.3. Classification tree illustrating the relationship between response types (No-response, Linear response, and Linear-plateau response, n = 94) and key explanatory variables: NO3 – pre-planting nitrate-N, SDI_4 – Shannon Diversity Index from VT to R6, and CPP_3 – Cumulative precipitation from V9 to VT. Thresholds at each node indicate the splitting criteria, directing observations down different branches. Colors refer to each yield response type. Below each terminal node, the percentage represents the proportion of the total dataset contained in that node.

Tables

Table 2.1. Descriptive statistics of the plant measurement used in this study, aboveground biomass, N concentration, and NNI at the VT stage, and grain yield by N application timing (Planting or Sidedress). Summary of data collected from (Ransom et al., 2021).

	Planting			Sidedress		
Variable, unit	**Mean**	**Q25[a]**	**Q75[a]**	**Mean**	**Q25[a]**	**Q75[a]**
Biomass, Mg ha^{-1}	9.06	7.39	10.55	8.66	7.01	10.16
N concentration, %	1.66	1.38	1.96	1.72	1.46	2
Yield, Mg ha^{-1}	12.1	9.4	13.9	12.5	10.1	14.2
NNI	1.1	0.89	1.31	1.11	0.94	1.3

a Q25 and Q75 represent the 25th and 075th quantiles, respectively.

Table 2.2. Soil and weather variables used and their information.

SOIL		
Variable	Units	Depth
Soil Organic Matter (SOM)	%	0-30 cm
Clay		
Silt		
Sand		
Pre-planting Nitrate (NO_3-N)	ppm	
WEATHER		
Variable	Units	Periods
CPP = Cumulative precipitation (sum)	mm	1 = 30 days before planting up to planting 2 = planting up to V9 3 = V9 up to VT 4 = VT up to R6
EPE = Extreme Precipitation Events (counts)	days[a]	
EPE = Extreme Temperature Events (counts)	days[b]	
SDI = Shannon Diversity Index	0-1[c]	
RAD = Incident Radiation (sum)	MJ m^{-2}	
TM = Mean Temperature	°C	

[a] number of days with precipitation greater than 25 mm
[b] number of days with a maximum temperature greater than 30 °C
[c] 0 – uneven, 1 – even precipitation

Chapter 3 - Assessing in-season N response and recovery of maize from mild N stress

Abstract

Efficient N management in maize is critical to achieving high yields while minimizing environmental impacts. Overapplication of N remains widespread, driven by concerns over yield losses from N stress, which increases the risk of environmental N losses. Understanding the ability of maize to tolerate early-season N deficiencies is key to developing effective in-season strategies. This study aimed to evaluate different in-season N fertilization strategies and their effects on crop growth rate (CGR), and plant N status during the vegetative period, and assess their impact on yield and yield components to determine maize's ability to recover from temporary N deficiencies without yield penalties. Two field trials were conducted in Kansas during the 2023 and 2024 growing seasons, with treatments comprising N rates from 0 to 180 kg ha^{-1}, all applied at planting or split between planting (90 kg N ha^{-1}) and V6, V10, or V14 (30–90 kg N ha^{-1}). Results showed that maize can tolerate early-season N deficiencies without yield penalties if plant N status is timely corrected. Plant N status was recovered even with N application as late as V14 stage. However, N deficiencies during the vegetative stage reduced CGR, increased inter-plant biomass variation, and negatively affected kernel number. Kernel weight was positively associated with CGR during grain filling, highlighting the importance of adequate N availability in this phase to optimize kernel development and maximize yield. These findings emphasize the need for timely in-season N strategies to mitigate N stress, and support yield formation balancing productivity and sustainability.

Introduction

Nitrogen (N) management in maize (*Zea mays* L.) production involves a complex interaction between plant demand and soil supply, and is modulated by weather, which creates a significant challenge for targeting the optimal seasonal crop N supply (Briat et al., 2020; Morris et al., 2018). Under this scenario, overapplication of N fertilizer is considered by farmers as "insurance" against yield loss, demotivating reductions in applied N rates (Houser, 2022). Thus, this practice leads to increases in N losses via nitrate (NO_3) leaching and greenhouse gas emissions (N_2O) (Bowles et al., 2018; Halpern et al., 2022). A better understanding of the capacity of the crop to recover from mild N deficiencies is critical to develop more effective tactical in-season interventions. This knowledge could help enhance late-season N fertilization, aiming to improve the effective use of N by the crop during the critical period for grain yield determination (i.e. close to flowering; Cerrudo et al., 2013) and reducing the overall environmental N footprint (Ciampitti et al., 2022).

Improving the synchrony between soil N supply and crop N demand can increase the effective use of N and enhancing N recovery (Raun and Johnson, 1999; Cassman et al., 2002; Ciampitti and Vyn, 2012). The rate of N uptake in maize is slow early in the vegetative phase but accelerates after sixth-leaf stage, peaking before flowering, when demand is highest to support leaf area expansion and biomass accumulation. Uptake then slows during reproductive stages as N is redistributed from vegetative tissues to kernels (Hanway, 1962). Timing in-season N application with crop N demand is very complex and challenging, with maize yield responses to N varying across the published literature. For instance, a few studies reported no yield penalty for delaying N application until V10 (Walsh et al., 2012) or V11 (Scharf et al., 2002), but with moderate to none yield losses as the timing gets closer to flowering (Scharf et al., 2002; da Silva et al., 2005; Mueller et al., 2017). Still, it is noteworthy to emphasize that applying N too late in the growing season can lead to yield penalties and decreased N fertilizer recovery efficiency (Jung et al., 1972). In a recent meta-analysis, Fernandez et al. (2020), demonstrated positive responses to yield with environmental conditions promoting favorable crop growth and N uptake during the reproductive period. However, a proper diagnosis of crop N status (e.g., via proximal and/or remote sensing) should be based on foundational knowledge and understanding of the crop ecophysiology and its N economy.

Late season N application to maize crop has been investigated in previous studies (Walsh et al., 2012; Scharf et al., 2002; da Silva et al., 2005; Ransom et al., 2021). However, a better understanding of the impacts of plant N status on the main physiological and yield formation processes is still needed. Under N-limiting conditions, maize will reduce leaf area, growth rate, and its overall N content (Gastal et al., 2015), impairing the ability of the plant to intercept light, produce photo-assimilates, and consequently impact grain yield (Uhart and Andrade, 1995a). In addition, insufficient N levels also shift dry matter allocation away from reproductive structures, leading to fewer kernel number, lower kernel weight, and a reduction on harvest index (HI) (Ding et al., 2005; Uhart and Andrade, 1995a). This highlights the importance of determining the critical point where agronomic, economic, and environmental considerations intersect to guide optimal N management decisions.

Given the importance of plant N status on maize yield (Ciampitti and Lemaire, 2022), characterizing early season changes in crop growth, and plant N status will be key to understand the ability of maize to tolerate temporary N deficiencies without suffering a yield penalty. Thus, the objectives of this study were to (i) explore different in-season N fertilization strategies and their impact on crop growth, and plant N status (by chlorophyll meters) during the vegetative period, and (ii) evaluate the effect of these N scenarios on yield (and its components) to assess the ability to tolerate (without yield penalty) early season and temporary N deficiencies.

Material and Methods

Management practices, experimental design, and treatments

Two field experiments were conducted during the 2023 and 2024 growing seasons at two sites in Kansas: The Kansas River Valley Experimental Field (Experiment 1, 39.07N, -95.76W) near Topeka, KS, and a farm in Junction City, KS (Experiment 2, 39.07N, -96.72W), both under irrigated condition. Both experiments were arranged as a randomized completed block design with four replications. The treatment structure was comprised of rates of urea (CH_4N_2O, 46-0-0) fertilizer ranging from 0 to 180 kg N ha^{-1}. These treatments were applied in either a single application at planting (90 or 180 kg ha^{-1}) or a split application in both sites. In the split application, 90 kg N ha^{-1} was surface broadcasted at planting, while the remaining fertilizer N (30, 60, or 90 kg N ha^{-1}) was broadcasted at six-leaf (V6), tenth-leaf (V10), or fourteen-leaf (V14) maize developmental growth stage (Abendroth et al., 2011) (Table 3.1). After application of the treatment-specific N, ~12 mm of water was added by irrigation to incorporate the urea and prevent volatilization losses.

Experiments were planted on April 16th in 2023 and on April 14th in 2024 with a final achieved plant density of 80,000 plants ha^{-1}. The plots were 16 rows, 12 m wide (76 cm row spacing) and 20 m in length in 2023, and, 24 rows, 18 m wide (76 cm row spacing) and 25 m in length in 2024. In both experiments, maize was preceded by soybean [*Glycine max (L) Merr.*], and a hybrid with a Comparative Relative Maturity (CRM) of 105 days (Beck's Hybrids, Atlanta, IN) was used across both experiments.

In each experiment, soil analyses were conducted prior to planting to characterize initial conditions. Eight soil cores (2 cm diameter) were collected from the 0 to 30 cm depth within each block and a composite sample was prepared. All samples were sent to KSU Soil Testing Lab (Kansas State University, Manhattan, KS) for determination of soil nitrate (NO_3-N) concentrations via KCl extraction (Keeney and Nelson, 1982), and soil organic matter content (OM) using the loss on ignition method (Nelson and Sommers, 1996). Soil texture was determined using the hydrometer method (Gee and Bauder, 1986), alongside pH (1:1 soil to water ratio) (Thomas, 1996), phosphorus concentration (Mehlich-3 extraction) (Mehlich, 1984), and potassium concentration (ammonium acetate extraction) (Helmke and Sparks, 1996) (Table 3.2).

Weather measurements and crop phenology

The weather data has been retrieved from the Kansas Mesonet website using the nearest weather station (Experiment 1 - Silver Lake E4, Experiment 2 - Manhattan) for the period of April 1st to October 1st. Daily values were collected for maximum and minimum temperature, incident solar radiation, as well as total precipitation (Figure 3.1). Total precipitation from planting to harvesting was 332 and 592 mm for experiment 1 and 2 respectively, pivot irrigation provided an additional 140 mm during the growing season to prevent drought stress in both experiments. The average temperature was 22°C in both experiments. Based on these variables, thermal time (°C day) after planting for each experiment was calculated using a base temperature of 8°C (Ritchie and Nesmith, 1991). Figure 3.1 shows the distribution of daily maximum and minimum air temperature, solar radiation, and total daily precipitation for each experiment and the phenological stages (V6, V10, V14, R1, R3, and R6). Phenology data were recorded from twenty tagged plants within each experimental unit.

Aboveground biomass, leaf area, grain yield, and yield components

Aboveground biomass was collected from each plot at V6, V10, V14, R1, R3, and R6 stages by harvesting nine whole plants at ground level (~ 1 square meter sampling area). Samples were dried in a forced air oven (60°C) until constant weight and dry matter was determined.

Aboveground biomass accumulation in maize over thermal time is characterized by an S-shaped curve and was described in this study employing a nonlinear Gompertz growth model (Equation 1), with thermal time (°C day) as the predictor, and aboveground biomass (W, g m^{-2}) as the response variable as follows:

$$W = W_{max} exp^{-exp^{-k(g-M)}} \quad (1)$$

Where W denotes the response variable, biomass, at a given time thermal time, W_{max} represents the upper asymptote, M refers to the thermal time at which the growth rate is maximized, and k represents the relative growth rate at M.

Leaf area was estimated at V6, V10, V14, and R1 stages using an LAI meter (Model LI-3100, Li-Cor, Inc., Lincoln, NE) following destructive sampling of all leaves from two consecutive, representative plants in each plot. Green leaf area was then converted into the leaf area index, considering the sampled area.

For each experimental unit, the center rows (8 and 9 in 2023 and 10-15 in 2024) were harvested by a combine to determine grain yield, weight was adjusted to 155 g kg^{-1}. In addition to that, yield components were estimated based on a 3 square meter area ear sampling, followed by threshing and kernel counting (Seedburo 801 Count-A-Pak® Seed Counters), and lastly, kernel weight (g) at 155 g kg^{-1} was obtained for each sample to determine thousand kernel weight (g).

Crop growth rate

Crop growth rate (CGR) was calculated for both the critical period (V14-R3) and the grain filling period (R3-R6) by dividing the increase in crop biomass (g m^{-2}) over each period by the thermal time interval between sampling dates. Daily thermal time values were obtained using a base temperature of 8˚C for maize (Ritchie and NeSmith 1991). For the critical period, CGR (g m^{-2} ˚C day^{-1}) was obtained as the quotient between the increase in shoot biomass per square meter (g m^{-2}) from V14 to R3, and the thermal time accumulated over this interval. The crop growth rate per kernel during the grain-filling period (mg ˚C day^{-1} $kernel^{-1}$), a proxy of post-flowering source-sink ratio, was calculated as the ratio between CGR during this period and the number of kernels per square meter.

Sufficiency index

Soil Plant Analysis Development (SPAD) meter (SPAD-502Plus) readings were taken in twenty representative consecutive tagged plants, at four maize growth stages, V6, V10, V14, and R1. Readings were collected on the uppermost fully expanded leaf (collar visible) at each stage, except at R1, where the ear leaf was used (Peterson et al., 1993). The SPAD sensor was placed on the leaf blade, centered between the margin and midrib (Blackmer et al., 1993) and readings were taken around the same time each day to maintain consistency and then, averaged. The N Sufficiency Index (NSI) was calculated as the ratio between a given SPAD reading and the maximum reading recorded across treatments for each sampling stage and experiment (Peterson et al., 1993).

Statistical analysis

Separate analyses were conducted for grain yield and each grain yield component (kernel number and kernel weight). Initial treatment factor analyses were performed first to discern if grain yield and grain yield components responded to N fertilization timing. These initial analyses considered the two experiments and only treatments with in-season N application (T1, T2 and T12 were not used). Simple linear models (Supplementary material - Equation S.1) were fit using the *lm* function of the base *stats* package, all treatment factors, interactions, and block were set as fixed effects. The fitted models were subjected to a type III analysis of variance (ANOVA) for each treatment factor and resulting interactions with the *car* package (Fox and Weisberb, 2019).

Linear mixed effect models (Supplementary material, Equation B.2) were employed with *nlme* package (Pinheiro and Bates, 2000) to assess N rate effect on maize grain yield and its components. Both experiments and all treatments were used. Fixed effects included N rate, experiment, and interaction, while block was considered a random effect. The fitted models were subjected to a type III ANOVA for each factor and resulting interaction. A Sidak test was applied to define significant differences ($p<0.05$) between the treatments using the *muticomp* package (Hothorn et al., 2008)

To investigate the relationships between kernel number and CGR, kernel weight and CGR per kernel, NSI and CGR, and grain yield and SI, simple linear models were fit using the *lm* function of the base *stats* package. Residuals graphs were checked for normality, homoscedasticity, and homogeneity of variance.

Relative CGR and relative NSI were calculated for specific periods, having the 180 kg N ha^{-1} treatment applied at planting as a reference. Lastly, in order to discern if relative CGR and relative NSI for each period responded to N fertilization timing, simple linear models were fit using the *lm* function of the base *stats* package, all treatment factors, interactions, and block were set as fixed effects (similar approach used for testing timing effect on yield). The fitted models were subjected to a type III analysis of variance (ANOVA) for each treatment factor and resulting interactions with the *car* package (Fox and Weisberb, 2019). All the analyses were performed using R software (R Core Team, 2024).

Results

Changes in crop growth rate (CGR) and N status (SPAD)

CGR was significantly influenced by the rate of N fertilization and experiment across all analyzed periods ($p < 0.05$; Supplementary material, Table S3.1). The control exhibited consistently lower CGR values at experiment 1 compared to experiment 2, with an average difference of 10% across all periods Notably, during the V14–R1 period, both the interactions between N rate and experiment, as well as timing and experiment, were statistically significant ($p < 0.05$). Higher N rates were associated with increased CGR in experiment 1, while in experiment 2, CGR levels were similar across all treatments and periods, closely matching the reference treatment (Figure 3.2). Split applications of 90 kg N ha^{-1} at planting followed by in-season applications at, V6, V10, or V14 in 2023 showed a potential for increasing CGR, indicating a response to additional N late in the season. The 120 kg N ha^{-1} rate with 30 kg N ha^{-1} applied at V6, presented approximately 20% lower CGR compared to the reference (average across all periods) (Figure 3.2).

Changes in N status over time were evaluated using the NSI from V6 to R1, with 180 kg N ha^{-1} at planting as the reference (Figure 3.3). Relative NSI was significantly affected by N rate across all stages ($p < 0.05$; Supplementary material, Table S3.2) and by application timing at V6 and V10 ($p < 0.05$). In experiment 1, split applications with 90 kg N ha^{-1} at planting and 30, 60, or 90 kg N ha^{-1} in season indicated potential recovery to a higher N status, even with late applications at V14.

Grain yield and yield components response to N rate and application timing

Grain yield, kernel number and kernel weight were not affected by the timing of N fertilization ($p > 0.05$; Supplementary material Table S3.3). Both grain yield and yield components increased with N fertilization ($p < 0.001$; Table 3.3). Regarding grain yield, experiment 2 exhibited a greater median yield of 15.89 Mg ha^{-1} ($IQR_{25-75} = 15.37 - 16.25$) while experiment 1 had a median yield of 9.73 Mg ha^{-1} ($IQR_{25-75} = 8.77 - 11.12$) (Table 3.2). The experiment 1 control treatment yielded approximately 59% of its experiment 2 counterpart. Notably, the reference treatment with 180 kg N ha^{-1} applied at planting resulted in a 70% higher yield in experiment 2 than in experiment 1.Table 3.2 also contains kernel number and kernel weight data, experiment 1 had a median kernel number of 3370 kernels m^{-2} ($IQR_{25-75} = 3022 - 3737$), and median kernel weight of 244 g 1000 seeds^{-1} ($IQR_{25-75} = 220 - 263$). On the other hand, experiment 2 presented a median kernel number of 4712 kernels m^{-2} ($IQR_{25-75} = 4456 - 5037$), and median kernel weight of 278 g 1000 seeds^{-1} ($IQR_{25-75} = 262 - 294$).

Relationship between crop growth rate (CGR) and yield components

Kernel number showed a positive relationship with CGR (g m^{-2} °C day^{-1}) during the critical period from V14 to R3 (Figure 3.4A). The median CGR for the critical period was 1.78g m^{-2} °C day^{-1} ($IQR_{25-75} = 1.6 - 1.99$), with experiment 2 showing a 21% increase compared to experiment 1. Kernel number had a median of 4,180 kernels m^{-2} ($IQR_{25-75} = 3{,}376 - 4{,}778$), with experiment 2 values being 42% greater than those of experiment 1. A similar pattern was observed when comparing the control and reference treatments. The CGR and kernel number in experiment 2 was double that of experiment 1 for the 0 N treatment. On the other hand, the reference treatment had a 24% higher CGR and a 44% greater kernel number in experiment 2 compared to experiment 1.

Regarding kernel weight (mg) relationship with CGR per kernel (mg ˚C day^{-1} $kernel^{-1}$) during the grain-filling phase, a positive linear relationship was observed (Figure 3.4B). The median CGR for the grain-filling was 0.25mg ˚C day^{-1} $kernel^{-1}$ ($IQR_{25-75} = 0.19 - 0.29$), with experiment 2 showing a 54% increase compared to experiment 1. Kernel weight had a median of 263.20 mg ($IQR_{25-75} = 235.48 - 284.42$), with experiment 2 values being 14% greater than those of experiment 1. The CGR per kernel and kernel weight in experiment 2 was 9% and 4% lower respectively, compared to experiment 1 for the 0 N treatment. On the other hand, the reference treatment had an 84% higher CGR per kernel and a 16% heavier kernel weight in experiment 2 compared to experiment 1.

Relationship between N status, crop growth rate (CGR), and yield

A positive linear relationship was observed between NSI at flowering (R1) and CGR during the critical period for grain yield determination (V14-R3) (Figure 3.5A). The median NSI at the R1 stage was 0.92 ($IQR_{25-75} = 0.83 - 0.95$), with experiment 2 showing a 10% increase compared to experiment 1. Comparing both experiments, experiment 1 showed a wider range of NSI values. Control had a median NSI of 0.65 ($IQR_{25-75} = 0.56 - 0.68$), on the other hand, reference treatment (180 kg N ha^{-1} applied at planting) exhibited a median of 0.94 ($IQR_{25-75} = 0.88 - 0.96$).

Regarding maize grain yield and NSI at the R1 stage relationship, a positive trend was observed (Figure 3.5B), with the dependent variables explaining 71% of the total variance in grain yield. Lower values of NSI at R1 were highly linked to yield penalization. For experiment 2, only the 0 and 90 kg N ha^{-1} applied at planting showed values of NSI lower than 0.90.

Discussion

Our findings provide insights on the ability of the maize to tolerate temporary and early-season N deficiency (lower NSI values) without any yield penalty, if plant N status is timely adjusted. Optimal N rate fluctuates year to year and environments, and utilization of in-season N technology can assist to better target crop N needs (Dhital and Raun, 2016). Well-distributed and sufficient rainfall increases plant growth and N demand, thereby increasing yield response N applications mainly in fine-textured soils (Tremblay et al., 2012). For increasing yields, delaying N applications without inducing severe N deficiency cannot only help to maximize productivity but to reduce N inputs (Raun and Johnson, 1999). From an environmental perspective, identifying effective N management strategies is essential for sustainable maize production (Ciampitti et al., 2022). By maximizing N capture and uptake efficiency per unit of N applied, we could increase profitability for farmers while reducing N inputs. Ultimately, contributing to a reduced N footprint, a critical global objective for enhancing agricultural sustainability and for a societal benefit (Goodkind et al., 2023; Tamagno et al., 2022)

Severe N deficiencies with substantial reductions in maize crop growth rate (CGR) lead to yield penalties (Gastal et al., 2015; Vega et al., 2001). For the in-season N interventions, if early-season plant N deficiency is not severe then N applications can be delayed still restoring maximum plant growth to attain maximum yields (Binder et al., 2000; Varvel et al., 1997). Scharf et al. (2002) reported similar findings, observing only a 3% yield loss when N application was delayed until the V16 stage. These results suggest a flexible management approach where strategic in-season N applications can offset early N deficiencies and sustain yield, even with a reduced initial N input (Ciampitti and Vyn, 2012). However, it is noteworthy mentioning that as early-season plant N deficiency is more severe, enough to prevent recovery of the sufficiency plant N status, the earlier N needs to be applied in order to correct this situation to avoid a yield penalty (Binder et al., 2000). Therefore, it is evident that for in-season N to be a tactical effective intervention, improved near-real time diagnosis tools focusing on plant N status are highly needed.

From a plant physiology perspective, N deficiencies decrease CGR and increase overall variation (inter-plant competition) of plant biomass during the vegetative period (Ciampitti and Vyn, 2011; Rossini et al., 2011). Past studies already demonstrated the negative effect of N deficiency on the definition of kernel number (McCullough et al., 1994a, 1994b; Uhart and Andrade, 1995b), mainly linked to reductions in GGR around flowering (as reported on this study) and for the overall partitioning to reproductive organs (Andrade et al., 2002; D'Andrea et al., 2008; Rossini et al., 2011). The effect on kernel weight has been reported to be mainly linked to the duration rather than the rate of the grain filling when the crop is exerted under different stressor such as shading (Tanaka and Maddonni, 2009), defoliation (Sala et al., 2007), and water (NeSmith and Ritchie, 1992). However, under severe N deficiency kernel weight might be reduced due to a similar effect in both duration and rate (Jones et al., 1996). In overall, similarly to our study, kernel weight was associated in a positive relationship with CGR during the grain-filling stage, with greater plant growth linked to heavier kernels (Gambín et al., 2008). The latter scenario suggests that while maize may tolerate temporary N deficiencies early in the season, maintaining adequate N levels during grain filling is essential for optimizing kernel weight and achieving maximum yield.

We acknowledge the limitations of our study, although we examined a substantial range of N rates and fertilization timings, our findings are limited due to the number of genotypes tested (influence of hybrid variation; D'Andrea et al., 2006). Future research should expand on testing advanced N sensing technologies, particularly innovative proximal and remote sensing methods, which can provide near real-time insights into plant N status throughout the growing season. Such approaches would enable more precise, stage-specific management decisions, enhancing crop performance while addressing the critical goal of reducing agriculture's N footprint.

Conclusion

This study highlights the potential for maize to tolerate early-season and temporary N deficiencies without yield penalties, provided that plant N status is promptly adjusted during the growing season. It also offers insights into the impact of N stress on crop growth, plant N status during the vegetative period, and yield components, highlighting the importance of timely N management. Our findings underscore the importance of in-season N fertilization strategies to optimize crop growth and yield, particularly in response to environmental variability. These findings reinforce the need for sustainable N management practices that align N availability with crop N demand, reducing the risk of overapplication and minimizing N footprints, shifting away from the traditional mindset of applying N rates as a form of "insurance" against potential yield losses. Future efforts should prioritize advanced N sensing technologies, such as proximal and remote sensing tools, to enable precise, stage-specific N management. This approach is essential for enhancing N management while addressing the urgent need to reduce agriculture's N footprint and support sustainable food production systems.

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Figures

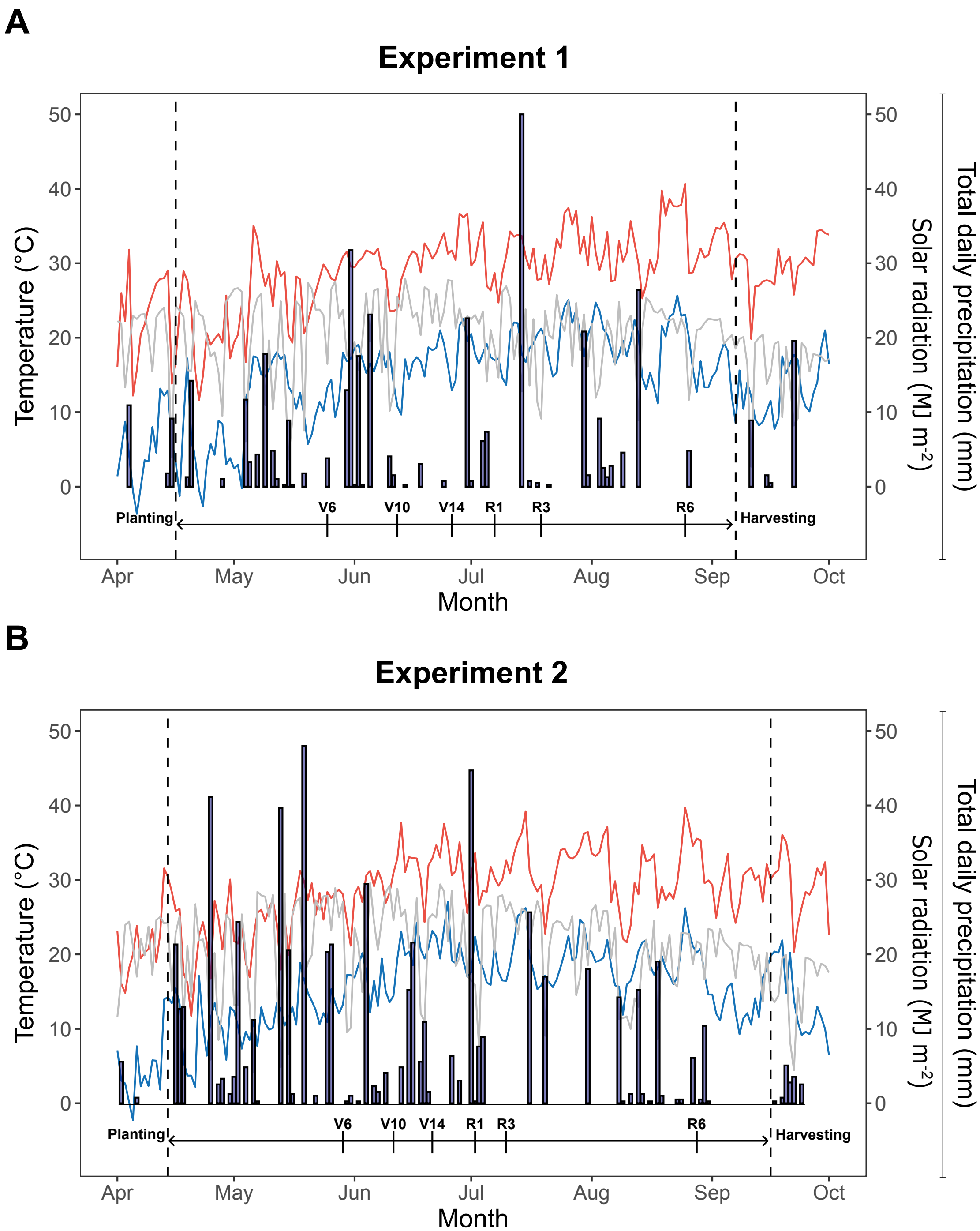

Figure 3.1. Daily maximum (red line) and minimum (blue line) air temperature (°C), solar radiation (MJ m^{-2}, grey line), and total daily precipitation (mm, blue bars) from April 1st to October 1st for (A) Experiment 1 (2023 at Kansa River Valley Experimental Field) and (B) Experiment 2 (2024 at Junction City, KS). Dashed vertical lines indicate planting and harvesting dates.

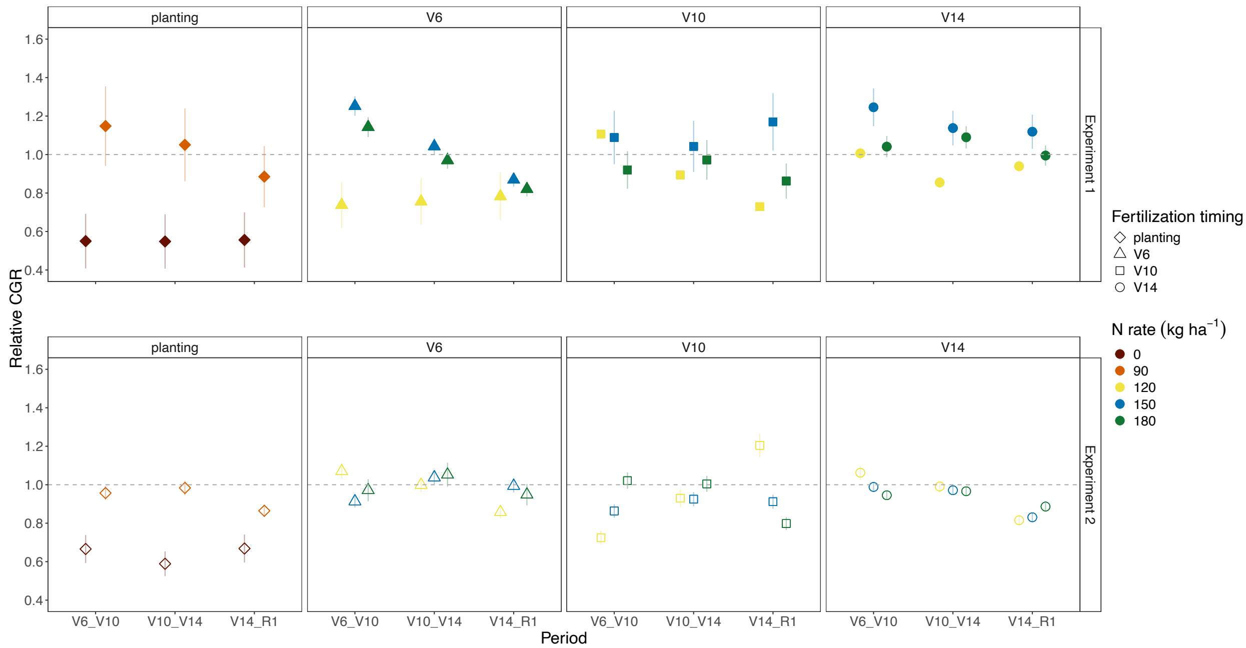

Figure 3.2. Relative crop growth rate (CGR) across three periods, V6 to V10, V10 to V14, and V14 to R1 for each N rate, timing, and experiment combination. Colors refer to N rate (brown – 0, orange – 90, yellow – 120, blue – 150, and green – 180 kg N ha^{-1}), and fertilization timing is differentiated by shape (diamond – planting, triangle – V6, square – V10, circle – V14). Top panel corresponds to experiment 1, while bottom panel corresponds to experiment 2. The treatment with 180 kg N ha^{-1} applied at planting serves as the reference level for comparison (dashed line, y = 1). The error bar represents the standard error.

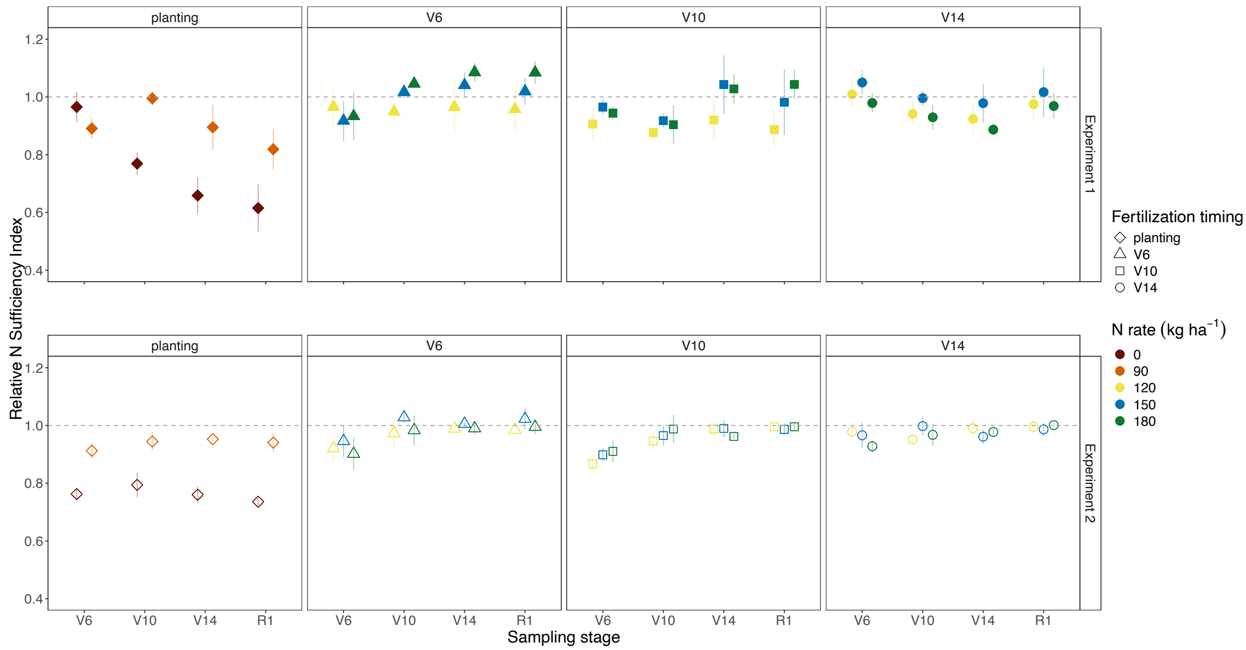

Figure 3.3. Relative N Sufficiency Index (NSI) at different maize growth stages (V6, V10, V14, and R1) for each N rate, timing, and experiment combination. Colors refer to N rate (brown – 0, orange – 90, yellow – 120, blue – 150, and green – 180 kg N ha^{-1}), and fertilization timing is differentiated by shape (diamond – planting, triangle – V6, square – V10, circle – V14). Top panel corresponds to experiment 1, while bottom panel corresponds to experiment 2. The treatment with 180 kg N ha^{-1} applied at planting serves as the reference level for comparison (dashed line, y = 1). The error bar represents the standard error.

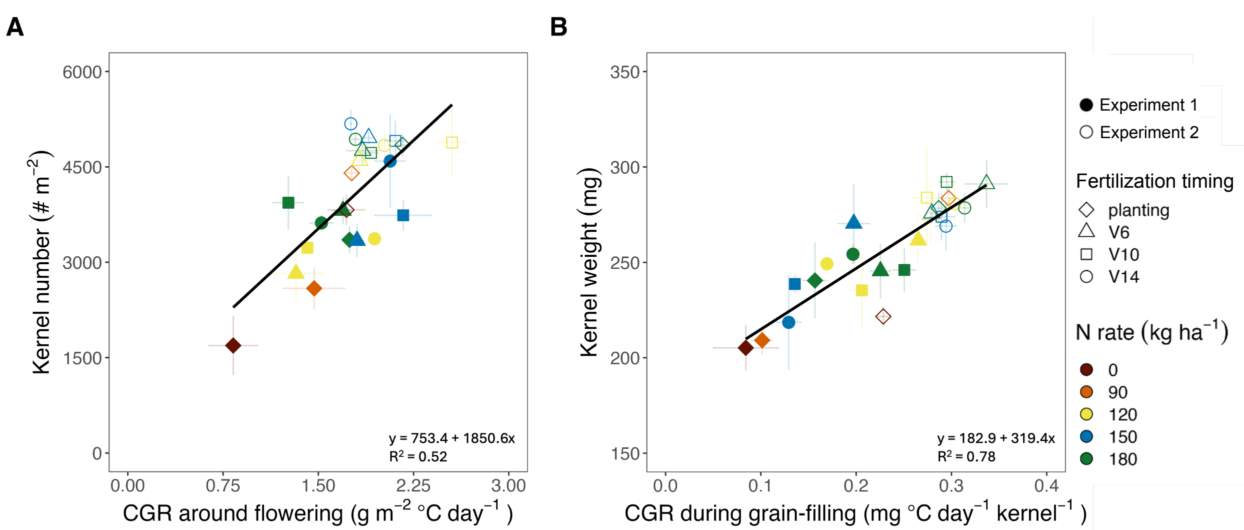

Figure 3.4. (A) Relationship between kernel number (# m^{-2}) and crop growth rate (CGR, g m^{-2} ˚C day^{-1}) around flowering (V14-R3 stage). (B) Relationship between kernel weight (mg) and CGR (mg ˚C day^{-1} kernel^{-1}) during grain filling (R3-R6 stage). Colors refer to the N rate,

fertilization timing is differentiated by shape, and the fill of the shapes (filled – experiment 1 and empty – experiment 2) distinguishes between the two experiments. Solid black lines represent the fitted linear regressions. Error bars for each observation (x- and y-axes) represent the standard error.

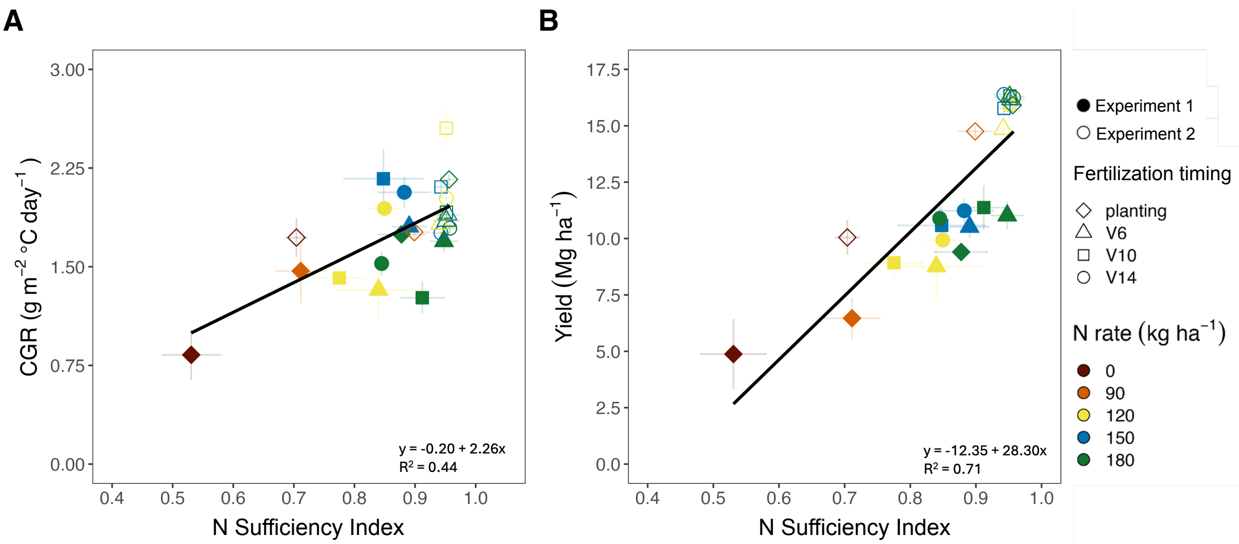

Figure 3.5. (A) Relationship between N Sufficiency Index (NSI) at flowering and crop growth rate (CGR, g m^{-2} °C day^{-1}) from V14 to R3. (B) Relationship between maize yield (Mg ha^{-1}) and NSI at flowering. Colors refer to the N rate, fertilization timing is differentiated by shape, and the fill of the shapes (filled – experiment 1 and empty – experiment 2) distinguishes between the two experiments. Solid black lines represent the fitted linear regressions. Error bars for each observation (x- and y-axes) represent the standard error.

Tables

Table 3.1. Treatment structure and rate of N applied, timing of fertilizer application, and total N rate used for both experiments.

	Timing of N application				
	planting	V6	V10	V14	
Treatment	kg N/ha applied				Total N rate (kg/ha)
T1	0	0	0	0	0
T2	90	0	0	0	90
T3	90	30	0	0	120
T4	90	0	30	0	120
T5	90	0	0	30	120
T6	90	60	0	0	150
T7	90	0	60	0	150
T8	90	0	0	60	150
T9	90	90	0	0	180
T10	90	0	90	0	180
T11	90	0	0	90	180
T12	180	0	0	0	180

Table 3.2. Pre-plant soil analysis values [Nitrate-N, organic matter content (OM), soil pH, potassium (K), and phosphorus Mehlich P-III (P)], in the uppermost 0.3 m of the soil profile, and phenological data [six leaves stage (V6), ten leaves stage (V10), fourteen leaves stage (V10), silking (R1), milk stage (R3), and physiological maturity (R6)], dates of planting and harvesting. The mean ± standard deviation (SD) is reported for each variable.

	Experiment 1	Experiment 2
SOIL		
Variable	Mean + SD	
Clay (%)	14.2 + 0.5	37.8 + 2.7
Sand (%)	49.5 + 4.6	3.8 + 2.3
Silt (%)	36.5 + 4.6	58.2 + 2.8
Nitrate-N (ppm)	4.6 + 1.6	5.0 + 1.3
OM (%)	1.5 + 0.1	3.3 + 0.2
pH units	6.8 + 0.2	7.0 + 0.1
K (ppm)	130.6 + 14.6	257.5 + 36.4
P (ppm)	49.6 + 8.9	92.5 + 15.1
PHENOLOGY		
Variable	Date (mm/dd/yy)	
Planting date	04/16/23	04/14/24
V6 stage	05/25/23	05/29/24
V10 stage	06/12/23	06/11/24
V14 stage	06/26/23	06/21/24
R1 stage	07/07/23	07/02/24
R3 stage	07/19/23	07/10/24
R6 stage	08/25/23	08/28/24
Harvesting date	09/07/23	09/16/24

Table 3.3. Maize grain yield (Mg ha^{-1}), kernel number (# m^{-2}), and a thousand kernel weight (g) for each N rate, timing, and experiment combination. Values represent the mean of estimated marginal means, and letters represent Tukey test results ($p < 0.05$), N rate and experiment interaction for grain yield and kernel weight, and N rate within experiment for kernel number.

Treatment	Grain yield		Kernel Number		Kernel weight	
N rate kg ha^{-1}	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2
0	4.9 C	10.0 B	1691 D	3825 B	235 BCD	222 D
90	6.5 C	14.7 A	2589 CD	4402 AB	209 D	284 ABC
120	9.2 B	15.6 A	3140 BC	4773 AB	249 BCD	280 AB
150	10.7 B	16.1 A	3890 A	4814 A	243 CD	273 ABC
180	10.6 B	16.2 A	3680 AB	5015 A	247 CD	285 A
Source of Variance	p-value		p-value		p-value	
N rate	***		***		***	
Experiment	***		***		ns	
N rate x experiment	*		ns		*	

***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; ns: not significant.

Chapter 4 - Final remarks

Optimizing N fertilizer management remains a critical challenge for sustainable maize production, with significant implications for enhancing both agronomic performance and environmental stewardship. This research provides valuable insights to guide future advancements in agronomic and environmental strategies aimed at improving the use of N fertilizer in maize cropping systems. It expands current knowledge by identifying different types of yield-N status relationships and uncovering the main drivers shaping these response curves. Additionally, it explores various in-season N strategies and their effects on crop growth rate (CGR), leaf area index (LAI), and plant N status. Lastly, it’s important to highlight the contribution of this research is the assessment of maize's ability to tolerate mild N deficiencies without incurring yield penalties, offering practical implications for more sustainable N management practices.

In Chapter 2, three distinct relationships between relative yield and N status (NNI) were identified. The majority of cases exhibited a linear-plateau response (60%), followed by a linear response (27%) and no response (13%). Regarding the main soil and weather variables driving these relationships, pre-plant nitrate-N emerged as a key factor in distinguishing non-responsive from responsive experiments, with higher pre-plant soil nitrate-N levels associated with non-responsive scenarios. Additionally, the distribution (SDI) and quantity of precipitation (CPP) from late vegetative stage to the end of the season were critical factors, collectively accounting for approximately 50% of the relative variable importance. Gaining a deeper understanding of the possible types of responses and the primary drivers behind each is essential for making informed in-season nitrogen management decisions.

In Chapter 3, we reviewed different in-season N strategies and their effects on key crop parameters, focusing on maize's ability to tolerate mild early-stage N deficiencies without compromising yield. Our results highlighted the ability of maize to recover its N status by late season N fertilization as measured by a SPAD meter. Treatments receiving 90 kg N ha^{-1} at planting, followed by late season N applications at V14, successfully recovered from lower N status to levels comparable to the reference treatment (180 kg N ha^{-1} applied entirely at planting). Most importantly, this recovery occurred without any yield losses.

Future research should prioritize the development of proximal and remote sensing approaches to monitor crop N status throughout the growing season. These methods can provide critical, real-time information to enable timely and precise N recommendations, improving decision-making for both N application rates and timing. The creation of decision-support models that integrate real-time N status data will be pivotal in addressing the dual challenge of increasing yields while reducing the environmental footprint of N fertilization. Lastly, upscaling from research plots to farmer fields will be critical to work assess the implementation of this technology for accounting for within field variation and developing timely interventions. As advancements in technology continue to accelerate and become more accessible to farmers, these innovations will play a key role in solving the long-standing N management puzzle, a challenge that has been the focus of extensive research for decades.

Appendix A - Chapter 2 Supplementary Material

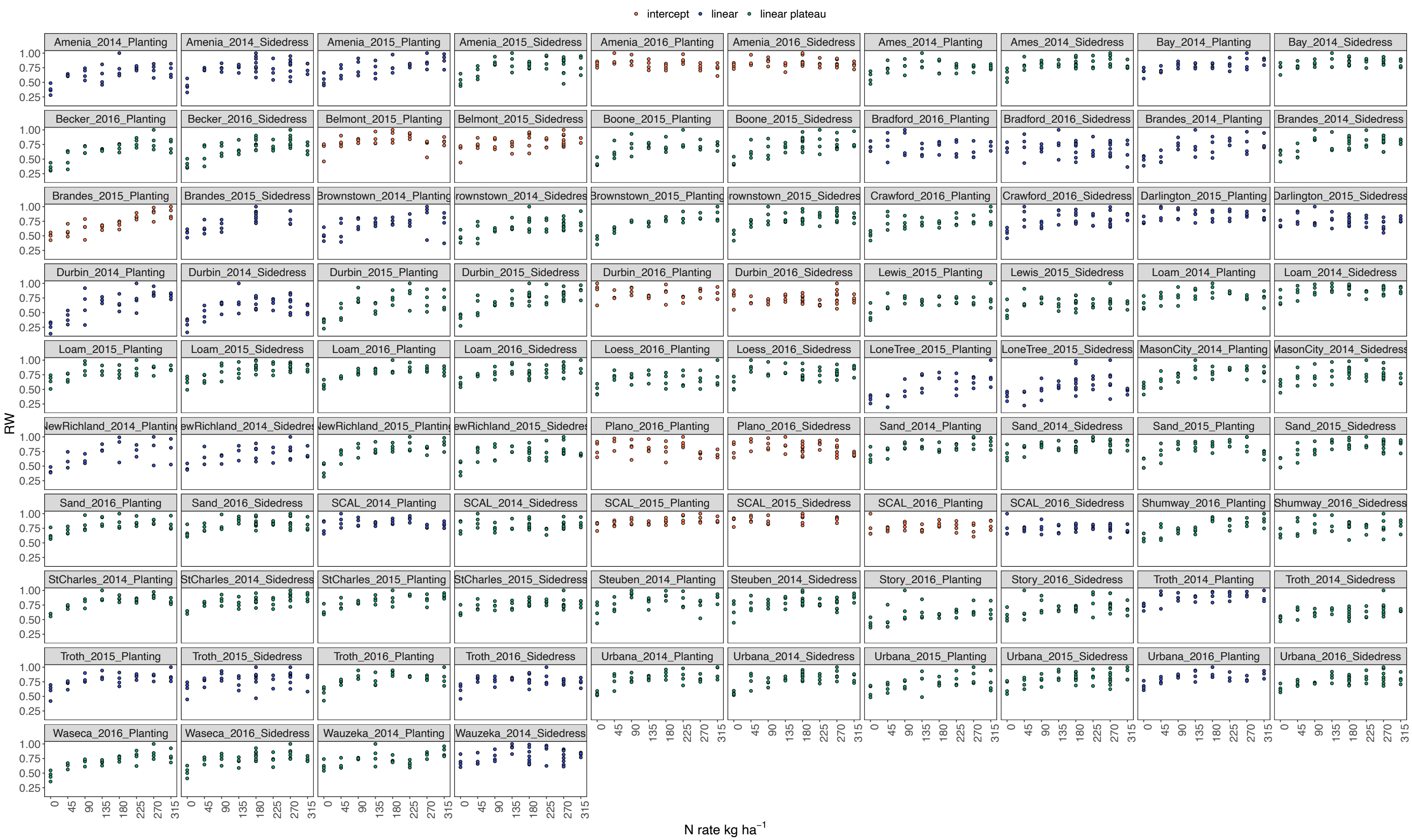

Figure S2.1. Relationship between relative biomass at VT stage (RW) and N rate (kg ha^{-1}) for each experiment (site-year-timing combination). Colors refer to each type of RY-NNI response (intercept – no-response, linear, and linear-plateau).

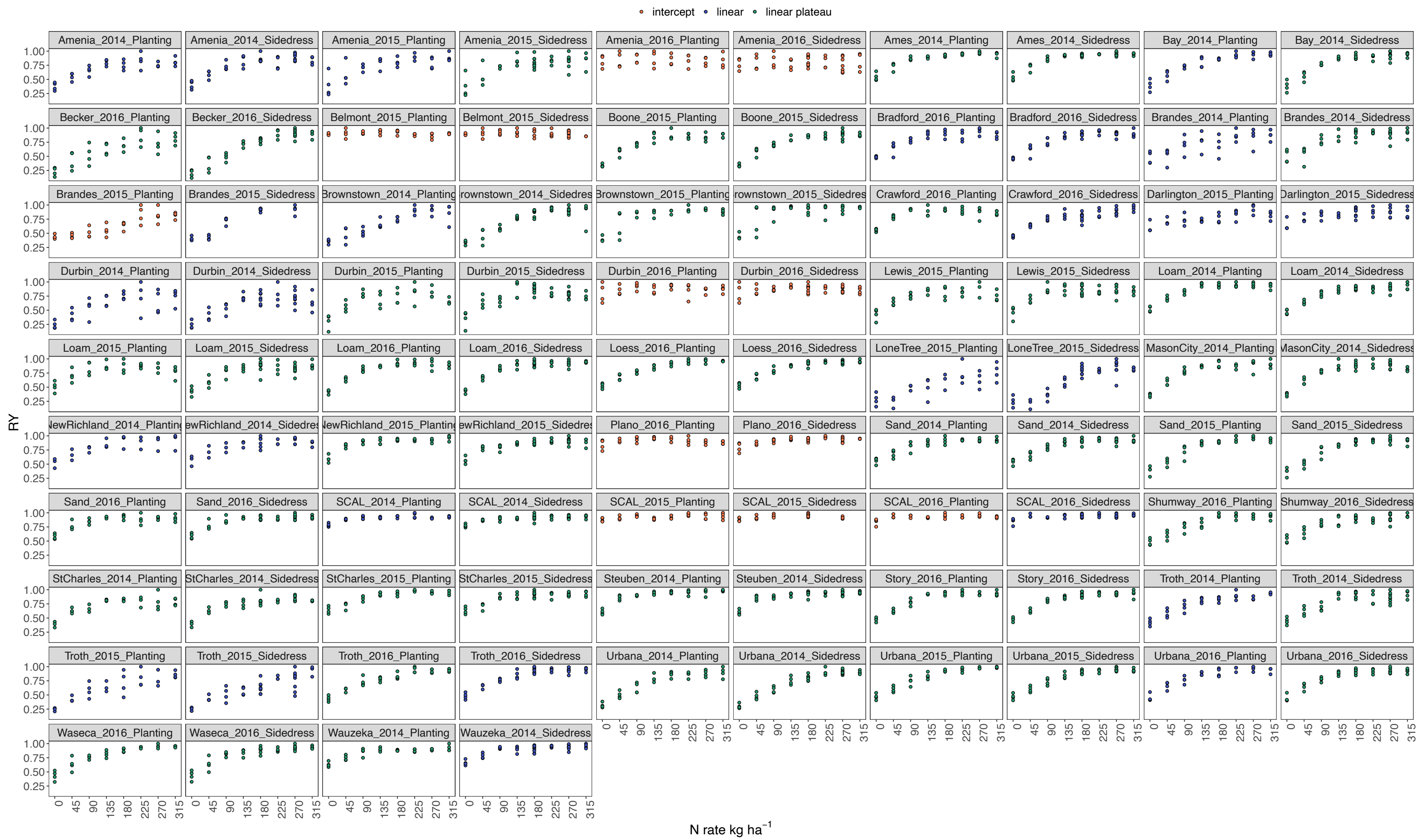

Figure S2.2. Relationship between relative yield (RY) and N rate (kg ha^{-1}) for each experiment (site-year-timing combination). Colors refer to each type of RY-NNI response (intercept – no-response, linear, and linear-plateau).

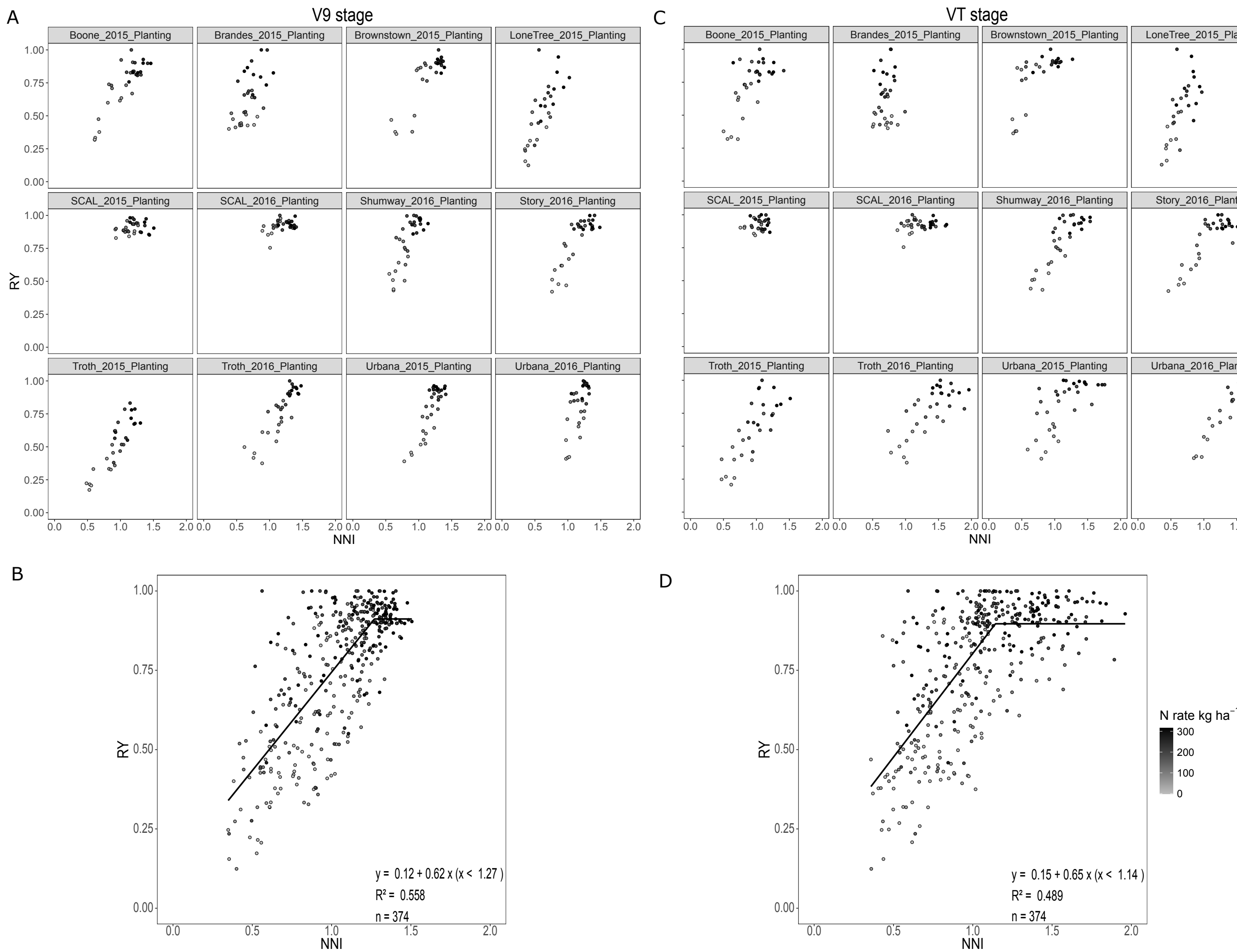

Figure S2.3. Relationship between RY and NNI across experiments with available V9 and VT data. (A and B – V9 growth stage; C and D – VT growth stage). The top panels show the relationship for each experiment, and the bottom panels pool data for each growth stage. Colors refer to N rate.

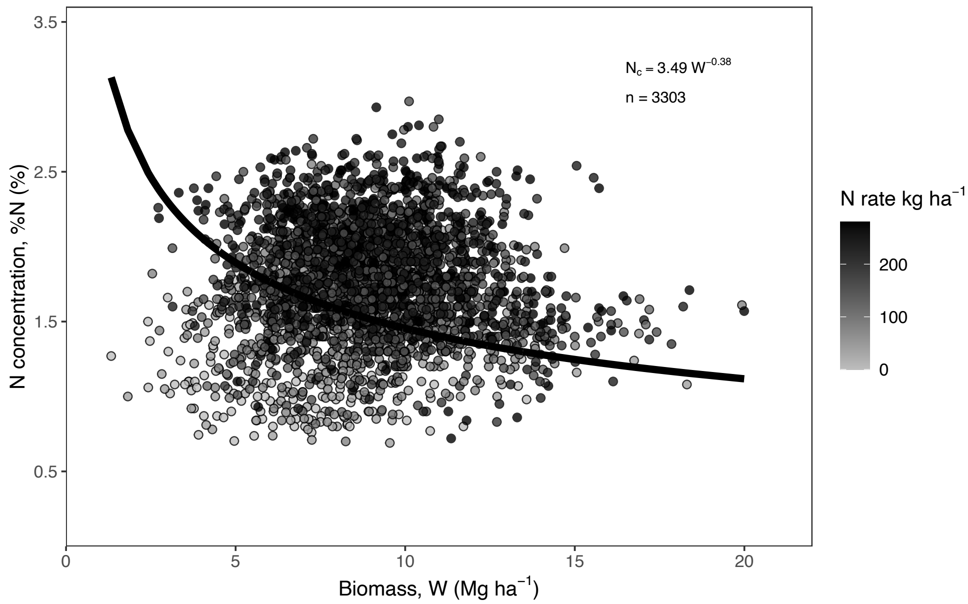

Figure S2.4. Relationship between plant N concentration (N, %) and aboveground biomass (W, Mg ha^{-1}) for maize at the VT stage based on the critical N dilution curve fitted by Ciampitti et al. (2021). Colors refer to N rate (0-315 kg N ha^{-1}).

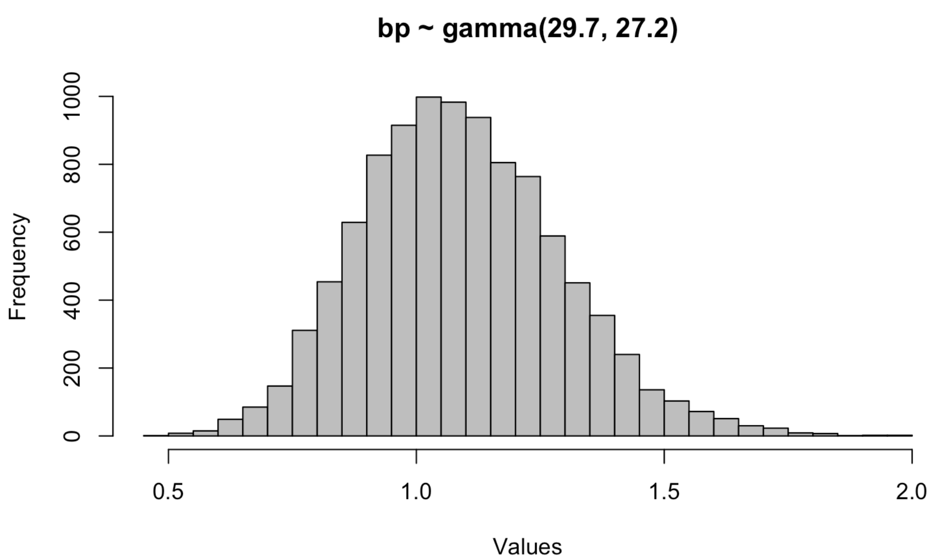

Figure S2.5. Visualization of the gamma distribution used as the prior for the breakpoint in the RY-NNI relationship.

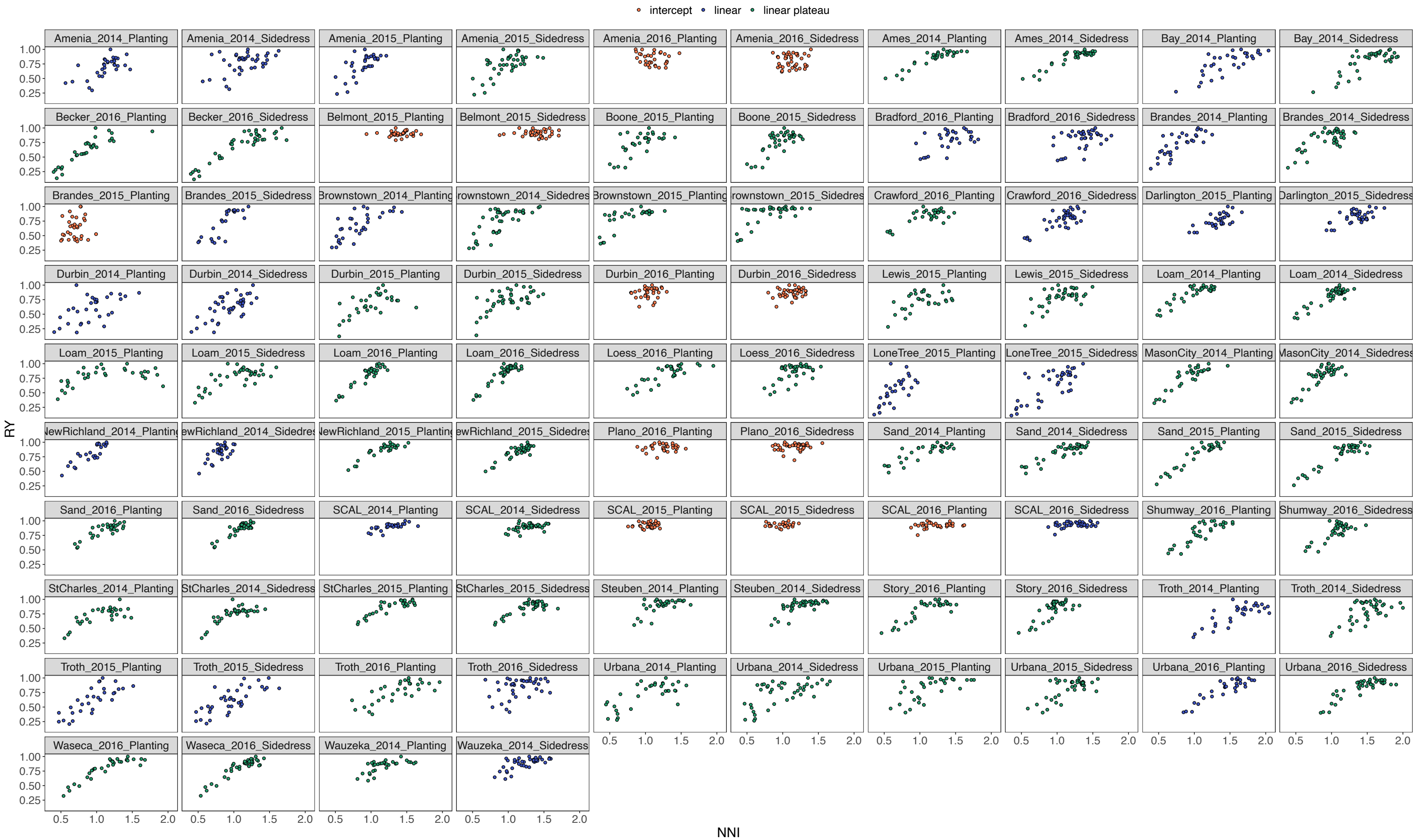

Figure S2.6. Relationship between relative yield (RY) and N nutrition index (NNI) for each experiment (site-year-timing combination). Colors refer to each type of RY-NNI response (intercept – no-response, linear, and linear-plateau).

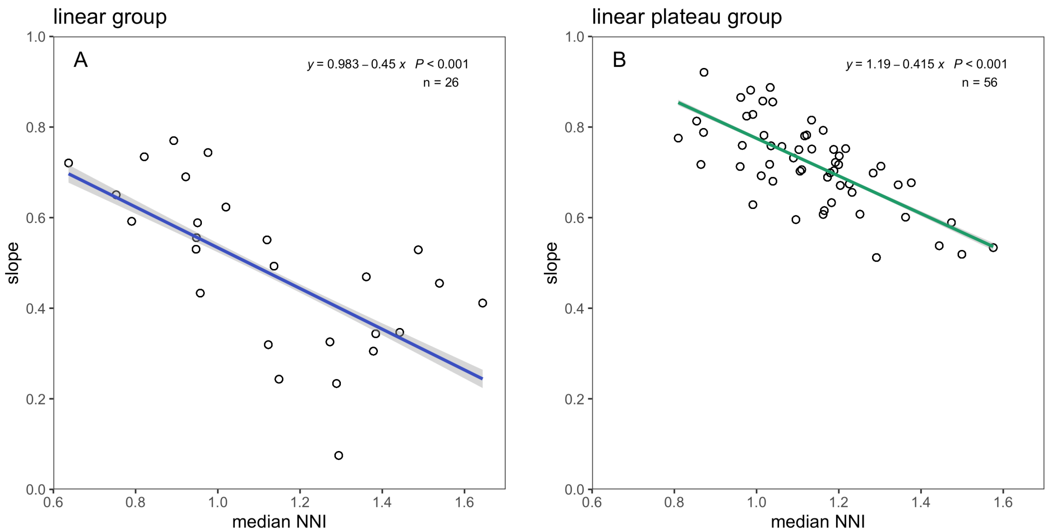

Figure S2.7. Relationship between slope and median N nutrition index (NNI) for the linear (A) and the linear-plateau (B) response groups.

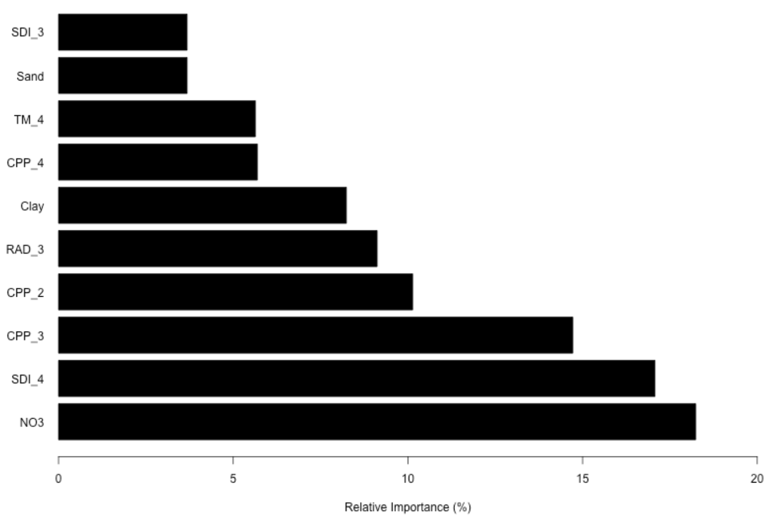

Figure S2.8. Relative variable importance (%) for the top 10 predictors included in the fitted model. NO3 – pre-planting nitrate-N, SDI_4 – Shannon Diversity Index from VT to R6, CPP_3 – cumulative precipitation from V9 to VT, CPP_2 – cumulative precipitation from planting to V9, RAD_3 – Incident radiation from V9 to VT, Clay – Clay content, CPP_4 – Cumulative precipitation from VT to R6, TM_4 – Mean temperature from VT to R6, Sand – Sand content, SDI_3 – Shannon Diversity index from V9 to VT.

Appendix B - Chapter 3 Supplementary Material

Equation B.1

$$y_{ijkr} = \alpha_i + \gamma_j + \delta_k + \beta_r + (\alpha\gamma)_{ij} + (\alpha\delta)_{ik} + (\gamma\delta)_{jk} + (\alpha\gamma\delta)_{ijk} + \varepsilon_{ijkr},$$

$$\varepsilon_{ijk} \sim N(0, \sigma^2).$$

Where:
y_{ijkr} is the observed yield for the i^{th} N rate applied in the k^{th} timing in the r^{th} block in the j^{th} experiment;
α_i is the main effect of the i^{th} level of N rate;
γ_j is the main effect of the j^{th} level of experiment;
δ_k is the main effect of the k^{th} level of timing;
β_r is the effect of the r^{th} of block;
All the combinations of α_i, γ_j, and δ_k indicate double and triple interactions between the different factors;
ε_{ijk} is the residual error;

Equation B.2

$$y_{ijr} = \alpha_i + \gamma_j + \alpha_i\gamma_j + \beta_r + \varepsilon_{ijr},$$

$$\beta_r \sim N\left(0, \sigma_\beta^2\right),$$

$$\varepsilon_{ijr} \sim N(0, \sigma_\varepsilon^2).$$

Where:
y_{ijr} is the observed yield for the i^{th} N rate applied in the r^{th} block in the j^{th} experiment;
α_i is the main effect of the i^{th} level of N rate;
γ_j is the main effect of the j^{th} level of experiment;
β_r is the random effect of the r^{th} level of block;
$\alpha_i\gamma_j$ indicate interaction between N rate and experiment;
ε_{ijr} is the residual error;

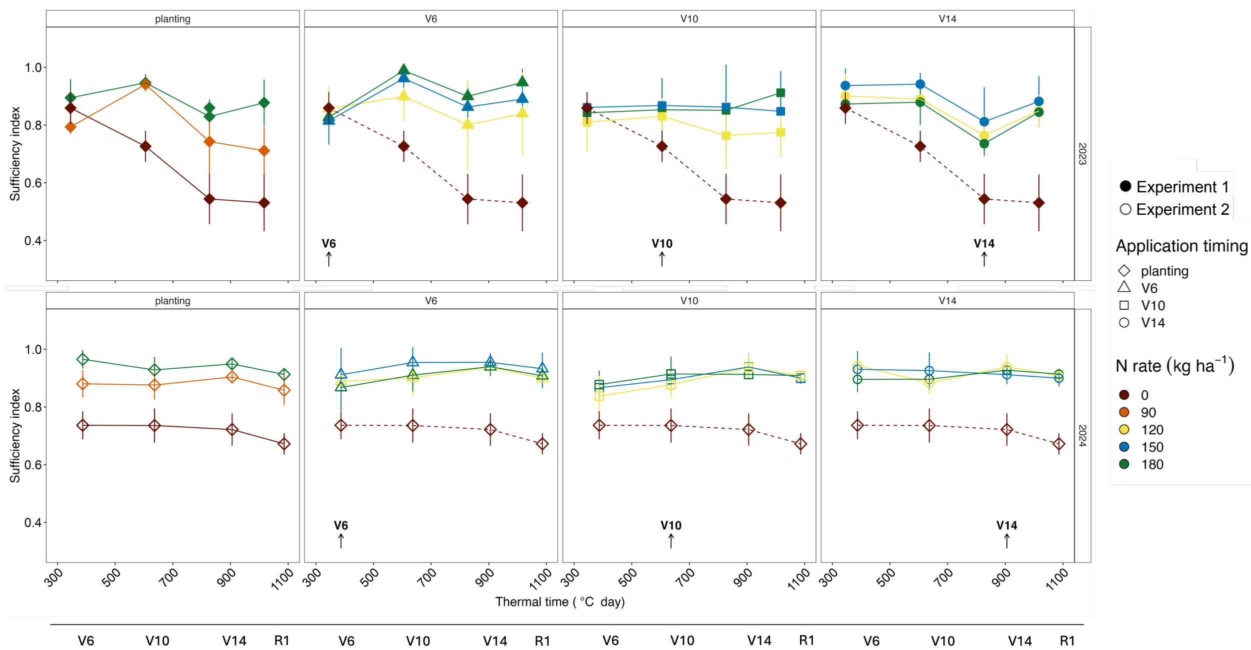

Figure S3.1. N Sufficiency Index (NSI) over time for each treatment and both experiments. Colors refer to the N rate, application timing is differentiated by shape, and the fill of the shapes (filled for experiment 1 and empty for experiment 2) distinguishes between the two experiments. Dashed lines within the V6, V10, and V14 panels represent the control treatment (0 N). Arrows indicate timing when N fertilizer was applied in-season.

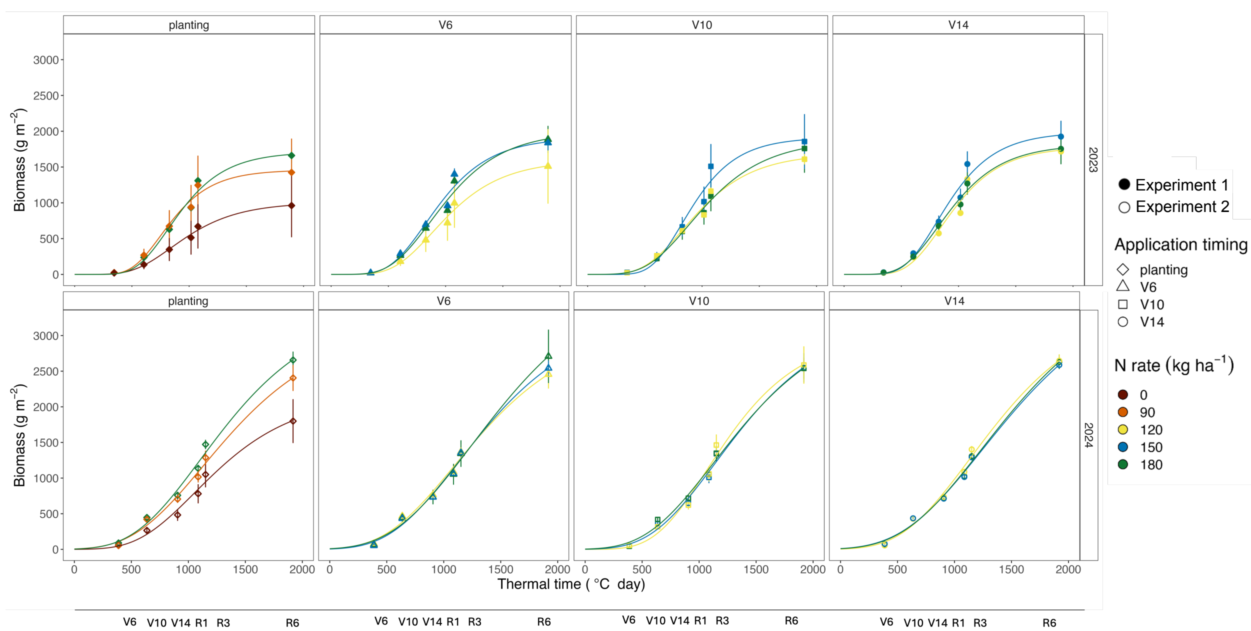

Figure S3.2. Biomass (g m^{-2}) evolution for each treatment and experiment. Colors refer to the N rate, application timing is differentiated by shape, and the fill of the shapes (filled for experiment 1 and empty for experiment 2) distinguishes between the two experiments.

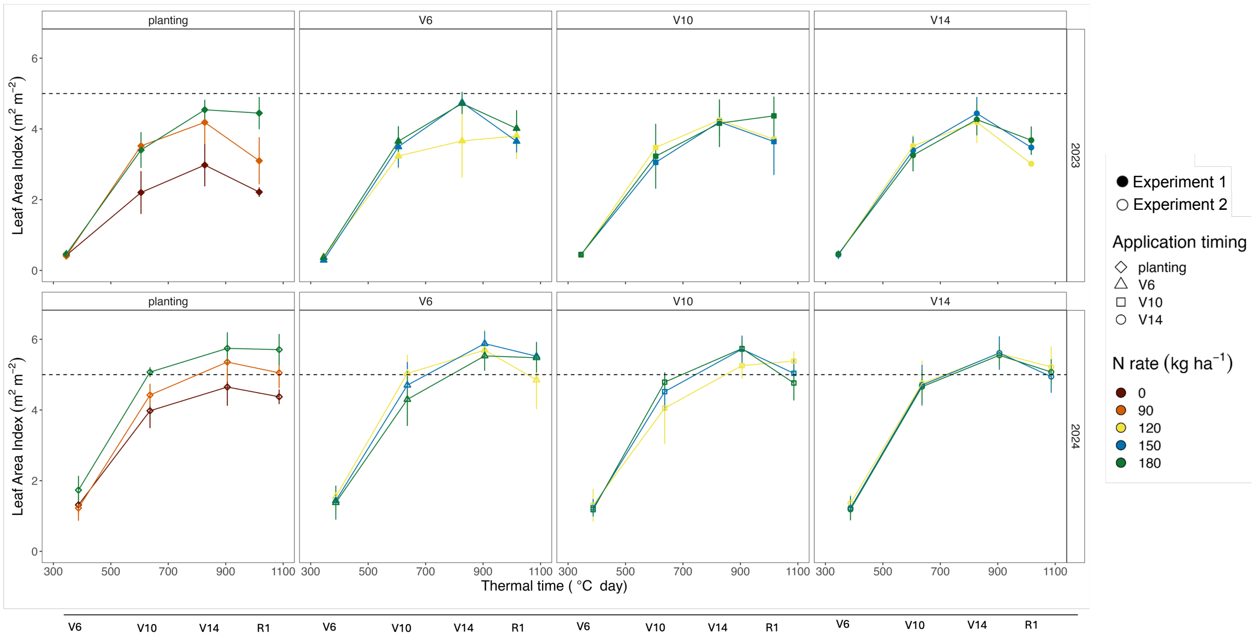

Figure S3.3. Leaf area index (LAI, m^2 m^{-2}) over time for each treatment and both experiments. Colors refer to the N rate, application timing is differentiated by shape, and the fill of the shapes (filled for experiment 1 and empty for experiment 2) distinguishes between the two experiments. Dashed horizontal lines represent the critical LAI (5 m^2 m^{-2}).

Table S3.1. ANOVA results for relative crop growth rate given treatments factors N rate (3 levels), timing (V6-V10 – 1 level, V10-V14 – 2 levels, V14-R1 – 3 levels), experiment (2 levels), and interactions for each period. Tested source of variance and p-values are presented.

Source of Variance	Period		
	V6 - V10	V10 - V14	V14 - R1
N rate	***	***	***
Timing	ns	ns	ns
Experiment	***	**	*
N rate x timing	ns	ns	ns
N rate x experiment	ns	*	*
Timing x experiment	ns	ns	*
N rate x timing x experiment	ns	ns	ns

***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; ns: not significant

Table S3.2. ANOVA results for relative N Sufficiency Index given treatments factors N rate (3 levels), timing (V6 – 1 level, V10 – 2 levels, V14 – 3 levels, V14 – 3 levels), experiment (2 levels), and interactions for each sampling stage. Tested source of variance and p-values are presented.

Source of Variance	Stage			
	V6	V10	V14	R1
N rate	***	***	***	***
Timing	*	**	ns	ns
Experiment	ns	ns	ns	ns
N rate x timing	ns	ns	ns	ns
N rate x experiment	ns	ns	ns	ns
Timing x experiment	ns	ns	ns	ns
N rate x timing x experiment	ns	ns	ns	ns

***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; ns: not significant

Table S3.3. ANOVA results for maize grain yield (Mg ha^{-1}), kernel number (# m^{-2}), and a thousand kernel weight (g) given treatments factors N rate (3 levels), timing (3 levels), experiment (2 levels), and interactions. Tested source of variance and p-values are presented.

Source of Variance	Grain yield	Kernel number	Kernel weight
N rate	***	***	***
timing	ns	ns	ns
Experiment	***	***	ns
N rate x timing	ns	ns	ns
N rate x experiment	ns	ns	ns
timing x experiment	ns	ns	ns
N rate x timing x experiment	ns	ns	ns

***$p < 0.001$; ** $p <0.01$; *$p < 0.05$; ns: not significant