

Nitrogen rate, timing, and fungicide interactions for corn production and assessment of plant
nutrient analysis

by

Andrew John Stammer

B.S., Iowa State University, 2011
M.S., Iowa State University, 2015

AN ABSTRACT OF A DISSERTATION

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Department of Agronomy
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Abstract

Ensuring appropriate crop nutrition is essential to optimize productivity and minimize environmental impact. To achieve this, we assessed the effect of nitrogen (N) application techniques during the early vegetative and early reproductive stages of corn (*Zea mays*) and interactions with the use of fungicides. New corn hybrids' yield potential and stay-green characteristics may benefit from late N fertilizer applications. Late N applications can also include foliar N applications in combination with management practices such as late fungicide applications. This combination of late N and fungicide may benefit corn and contribute to “stay green” conditions until much later in the grain filling period, contributing to yield. Our study evaluating interactions of N rate, timing, and fungicide found that delayed N fertilizer application of a significant portion of the total N fertilizer to vegetative stages may be too late in some cases and result in yield penalty. Late N application was beneficial, but only when initial fertilizer rates were sufficient to meet corn growth and development needs until the reproductive stages. The overall response to fungicides was significant for specific site years, showing likely driven by given conditions favoring the development of the disease.

Another study compared a range of N fertilizer rates and timings for corn. The experiment was conducted with a zero N control and a range of five N rates. The Kansas State University recommendation was used for each study site; plus and minus increments of 28 and 56 kg N/ha were established, either all at planting to V4 or 60% at planting and 40% at the VT growth stage. The Kansas State University nitrogen recommendations were not low enough to reduce yield, even at high-yielding sites. Therefore, the site-specific recommendation is likely at least 56 kg N/ha over the optimum agronomic rate for a specific location, either pre-plant or split-applied.

Analysis of plant nutrient content is a common diagnostic tool to assess plant nutritional status. We evaluated different methods and determined that dry ashing and nitric perchloric digestions gave similar results and repeatability for several nutrients. However, a new closed tube method had a lower recovery, likely due to lower digestion temperatures and less complete reaction than the established methods and other similar methods that have proven successful.

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Dorivar Ruiz Diaz

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CHAPTER 1 - GENERAL INTRODUCTION

Plant nutrition is essential to producing the food, feed, fiber, and fuel our society depends on. This dissertation builds on centuries of previous research in this area to expand knowledge of impacting and measuring crop nutrition both in the field and lab. This work seeks to evaluate how late-season nitrogen application might impact the production of corn in Kansas, and also evaluate the need for nitrogen application as both an agronomic and environmental consideration. Late-season nitrogen applications using high-clearance self-propelled sprayers are an emerging tool that warrants further exploration. These methods can address nitrogen deficiencies that emerge because of higher-than-normal rainfall, weather delays during traditional periods of fertilizer application, or misapplication of nutrient sources. Plant tissue analysis is a tool used for in-season evaluation of plant nutrient status. An evaluation of common methods will help evaluate similarities and differences between methods.

Dissertation organization

This dissertation contains five chapters. Chapter 1 is a general introduction with an overview of the following chapters. Chapter 2, titled “Nitrogen use efficiency in Corn with split nitrogen application and late fungicides, " explores the physiological impacts of late-applied nitrogen fertilizer on the soil and plant and interactions with foliar fungicide application. Chapter 3 evaluates late applied N fertilizer to corn and is titled “Can split applied nitrogen fertilizer at the late vegetative growth stage impact the optimum nitrogen rate?”. In Chapter 4, the focus shifts from field fertilizer applications to lab methods related to the digestion of plant material. The title of Chapter 4 is “Assessment of micro-scaled digestion of plant materials for plant tissue analysis”. Chapter 5 contains general conclusions.

In Chapter 2 the work focuses on emerging late nitrogen applications to the corn crop, fungicide, and foliar fertilizer treatments. Changes in corn hybrids with higher yield potential and stay-green characteristics can benefit from late nitrogen (N) fertilizer applications. Approximately 20% of the total nitrogen uptake by corn plants occurs after the R1 stage (Woli et al., 2016; Ciampitti & Vyn, 2012), highlighting the significance of late N supply. In addition, around 50% of all grain nitrogen is from the corn plant's uptake of new nitrogen after the R1 stage (Ciampitti, Zhang, Friedemann, & Vyn, 2012). Adequate nitrogen nutrition during later growth stages is essential to optimize corn yield, and when combined with foliar fertilizer and fungicides, new insights into plant performance may become evident.

Chapter 3 evaluates late split nitrogen fertilizer applications and compares them to land grant recommendations in Kansas. While traditionally, work in the Midwest has focused on side-dress application on early vegetative corn soon after planting to waist-high (Robertson & Ohlrogge, 1952; Killorn & Zourarakis, 1992), and pre-plant to V10 (Sweeney & Ruiz Diaz, 2021; Walsh, Raun, Klatt, & Solie, 2012). However, other work on different soils has determined no economic return to late split N applications (Ruiz Diaz, Hawkins, Sawyer, & Lundvall, 2008) (Nafziger & Rapp, 2021). Previous work demonstrated no impact of splitting nitrogen application on yield (Mueller et al., 2017; Fernandez, DeBruin, Messina, & Ciampitti, 2020). Evaluating these emerging techniques alongside existing Kansas State University recommendations will provide additional insight into how these tools can impact corn productivity.

Chapter 4 provides additional information on evaluating the nutrient status of plants. Plant tissue analysis is an important tool utilized in various crop systems. Comparing the effectiveness of various lab methods across a wide range of plant materials and elements gives

more confidence to methods used in labs. It may provide insight into situations where data could be biased by the digestion method. Chapter 5 provides a summary of the contents of the dissertation.

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CHAPTER 2 - NITROGEN USE EFFICIENCY IN CORN WITH SPLIT NITROGEN APPLICATION AND LATE FUNGICIDES

ABSTRACT

Changes in commercially available sprayers offer ways of applying nitrogen (N) fertilizer, such as Urea Ammonium Nitrate (UAN) fertilizer, to corn (*Zea mays* L.) at early reproductive growth stages that rarely receive N fertilizer. Additionally, fungicide use in corn production has increased. The stay-green characteristics that fungicides may provide could impact N uptake and nitrogen recovery efficiency (RE). This study evaluated the interaction effect of three factors (N fertilizer rate, fungicide application, and fertilizer application time/method). The experiment included a combination of two N rates (R) 180 and 225 kg N/ha, two fungicide treatments (F) (+/-) azoxystrobin and propiconazole applied at R1, and three N fertilizer application method/timing combinations (M). These consisted of all N applied at planting, late split-applied N with 45 kg N/ha withheld and applied at the R1 stage, and late split-applied N plus foliar-applied urea-formaldehyde/triazone N at 5 kg N/ha at the R1 growth stage. No three-way interaction was observed for any variable across sites. Significant interactions were observed for Rate x Method for Grain N uptake, plant nutrient uptake, and recovery efficiency. Lower N Rates contributed to yield declines in late split applications relative to identical rates of N pre-plant applications. However, the late-split N application increased corn N response for the higher N rate. This shows that withholding a significant N fertilizer amount until the early reproductive stage can reduce yield if the early season N supply is inadequate.

Abbreviations: Fungicide (F), Grain Yield (GY), N application Method (M), N Rate (R), Recovery Efficiency (RE), Whole Plant N Uptake (PNU) Urea Ammonium Nitrate (UAN)

INTRODUCTION

New corn hybrids with higher yield potential and stay-green characteristics can benefit from late nitrogen (N) fertilizer applications. Late N application can be achieved through various methods, such as soil-applied urea, urea ammonium nitrate (UAN) fertilizer, or foliar N applications. Combining these late N applications with management practices like late fungicide applications may further enhance potential corn response. In addition, this combination of late N application and fungicide treatment may benefit corn crops by promoting "stay green" conditions throughout the grain filling period, ultimately contributing to higher yields.

Existing studies indicate that approximately 20% of the total nitrogen uptake by corn plants occurs after the R1 stage (Woli et al., 2016) (Ciampitti & Vyn, 2012), highlighting the significance of late N supply. Moreover, research suggests that around 50% of all grain nitrogen can be attributed to the corn plant's uptake of new nitrogen after the R1 stage (Ciampitti, Zhang, Friedemann, & Vyn, 2012). These findings emphasize the importance of adequate nitrogen nutrition during later growth stages to optimize corn yield.

The effect of late fungicide applications to help maintain leaf health and its impact on N use has not been evaluated extensively. Previous work indicates fungicides can improve corn yield (Paul et al., 2011). Although there is interest in applying fungicides for plant health effects, there are concerns regarding developing resistance to plant diseases (Hahn, 2014). Fungicides may impact corn yield beyond disease suppression with additional physiological changes, including changes in fiber composition, lignin, and other physiological changes (Cardoso, 2020). Fungicides can influence wheat NUE; perhaps corn recovery efficiency can also be influenced.

High-clearance equipment is becoming widely available. Producers and retailers can utilize this equipment for spraying pesticides and applying fertilizers at late-vegetative and early

reproductive growth stages. Using this equipment for multiple purposes (such as various chemicals and fertilizers) during the growing season can help maximize the economic return on this investment. Additionally, this equipment provides capacity for N fertilizer application beyond dry spreaders and anhydrous bars, which could be helpful in locations with restricted applications of fall N fertilizer. Since UAN contains nitrate-N, ammonium-N, and urea-N, early applications are vulnerable to leaching losses earlier than ammonium or urea-based fertilizer products. This fertilizer material may benefit from more intensive management with split applications compared to ammonium or urea-based fertilizers.

Applying foliar fertilizers and/or fungicides during a time of high nutrient demand and photosynthetic activity may contribute to yield increase and improved nitrogen use efficiency (NUE). This increase in yield and NUE may be important for high-yield environments; however, "average" yielding corn could also show substantial improvements in yield and NUE. Some corn acres may be considered low yield potential; yield limitations in some parts of eastern Kansas and parts of Missouri are due to poor soils with a high potential for N loss. Soils prone to N loss can limit yields; however, with the proper fertilizer N management (time and source), yields can be improved, generating economic returns (Scharf, Wiebold, & Lory, 2002).

Additional questions regarding late N applications may involve using nitrogen additives or slow-release N sources. It is also suggested that other nutrients can be in high demand during early reproductive stages in corn, particularly some micronutrients with key roles during the flowering/reproductive stage. However, N supply during early reproductive stages is often one of the main limiting factors, and the application of fungicides during this period can open an opportunity for additional foliar N applications cost-effectively. The overall objective of this study was to assess the interacting effects of three management treatments i) fungicide

applications at the early reproductive stage, ii) nitrogen application rates, and iii) different N fertilizer application time/methods; on grain yield and grain N concentration, grain N uptake, whole plant N uptake, and nitrogen use efficiency.

MATERIALS AND METHODS

Experimental design

We conducted the experiment at three locations from 2016-2018, for a total of five site-years. Sites were across central and eastern Kansas. Site years in 2016 were located near Scandia, Rossville, and Neosho Rapids. In 2017, the study was near Scandia, and in 2018, it was in Rossville (Table 2.1). The study was no-tilled at Sites 1, 3, and 5; sites 2 and 4 were tilled. All sites were irrigated except site 3. Corn was grown after soybeans at all sites. Plots were four rows wide and ranged from 4.5m to 11.2m long, depending on the site year.

The study was designed with the combination of three factors: i) two N rates 180 and 225 kg N/ha; ii) two fungicide treatments (with and without) Quilt Xcel (Azoxystrobin and Propiconazole) applied at R1; and iii) three N fertilizer application method/timing that included: a) all N applied at planting, b) late split-applied N with 45 kg N/ha withheld and applied at the R1 stage; and c) late split-applied N as previously described plus foliar-applied N at 5 kg N/ha at the R1 growth stage. The factors were arranged in a randomized complete block design with a split plot whole plot for the fungicide treatment with four replications. One additional treatment included a control with no N fertilizer application to assess the response to N at each site.

Soil applied nitrogen fertilizer used UAN (28-0-0) streamed on the soil the surface between the rows. The foliar nitrogen product (28% N) was urea triazone-based (N-Sure) to avoid leaf burn applied at the R1 growth stage. Soil and foliar applications were made using a

high-clearance sprayer, drop nozzles for soil application, and flat-fan nozzles above the canopy for foliar applications. Fungicide and foliar N were sprayed in separate applications for treatments that received both treatments.

Precipitation, relative humidity, and air temperature were measured with automated weather stations within one km of experimental sites with the exception of site 3, which did not have a local weather station. Site 3 was within 50 km of the weather station.

Soil analyses

Before site establishment, a soil sample composed of 12 cores was collected from the soil surface 0-15 cm and profile 0-60 cm. The surface samples were analyzed for soil pH (Peters, 2012), soil organic matter (OM) by loss on ignition (Combs, 1998), soil test P by Mehlich-3 colorimetric, soil test K by ammonium acetate (Warnke, 1998), and Zn by DTPA extraction (Whitney, 1998). Profile samples were analyzed for nitrate and ammonia by the KCl extract by cadmium reduction (Gelderman, 1998). Phosphorus fertilizer was blanket-applied in the study area at 45 kg P₂O₅/ha using triple superphosphate. Pre-plant soil test data is available in Table 2.1.

Plant and soil measurements

Plant measurements included whole plant biomass and N concentration at physiological maturity, which was used to calculate plant nitrogen uptake (PNU). Whole plant samples were composited from 6 plants from the two center rows in each plot with the ears removed. Whole plant samples were weighed, chipped, and subsampled for dry matter and nitrogen. Plant samples were digested by sulfuric acid peroxide digestion and analyzed for N content (Thomas, Sheard, & Moyer, 1967). Harvest was conducted using plot combines or by hand at site 3. The grain

sample was analyzed for total N content by sulfuric acid peroxide digestion. Grain N uptake was calculated using grain yield biomass and N concentration.

Nitrogen use efficiency was calculated as the recovery efficiency (RE). The formula used was $RE = (\text{Total Plant N Uptake} - \text{Control Plant N Uptake}) / (\text{N Rate applied})$.

Statistical analysis

Statistical analysis was conducted with the GLIMMIX procedure in SAS 9.4. Analysis was completed across site years, with site year, fungicide, N rate, and N timing as fixed effects in the model. Only replication was considered random. Mean separation was conducted with LSMeans. The Kenward Roger estimate was used to calculate degrees of freedom. Results were interpreted at $p < 0.10$.

RESULTS AND DISCUSSION

Corn response to management treatments such as fungicides, N rate, and N fertilizer application time and method is highly dependent on specific soil and environmental conditions. Our study was conducted primarily under irrigated conditions (Table 2.1); however, the range of sites offered contrasting weather conditions across site years (Figure 2.1). Therefore, the potential for fungal disease pressure and N loss potential varies by site, as is typically expected in production fields. Our primary discussion will focus on the analysis across sites to further examine the types of interactions that may be expected between the main treatments/factors across environments.

The four-way interaction for the factors Site year (S), N rate (R), fungicide (F), and method (M) did not show a significant effect for GNC, GNU, WPU, GY or RE.

The three-way interactions of the main factors of S, R, F, and M were not significant for GNC, GNU, WPU, GY, and RE (Table 2.2). However, examining the interaction between FxM across sites showed significant interactions for GNU and GY (Table 2.2). When fungicide is applied, grain N uptake increases for all fertilization methods; however, when no fungicide is applied, there was only an increase in grain N uptake for the split applied N + foliar N (Figure 2.2B). The difference of 10 kg N/ha in N uptake exceeds the amount of N applied with the foliar fertilizer of 5 kg N/ha. This suggests that fungicides may promote additional N allocation to the grain later in the growing season. For this study, we did not collect plant disease scores; therefore, whether plant health or disease suppression effects were driving this effect is unclear. Some sites did have disease presence, including common rust and, to a lesser degree, gray and northern leaf spots. In some sites with minimal disease pressure, perhaps stimulation of above-ground N supply by foliar N application maintains N uptake from soil or supports the remobilization of N within the plant. Previous work has shown plant health benefits from applying foliar phosphates for several diseases present, such as common rust (Reuveni & Reuveni, 1998). Perhaps foliar N fertilizers reduced disease impact.

When RxM interactions were compared, significant interactions were observed for Grain N Uptake, Whole Plant N uptake, and RE (Table 2.2). Previous work has shown increased plant N uptake from late split N applications (Mueller et al., 2017). Whole plant uptake increased when Split N and Split-N + Foliar treatments were only applied at the 225 kg N/ha rate (Figure 2.3C). Grain N concentration and grain yield showed a similar pattern (Figures 2.3A and 2.3D), although the interaction was not significant. However, for the 180 kg N/ha rate, the response to the fertilization method was the opposite, with a higher response observed for the pre-plant N fertilizer method (Figure 2.3). This overall trend suggests that with a lower total N application

rate (180 kg N/ha), the additional N applied as a late split application at the R1 stage is likely too late to provide additional benefit to the crop. Perhaps an early season deficiency, with an early season rate of 135 kg N/ha available before the split application, reduced crop response and yield. This effect suggests that an ideal timing for the second N split application is earlier in the growing season than in the reproductive growth stages.

Previous work has shown that UAN at early vegetative stages can be a more effective form of fertilizer in relatively dry years (Gagnon, Ziadi, & Grant, 2012). Also, adequate N supply for most of the growing season is essential to develop key corn yield components and perhaps better position the crop to take advantage of additional applications at reproductive growth stages, given good growing conditions during the grain filling period.

Note that the SxF interaction was not significant. The lack of significance for this interaction suggests that individual site differences may not necessarily drive response to fungicide even though sites had a variety of weather conditions (Figure 2.1) and likely different disease pressures.

The interaction of Rx F was not significant for any of the factors of interest (Table 2.2). Previous work with several fungicides at V5 which showed no increases in grain or silage yield at several sites in Wisconsin (Mourtzinis et al., 2017).

Nitrogen rate (R) showed significant effects for GNU, PNU, and GY, but not for GN or RE (Figure 2.5). These results show that the application of 180 kg N/ha was not sufficient to maximize uptake and yield across sites.

The main effect of fungicide application showed a significant increase in Grain N uptake, Whole Plant N uptake, and grain yield (Figure 2.4). Grain N concentration showed a small average increase but was not statistically significant, so the primary driver of grain N uptake was

increased grain yield. Previous work has shown that fungicides can increase corn yield depending on specific conditions conducive to disease development (Paul et al., 2011).

The main effect of nitrogen rate was statistically significant for plant N uptake (PNU), whole plant N uptake, and grain yield (Figure 2.5). This is consistent with previous work that shows increases in nitrogen fertilizer rate showed a significant interaction with the fertilizer application method on RE (Figure 2.6A) (Mueller 2017). At 180 kg N/ha, split applications resulted in a reduced recovery efficiency, while at 225 kg N/ha, split applications, and foliar N increased recovery efficiency. This indicates that late split applications with lower total N rates may not supply adequate N during the vegetative period and decrease RE. Rate effects on RE were not significant when compared across rates (Table 2.2). Fungicide applications did increase recovery efficiency (Figure 2.6C).

Grain N uptake, PNU, and GY were positively impacted by N rate and fungicide main effects. The nature of these relationships suggests that reductions in disease pressure and possible plant health effects may contribute to increased PNU, RE, and GY in corn production. Whether these impacts are due to disease reduction is not easily determined from this dataset. Still, some sites that showed significant responses to fungicide applications did have noticeable visual disease pressure.

CONCLUSIONS

Increases in grain N uptake, plant N uptake, and Grain Yield from both N rate and Fungicide application suggest that split application has the potential to increase corn N uptake, especially in site years with the potential for in-season N loss. The RxM interactions suggest that late split applications of N may increase plant N uptake in the crop. However, results from our study also showed the risks associated with withholding a significant portion of the total N

fertilizer needs, resulting in N deficiency for most of the corn growing season that may not be able to recover with N applications at the R1 stage fully. Results also showed that using management factors such as fungicide applications might require additional refinement of N recommendations and management under certain conditions.

Foliar N applications combined with late-split N fertilizer application (R1 growth stage) may benefit the crop under specific conditions by preventing disease, rather than simply providing additional N for grain filling during the reproductive stage.

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TABLES AND FIGURES

Table 2.1. Site description, soil types, and selected soil properties for the 0-15 and 0-60 cm sampling depth.

Site	Year	County	Soil	Soil Texture	Irrigation	0-15 cm depth					0-60 cm depth		
						pH	OM	P	K	Zn	NH ₄ -N	NO ₃ -N	
							g kg			mg kg			
1	2016	Republic	Crete	Silt Loam	Irrigated	6.7	33	4	447	1.1	3	5	
2	2016	Shawnee	Eudora	Silt Loam	Irrigated	7.2	13	18	201	1.1	1	3	
3	2016	Lyon	Chase	Silty Clay Loam	Rainfed	7.2	29	11	119	1.1	13	3	
4	2017	Republic	Crete	Silt Loam	Irrigated	6.2	32	11	487	1.4	6	6	
5	2018	Shawnee	Eudora	Silt Loam	Irrigated	6.8	16	44	198	1.9	*	17	

* NH₄-N was not measured at this location

Table 2.2. Statistical significance of the main factors of site year, nitrogen rate, fungicide application, and nitrogen application method, and interactions for grain N concentration, grain N uptake, whole plant N uptake, grain yield and recovery efficiency across sites.

Effect	Grain N concentration	Grain N uptake	Whole plant N uptake	Grain yield	Recovery Efficiency
	----- Pr > F -----				
Site Year (S)	<0.001	<0.001	<0.001	<0.001	<0.001
Fungicide (F)	0.055	0.001	0.001	0.003	0.013
Rate (R)	0.159	0.015	0.002	0.021	0.204
Method (M)	0.100	0.466	0.266	0.863	0.935
S×F	0.160	0.542	0.795	0.318	0.935
S×R	0.109	0.175	0.120	0.401	0.522
F×R	0.914	0.213	0.696	0.158	0.919
S×M	0.131	0.331	0.230	0.658	0.475
F×M	0.249	0.039	0.326	0.085	0.491
R×M	0.282	0.118	0.024	0.150	0.065
S×F×R	0.802	0.600	0.545	0.507	0.832
S×F×M	0.580	0.651	0.508	0.827	0.856
S×R×M	0.685	0.380	0.409	0.205	0.538
F×R×M	0.276	0.175	0.154	0.388	0.312
S×F×R×M	0.191	0.791	0.724	0.786	0.894

† The 0 N fertilizer control was not included in this analysis.

Table 2.3. Total water and irrigation water per site by month

Site	1	2	3	4	5
Month	Water (mm)				
April	113 (0)	262 (0)	143	59 (0)	35 (0)
May	158 (0)	168 (0)	88	131 (0)	116 (83)
June	27 (3)	140 (82)	2	111 (32)	151 (76)
July	160 (10)	207 (83)	134	120 (64)	55 (24)
August	197 (3)	208 (0)	141	111 (64)	101 (0)
September	63 (0)	280 (0)	148	58 (0)	67 (0)

The table shows precipitation and irrigation, with irrigation in parentheses.

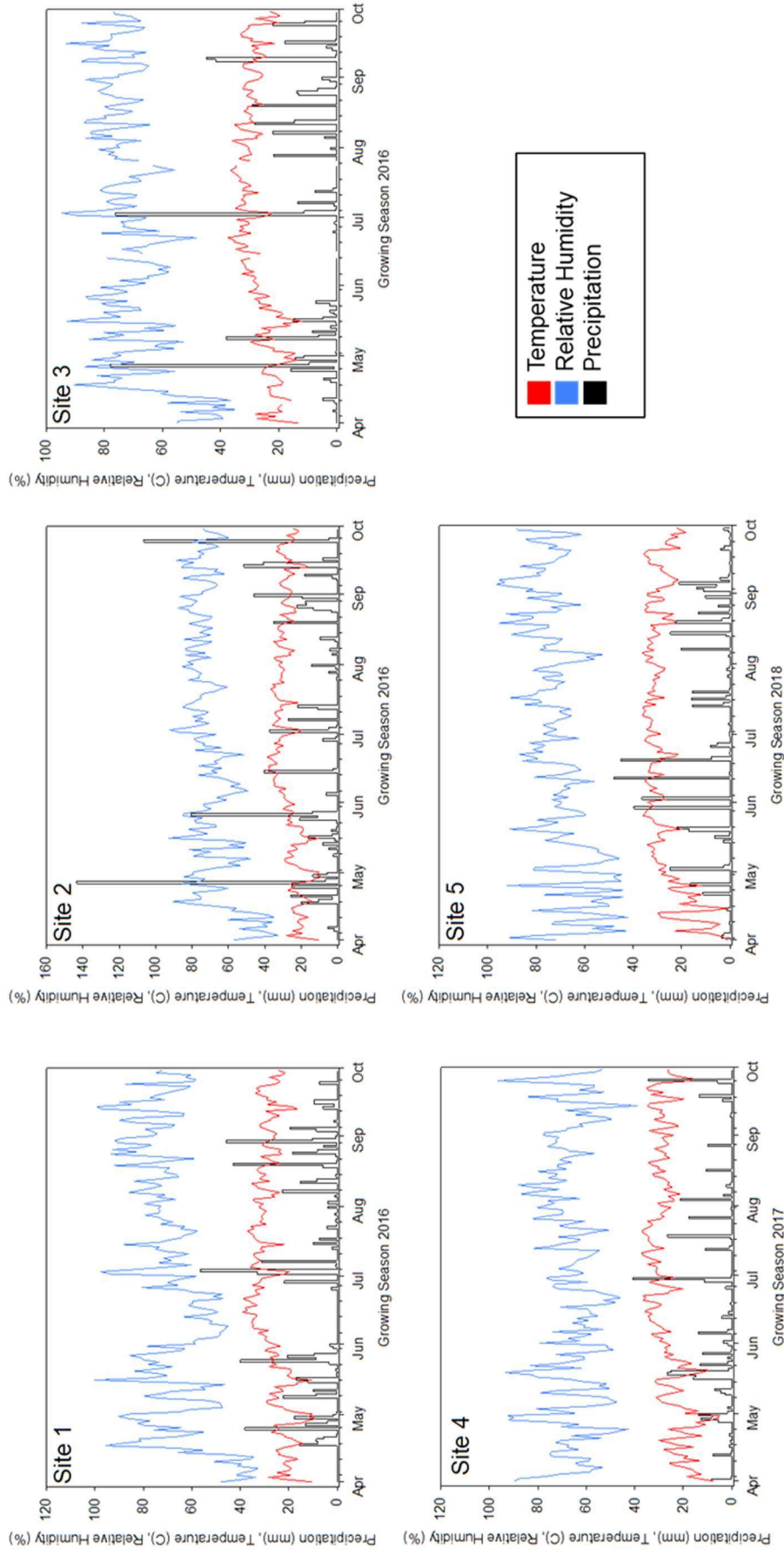


Figure 2.1 Weather across sites. Black lines represent rainfall, red lines represent temperature, and blue lines represent relative humidity. All data is daily or daily totals.

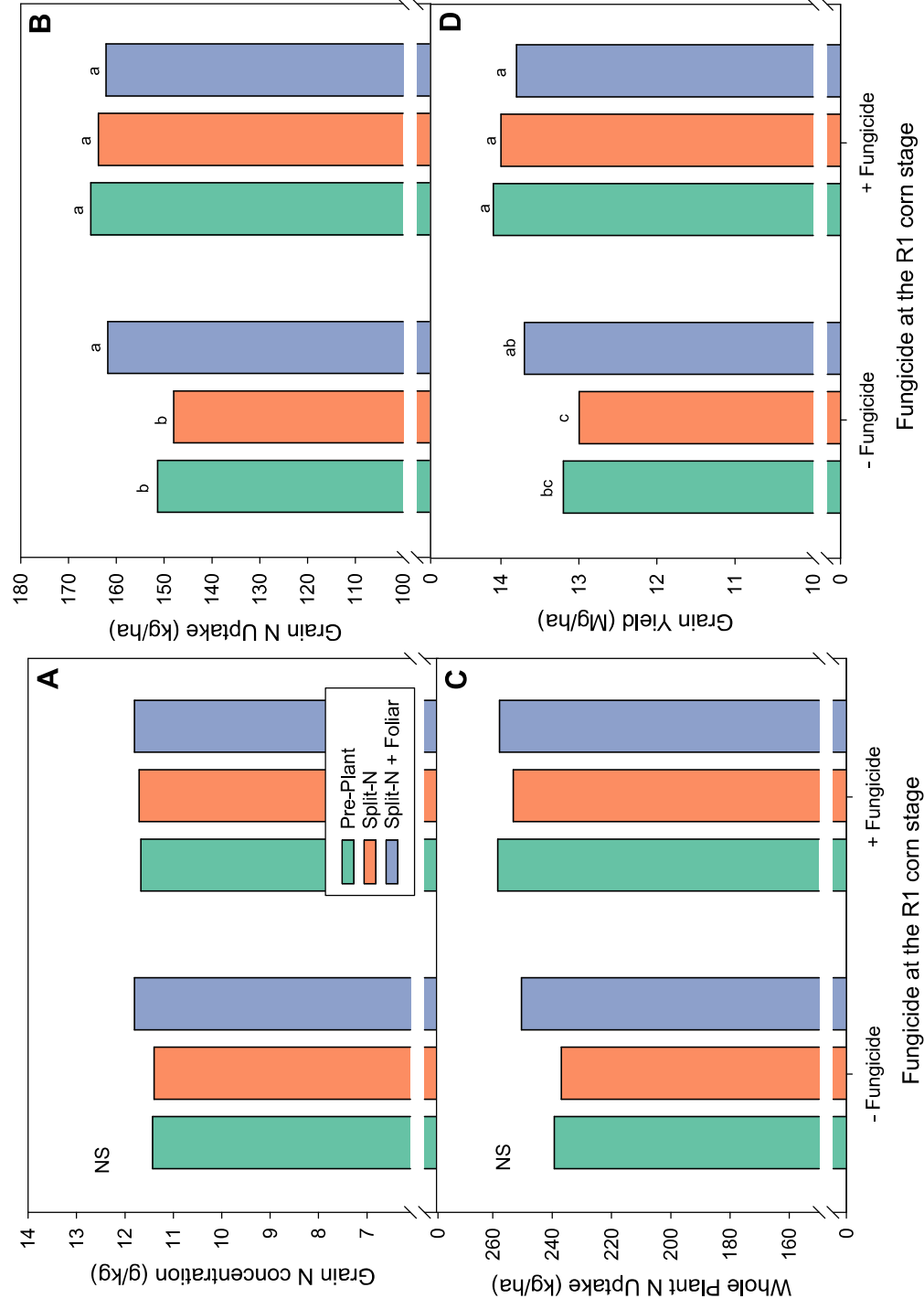


Figure 2.2 Interaction of Fungicide application and Method of Nitrogen application on Grain N concentration (A), Grain N Uptake (B), whole plant N uptake (C), grain yield (D). The significance of interaction at $\alpha=0.10$ is indicated by the presence of confidence interval bars, and the lack of interaction significance is indicated by NS (not significant).

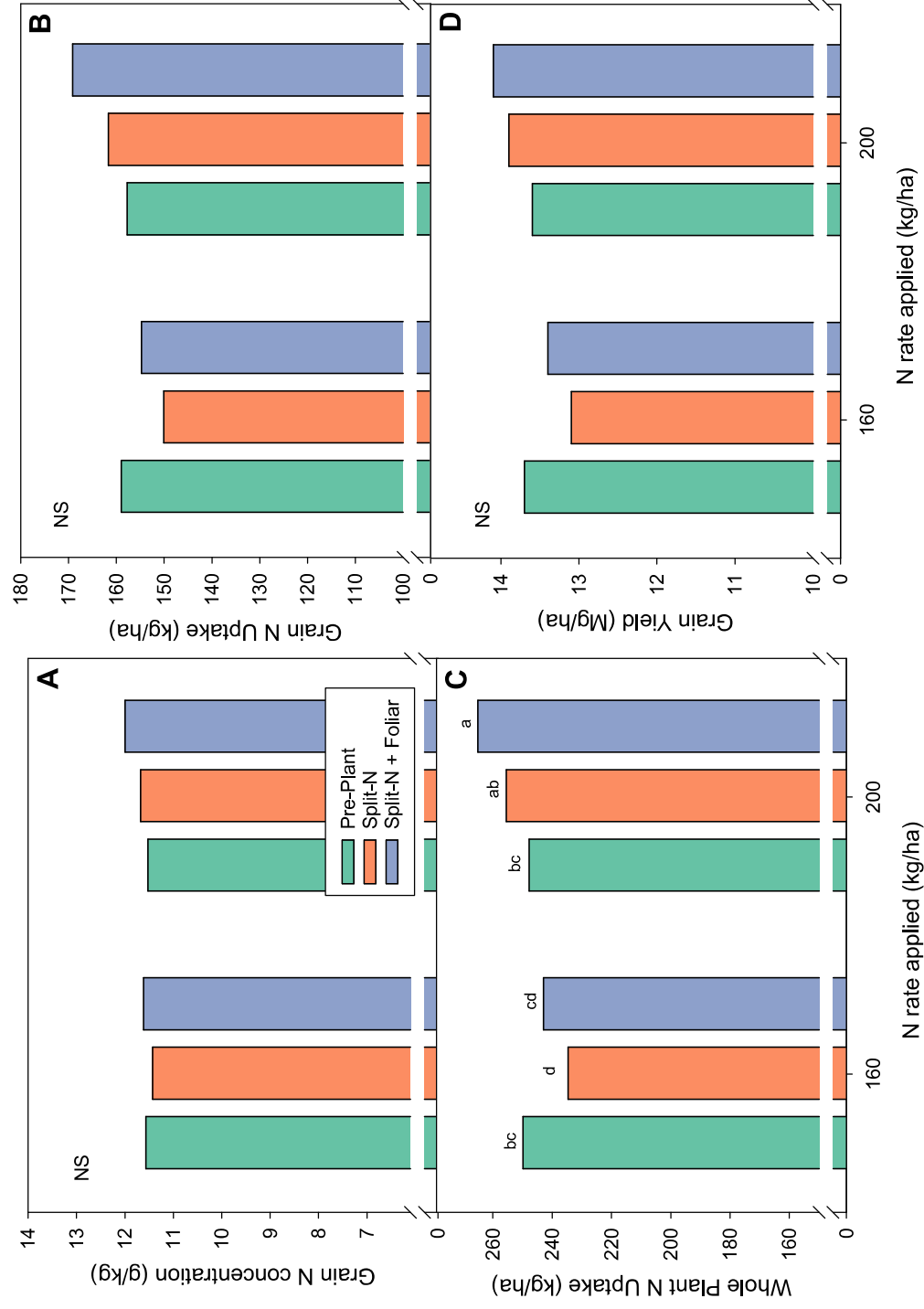


Figure 2.3. Interaction of N rate and N method on grain N concentration (A), grain N uptake (B), whole plant N uptake (C), and grain yield (D). Significance of interaction at $\alpha=0.10$ is indicated by the presence of confidence interval bars and lack of interaction significance is indicated by NS (not significant).

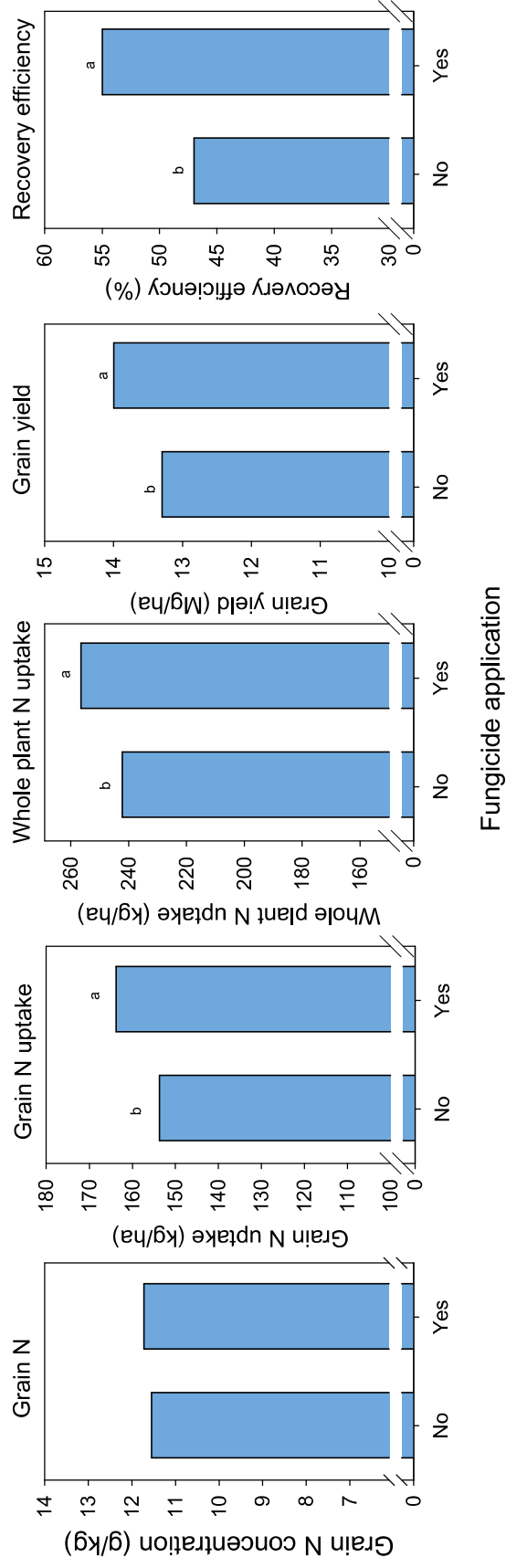


Figure 2.4. Fungicide application main effects on grain N, grain N uptake, whole plant N uptake and grain yield and recovery efficiency. Confidence intervals indicate significance at $\alpha=0.1$. Lack of significance is indicated by NS (not significant).

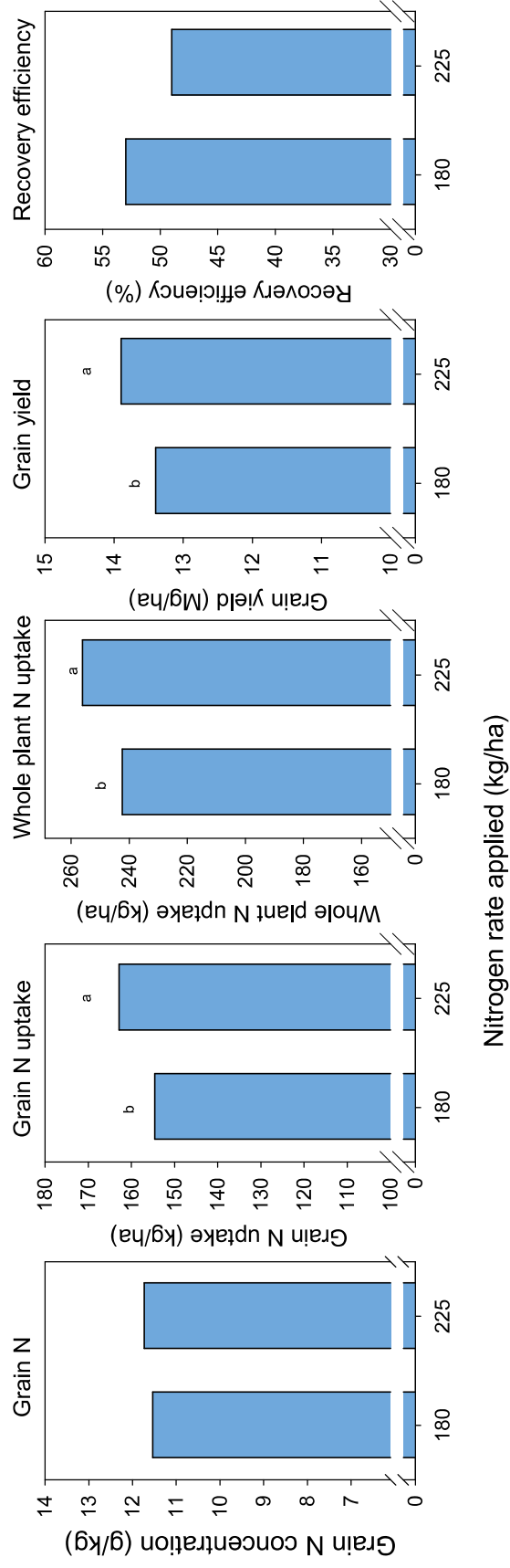


Figure 2.5. Main effects of N rate applied on (A) Grain N (not significant), (B) Grain N uptake, (C) Whole plant N uptake, (D) Grain yield and (E) Recovery efficiency (not significant). Bars separated are significantly different at $\alpha=0.1$.

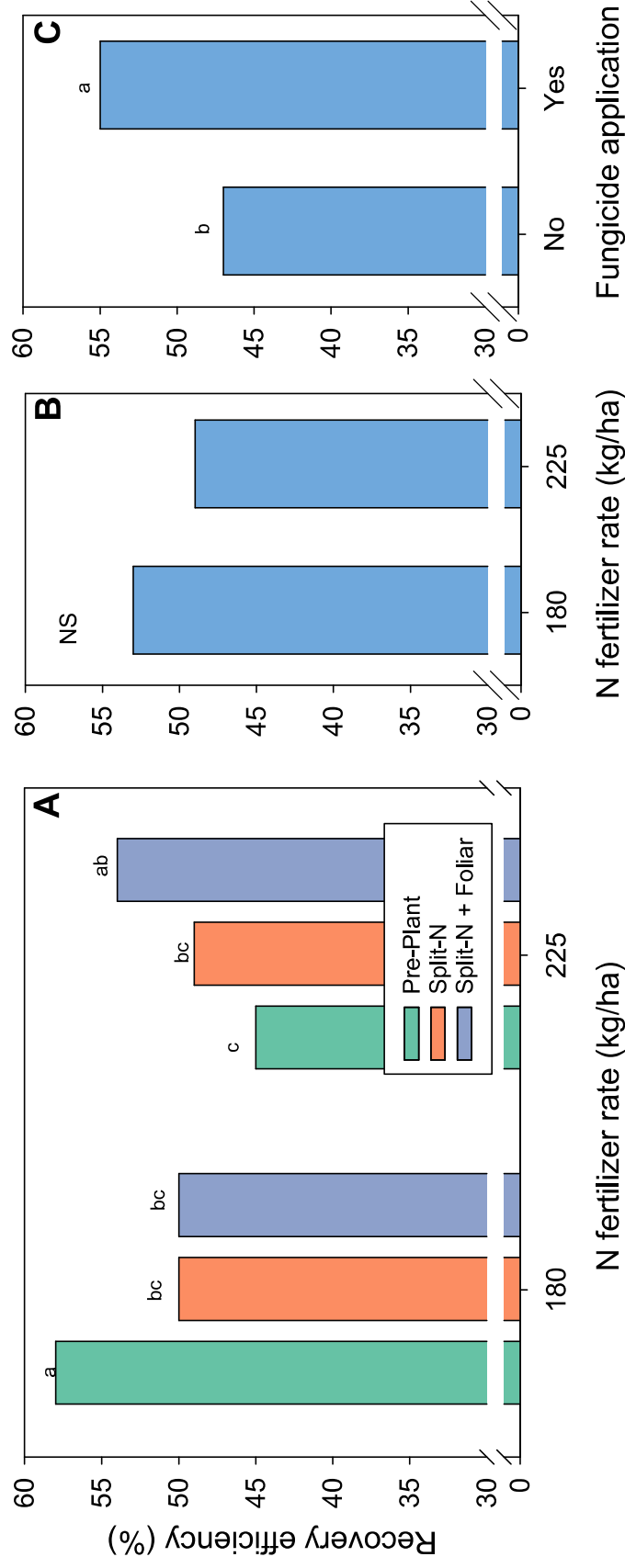


Figure 2.6. Nitrogen (N) recovery efficiency as affected by N application method interaction (A), N fertilizer rate (B) (not significant), and fungicide application (C). Bars separated are significantly different at $\alpha=0.1$.

CHAPTER 3 - CAN SPLIT APPLIED NITROGEN FERTILIZER AT THE LATE VEGETATIVE GROWTH STAGE IMPACT THE OPTIMUM NITROGEN RATE?

ABSTRACT

Corn (*Zea mays* L.) growth is highly responsive to nitrogen (N) fertilization. New technologies such as high clearance sprayers are changing the methods and timing of N application possible at scale in production agriculture. Many N applications rely on recommendations from land grant universities, and most do not have provisions for N timing. Thus, a study was designed to compare a range of nitrogen fertilizer rates and timings. An experiment was conducted with a zero N control and a range of five nitrogen rates at the Kansas State University recommendation. Nitrogen fertilizer was applied in intervals of greater and less than 56 and 28 kg N/ha. Treatments were applied either all at planting or 60% at planting and 40% at the VT growth stage. All sites showed grain yield (GY) increases between the fertilized and unfertilized treatments. There was no consistent pattern when equivalent rates of N fertilizer were compared between the all-at-plant application and the split late vegetative treatment. Kansas State University N recommendations for corn were not low enough to reduce yield, even at lower yield goal sites. The site-specific agronomic optimum N rate was more than 56 kg N/ha less than the recommended rate treatments, whether surface banded at planting or applied split between V4 and VT/R1. Improving recommendations for changes in nitrogen timing may be warranted, particularly in environments with a high risk of N loss; however, our study shows that late N application (VT/R1 growth stage) may be too late to provide additional benefit across broad conditions.

Abbreviations: ELN, ear leaf nitrogen; GY, grain yield; PNU, plant nitrogen uptake, UAN, urea ammonium nitrogen fertilizer.

INTRODUCTION

The widespread introduction of high-clearance sprayers allows for the application of agricultural chemicals late in the growing season. For example, this adoption allows for the application of urea ammonium nitrate (UAN) fertilizer solutions from early to late into the growing season using drop nozzles. Nitrogen fertilizer for corn is normally applied before the crop is planted in the Midwest and Great Plains regions of the United States. Although side-dress applications early in the growing season have allowed for an extended period of fertilizer application, high clearance sprayers, wide availability of liquid fertilizer solutions, interest in improving the efficiency of N fertilizer use, and fertilizer impacts on groundwater quality are contributing to increased interest in applying N later in the growing season than previously possible.

Work in the Midwest has focused on side-dress application on early vegetative corn soon after planting to waist high (Robertson & Ohlrogge, 1952) (Killorn & Zourarakis, 1992), and pre-plant to V10 (Sweeney & Ruiz Diaz, 2021) (Walsh, Raun, Klatt, & Solie, 2012). Other work has explored later N timing applications for corn crop growth. For example, work on claypan soils determined that nitrogen fertilizer applications as late as R1 increased corn yield when applied as a rescue treatment (P. C. Scharf, Wiebold, & Lory, 2002). However, other work on different soils has determined no economic return to late split N applications (Ruiz Diaz, Hawkins, Sawyer, & Lundvall, 2008) (Nafziger & Rapp, 2021). Other work in the Midwest has demonstrated no impact of splitting nitrogen application on yield but impacts on nitrogen uptake

(Mueller et al., 2017). An international meta-analysis also reached the same conclusion (Fernandez, DeBruin, Messina, & Ciampitti, 2020).

Nitrogen recommendations used by land grant universities in the north-central region tend to be empirical or mechanistic. The empirical approach is exemplified by the Maximum Return to Nitrogen (MRTN) (Vanotti & Bundy, 1994). Land grant universities have adopted MRTN in seven corn belt states (Sawyer & Nafziger, 2005). This approach uses aggregated data from nitrogen rate trials to build a yield-to-nitrogen input response curve to calculate a range of nitrogen rates within an economic return of one dollar per acre, incorporating crop and fertilizer prices. The MRTN approach is helpful in its ability to incorporate nitrogen prices and crop prices. However, it has not been adapted to site or year-specific conditions outside of broad-scale groupings of soil regions or rotation sequences.

Mechanistic recommendations consider plant N uptake and sources of plant available N as individual factors (Stanford, 1973). A final nitrogen recommendation is calculated as an N requirement based on yield minus nitrogen credits from sources such as residual soil nitrogen, organic matter decomposition, and previous crop adjustments. This approach can be a helpful educational tool for practitioners because the calculations may provide a detailed breakdown of the different sources of N supply and N uptake by plants. The mechanistic system can be gradually improved as additional research data becomes available for a given factor, such as crop nitrogen uptake, soil organic matter mineralization, or nitrogen contributions from legume crops or manure. A fundamental weakness can be when nonlinear relationships are expressed as a linear factor; the recommendation can be over or under-expressed at extreme values. This can lead to recommendations that may exceed the actual N requirements of the crop, mainly when expected yields are outside the range used to develop the recommendations. Some

recommendations include maximum recommendation rates as a preventative measure against N over application. Nitrogen mineralization can be challenging to model across soil types when mineralization factors are developed on soil with different characteristics than the site of interest.

Kansas State University N recommendation (MF 2586) (Leikam, 2003) is mechanistic, with a yield goal-based N requirement (11.56 Kg N/ha per Mg corn grain yield). This N requirement is adjusted to provide a final N recommendation based on several factors such as residual soil nitrate, previous crop, an organic matter factor that estimates mineralization from the soil, and N from irrigation water. A legume “credit” from the preceding crop is due to a combination of reduced C:N ratio of soybean residue due to grass crops and residual soybean N from symbiotic fixation (Gentry, Below, David, & Bergerou, 2001). The recommendation equation provides this information as a 45 kg N/ha soybean credit. Residual soil nitrate is subtracted from the N requirement. For warm-season crops such as corn, organic matter contributes a credit of 45 kg N/ha per percent organic matter. Previous work in Kansas has shown that irrigated soils may need less N than the Kansas State Recommended rates (Gehl, Schmidt, Maddux, & Gordon, 2005).

While there is interest in using nitrogen timing as a component in recommendations (effect on fertilizer use efficiency), Kansas State University recommendations do not currently include this factor. Theoretically, as one brings the applications of N closer to the time of crop uptake, there is likely to be better utilization. However, recent research results vary depending on year-to-year losses (Clark, 2020). This study aimed to compare nitrogen rates and timings for surface-banded UAN in corn production at optimum N rates. Also, it provided an evaluation of Kansas State University nitrogen recommendations for corn.

MATERIALS AND METHODS

We conducted the experiment at five site-years in 2016 and 2017. Sites were located across central and eastern Kansas. In 2016 studies were located in Republic, Shawnee, and Lyon counties, and in 2017 two additional sites in Republic and Shawnee counties (Table 3.1). Studies were established on a variety of soils. Silt loams dominated the soil textural classes with the exception of one silty clay loam at site 3 (Table 3.1). Three additional sites were not included in the analysis because of severe hail damage, harvest data inaccuracies, and weed control failure.

Before the study establishment, soil samples were collected from the soil surface (0-15 cm) and profile (0-60 cm). The surface samples were analyzed for soil pH (1:1 soil to water) (Peters, 2012), organic matter by loss on ignition (Combs, 1998), Mehlich-3 colorimetric phosphorus (Frank, 1998), ammonium acetate potassium (Warnke, 1998), and Zn and Fe (DTPA) (Whitney, 1998). Profile samples (0-60 cm) were analyzed for nitrate and ammonia (KCl extract and analyzed by cadmium reduction) (R. H. Gelderman, & Beegle, D.B., 1998), sulfate (calcium phosphate) (Franzen, 2015), and chloride (R. H. Gelderman, Denning J.L., & Goos R.J., 1998). A blanket application of 45 kg P₂O₅/ha was applied before planting.

The study was no-tilled at sites 1, 3 and 4. Sites 2 and 5 received vertical tillage before planting. Sites were irrigated except for site 3, and soybeans preceded the study at all site years (Table 3.2). Nitrogen rates for this study were based on Kansas State University recommendations (Leikam, 2003). With the expectation that the current KSU recommendations would not differ from the agronomic optimum rate, we applied a site year-specific N rate as our fertilizer N target rate. The recommended N rates at each site were calculated by using a “yield goal” times a coefficient to estimate total plant N demand (estimated plant N uptake), and

subtracting N from organic matter credit, measured soil nitrate levels, and a previous crop adjustment (Table 3.2).

A zero N fertilizer control and ten N fertilizer rate and timing combinations were applied for a total of eleven treatments. After establishing the target rate for each site, additional rates were either -56, -28, or +28, and +56 kg N/ha from the target rate (Table 3.3). These five rates were applied either entirely at planting or 60% at planting and 40% at the VT growth stage. A 0 kg N/ha control was the final treatment for 11 treatments in the study. Treatments were applied as 28% N UAN using a high-clearance sprayer and dribbled on the soil surface between the rows. Treatments were arranged in a randomized complete block design with four blocks per site year with the exception of site 3, which had only three blocks because the first block was damaged by flooding. Plot lengths ranged from 4.57-11.27 meters.

A variety of chemical, physical, and soil measurements were collected. Plant measurements included R1 ear leaf N (ELN), whole plant biomass and N content at the R6 growth stage, grain yield, and post-harvest soil samples. Ear leaves were collected from 10 plants in the plots, dried to constant weight at 65 degrees C, ground to pass through a 2mm sieve, and measured for total N. Whole plant samples were composed of six whole plants, excluding grain. Whole plant samples were weighed and then processed through a wood chipper. Subsamples were measured for moisture content before the samples were dried at 65 degrees C and ground to pass through a 2mm screen. Ear leaves, whole plant samples, and grain were analyzed by a sulfuric acid hydrogen peroxide digestion (Thomas, Sheard, & Moyer, 1967). Post-harvest residual soil N (0-60 cm) was collected soon after harvest and measured for nitrate and ammonium (R. H. Gelderman, & Beegle, D.B., 1998). Precipitation data were collected for each site from the nearest Mesonet station.

SAS 9.4 was used to analyze data by PROC GLIMMIX for mean separation to determine treatment effects. Independent variables analyzed included ELN, PNU, GY, and soil nitrate. Each variable was analyzed individually, with replications set as a random factor. The Kenward Roger estimate was used for degrees of freedom. Significance was calculated using $\alpha=0.10$. Sites were analyzed individually because of the site-specific nitrogen rates.

RESULTS AND DISCUSSION

The plant measurements of the study showed significant differences between fertilized and unfertilized treatments. However, this pattern was not uniform when residual soil nitrate is considered. Sites 1-4 had statistically significant differences in ELN concentration between all fertilized treatments and unfertilized controls with the exception of site 5 (Figures 3.1, 3.2, 3.3, 3.4, and 3.5). Site 5 had differences between some treatments, but not all. An established ear leaf N sufficiency range of 2.8 g/kg (Bryson & Mills, 2014) was above the ear leaf concentration of all sites, excluding site year 4, although the sites were only below the sufficiency range by .1-.4 g/kg.

All five sites had statistically significant yield responses to N fertilizer when fertilizer treatments were compared to the unfertilized controls. Although grain yield responded to the addition of nitrogen fertilizer, rates that showed significant differences were at least 56 kg N/ha lower than our target N rate. Preplant and split applications of fertilizer showed no grain yield differences. This is consistent with other work in the north-central region, which has not found a clear benefit to late applied N under growing conditions without high levels of N loss or close to the optimum N rate (Ruiz Diaz et al., 2008; P. C. Scharf et al., 2002).

All sites showed a significant whole plant N uptake (PNU) response to N fertilizer. Individual sites showed mixed uptake trends, with some sites showing increased uptake from early N applications and others showing increased uptake from split applications. This is likely due to differences in weather patterns (Figure 3.6).

Soil ammonium values had mixed statistically significant trends at levels that were not likely agronomically significant (not shown). However, many fertilized treatments had ammonium-N trend levels similar to the unfertilized plots, suggesting that the soil ammonium may have been more reflective of site conditions rather than fertilization.

Site one (Figure 3.1) had statistically significant differences between fertilized and unfertilized treatments in ELN, GY, and PNU. The equivalent yield responses across treatments show no response to additional N beyond the lowest N rate applied at the study (140 kg N/ha), and no yield response to a split application compared to the preplant to V4 timing. Soil nitrate levels sporadically increased with nitrogen rate, indicating an increased risk of post-harvest N loss to the environment as N fertilizer rates increased. This is consistent with previous research suggesting that residual nitrate increases as N rates increase past the optimum N rate (Hong, Scharf, Davis, Kitchen, & Sudduth, 2007). High yield goals at this site lead to higher nitrogen rates that may have contributed to the residual soil nitrate. These trends occurred in a year with relatively sporadic rainfall events (Figure 3.6) and significant irrigation in July, which led to residual soil nitrate.

At site two there were similar statistically significant differences in ELN, GY, and PNU between unfertilized and fertilized treatments (Figure 3.2). Despite relatively high rates of nitrogen (Table 3.3) soil nitrate levels were low across treatments, with few differences between

treatments and unfertilized control, likely due to more permeable soils at this site. Low nitrate levels at site 2 are likely related to the intense rainfall present at this site (Table 3.5, Figure 3.6).

Site three (Figure 3.3) had lower yield potential than many of the other sites so the applied N rates were significantly lower (65-154 kg N/ha). Significantly different levels of ELN, GY, and PNU were observed between fertilized and unfertilized treatments. Generally, low soil nitrate levels are likely due to the poorly drained soil at this site and other factors leading to denitrification and/or nitrate leaching. Low soil nitrate at site 3 may be related to higher denitrification losses after rains at this site.

Site four (Figure 3.4) had statistically significant differences between fertilized and unfertilized ELN, GY and PNU treatments. Post-harvest soil nitrate increased with rates in the pre-plant fertilized treatments but showed a non-statistical numerical trend in the split N treatments.

Site 5 (Figure 3.5) showed statistically significant differences in PNU, GY, and soil nitrate between fertilized and unfertilized treatments. Soil nitrate was likely higher for two high split application treatments because of a high rainfall (Figure 3.6) event between the first and second applications of fertilizer and resulting losses from leaching or denitrification.

Although grain yield responded to the addition of nitrogen fertilizer, our target rate was at least 56 kg/ha above the agronomic optimum rate for each site because of the consistent response to N fertilization and the lack of grain yield difference between rates. Preplant and split applications of fertilizer showed no grain yield differences. This is consistent with other work in

the north-central region, which has not found a clear benefit to late applied N under growing conditions without high levels of N loss (Ruiz Diaz et al., 2008; P. C. Scharf et al., 2002).

Since our target rates showed no consistent reductions in yield, examining how they were calculated may provide insight into which mechanistic factors are contributing to errors in these target rates. Organic matter contribution was not measured for each site, but the credit used in the equation is similar to that used in neighboring Missouri for similar textured soils (Buchholz, 1983). Many soil factors influence the relationship between soil organic matter and nitrogen credits, including quantity of organic matter, texture, and organic matter properties (Ros, 2012). Nitrogen credits from organic matter mineralization could be improved by more complex and site-specific modeling of biogeochemical properties (White, Finney, Kemanian, & Kaye, 2021). Examples in the literature with similar textural classes support the mineralization rate for sites in this study (Buchholz, 1983). Soil nitrate levels were measured before planting and are not a likely source of error, except for potential leaching or denitrification losses between sampling and corn N uptake. The previous crop adjustment (soybean) of 35 kg N/ha is similar to both adjustments in use by Nebraska (40 kg N/ha) (Shaver, 2014), and Missouri (27 kg N/ha) (P. C. Scharf, J. Lory, 2006). In addition, subtraction of the differences between continuous corn recommendations and corn following soybean recommendations from the MRTN recommendations using the Iowa database provides a range of 35 to 40 kg N/ha ("Corn N Rate Calculator," 2023) (Lory, Russelle, & Peterson, 1995). While these factors play a role depending on specific soil and environments, neither of these factors seems likely the main driver of the agronomically nonresponsive levels of nitrogen recommended by Kansas State University Recommendations for irrigated corn.

One possible source of error in the nitrogen recommendation formula is the absence of an internal efficiency component for soil nitrate. The crop requirement assumes 50% efficiency which could vary widely across different combinations of fertilizer timing, fertilizer source, and weather conditions. Internal efficiency is not considered for soil nitrate. This could potentially overestimate the impact of soil N on N requirements. Although the impact of the nitrate component on the nitrogen recommendation is relatively small in many situations, years with high residual nitrate could be years where this factor greatly impacts the recommendation.

We should examine the components of yield goal and plant nutrient uptake. The yield goals and achieved yield are shown in Table 3.4. The yield was equal to or exceeded the yield goal for each site except site 1. This suggests that an error in yield goal prediction was not a major component of the lack of response to the last 28 kg N/ha. The combination of realistic yield goals and lower-than-expected PNU across site years suggests that the plant nitrogen uptake factor is a significant component of high nitrogen rates in these studies. New corn genetics are more efficient in converting N in the biomass to grain yield (Ciampitti & Vyn, 2012) and are likely affected by management and growth conditions such as plant stress during the growing season. Further adjustment of the yield factor of the equation will likely lead to additional improvements in the recommendations. One component not evaluated here (and contained in the current coefficient) is the fertilizer recovery efficiency. The lack of response to the split application at most sites is likely a result of adequate N supply for both application timings and did not allow for the evaluation of efficiency improvements due to split applications in this study that would require rates at or below the agronomic optimum N rate.

CONCLUSIONS

The Kansas State University N recommendations showed no yield differences versus rates that were reduced by 56 kg N/ha when applied preplant or as a split application. The main sources of error seem likely from nitrogen uptake and variable use efficiency, especially at high yield levels under the mostly irrigated sites in our study. Further research to evaluate rates and timing of split N fertilizer will help provide targeted recommendations to inform agronomic decisions. The recommendations were at least 56 kg/ha higher than rates that would have shown yield differences. Adapting N fertilizer recommendations to accommodate a late vegetative split nitrogen application will require additional research with additional timings and rates, particularly for earlier split applications that may have a bigger impact on nitrogen use efficiency than late applications such as VT/R1 growth stages.

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TABLES AND FIGURES

Table 3.1. Selected soil properties and analysis for the 0-60 and 0-15 cm sampling depth.

Site	County	Soil Series	Soil Texture	0-60 cm depth					0-15 cm depth				
				NH ₄ -N	NO ₃ -N	Cl ⁻	SO ₄ -S	pH	OM	P	K	Fe	Zn
				-----mg/kg-----					g/kg	-----mg/kg-----			
1	Republic	Crete	Silt Loam	2	6	10	10	6.4	3.2	5	375	50	0.9
2	Shawnee	Eudora	Silt Loam	1	3	3	1	7.2	1.3	18	201	23	1.1
3	Lyon	Chase	Silty Clay Loam	13	3	4	4	7.2	2.9	11	119	47	1.1
4	Republic	Crete	Silt Loam	6	6	8	11	6.5	3.1	9	504	52	1.4
5	Shawnee	Eudora	Silt Loam	8	5	6	2	6.9	1.6	36	245	28	2.2

Table 3.2. Management information and components of KSU recommended N rate by site

Site	Tillage	Irrigation	Previous Crop	N Before Credits	SOM Credit	Profile N	Previous crop adjustment	Target N fertilizer rate
-----kg N/ha -----								
1	No-Till	Irrigated	Soybeans	395	72	46	45	231
2	No-Till	Irrigated	Soybeans	359	30	23	45	261
3	Vertical Tillage	Dryland	Soybeans	269	65	22	45	137
4	No-Till	Irrigated	Soybeans	395	70	32	45	248
5	No-Till	Irrigated	Soybeans	359	35	42	45	237

Table 3.3. Nitrogen rates applied by site

Site	Nitrogen Rates				
	Control	-56	-28	KSU Rec	+28 +56
	----- kg N/ha-----				
1	0	175	203	231	259 287
2	0	205	233	261	289 317
3	0	81	109	137	165 193
4	0	192	220	248	276 304
5	0	181	209	237	265 293

Table 3.4. Comparison of yield goal and, yield

Site	Yield Goal	Yield
----Mg/ha----		
1	13.81	11.23
2	12.55	14.84
3	9.42	9.40
4	13.81	14.65
5	12.55	14.29
Yield achieved based on the average of highest yielding site years		

Table 3.5. Total water and irrigation water per site by month

Site	1	2	3	4	5
Month	Precipitation + Irrigation mm (Irrigation mm)				
April	113 (0)	262 (0)	143	59 (0)	139 (0)
May	158 (0)	168 (0)	88	131 (0)	166 (0)
June	56 (32)	140 (82)	2	111 (32)	223 (58)
July	246 (95)	207 (83)	134	120 (64)	137 (66)
August	225 (32)	208 (0)	141	111 (64)	125 (20)
September	63 (0)	280 (0)	148	58 (0)	32 (0)

Table shows combined precipitation and irrigation, with irrigation in parentheses

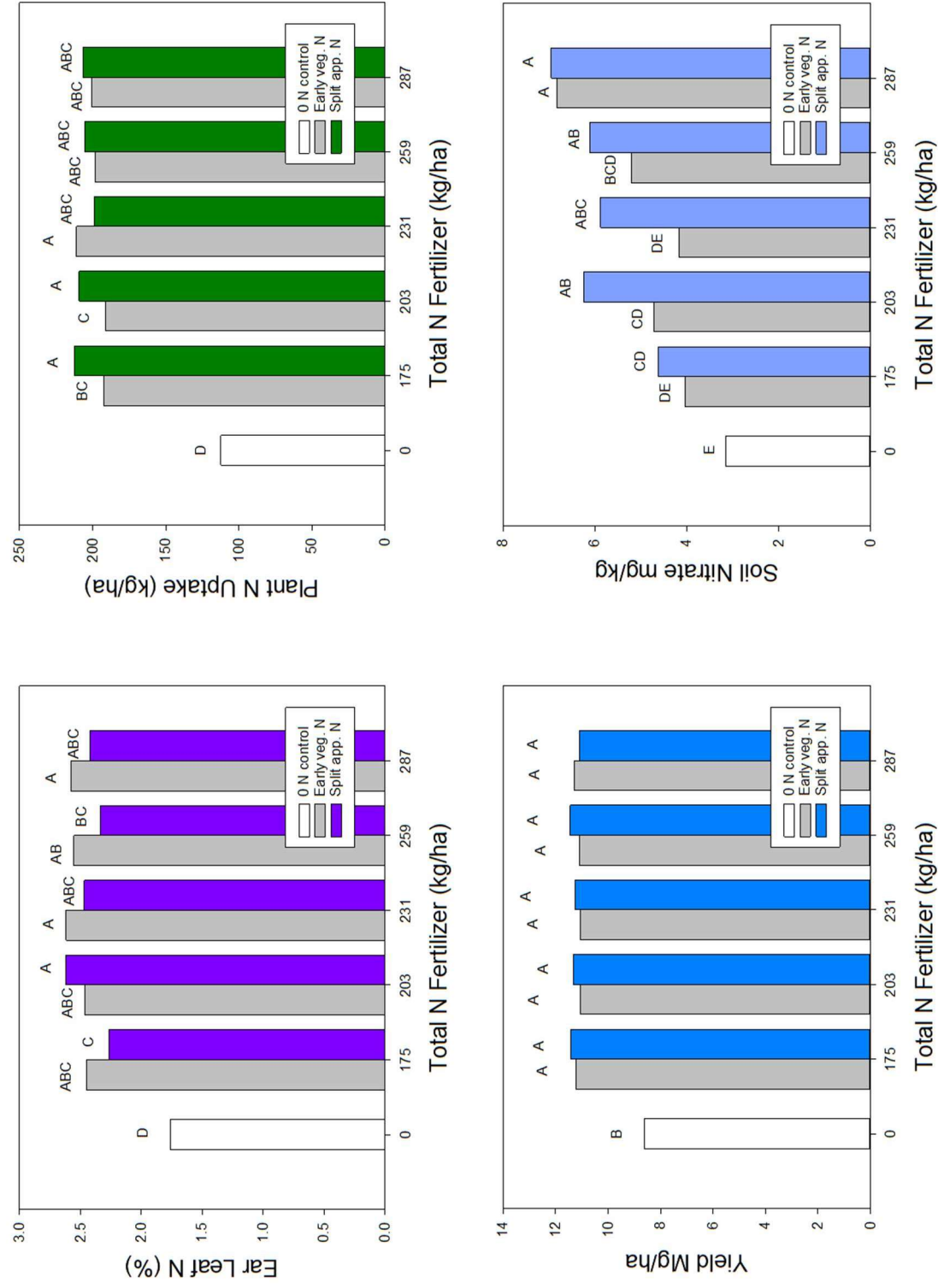


Figure 3.1.1. Ear leaf nitrogen, plant nitrogen uptake, yield, and soil nitrate after harvest by fertilizer rate and timing at Site 1.

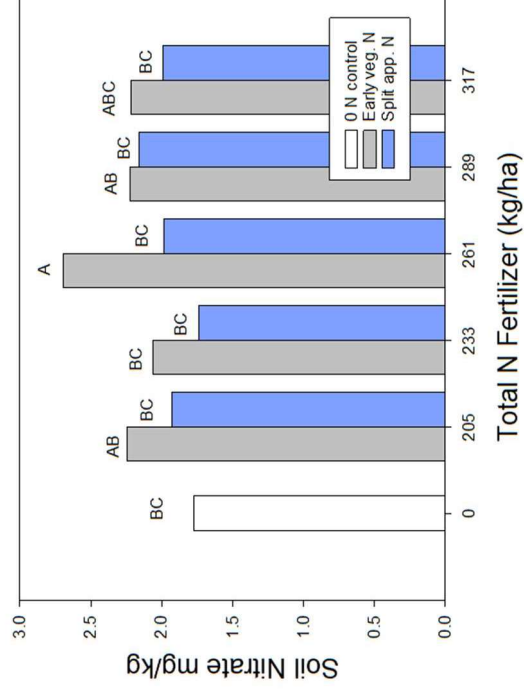
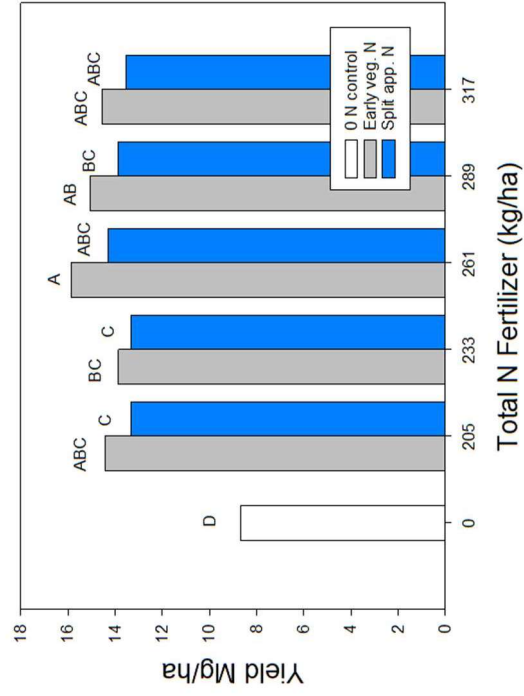
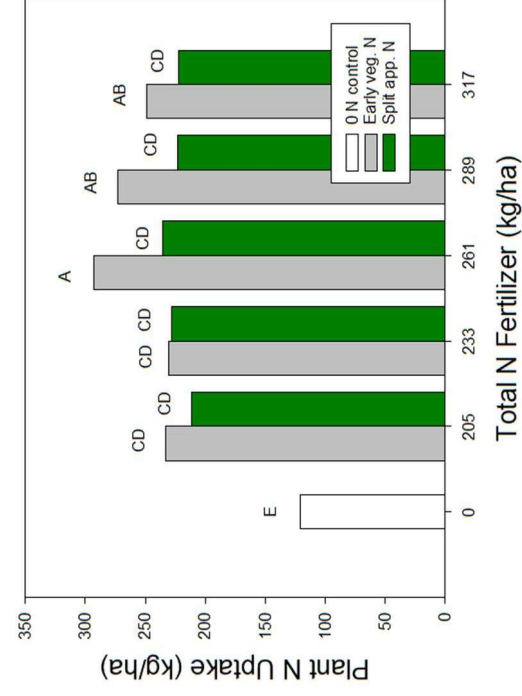
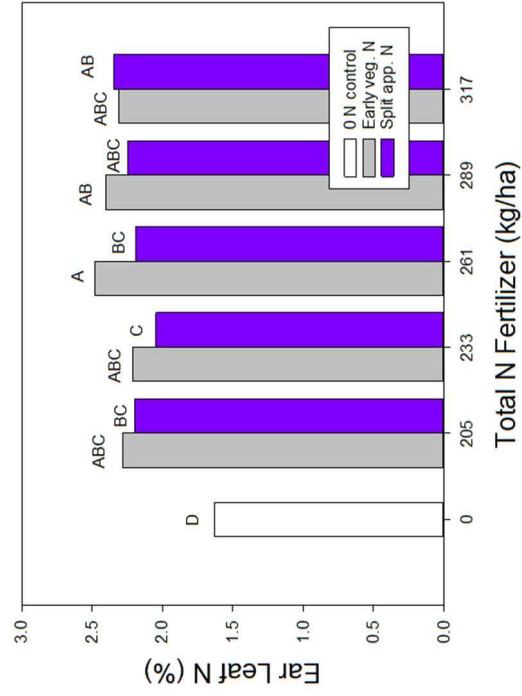


Figure 3.2. Ear leaf nitrogen, plant nitrogen uptake, yield, and soil nitrate by fertilizer rate and timing at Site 2.

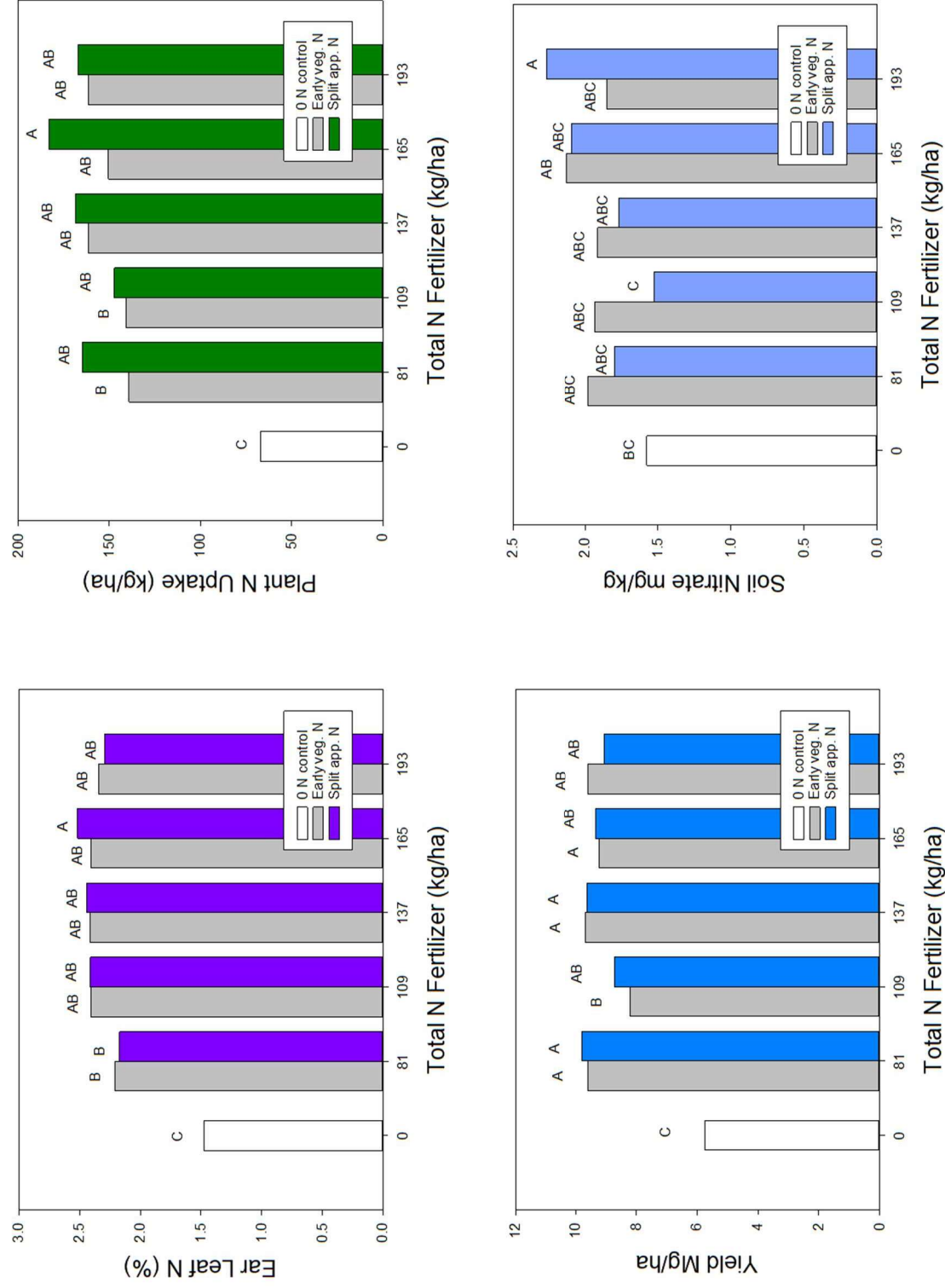


Figure 3.3. Ear leaf nitrogen, plant nitrogen uptake, yield, and soil nitrate by fertilizer rate and timing at Site 3.

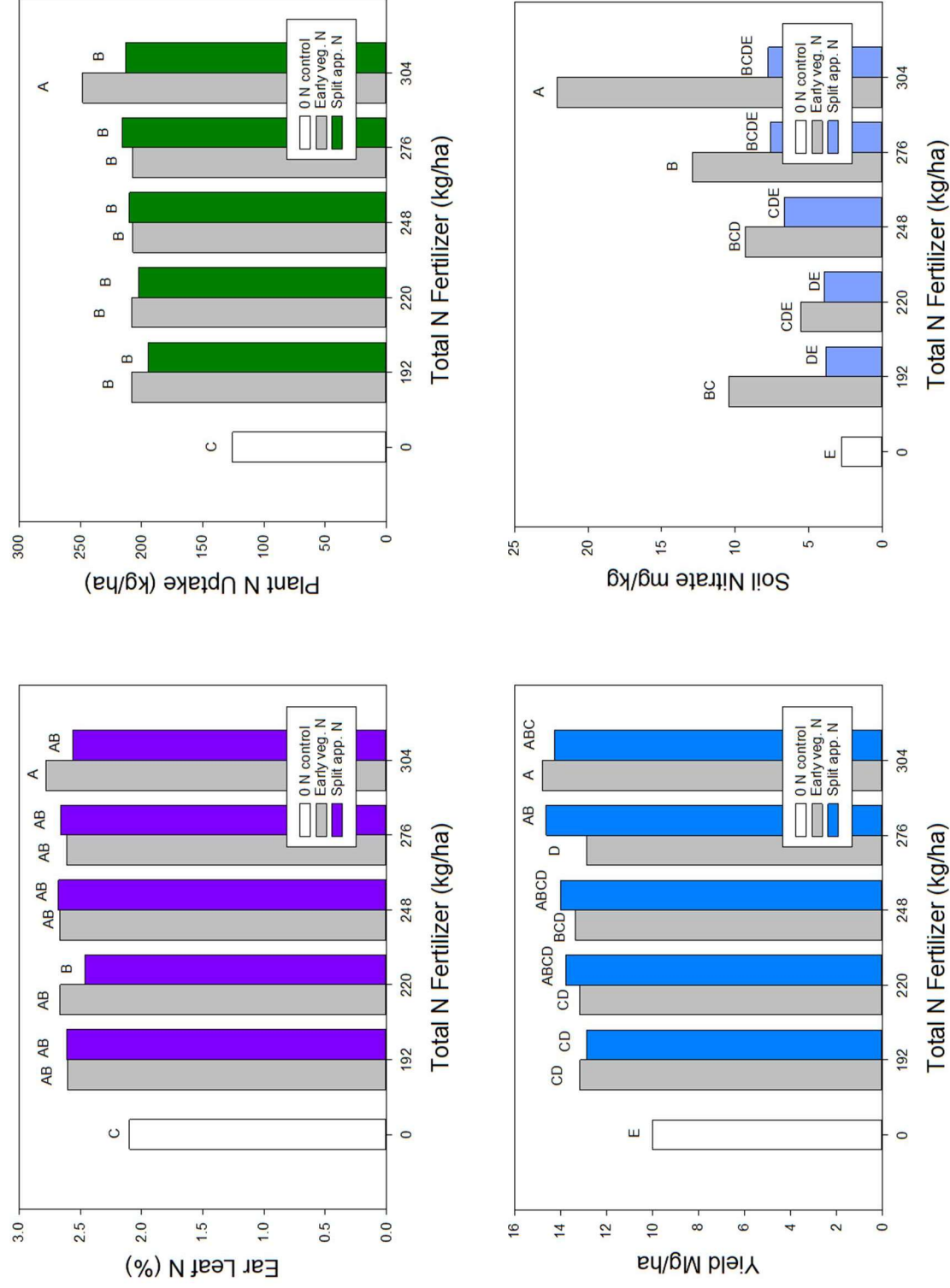


Figure 3.4. Ear leaf nitrogen, plant nitrogen uptake, yield, and soil nitrate by fertilizer rate and timing at Site 4.

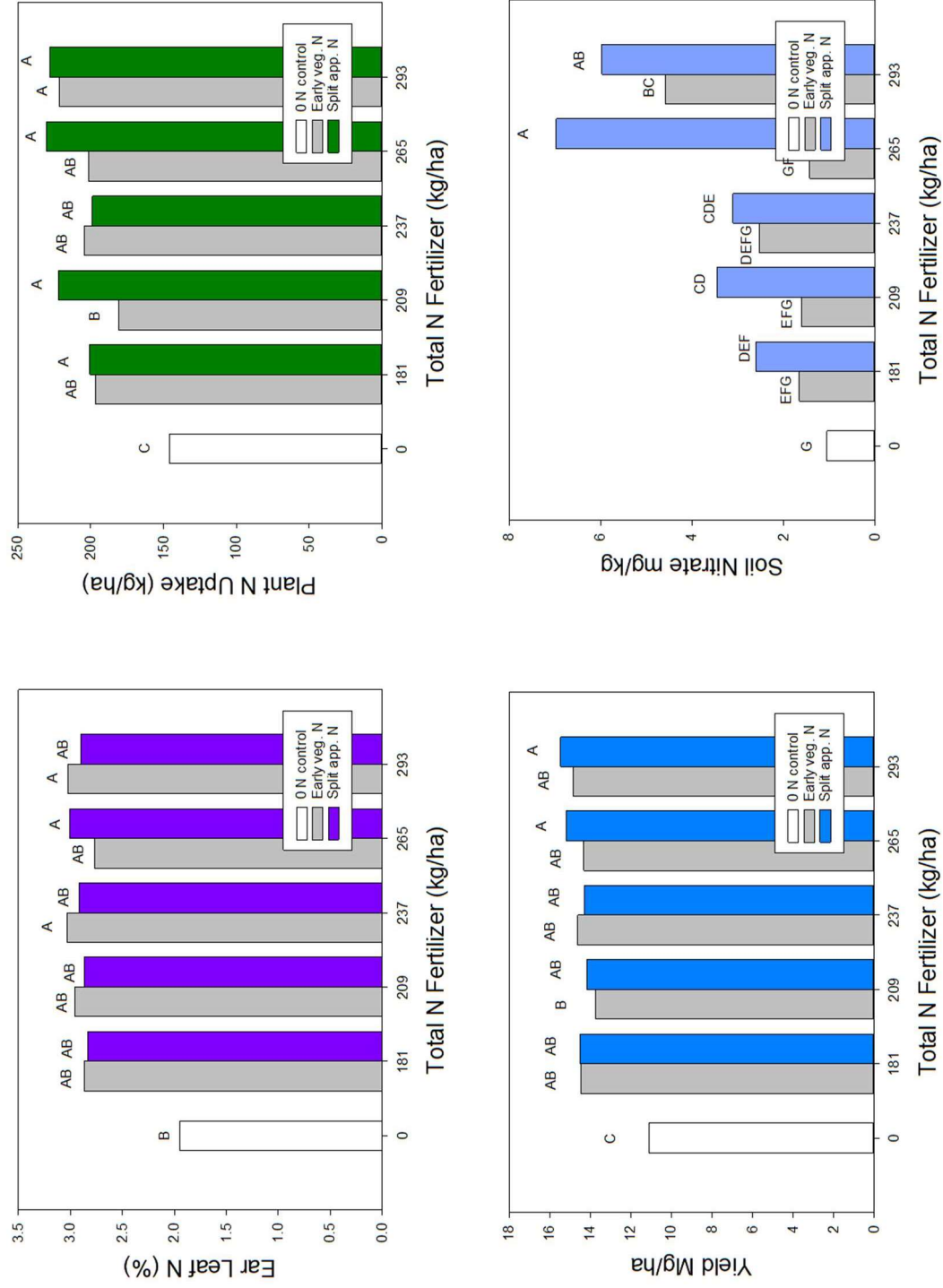


Figure 3.5. Ear leaf nitrogen, plant nitrogen uptake, yield, and soil nitrate by fertilizer rate and timing at Site 5.

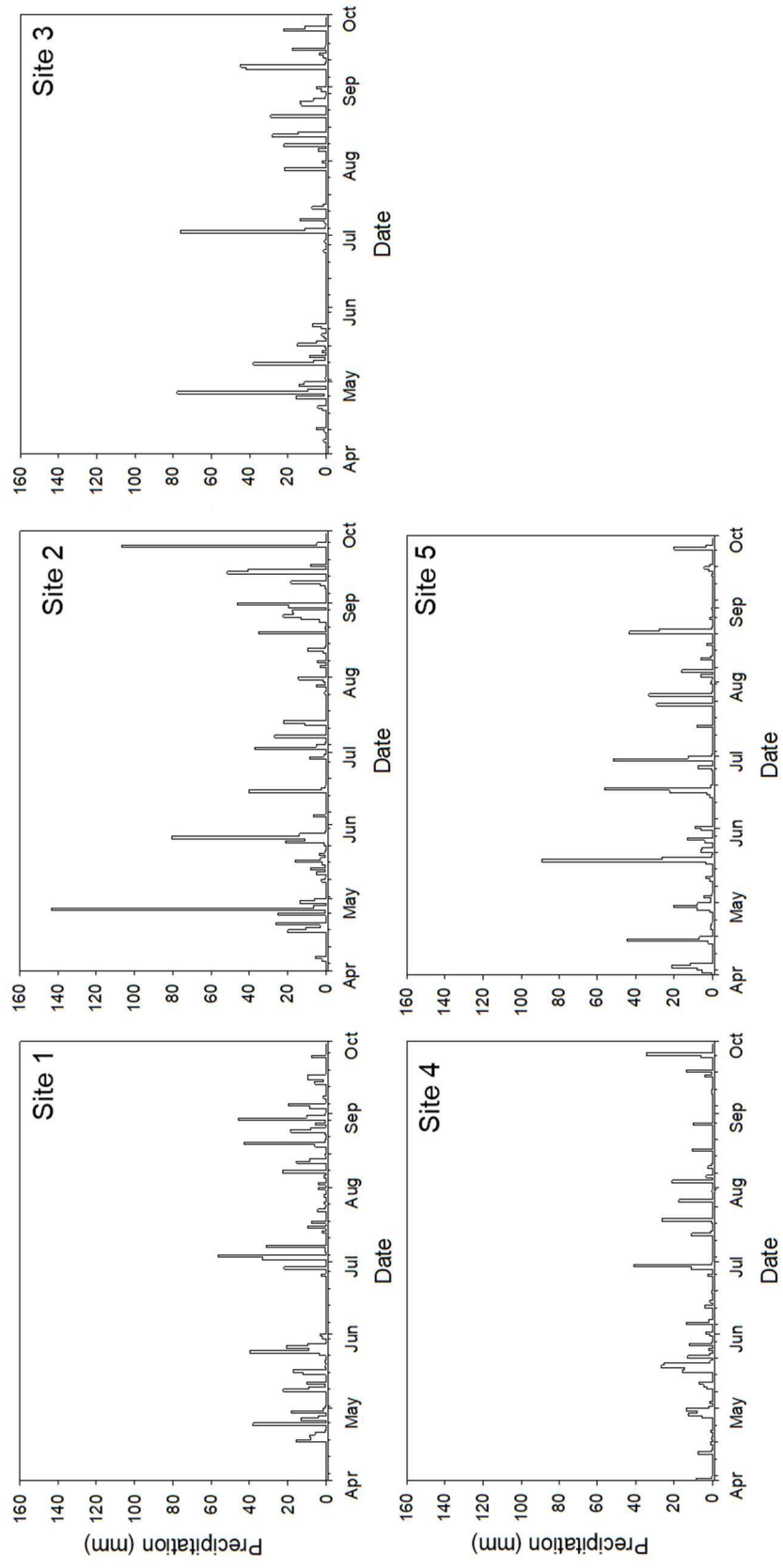


Figure 3.6. Precipitation from April 1 to October 1 for all sites based on the nearest Kansas Mesonet station.

CHAPTER 4 - ASSESSMENT OF MICRO-SCALED DIGESTION OF PLANT MATERIALS FOR PLANT TISSUE ANALYSIS

ABSTRACT

Small samples, even smaller than the .5g samples often used for micro digestion of plant analysis samples, are of interest for lab work since they can allow an analysis of smaller portions of plants, which could be necessary for small-scale greenhouse research or other projects when large sample size is difficult. They also may require less oxidizing agents to complete the digestion process, which can reduce the reagent costs for laboratories. However, smaller sample sizes might induce additional error in the analytical process because of increased sampling error, especially with larger sample particle sizes. While differences in recoveries of different plant analysis methods have been documented in the past, applying these efforts to uniform sample sizes and methods provides insight into the differences between them. Differences in elemental recovery between methods of plant digestion may lead to differences in agronomic test interpretation. An experiment was designed with digestion methods, run dates, and plant materials. Plants were digested with nitric perchloric, dry ash, and nitric tube methods for several days. The plant parts in the study were corn grain, corn ear leaf, soybean grain, soybean leaf, wheat grain, and wheat flag leaf. Plant parts were measured for Ca, Cu, Fe, K, Mg, Mn, P, S, and Zn by ICP-OES. When comparing methods, we determined that all methods had similar recoveries for elements other than S, Fe, Mn, and K, and that a new closed tube method had lower recovery of K, Fe, and Mn, likely due to lower digestion temperatures and less complete reaction than the established methods.

INTRODUCTION

The dissolution of plant matter to determine nutrient content dates back to the early days of plant study, often with interest in ash nutrient content (Hall, 1905; Tyner, 1947). Early expression of plant nutrient content as a percent of ash gave way to the modern expression of minerals on a dry matter basis (Tyner, 1947). Expressing the nutrient content on a dry matter basis rather than ash allowed for less error associated with soil contamination. Early plant analysis systems utilized combinations of lab methods and apparatus to determine the desired analytes. One methodology developed in Sweden utilized Kjeldahl Nitrogen, molybdate blue phosphorus, and flame spectrographic analysis with the spectra read by a film for several cations, electrical arc and spark methods for a variety of elements, including zinc and boron (Lundegardh H., 1951).

Single elemental analysis methods gave way to inductively coupled plasma (ICP) spectroscopy analysis, allowing for many analytes to be simultaneously measured, leading to increased speed and efficiency and lower cost/sample that help make these tests increasingly accessible to ag producers and consultants. Modern ICP spectrophotometry can run over 60 plant samples per hour across a wide range of elements.

Plant digestion began as a variety of dry ash and wet acid digestion methods, utilizing heat, strong acids, and other oxidizers to break down plant material. Dry ashing oxidizes the organic components of the material at high temperatures in a muffle furnace, allowing for less use of hazardous chemicals. Another approach commonly used is wet digestion, utilizing a reaction of dried plant material, strong acids such as concentrated nitric or sulfuric acid, and often other oxidizers or digestion aids. As laboratories increased their throughput capacity with wet digestion methods, individual digestion vessels gave way to racks of test tubes set in milled

aluminum blocks heated on top of hot plates. Commercially produced blocks with internal heating elements and programmable computer control were joined by microwave heating of sealed Teflon vessels to speed digestion (Wu, Feng, & Wittmeier, 1997). A wet digestion without a block digester could potentially offer the benefits of wet digestion without the need for specialized equipment, reducing bottlenecks in the lab. Digestion in closed containers can prevent the loss of sample material, contain splashing, and reduce the amount of fumes to be dealt with (Wheal, Fowles, & Palmer, 2011). Early efforts at closed tube method development came from the 1980's but utilized glass rather than plastic tubes (Anderson & Henderson, 1986). While the recovery of elements should be similar, the digestion method can induce interferences. Digestion with sulfuric acid masks sulfur content, and stripping of boron from digestion tubes that have not been treated with acid may contaminate samples (Green, 1976). Plastic or teflon digestion tubes may prevent contamination from boron (Wheal et al., 2011). Sealed digestion vessels also help prevent additional contamination (Hansen et al., 2009). The objective of this study was to evaluate the possibility of using an alternative heating method, a lab oven with a novel capped polypropylene tube plant digestion. This digestion was tested to compare it to nitric perchloric and dry ashing digestions.

MATERIALS AND METHODS

A study was conducted with three digestion methods, six run dates, and six plant materials. The factors of digestion method and plant materials were arranged factorially in a complete randomized design with six replicates. These run dates were repeated six times for each method. Three different digestion methods were compared: a nitric perchloric digestion, which served as the baseline, a dry ashing method, and a novel nitric digestion in closed polypropylene

tubes. For each digestion method, samples were treated similarly with similar weights of plant material (0.2 to 0.3 g), and identical final volumes. After digestion, samples were diluted to 50 ml volume, and standard curves using identical concentrations of the unknown elements were used. All samples were measured via ICP OES. We calibrated the instrument with matrix-matched standards using identical elemental concentrations.

Samples of wheat grain, wheat flag leaves, corn grain, corn ear leaf materials, soybean grain, and leaves were collected from the field, dried to constant weight at 60 °C and ground to a 1mm size by Wiley Mill in the case of the leaves, or ground using a burr style coffee grinder to a fine powder less than 1mm in diameter for grain material. The samples ranged in size from 100-4,000g and were thoroughly homogenized before use. Then, samples were digested by the following methods before reading the solution for Ca, Cu, Fe, K, Mg, Mn, P, S, and Zn. The general description of plant materials is shown in Table 4.1.

Dry Ashing

Dry ashing is a relatively simple method of sample preparation. The dry ashing method was adopted from a Southern regional publication (Plank et al., 1992). A sample of .2-.3g was weighed into a 10 ml pyrex beaker before samples were ashed. Samples were ashed for six hours at 550°C in 10 ml borosilicate glass beakers before analytical transfer to 50 ml digestion tubes and brought to volume using 1% vol/vol with concentrated nitric acid. The capped tubes were inverted to mix the samples. Any undissolved ash settled to the bottom of the tube overnight before the solution was read via ICP OES.

Nitric Perchloric Digestion

Digestion of plant material with a combination of nitric and perchloric acid is a long-standing method. The present study modified the nitric perchloric digestion method (Geiseking,

1935) to work at a micro-scale. Between .20 and .30g of sample was weighed on an analytical balance, before 5 ml nitric acid and two boiling chips were added to the digestion tube. An overnight predigestion preceded heating the samples. The next day, 5 ml of perchloric acid was added to each tube. Tubes were then inserted into a cool digestion block. The temperature was set to 200°C, and the block was turned on. The rack was rotated in the block every 30 minutes and digested for 3 hours. Then, the sample rack was removed from the block and allowed to cool for 20 minutes. Samples were diluted with distilled water to the 50 ml mark and mixed by inverting the tube several times.

Nitric Tube Digestion

A digestion method utilizing polypropylene centrifuge tubes heated in a lab oven was developed based on several existing methods. Between .20 and .30g of plant material was weighed into a centrifuge tube. Next, a 5-ml aliquot of concentrated nitric acid was added, and the sample was predigested overnight. Following predigestion, capped tubes were heated in a lab oven at 60°C for 4 hours. After cooling, samples were brought to volume (50ml) using DI water and read via ICP OES.

Statistical analysis

Statistical analysis was performed using the generalized linear mixed modeling procedure (PROC GLIMMIX) in SAS 9.4 (SAS Institute, 2020). Concentrations of Ca, Cu, Fe, K, Mg, Mn, P, S and Zn were treated as independent variables. The analysis method and plant material were treated as fixed effects, and the run (date of sample run) was treated as a random effect. Degrees of freedom were corrected by Kenward Roger. Confidence limits were calculated using $\alpha=0.10$, and means were separated by a 1:1 line to evaluate the relationship between analysis methods. To determine the effect of run on variation in plant nutrient concentration, variance components

were determined with PROC GLIMMIX using the method of restricted maximum likelihood (REML) (SAS Institute, 2020).

RESULTS AND DISCUSSION

Generally, results compared well between digestions with the notable exception of S, Fe, Mn, K and to a lesser extent P, Mg, and Zn (Table 4.2 and Figures 4.1-4.3). Sulfur was the only element with an interaction of methods and materials, meaning that some materials reacted differently with this method. Sulfur results differed greatly because differential levels of ashing across material types lead to different residual levels of S remaining in the samples. Under this ashing regime, some samples were ashed into a white powder, while others were visibly charred but not completely converted into white ash. Dry ashing of plant material can lead to sulfur losses through gaseous losses during the ashing process. Previous work has shown similar relationships, with approximately a 30% reduction in S concentration in dry-ashed samples relative to wet digestions (Blanchar, Rehm, & Caldwell, 1965; Soon, Kalra, & Abboud, 1996).

Significant differences in iron recovery between the nitric perchloric and nitric tube methods may be related to the lack of additional oxidizers in the nitric tube digestion leading to a partial extraction at higher concentrations, since iron can be extracted from many organic materials (Gorsuch, 1970). Another possibility is that silica is less completely dissolved in the less intense nitric tube digestion, which could interfere with Fe measurement (Sahrawat, Ravi Kumar, & Rao, 2002).

Potassium recovery was similar between the nitric perchloric and dry ash methods, while the nitric tube method recovered less than other methods. This is especially surprising since K is

commonly found as a relatively soluble anion in fresh plant material. In addition, previous work has shown similar recoveries between wet and dry ashing for K (Sahrawat et al., 2002).

Dry ashing recovered less Mn than wet digestion, and generally, there was a high variance within the dry ash procedures relative to other methods. This differs from previous work that showed similar recoveries for Mn between methods across a wide variety of materials (Gorsuch, 1970; Rechcigl & Payne, 1990; Sahrawat et al., 2002).

The dry ash digestion recovered phosphorus at the highest levels, followed by the nitric perchloric digestion method, and then by the nitric tube method. This suggests that the nitric tube digestion method is less complete in its recovery than the other two methods.

Zinc had a significantly higher recovery for dry ashing than the other methods.

Magnesium was significant in the ANOVA, but only one sample had a significant difference between the dry ash and nitric perchloric digestion.

Variance based on the impact of material and a combination of run sequence and the material was calculated by element and method component (Table 4.3). Dry ash Mn had a high variance. This may be related to the formation of complex insoluble silicates (Gorsuch, 1970).

CONCLUSIONS

Although destroying organic matter may seem like an unimportant step in plant analysis, different digestion methods may significantly contribute to differences in measured elemental values in unknown samples. This is especially pronounced in the case of certain analytes, including S, Fe, Mn, and K, and to a lesser extent P, Zn, and Mg. Differing levels of elemental recovery between methods may risk misdiagnosing crop nutritional status in situations where the methods utilized to digest plant material do not match the methods used to establish interpretive

values. The dry ash method showed the highest variance for most plant nutrients, suggesting lower repeatability. The nitric perchloric, and nitric tube digestion showed similar variance, offering a more consistent value over multiple runs. Further refinement of the nitric tube digestion may offer a good alternative that may work well with equipment and daily work schedules at commercial labs if recovery challenges with select elements are overcome.

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TABLES AND FIGURES

Table 4.1. Plant species, plant parts and C and N

Plant	Plant part	Total N ----- g kg ⁻¹	Total C -----	C:N Ratio
Corn	Ear Leaf	23.9 (0.22) †	433.2 (0.53)	181.5 (.17)
Corn	Grain	12.8 (0.19)	438.1 (0.48)	341.3 (.51)
Soybean	Leaf	56.8 (0.36)	443.3 (1.15)	78.0 (.03)
Soybean	Grain	58.3 (0.26)	495.2 (0.50)	84.9 (.03)
Wheat	Flagleaf	37.3 (0.01)	429.7 (1.40)	115.3 (.04)
Wheat	Grain	21.9 (0.22)	432.8 (1.35)	197.8 (.23)

† Values in parenthesis indicate the standard error of the mean

Table 4.3. Estimate of the variance (s^2) components associated with plant tissue nutrient concentrations by digestion methods.

Nutrient	Variance component†	Method*		
		DA	NP	NT
		----- S^2 (SE) -----		
Ca	RunSeq(Material)	2.7 (1.6)	0.3 (0.1)	0.2 (0.1)
Cu	RunSeq(Material)	2.4 (0.8)	0.8 (0.3)	0.3 (0.1)
Fe	RunSeq(Material)	363 (123)	86 (34)	232 (63)
K	RunSeq(Material)	10 (3.4)	2.6 (0.8)	0.9 (0.3)
Mg	RunSeq(Material)	0.14 (0.08)	0.05 (0.02)	0.02 (0.01)
Mn	RunSeq(Material)	2000 (609)	119 (33)	46 (15)
P	RunSeq(Material)	0.25 (0.08)	0.12 (0.04)	0.08 (0.02)
S	RunSeq(Material)	0.22 (0.1)	0.15 (0.04)	0.02 (0.01)
Zn	RunSeq(Material)	15 (7)	24 (7)	4 (1)

†Variance component Run Sequence Material was examined variance by individual digestion run across materials.

*Methods were dry ash (DA) nitric perchloric (NP) and nitric tube (NT) digestions.

Table 4.4. LS Mean concentrations of P, K and Ca for different plant materials by digestion methods

Plant material	P			K			Ca		
	DA	NP	NT	DA	NP	NT	DA	NP	NT
	----- g kg ⁻¹ -----								
Corn Grain	3.02	2.79	2.62	4.77	3.17	2.48	2.32	0.08	0.08
Corn Leaves	3.39	2.99	2.84	17.26	17.23	15.22	4.92	4.80	4.78
Soybean Grain	4.06	3.86	3.71	16.69	16.06	14.32	4.06	3.05	3.39
Soybean Leaves	4.69	4.53	4.42	22.16	22.16	20.26	9.14	9.56	9.50
Wheat Grain	3.88	3.67	3.45	3.60	3.37	2.61	0.57	0.38	0.36
Wheat Leaves	2.62	2.17	2.02	14.83	13.72	11.60	6.79	6.51	6.26

*Methods were dry ash (DA) nitric perchloric (NP) and nitric tube (NT) digestions.

Table 4.5. LSMean concentrations of Mg, S and Zn for different plant materials by digestion methods

Plant material	Mg			S			Zn		
	DA	NP	NT	DA	NP	NT	DA	NP	NT
	----- g kg ⁻¹ -----			----- mg kg ⁻¹ -----					
Corn Grain	1.38	0.95	0.86	0.80	0.86	0.66	21.0	18.1	17.2
Corn Leaves	1.78	1.67	1.60	0.91	1.42	1.33	21.6	16.7	16.9
Soybean Grain	2.45	2.31	2.25	1.59	2.81	2.66	32.5	31.3	30.9
Soybean Leaves	3.32	3.44	3.29	1.71	3.01	2.91	43.6	40.6	39.0
Wheat Grain	1.48	1.45	1.32	0.26	1.38	1.24	35.9	34.2	31.4
Wheat Leaves	2.20	2.06	1.92	1.71	2.84	2.67	19.0	13.7	14.0

*Methods were dry ash (DA) nitric perchloric (NP) and nitric tube (NT) digestions.

Table 4.6. LSMean concentrations of Fe, Cu and Mn for different plant materials by digestion methods

Plant material	Fe			Cu			Mn		
	DA	NP	NT	DA	NP	NT	DA	NP	NT
	-----mg kg ⁻¹ -----								
Corn Grain	27.21	20.54	16.18	2.27	1.71	1.76	15.11	4.88	4.83
Corn Leaves	106.33	140.60	98.94	7.32	7.93	7.93	36.03	38.89	38.66
Soybean Grain	62.50	59.58	54.36	10.52	10.81	10.59	44.11	32.94	37.48
Soybean Leaves	118.78	144.65	104.12	11.41	11.74	11.22	75.77	116.70	116.81
Wheat Grain	39.63	43.56	35.52	2.38	4.62	4.48	43.57	53.29	51.91
Wheat Leaves	82.40	93.44	71.63	4.79	5.66	5.58	116.06	203.91	199.49

*Methods were dry ash (DA) nitric perchloric (NP) and nitric tube (NT) digestions.

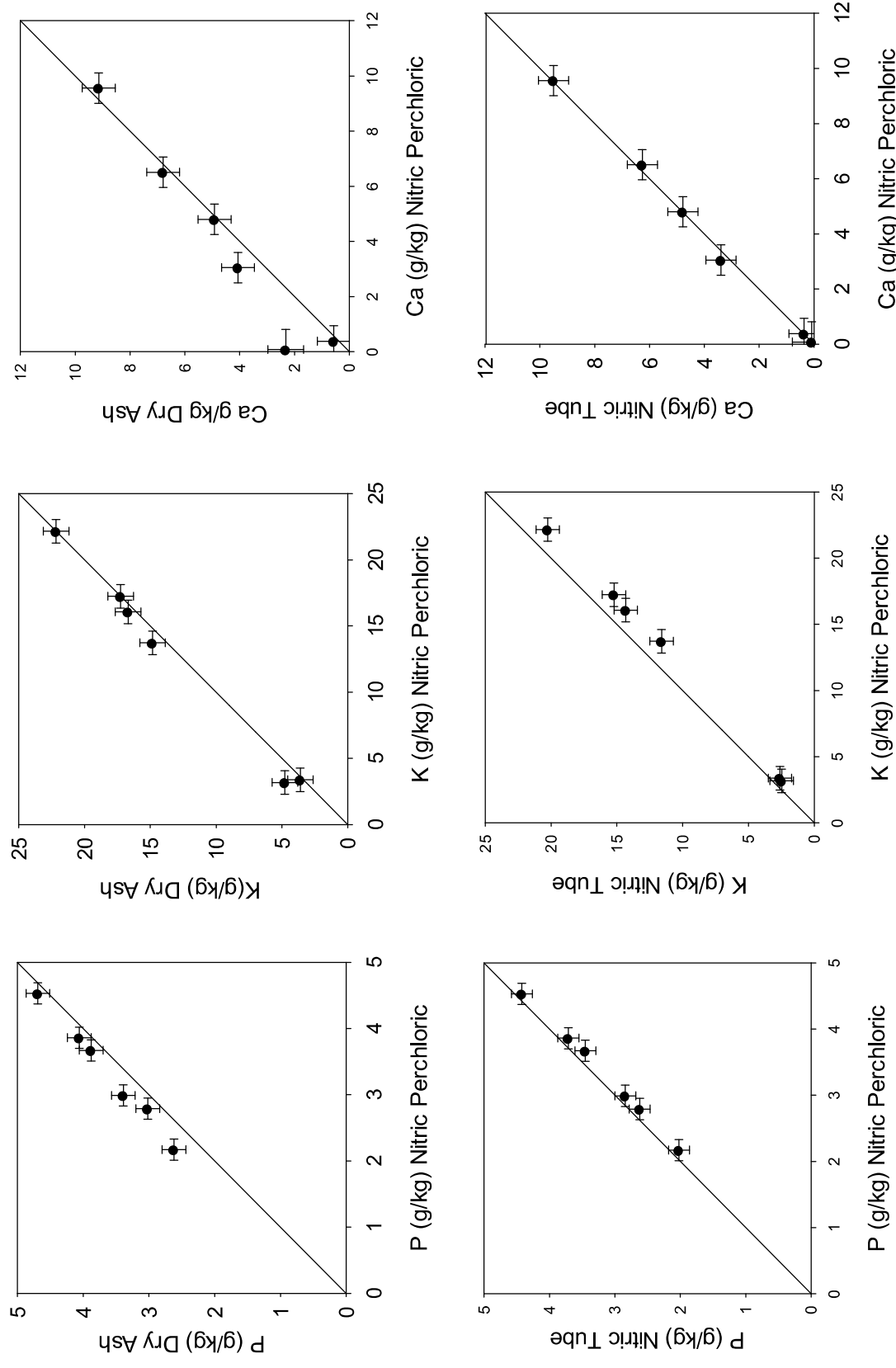


Figure 4.1. Elemental concentration of samples by combinations of digestion methods for phosphorus, potassium and calcium. Error bars show standard error.

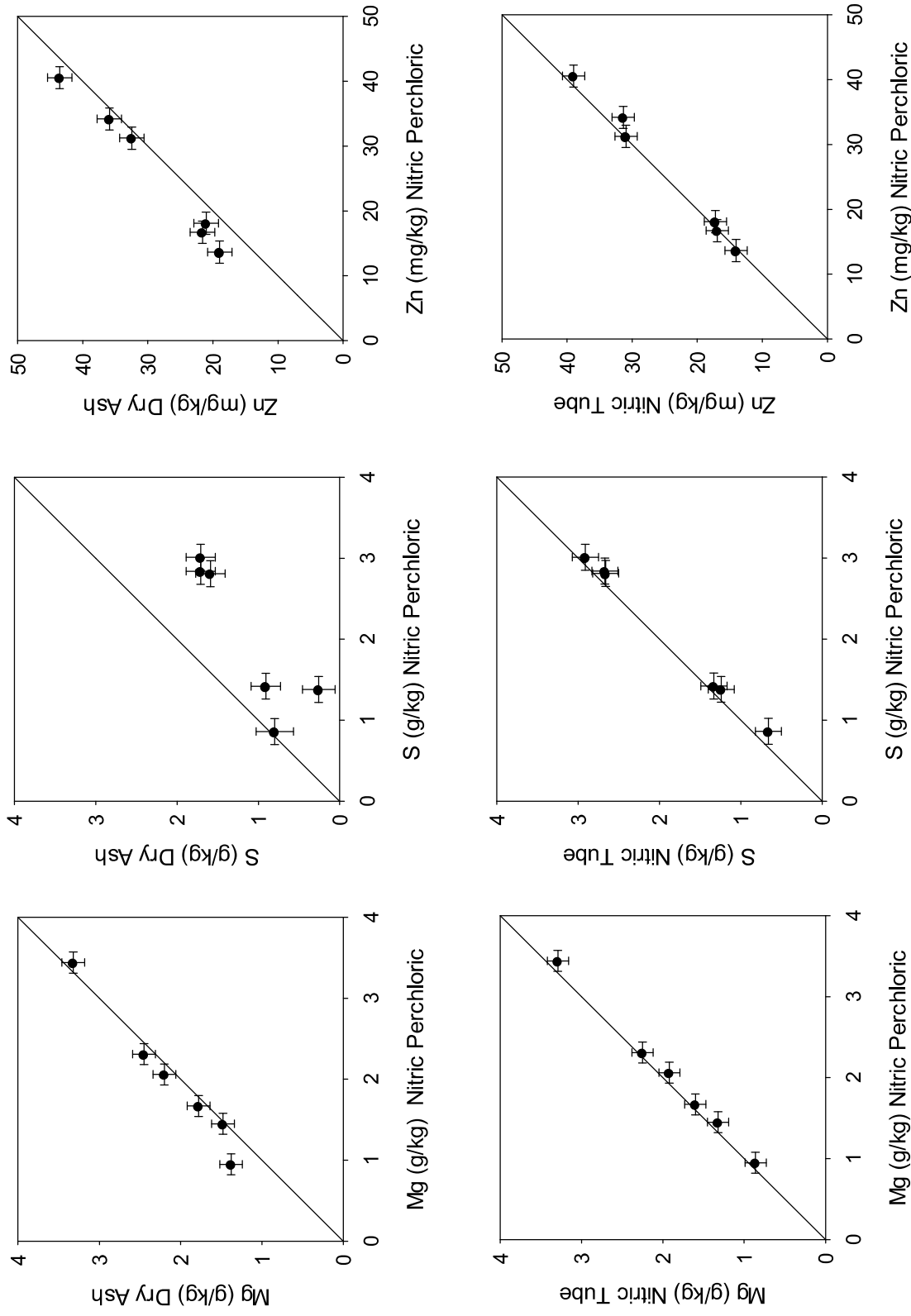


Figure 4.2. Elemental concentration of samples by combinations of digestion methods for magnesium, sulfur, and zinc. Error bars show standard error.

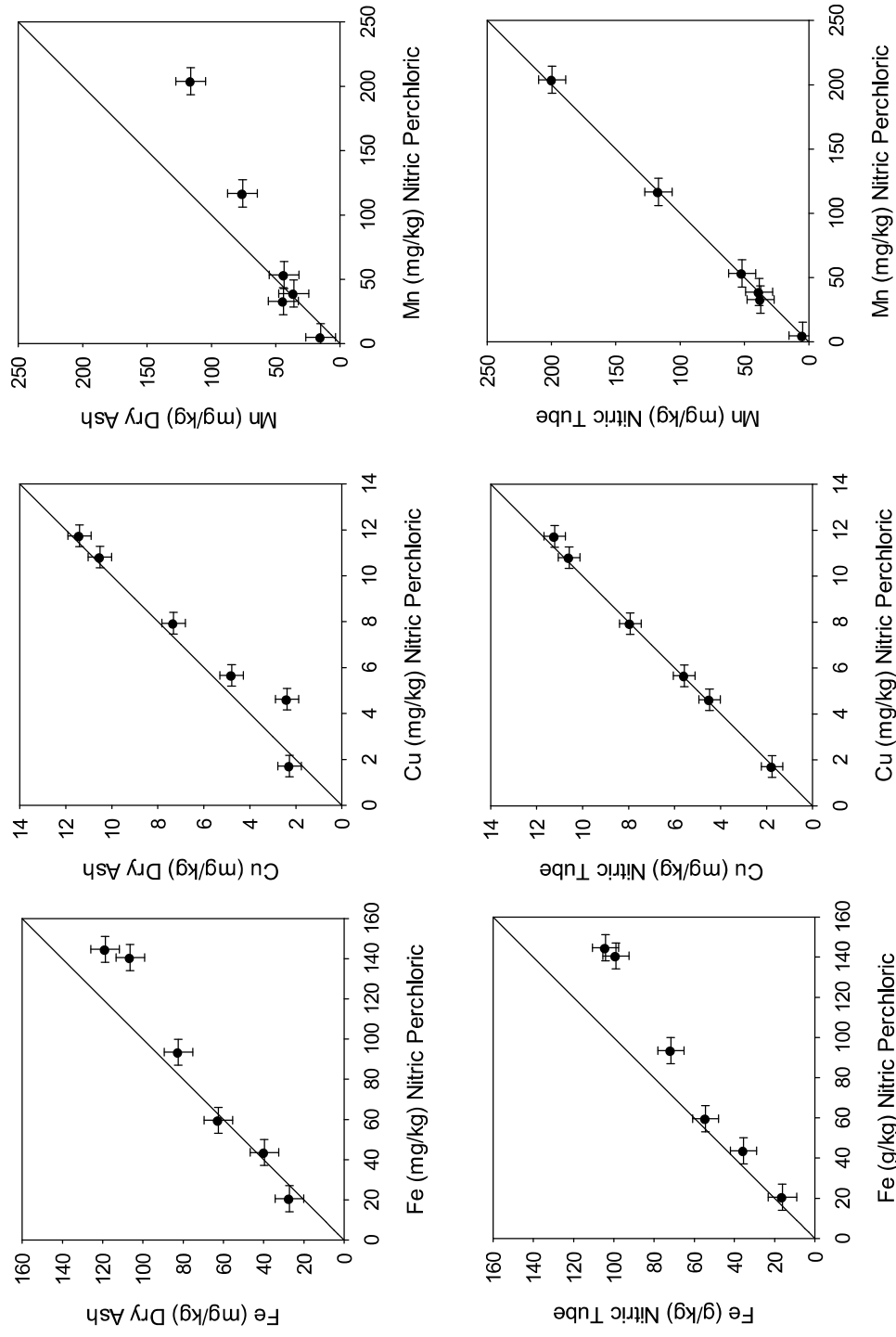


Figure 4.3. Elemental concentration of samples by combinations of digestion methods for iron, copper, and manganese. Error bars show standard error.

CHAPTER 5 - GENERAL CONCLUSIONS

Results from the work presented in chapter 2 showed that increases in grain N uptake, plant N uptake, and grain yield from both N rate and fungicide application suggest that split application can increase corn N uptake, especially in environments with the potential for in-season N loss. The interactions of N rate by application method (RxM) for N uptake suggest that late split N applications may increase corn's plant uptake. However, this work also showed the risks associated with withholding a significant portion of the total N fertilizer needs. This resulted in N deficiency for critical periods of the growing season, leading to yield impacts that the crop did not recover from with N applications at the late R1 stage. Fungicide applications or foliar nitrogen applications may require additional refinement of N recommendations and management. Foliar N applications combined with late-split N fertilizer application (R1 growth stage) may benefit the crop under specific conditions by preventing disease, rather than simply providing additional N for grain filling during the reproductive stage.

A key takeaway from chapter 3 was that the Kansas State University N recommendations showed no yield differences versus rates that were reduced by 56 kg N/ha when applied preplant or as a split application. This shows opportunities to improve recommendations for irrigated sites where lower N rates may be appropriate given likely higher N use efficiency per unit of grain yield. Further research to evaluate rates and timing of split N fertilizer will help provide targeted recommendations to inform agronomic decisions across both irrigated and dryland sites.

As documented in chapter 4, destruction of organic matter in the lab is a key step in plant tissue analysis and may contribute to recovery differences between several key elements such as S, Fe, Mn, K, P, Zn and Mn. Differing recovery levels could contribute to potential misdiagnosis of crop nutritional status if interpretation data is based on a different method of analysis than the

test performed by the lab. Dry ash showed a higher variance than wet digestions with nitric acid.

Understanding these differences could inform the best methods for analysis and provide perspectives for interpretation of results for agronomists.