TIME AND RATE OF NITROGEN FERTILIZATION INFLUENCE MAIZE NITROGEN USE EFFICIENCY AND SOIL ENZYME ACTIVITY

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Abstract

Experiments were conducted from 2014 to 2016 comparing single (fall and spring) and split applications of differing nitrogen (N) rates for maize (*Zea mays* L.) on an irrigated Hubbard-Mosford loamy sand complex at Becker, a non-irrigated Normania loam soil at Lamberton, and a non-irrigated Nicollet clay loam soil at Waseca, MN. Fall and spring treatments were applied at recommended and 125% of recommended rates based on University of Minnesota guidelines for the different locations. Split-application treatments consisted of Sp, a two-way split (one-half of the N applied before planti and one-half applied at the six-leaf collar stage of maize phenological development (V6) and TSp, a three-way split (one-third of the N applied pre-plant, one-third at the V6 stage, and one-third at the silking stage of maize phenological development (R1) stage. Nitrogen rates varied by location and were based on University of Minnesota guidelines. All sites were planted to soybean [*Glycine max* L. (Merr.)] in 2013 and to maize in 2014 to 2016. At Becker, applying N fertilizer at the recommended rate as a three-way split improved maize grain and biomass yield, maize nutrient uptake, and nitrogen use efficiency (NUE). At Lamberton, grain yield, nutrient uptake, and NUE parameters did not differ among treatments applied at recommended rates, regardless of application time. At Waseca, applying the recommended N rate as either a two- or three-way split improved grain yield and NUE compared with fall or pre-plant application, while recommended N rates maximized nutrient uptake. Soil enzyme activity fluctuated across the growing season and decreased over time, particularly in the coarse-textured soils at Becker. Although microbial activity declined annually, there was no significant change in glucosidase activity. There was a decline in acid phosphatase activity in coarse- but not finer-textured soils. At Becker, applying N fertilizer as a three-way split increased sulfatase activity compared with applying N fertilizer in the fall or pre-plant. Enhanced understanding of how site-specific soil and weather characteristics influence these responses could increase maize yield and nutrient uptake while reducing the potential for nitrogen loss to the environment.

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CHAPTER 1

Timing and rate of urea influence maize yield and NUE

Abstract

The timing and rate of nitrogen (N) fertilizer application can influence maize (*Zea mays* L.) grain yield and nitrogen use efficiency (NUE), but results have been inconsistent across locations and growing seasons. This study was conducted to compare single (fall and spring) and split applications of differing N rates for maize and soil enzyme activity. This study was conducted on an irrigated Hubbard-Mosford loamy sand complex at Becker, a rainfed Normania loam soil at Lamberton, and a rainfed Nicollet clay loam soil at Waseca, MN. Fall and spring treatments were applied at recommended and 125% of recommended rates (RN) based on University of Minnesota guidelines for the different locations. Split-application treatments consisted of Sp, a two-way split and TSp, a three-way split. Nitrogen rates varied by location and were based on University of Minnesota guidelines. All sites were planted to soybean [*Glycine max* L. (Merr.)] in 2013 and to maize in 2014 to 2016. At Becker, applying fertilizer at RN as a three-way split application produced 12.6 to 15.7 Mg ha^{-1} of maize grain annually, significantly greater grain yields than all fall or pre-plant applications regardless of rate. At Becker, the TSp treatment improved agronomic efficiency (AE) and partial factor productivity (PFP) over fall or spring applications but did not impact nitrogen recovery efficiency (RE). At Lamberton there was a lack of differences in maize grain yield and NUE parameters between treatments applied at RN, regardless of timing of application. However, applying

75% of the recommended rate as a TSp improved AE and PFP while producing comparable yields to fall and preant treatments. At Waseca, applying the recommended N fertilizer rate as either a two- or three- way split N fertilizer application improved maize grain yields and NUE compared with fall or preplant applications. Similar to Lamberton, applying 75% of the recommended N fertilizer rate as either a two- or three-way split application produced comparable yields to fall or preplant applications applied at recommended N fertilizer rates. Findings demonstrate that split-applications of N can increase maize grain yield on irrigated coarse-textured soils and there is potential for improved N management on non-irrigated clay loam soils. Enhanced understanding of site-specific soil and weather characteristics that influence such responses could increase maize yield and N uptake while reducing the potential for N losses to the environment

ABBREVIATIONS

AE, agronomic efficiency; BMP, best management practice; F, fall application; N, nitrogen; NUE, nitrogen use efficiency; PFP, partial factor productivity; PP, preplant application; RE, recovery efficiency; RN, recommended rate; R1,silking stage of maize phenological development; SB-C-C-C, soybean-maize-maize-maize; Sp, two way split application; TSp, three way split N application; V2, two leaf-collar stage of maize phenological development; V4, four leaf-collar stage of maize phenological development; V6, six leaf-collar stage of maize phenological development; V8, eight leaf-collar stage of maize phenological development; V12, twelve leaf collar-stage of maize phenological development.

Introduction

The rate and timing of N application are important management decisions that farmers make for maize (*Zea mays* L.) production. The goal is to minimize loss and increase uptake (Jokela and Randall, 1989). Nitrogen use efficiency (NUE) parameters have become useful in determining the efficiency of different N management strategies and potential environmental impact. In Minnesota, a 2009 survey of nearly 1500 growers found that 33% of growers applied N in the fall, 59% applied in the spring and only 9% side dressed the majority of their N after maize emergence (Bierman et al., 2012). Although growers are drawn to the convenience of applying N in the fall, a spring preplant N strategy often provides greater economic return and less N loss (Vetsch and Randall, 2004; Randall and Vetsch, 2005). Post emergence N application has been shown to increase grain yields compared with fall or preplant applications in course textured soils or excessively wet springs in finer textured soils (Jaynes, 2013; Rubin et al., 2015). However, other studies have not found a significant difference in yield between N fertilizer applied near the time of planting or as a split application (Jaynes, 2013; Fernandez et al., 2016; Venterea et al., 2016). Maize has low demand for N during the early growth stages but the demand increases and remains high several weeks into the growing season (Abendroth et al., 2011; Venterea and Coulter, 2015). If the timing of N application can be more closely matched to maize uptake, the risk of N loss through leaching and denitrification can be reduced.

Comparisons of timing of N fertilizer application have produced mixed, and often site specific results. In Iowa, a study comparing the application of N fertilizer at the 2nd leaf stage (V2) or equally split between the V2 and V6 or V12 in a maize-soybean rotation found no consistent difference in maize grain yields (Jaynes, 2013). This is in contrast to an earlier study where a split application of post emergence (V1-V3) and midseason (V16) application of liquid urea-ammonium nitrate (UAN) yielded significantly less maize grain than if the same amount of N had been applied all at postemergence (Jaynes and Colvin, 2006). Similar conclusions were reached in a three-year study in southwestern Onterio, Canada where the application of urea at planting increased maize grain yields by 10.7% compared with split fertilizer application where 22 kg N ha^{-1} was applied at planting and 130 kg N ha^{-1} applied at V6 (Drury et al., 2011). Results across Minnesota have also been mixed. Venterea and Coulter (2015) concluded that applying urea at planting, or equally split between planting and V6 or V14 did not significantly impact maize grain yield on a naturally-drained Waukegan silt loam. However, a seven-year average of split N applications on a poorly-drained Canisteo clay loam soil increased maize grain yields by 0.4 Mg ha^{-1} compared with N applied at preplant (Randall et al., 2003).

Creating conditions that are favorable to improving maize grain yield could potentially also improve N use efficiency (NUE). Nitrogen use efficiency in maize production systems is estimated to be approximately 33% globally, in part caused by the loss of fertilizer N from leaching below the root zone, denitrification, as well as, soil and plant derived volatilization (Raun and Johnson, 1999; Sindelar et al., 2015). The low

NUE in maize systems has contributed to surface water pollution in the Mississippi watershed and the hypoxic zone in the Gulf of Mexico (Porter et al., 2015). Results from studies assessing the impact of timing of N fertilizer application on NUE suggest that applying N fertilizer as a split application or preplant application, often result in improved NUE compared with N fertilizer applied in the fall (Cassman et al., 2003; Wortmann et al., 2011).

In Argentina, a four-year study on no-till maize reported that a split application of N fertilizer applied at planting and at V6 improved NUE over a single application at planting in three of the four study years, particularly at lower N fertilizer rates (Sainz Rozas et al., 2004). Similarly, in Pakistan a split application of urea was reported to increase NUE by 15 to 18% compared with a single full application at planting (Abbasi et al., 2013). In Minnesota, a six-year study was conducted to determine the effects of anhydrous ammonia (134 kg N ha^{-1}) applied in the fall or spring in combination with the nitrification inhibitor nitrapyrin (2-chloro-6-(trichloromethyl) pyridine) on maize production (Randall and Vetsch, 2005). The authors reported that N recovery, averaged across six years, was 47% for fall applied N without nitrapyrin (NP), 56% for both fall N with NP and spring N without NP, and 61% for spring N without NP (Randall and Vetsch, 2005). The N recovery reported by Randall and Vetsch (2005) was greater than a previous study by Randall et al. (2003), which applied 150 kg N ha^{-1} and produced lower yields. From 2002 through 2004, nitrogen response trials at 11 or 12 locations per year in Nebraska found that for continuous maize (CM) and maize-soybean (CS) rotation, the mean maize N recovery efficiency was between 70 to 85% at 112 kg N ha^{-1} for CM and

56 kg N ha^{-1} for CS (Wortmann et al., 2011). However, mean recovery efficiency was 40% when applying 336 kg N ha^{-1} for CM and 280 kg N ha^{-1} for CS (Wortmann et al., 2011).

Nitrogen fertilizer management can also influence soil nutrient content and maize N uptake. A greenhouse comparison of in-furrow starter fertilizer on clay loam, silt loam and fine sand textured soils determined that plant nutrient concentration and uptake were significantly affected by fertilizer application and differed by soil type (Kaiser and Rubin, 2013). Plants growing on the silt loam textured soil always had a greater N concentration than those on the fine sand (Kaiser and Rubin, 2013). Results from the study indicated that the nutrient status of the soil should be considered for N fertilizer management to account for the different physical or chemical properties of individual soils (Kaiser and Rubin, 2013). The time of maximum N accumulation in relation to maize development is dependent on available N supply in the soil once the maize crop has entered a rapid phase of vegetative growth. Wortmann et al. (2011) demonstrated that when using economic optimum N rates (EONR), defined as the N rate that returns the most profitable economic yield rather than maximum yield, approximately 60% to 70% of the applied N could be recovered. The use of EONR was found to produce low residual soil N levels compared with increased N rates, resulting in greater agronomic efficiency (AE), defined as the yield increase obtained per unit of fertilizer applied, and partial factor productivity (PFP), defined as the yield produced per unit of fertilizer applied (Wortmann et al., 2011). By achieving a PFP of 83 kg kg^{-1}, exceeding the global estimate of 40 kg kg^{-1} and US maize estimate of 58 kg kg^{-1}, Wortmann et al. (2011) demonstrated that high NUE and high yield maize can be achieved, but could be improved by using in-season N applications. Possible benefits of splitting N application compared with a single large N application is improved NUE and the possibility for lower N rates to achieve optimum yield at a given location. This may result in less N being available for potential loss through leaching, denitrification or volatilization. An important component of NUE is the grain yield per unit of applied N fertilizer, which can be determined by agronomic efficiency (AE), the increase in grain yield per unit N applied, the crop partial factor productivity (PFP), as the total grain yield relative to the amount of N applied, and recovery efficiency (RE), the increase in aboveground N biomass per unit N applied (Wortmann et al., 2011). These parameters provide growers an integrative index that quantifies total economic output relative to the utilization of nutrient resources in the system (Yadav, 2003). From 1992 to 2010, the ratio of maize produced per kg of N fertilizer applied has steadily increased from 20 to 38 kg of maize grain produced per kg of N applied (Murrell, 2011).

Abendroth et al. (2011) demonstrated that about 50% of the total N uptake in maize occurs by the time maize biomass production is about 25% of the maximum. Based on the relationship between N uptake and maize growth, it appears that one could break up the total N fertilizer input for maize into two, three, or even four application timings that coincide with specific times when maize plants need it most. Recommendations to improve N management include a reduction in fall applied N fertilizer and a shift to applying N fertilizer in the spring months or at planting, and greater use of split N fertilizer applications during the growing season (Bundy et al., 1999; Cassman et al., 2002). By better timing N application to key maize N uptake stages, farmers may be able to apply less N fertilizer, improve NUE and still maintain high yields.

Since N can be lost from cropping systems in a number of ways, a single solution to the N management dilemma is unlikely (Binder et al., 2000). There still is a given need for a more enhanced understanding of NUE in maize. Research is needed that evaluates maize growth, yield, and N uptake when N fertilizer is applied as split applications that match maize N requirements throughout the growing season in both irrigated and non-irrigated soils. The objective of this study was to examine how different N rates and timing of application of in SB-C-C-C in both irrigated and rainfed soils over three consecutive growing seasons at three locations across Minnesota. In the irrigated coarse textured soils, split applications are expected to improve maize grain yields and NUE in relation to fall applications while in the non-irrigated coarse textured soils applying urea either at planting or as split applications will improve yields and NUE in relation to fall applications.

Materials and Methods

Site Description and Experimental Design

Field experiments were conducted at University of Minnesota Research and Outreach Centers near Lamberton, MN (44° 24’ N, 95° 30’ W), Waseca, MN (44° 07’, 93° 52’ W) and at the Sands Plain Research Center at Becker, MN (45° 39’ N, 93° 89’ W) from 2014 to 2016. The rainfed sites at Lamberton had a Normania loam soil (Fine-loamy, mixed, superactive, mesic Aquic Hapludolls) and at Waseca a Nicollet clay loam soil (Fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquolls); while the irrigated site at Becker, MN had a Hubbard-Mosford loamy sand complex (Sandy, mixed, frigid Typic Hapludolls or frigid Entic Hapludolls). The experimental design was a randomized complete block design with four replications. Plot sizes at Becker and Waseca, MN were 4.6 m (6 rows) by 15 m and Lamberton was 6 m by 12 m, the differences in plot sizes were due to the differences in implements available at each location for tillage and planting operations. All sites were planted to soybean [*Glycine max* L. (Merr.)] in 2013 and to maize from 2014 to 2016. For each year, the tillage system involved field cultivating in the spring prior to planting, and stalk chopping followed by disk ripping in the fall after maize harvest. Pest management followed best management practices (BMP) and varied by location and year.

The selected recommended N rate (RN) in 2014 for maize following soybean at Becker, MN was 168 kg ha^{-1}, while at Lamberton and Waseca it was 135 kg ha^{-1}. For 2015 and 2016 the RN for maize following maize at Becker was 235 kg ha^{-1} while at Lamberton and Waseca, MN it was 202 kg ha^{-1}. All rates were based on University of Minnesota guidelines (Kaiser et al., 2012). The N source used for fall applications was SuperU® (46-0-0), a urea based granule with dicyandiamide and N-(n-butyl)thiophosphoric triamide to inhibit nitrification and urease. For the pre-plant and in-season applications the N source was urea (46-0-0).

All fertilizers were hand-applied and incorporated with tillage immediately after application in the fall and prior to planting. In-season applications were incorporated through irrigation at Becker. At Lamberton and Waseca, fertilizer was placed in 5 cm deep furrows in the inter-rows made using a hoe, after fertilizer application, furrow trenches were closed with soil. Treatments were applied to the same experimental plots in all years. The treatments tested in this study consisted of a non-fertilized control plus nine N management systems: F100, single SuperU application in the fall at 100% of RN; F125, single SuperU application in the fall at 125% of the RN; PP100, single urea application applied at planting at 100% of the RN; PP125, single urea application applied at planting at 125% of the RN; Sp75, two-way split urea application at planting (one half) and V6 (one half) applied at 75% of the RN; Sp100, two-way split urea application at planting (one half) and V6 (one half) applied at 100% of the RN; TSp50, three-way split urea application at planting (one third), V6 (one third), and silking (one third) applied at 50% of the RN; TSp75, three-way split urea application at planting (one third), V6 (one third), and silking (one third) applied at 75% of the RN; TSp100, three-way split urea application at planting (one third), V6 (one third), and silking (one third) applied at 100% of the RN. The growth stages selected for N application in treatments TSp50, TSp75, and TSp100 were based on the literature information regarding nutrient requirement and time of maximum uptake of the crop (Hanway, 1962; Jokela and Randall, 1989; Abendroth et al., 2011). This strategy for split N application aimed to provide the crop with N at the times throughout the growing season when it was most needed.

Grain Yield and Aboveground N Uptake

In-season maize tissue sampling was performed seven times during the growing season to assess nutrient uptake by the crop and total biomass yield during each sampling time. A staggered sampling was used to minimize the potential interference of removing plants on the development of crop and that of the plants which would be used for yield estimate. Stover sampling was based on the fertilizer application for three-way split treatments and collected at physiological maturity. Stover samples collected at physiological maturity were weighed wet in the field, chopped in the field, and a sub sample was collected for moisture content. Maize ears were separated in the field, dried, shelled, and weighed. At Lamberton and Waseca, MN, crop grain yield was determined at harvest using a plot combine and a subsample of the grain was also collected for determination of total nutrient uptake at harvest. At Becker a total of 6 m from the center of rows three and four in each plot were hand harvested and a subsample of the grain was used for determination of total nutrient uptake at harvest.

Stover samples from all sampling and a grain subsample were dried in a forced-air dryer at 60ºC until constant mass and the dry weight was then collected for each subsample. The dry stover and grain samples were then ground to 1mm and sent to Lamberton, MN for chemical composition analysis with a CNS analyzer (Vario MACRO, Elementar, Langenselbold, Germany).

Nitrogen use efficiency was calculated as AE, PFP, and RE as described by Wortmann et al. (2011), Sindelar et al. (2015) and Cassman et al. (1996). Maize yield and

N fertilizer rates were input into the equations as kg ha^{-1}. Agronomic efficiency is the difference in grain yield between the fertilized treatment and the nonfertilized control per unit of fertilizer N applied. The increase in grain per unit of N applied, was calculated according to the following equation:

$$AE_i = (Y_{Ni} – Y_0) / N_i \text{ rate} \quad [1]$$

where Y_{Ni} is the grain yield at physiological maturity for the *i*th N fertilizer treatment and Y_0 is the mean grain yield at physiological maturity for the unfertilized treatment.

Partial factor productivity, the total grain yield relative to the amount of N applied, was calculated according to the following equation:

$$PFP_i = Y_{Ni} / N_i \text{ rate} \quad [2]$$

where Y_{Ni} is the grain yield at physiological maturity for the *i*th N fertilizer treatment.

Recovery efficiency, the increase in nitrogen uptake per unit of N fertilizer applied, was calculated according to the following equation:

$$RE_i = (UN_{Ni} – UN_0) / N_i \text{ rate} \quad [3]$$

where UN_{Ni} is the total N in above ground biomass at physiological maturity for the *i*th N fertilizer treatment and UN_0 is the mean total N in above ground biomass at physiological maturity for the unfertilized treatment.

Statistical Analysis

Maize grain yield was analyzed by repeated measure ANOVA using the GLIMMIX Procedure in SAS at $P \leq 0.05$. Treatment, location and year were considered fixed effects, while replication was considered random. Year was the repeated variable in the model and the covariance structure that best fit the model was CSH for grain yield and RE, CS for PFP, and UN for AE. Tests for normality were performed prior to making inferences on main effects. When appropriate, pairwise mean comparisons (at $P \leq 0.05$) were made using the lines option in the GLIMMIX procedure of SAS.

Results and Discussion

Weather

Becker

In 2014, total rainfall during the months of April, May and June were greater than the historical averages for those months (Table 1.1). In April, 178 mm of precipitation was recorded at Becker, 118 mm above historical averages; the month of May recorded 184 mm of precipitation, 103 mm above historical averages; while June received 160 mm of precipitation, 48 mm above historical averages for the month (Table 1.1). However, precipitation dropped below historical averages by 13 mm in September and 44 mm in October 2014 (Table 1.1). In 2015, Becker had a slightly drier March and April, receiving 34 and 23 mm less precipitation than historical averages for those months (Table 1.1).

However, May, July and August saw an increase of 67, 61 and 105 mm of precipitation compared with historical averages (Table 1.1). In 2016, precipitation was similar to historical averages except in July, September and October where Becker received an additional 87, 25, and 74 mm, respectively, more precipitation compared with historical averages (Table 1.1).

Lamberton

In 2014, Lamberton received its precipitation throughout the growing season in an alternate manner of dry (May), wet (June), dry (July), wet (September) (Table 1.1). More specifically, May received just 46 mm of precipitation, 42 mm less precipitation compared with historical averages (Table 1.1). June was considerably wetter, receiving 188 mm of precipitation, 84 mm more rain than historical averages (Table 1.1). Dry conditions in Lamberton returned in July, 2014 where only 30 mm of precipitation fell, 61 mm less precipitation than historical averages for the month (Table 1.1). September saw a return of wet conditions, with 154 mm of precipitation falling, 7.6cm more precipitation than historical averages for the month (Table 1.1). In 2015, Lamberton received precipitation closer to historical averages. April received just 31 mm of precipitation, 39 mm less than historical averages, however, in May Lamberton received 139 mm of precipitation, 51 mm more than historical averages (Table 1.1). August also saw a slight increase in precipitation, receiving 113 mm, 34 mm more than the historical average. 2016 was the wettest of the three years for Lamberton (Table 1.1). March, April and May received 14, 15, and 53 mm more precipitation than the monthly historical averages, while only June saw less than average monthly precipitation, receiving 38 mm less precipitation than the historical average (Table 1.1). July, August, and September saw wetter conditions return, receiving 176, 135, and 134 mm of precipitation, representing 85, 55, 55 mm, respectively, more precipitation than the historical averages (Table 1.1).

Waseca

In 2014, April received 142 mm of precipitation, 60 mm more than historical averages for the month (Table 1.1). The month of May received 27 mm of precipitation less than historical averages, however June was an exceptionally wet month, receiving 329 mm of precipitation, 210 mm more than historical averages. The rest of the year was drier, with July receiving 82 mm less precipitation than historical averages and August, September, and October receiving 40, 34, and 33 mm less precipitation than historical averages, respectively (Table 1.1). Greater than historical averages rainfall was observed in 2015 during June through September (Table 1.1). June received 194 mm of precipitation (74 mm more than average), July received 188 mm of precipitation (76 mm greater than average), August received 152 mm of precipitation (32 mm greater than average), and September received 149 mm of precipitation (56 mm greater than average) (Table 1.1). In 2016, precipitation was slightly lower than average from January through June; however July received, 227 mm of precipitation, 115 mm above historical averages, August got 297 mm of precipitation, 177 mm above historical averages, and September received 376 mm of precipitation, 283 mm more than historical averages for the month (Table 1.1).

Maize grain yield

Becker

In 2014, maize following soybean, TSp100 yielded 12.6 Mg ha^{-1} of grain, a greater yield than all other treatments (Table 1.2). The other split treatments, TSp75, TSp50, Sp100, and Sp75, also produced greater grain yields than all PP or F treatments regardless of N rate (Table 1.2). Given that Becker is a coarse-textured soil, it is likely that the high amount of precipitation received in April, May and June in 2014 resulted in high amounts of N applied with the F and PP to leach below the root zone (Table 1.2). In 2015, maize following maize, the highest yielding treatments at Becker were Sp100 and TSp100, producing 15.2 and 14.5 Mg ha^{-1} of grain, respectively (Table 1.2). Urea applied at 125% recommended rate as PP yielded 11.7 Mg ha^{-1}, similar to treatments with 75% recommended rate (Sp75 and TSp75) which produced 12.0 and 12.8 Mg ha^{-1} , respectively (Table 1.2). Fall application of N, regardless of rate, yielded significantly lower than any PP, Sp or TSp treatments (Table 1.2). Similar to 2014, high rainfall in May 2015 likely led to high N leaching in F treatments compared with other treatments. In 2016, TSp100 treatment yielded significantly more grain compared with F applications, PP100, or the split applications at the reduced recommended rates (Table 1.2). Both Sp100 and PP125 produced significantly more grain than TSp50, PP100, and F applications (Table 1.2). Weather patterns were close to historical averages except for high rainfall in July 2016 (Table 1.1). This did not seem to impact yields for Sp and TSp treatments (Table 1.2).

Results over the three years differ from Venterea et al. (2016) who found no difference in grain yield between single and split N applications on a non-irrigated Waukegan silt loam in the upper Midwest. However, the results of our study do corroborate to findings from sandy textured soils in Kansas where a split application of 185 kg N ha^{-1} provided similar, and sometimes greater maize grain yields than 250 to 300 kg N ha^{-1} preplant applications (Gehl et al., 2004). Similarly, in irrigated sandy soils in Minnesota, Rubin et al. (2015) reported that a preplant-V4 urea application increased maize grain yield by 0.63 Mg ha^{-1} compared with a single preplant application of enhanced efficiency fertilizers. Relatedly, a 40% preplant and 60% V8 split application of anhydrous ammonia yielded significantly more thanfall applications with or without the nitrification inhibitor nitrapyrin [(2-chloro-6-trichloromethyl) pyridine] (Randall and Vetsch, 2005). The results of the current study support the BMPs set out by the University of Minnesota for irrigated sandy soils, which recommend that a split-application of urea is superior to a single application (Lamb et al., 2015).

Lamberton

In 2014, when the same rate of N was applied in the fall, pre-plant, or as a split application, the same grain yield was observed (Table 1.2). The PP125 was the best treatment in 2014 at Lamberton and produced 14.7 Mg ha^{-1} of grain, a significantly greater yield than C (9.5 Mg ha^{-1}), Sp75 (13.2 Mg ha^{-1}), and the TSp treatments (12.2-

13.2 Mg ha^{-1}), respectively (Table 1.2). In addition, the treatment Sp75, which is a 25% reduction of the recommended fertilizer rate, led to the same yield as applying 125% of the recommended rate in the fall (F125) (Table 1.2). Although May 2014 was a relatively dry month receiving just 46 mm of precipitation, June received 188 mm of precipitation, just over 80 mm more than the 35 year average, while July only received 30 mm of precipitation (Table 1.1). It is likely that the rain in June improved soil N mineralization for Sp75 and the plants were able to utilize most of the N applied at the preplant and V6 applications. While the dry conditions in July could have hindered N uptake for N applied in July for the TSp treatments. In 2015, similar results were observed in comparison with 2014 although no differences in yield were observed between the F, PP, Sp100, Tsp75 and Tsp100 split treatments (Table 1.2). In 2016, the F125 treatment yielded 15.5 Mg ha^{-1} of maize grain; however, no differences were observed between F100, PP100, PP125, Sp75, Sp100, Tsp75, and Tsp100 (Table 1.2). The lack of differences between treatments that received from 75% to 125% of the recommend rates corroborates findings by Venterea and Coulter (2015) and Venterea et al. (2016) who reported no differences in maize grain yield between pre-plant and split applications.

The results observed for the split treatments at Lamberton show that yields increased in the Sp100 and TSp75 and TSp 100 the longer the system was kept in a maize on maize cropping system (Table 1.2). Less rainfall in 2014 compared with the long-term average suggests that some of the 2014 applied N in the split treatments was utilized in 2015. This is could be due to the split application fertilizer being applied to soil manually by opening a 5-cm deep trench, adding the fertilizer, and then covering the fertilizer to avoid volatilization losses. When contact between fertilizer and soil is present little loss to volatilization is observed (Engel et al., 2011); in addition, dry soil also tends to inhibit N volatilization (Ma et al., 2010). Little rainfall was observed after the third and final N application at Lamberton (Table 1.1). The lower yields observed for the Tsp treatments in 2014 suggest that not all the applied N was utilized by the crop in these treatments (Table 1.2). The increase in yields from 2014 to 2016, particularly in Sp100, Tsp75, and Tsp100 suggests that improved nutrient management is possible by managing N differently at Lamberton.

Waseca

In 2014, PP125 produced 11.2 Mg ha^{-1}, a greater yield compared with all F treatments, Sp75, and TSp treatments (Table 1.2). When comparing similar N rates in 2014, PP100 and Sp100 similarly yielded 10.3 Mg ha^{-1} and 9.5 Mg ha^{-1}, respectively, which were greater than for F100 (8.6 Mg ha^{-1}) (Table 1.2). In contrast, comparing similar N rates in the split applications shows that the application of 75% of the recommended rate was more effective when applied in two applications than applied in three applications (Table 1.2). In 2014, there was a clear N rate effect at Waseca regardless of time of N application, in the F and TSp treatments (Table 1.2). The F125 had a greater yield than F100, and Tsp100 had greater yield than Tsp75 and Tsp50 (Table 1.2); in contrast, yield was similar for PP100 and PP125, and Sp100 and Sp75 (Table 1.2). The wet June in 2014 (329 mm of rainfall) likely enhanced losses of fertilizer N applied up to planting, possibly restricting root development thus limiting crop N uptake and lowering yields. That no yield differences were observed between Sp75 and Sp100, but there was yield difference between Sp75 (9.2 Mg ha^{-1}) and Tsp75 (7.9 Mg ha^{-1}) suggests that the greater moisture in June could have helped N utilization by the crop in the Sp75 treatment, while the final fertilizer application for TSp did not receive enough moisture to incorporate from the 30 mm of precipitation recorded in July 2014 (Table 1.2). Similar results were observed in a study located approximately 130km north of Waseca, MN where there was no difference in yield between fertilizer applied at 146 kg N ha^{-1} (recommended rate) and 124 kg N ha^{-1} (85% of recommended rate) (Venterea et al., 2016).

In 2015, splitting N rates into two or three times led to the largest yields observed in Waseca, especially for the treatments that received 100% the recommended N rate (Table 1.2). Furthermore, application of Sp75 and Tsp75 had as good a yield as applying as much as 125% of the recommended rate in a single application in the fall or spring (Table 1.2). In 2016, there were no differences in yield between F125, PP treatments, Sp treatments, and TSp75 and TSp100. It was observed that applying 75% of the recommended rate in either two or three split applications resulted in the same yield as applying 125% of the recommended rate in one full application either in the fall or spring (Table 1.2). For example, Sp75 (75% of the recommended rate) had the same yield as F125 and PP125, both 125% of the recommended rate (Table 1.2). Applying 50% of the recommended rate as TSp resulted in similar maize grain yield to one full application at recommended rate or 125% recommended rate in the fall (Table 1.2). It is possible that the greater moisture in July, August, and September of 2016 could have helped N utilization by the crop in the split treatments (Table 1.1). For example, the yield in 2016 for the TSp50 treatment was reduced by 6% compared with 2015; however, in the F125 treatment the maize grain yield was 26% lower in 2016 compared with 2015 (Table 1.2). The significant drop in maize grain observed for the treatment F125 could have been a result of increased leaching due to a warmer soil conditions over the winter in 2015 and a wetter early summer in 2016. The lower N application in TSp50 likely resulted in less leaching potential that did not impact soil N content. This was demonstrated by modeled findings by Gowda et al. (2008) from a 365-ha watershed in Iowa where nitrate-N losses were reduced by 21 kg ha^{-1} (17%) when there was a 20% reduction in spring-applied fertilizer rate. Furthermore, Randall et al. (2003b) found that 62% of the annual subsurface drainage occurred in April through June, accounting for 68% to 70% of the nitrate lost annually to subsurface drainage. As precipitation often can exceed evapotranspiration during these months, if significant nitrification of fall-applied N occurs in either the fall or spring before crop uptake of N, then losses of nitrate in drainage water could be largely due to leaching (Randall et al. 2003b).

Overall, the year-to-year trend at Waseca suggests that a split application, when applied at recommended rates, tended to maximize grain yield (Table 1.2). This supports the conclusion of the modeled annual findings of Randall et al. (2003) where a seven-year average of maize grain yields found split N treatments (40% preplant and 60% sidedress at V8) produced the greatest maize grain yields compared with fall or preplant N fertilizer applications using anhydrous ammonia at 150 kg N ha^{-1}. Although Vetsch and Randall (2003) did not observe any difference in maize grain yield in two of three study years when comparing the effect of 123 kg N ha^{-1} of anhydrous ammonia applied in the fall or at preplant; it was noted that when wet and warm spring conditions follow a late fall N application (after October 20), maize grain yield can decrease by 20% and N uptake will decrease by 27%. As similar weather patterns were observed in the winter of 2015 and spring of 2016, the yield from 2016 supports this observation.

Nitrogen Use Efficiency Parameters

Efficient N use often results in improved N recovery due to minimal losses of N to denitrification, leaching, and volatilization, while improving grower profitability, and water and atmospheric conditions (Wortmann et al., 2011). However, as inorganic N in the soil profile increases the potential for N loss also increases (Wortmann et al., 2011). This is primarily due to the over application of N fertilizer. An important component of NUE is the grain yield per unit of applied N fertilizer, which can be determined by agronomic efficiency (AE), the increase in grain yield per unit N applied, the crop partial factor productivity (PFP), as the total grain yield relative to the amount of N applied, and recovery efficiency (RE), the increase in aboveground N biomass per unit N applied (Wortmann et al., 2011). These parameters provide growers an integrative index that quantifies total economic output relative to the utilization of nutrient resources in the system (Yadav, 2003). From 1992 to 2010, the ratio of maize produced per kg of N fertilizer applied has steadily increased from 20 to 38 kg of maize grain produced per kg of N applied (Murrell, 2011).

Agronomic efficiency

Agronomic efficiency, calculated in units of yield per unit of nutrient applied, closely reflects the production impact of an applied fertilizer and relates directly to economic return (Fixen et al., 2014). This makes it a good short-term indicator of the impact of applied nutrients on productivity (Fixen et al., 2014). For N, the typical levels of AE for cereal crops globally tend to range from 15 to 30 kg kg^{-1} with lower levels suggesting that changes in management could increase crop response or reduce input costs (Fixen et al., 2014).

Becker

In 2014, the agronomic efficiency was greater for TSp treatments compared with Sp, PP, and F treatments regardless of rate (Table 1.3). There were no differences between the F and PP treatments (Table 1.3). This follows the yield response in 2014, where all split treatments, regardless of rate, yielded more than the PP and F treatments (Table 1.2). In 2015, the F treatments, regardless of rate, produced the lowest AE, followed by the PP treatments (Table 1.3). There was also no difference in AE between TSp and Sp treatments, regardless of rate, but both were significantly greater than the PP and F treatments, respectively (Table 1.3). The ability of Sp75 and TSp75 to produce yields similar to PP125 resulted in a greater AE for split applications at reduced rates compared with PP treatments at recommended or increased rates (Table 1.2). In 2016, there was no differences in AE between reduced rate split applications (50% and 75% recommended rate); however, TSp50 and TSp75 were greater than Sp100 even though Sp100 yielded more grain than TSp50 (Table 1.2, Table 1.3). Similar findings were reported by Rubin et al. (2016) when comparing the effect of a split application of urea and a single preplant application of an enhanced efficiency fertilizer. There, AE increased by 12% when using a split urea application compared with a preplant application of an enhanced efficiency fertilizer (Rubin et al., 2016). Under continuous maize, split applications at Becker, MN produced an agronomic efficiency ranging from 40 to 53 kg kg^{-1} (Table 1.3). These levels of NUE's are greater than mean 27 to 29 kg kg^{-1} reported by Rubin et al. (2016) when comparing different enhanced efficiency fertilizers applied as a preplant or split application in sandy soils. The AE achieved when using a split application demonstrates the potential for improved NUE under continuous maize production in sandy soils. With three years of consistently greater AE compared with F or PP treatments, these results confirm that the most efficient BMP for irrigated sandy soils is the use of split N application (Lamb et al., 2015).

Lamberton

Unlike in Becker, AE in Lamberton was influenced more by rate of urea application rather than timing of application (Table 1.3). Across the three years, agronomic efficiency tended to decrease as the fertilizer N rate increased (Table 1.3). This was also reported by Sindelar et al. (2015) when researching the impact of N management, stover and tillage management in continuous maize. In 2014, there was no difference in AE between treatments receiving at least 75% of the recommended N fertilizer rate (Table 1.3). The lack of differences between AE among treatments receiving recommended rates would be expected given the similar grain yields reported between similar treatments. However, given that there was no yield difference between Sp75 and F125, one would expect Sp75 (37 kg kg^{-1}) to have a greater AE than F125 (30 kg kg^{-1}) (Table 1.3). Only TSp50 (41 kg kg^{-1}) had a greater AE than TSp100 (28 kg kg^{-1}) (Table 1.3). In 2015, TSp50 (59 kg kg^{-1}) had a greater AE over all other treatments except TSp75 (53 kg kg^{-1}) (Table 1.3). Similarly, TSp75 also had a greater AE than all other treatments except Sp75 (46 kg kg^{-1}) (Table 1.3). There was also no differences between F, PP, Sp treatments and TSp100 (36 to 46 kg kg^{-1}) (Table 1.3). Given that TSp75 produced grain yields similar to maize receiving recommended or increased N fertilizer rates, the improved AE was expected. In 2016, the highest AE was Sp75 with 51 kg kg^{-1}, which was greater than F, PP treatments regardless of rate and TSp100 (Table 1.3). TSp50 (50 kg kg^{-1}) was also greater than all F and PP treatments (Table 1.3). Given the high grain yields reported across treatments in Lamberton (Table 1.2), it is logical that the treatments receiving lower N rates would produce greater agronomic efficiency. With AE ranging from 28-59 kg kg^{-1} at Lamberton, the AE achieved was greater than the 10-30 kg kg^{-1}AE recorded in over 700 data points across Sub-Saharan Africa by Vanlauwe et al. (2011) but similar to the 38 kg kg^{-1} reported from a 15 year maize-wheat cropping system in China (Duan et al., 2014). For treatments receiving recommended rates of N fertilizer, the average AE ranged from 33 to 42 kg kg^{-1} per year, while treatments receiving less than recommended rates produced an average agronomic efficiency ranging from 37 to 53 kg kg^{-1} (Table 1.3). Both of these ranges are greater than the average AE of 29 kg kg^{-1} recorded in continuous maize systems in Nebraska (Wortmann et al., 2010). The high agronomic efficiency at Lamberton suggests that high yielding and efficient continuous maize production is achievable.

Waseca

In 2014, Sp75 had an AE of 46 kg kg^{-1}, greater than all F treatments, TSp50, and TSp75, respectively (Table 1.3). There was no difference between PP treatments, Sp treatments and TSp100 (Table 1.4). Surprisingly, there was no difference in AE between F100, F125, PP125 and TSp50 (Table 1.4). This is likely due to TSp50 yielding just 6.6 Mg ha^{-1} in 2014 while F100, F125, and PP125 yielded 8.6 Mg ha^{-1}, 9.6 Mg ha^{-1}, and 11.2 Mg h^{-1}, respectively (Table 1.2). Given that TSp50 tends to produce greater AE due to the lower amount of fertilizer applied, the similarity in AE between TSp50 and F100, F125, and PP125 can be contributed to the low yield produced by TSp50. In 2015, there was no significant difference between F100 and all Sp and TSp treatments, regardless of rate and timing of application (Table 1.3). There was no difference in AE between treatments receiving recommended rates, regardless of timing of application (Table 1.3). This is likely due to the high yield of 15.3 Mg ha^{-1} produced by F100 and similarly high yields produced using Sp or TSp treatments. In 2016, there was no significant difference in AE between treatments receiving less than 100% recommended rates (Table 1.3). Fall treatments, regardless of rate, had an AE significantly lower than all other treatments (Table 1.3). This is likely due to F100 and F125 yielding 11.1 Mg ha^{-1} and 12.1 Mg ha^{-1}, respectively, compared with the 13.0-13.3 Mg ha^{-1} recorded for PP100, Sp100 and TSp100 (Table 1.2). The difference in yield reduced the AE for the fall treatments compared with the other treatments. There was no difference in AE between TSp100, Sp100 and PP100 (Table 1.3). Results in 2016 suggest that the application of N applied at PP or applied in season near to or at the time of high N uptake can produce high AE. Wortmann et al. (2010) came to similar conclusions, reporting that the application of N near to economic optimum nitrogen rates improved NUE in Nebraska. Similarly, treatments receiving recommended rates (F100, PP100, Sp100, and TSp100) produced an annual mean AE ranging from 33 to 40 kg kg^{-1} per year, while treatments receiving 50 to 75% of recommended rates produced a mean AE of 37 to 47 kg kg^{-1} per year, both greater than the mean 29 kg kg^{-1} reported by Wortmann et al. (2010) across continuous maize systems in Nebraska and the average 10-30 units of cereal grain per unit of N applied reported by Snyder and Bruulsema (2007).

Partial factor productivity

Crop partial factor productivity (PFP) provides growers an efficient measure of the total grain yield relative to the amount of N fertilizer applied. Easy to estimate, it can be used across different spatial scales to assist growers in tracking fertilizer N use efficiency. The use of PFP allows growers to track their efficiency and potentially improve their fertilizer management to maximize economic and environmental indicators. For N, the global range for cereal crops is 40 to 90 kg kg^{-1} with lower levels suggesting less responsive soils or the over application of nutrients, while greater levels suggest that nutrient supply is likely limiting productivity (Fixen et al., 2014).

Becker

In 2014, TSp50 had a greater PFP than all other treatments (Table 1.4). Although there was no difference in PFP between TSp75 (83 kg kg^{-1}) and TSp100 (75 kg kg^{-1}), TSp75 had a greater PFP compared with Sp75 (71 kg kg^{-1}) (Table 1.4). The lowest PFP was recorded in F125 with 28 kg kg^{-1} but was not statistically different from F100 (34 kg kg^{-1}) and PP125 (36 kg kg^{-1}) (Table 1.4). The low PFP recorded for the fall and increased N fertilizer treatments is not unusual, given their low yields on the coarse textured soil (Table 1.2). In 2015, TSp50 again had a greater PFP compared with all other treatments (Table 1.4). TSp75 (73 kg kg^{-1}) recorded a greater PFP than TSp100 (62 kg kg^{-1}) and Sp100 (64 kg kg^{-1}) but there was no difference between Sp75, Sp100, and TSp100 (Table 1.3). Although the PP treatments had a greater PFP than the F treatments, rate did not have an effect in F or PP treatments, with no difference between F100 (29 kg kg^{-1}) and F125 (28 kg kg^{-1}) and PP100 (43 kg kg^{-1}) and PP125 (40 kg kg^{-1}), respectively (Table 1.4). In 2016, TSp50 (94 kg kg^{-1}) had a greater PFP than all other treatments (Table 1.4). There was no difference between TSp75 and Sp75, respectively (Table 1.3). Similarly, there was no difference between TSp100 and Sp100 (Table 1.4). In both F and PP treatments rate did not influence PFP. F treatments, regardless of rate, had the lowest PFP with 34 and 35 kg kg^{-1}, while PP100 recorded 54 kg kg^{-1} and PP125 49 kg kg^{-1}, respectively (Table 1.4).

Across all three years, the results observed suggest that PFP tended to decrease with increasing rate of N fertilizer, however TSp100 and Sp100 produced a PFP similar to TSp75 and Sp75. This is primarily due to the greater yields the Sp100 and TSp100 treatments produced during the duration of the study (Table 1.2). The PFP values for treatments applied at PP or as a split application were typically in the range of 40-80 units of grain per unit of N, as suggested by Snyder and Bruulsema (2007), however fall applications produced a lower PFP range of 28 to 35 kg kg^{-1}. The annual PFP range of 59 to 79 kg kg^{-1} for Sp100 and TSp100 at Becker, is slightly lower than the average 83 kg kg^{-1} reported for the EONR for high yielding irrigated maize in Nebraska (Wortmann et al., 2011). However, the PFP recorded in our study is similar to the national average of 58 kg kg^{-1} and the 68-69 kg kg^{-1} recorded for either a split half at preplant and half at V4 application or as single preplant urea application in the upper Midwest (Wortmann et al., 2011; Rubin et al., 2015).

Lamberton

In 2014, TSP50 had the highest PFP with 181 kg kg^{-1}, greater than all other treatments (Table 1.4). There was no difference between TSp75 (126 kg kg^{-1}) and Sp75 (131 kg kg^{-1}), however both were greater than all treatments receiving at least 100% of the recommended rate (Table 1.4). For treatments receiving 100% of the recommended rate, there was no difference except between PP100 (107 kg kg^{-1}) and TSp100 (98 kg kg^{-1}) (Table 1.4). The treatments F125 and PP125 recorded the lowest PFP with 86 and 88 kg kg^{-1}, respectively (Table 1.4). In 2015, N application rate had the biggest effect on

PFP, regardless of timing of application (Table 1.4). Here, TSp50 (109 kg kg^{-1}) had the greatest PFP, followed by Sp75 (79 kg kg^{-1}) and TSp75 (79 kg kg^{-1}), respectively (Table 1.3). There was no difference between F100 (67 kg kg^{-1}), PP100 (67 kg kg^{-1}), Sp100 (68 kg kg^{-1}), and TSp100 (66 kg kg^{-1}), respectively (Table 1.4). The lowest PFP was recorded in F125 and PP125 with 56 kg kg^{-1} (Table 1.4). In 2016, the PFP for each treatment increased however trends remained the same as in 2015 (Table 1.4). Rate had the biggest effect on PFP with the lowest rate recording the highest PFP while treatments receiving 125% recommended rate had the lowest PFP, respectively (Table 1.3).

The findings from Lamberton support findings by Halverson and Bartolo (2014) where PFP also decreased with increasing N rate. The average PFP for all treatments receiving recommended rates was approximately 81 kg kg^{-1}, slightly lower than the average 83 kg kg^{-1} reported by Wortmann et al. (2011), but greater than the national average of 58 kg kg^{-1}. Overall, the findings from Lamberton further confirm the importance of applying N fertilizer at recommended rates and not over applying in order to improve NUE measures.

Waseca

In 2014, TSp50 (99 kg kg^{-1}) and Sp75 (91 kg kg^{-1}) were greater than all other treatments (Table 1.4). There was no difference between TSp75 (78 kg kg^{-1}), PP100 (77 kg kg^{-1}), Sp100 (74 kg kg^{-1}), and TSp100 (71 kg kg^{-1}) (Table 1.3). However, TSp100, PP125 (67 kg kg^{-1}), and F100 (64 kg kg^{-1}) were also not different (Table 1.4). Lastly, there was no difference between F125 (58 kg kg^{-1}), F100, and PP125 (Table 1.4). In

2015, TSp50 (115 kg kg^{-1}) produced a greater PFP than all other treatments (Table 1.4). This was followed by TSp75 (93 kg kg^{-1}) and Sp75 (92 kg kg^{-1}) (Table 1.4). There was no difference between TSp100 (77 kg kg^{-1}), Sp100 (76 kg kg^{-1}), and F100 (76 kg kg^{-1}), although all three were greater than PP100 (67 kg kg^{-1}) (Table 1.4). Lastly, PP100 was greater than F125 (56 kg kg^{-1}) and PP125 (56 kg kg^{-1}) (Table 1.3). In 2016, TSp50 had the greatest PFP with 107 kg kg^{-1}, respectively (Table 1.4). Similar to 2015, there was no difference between TSp75 (84 kg kg^{-1}) and Sp75 (85 kg kg^{-1}) (Table 1.4). Unlike 2015, there were no differences between PP100 (66 kg kg^{-1}), Sp100 (65 kg kg^{-1}), and TSp100 (66 kg kg^{-1}); which were greater than F100 (56 kg kg^{-1}), F125 (48 kg kg^{-1}) and PP125 (54 kg kg^{-1}) (Table 1.3).

Similar to findings at Lamberton, increasing the rate of fertilizer reduced PFP in continuous maize (Table 1.4). However, it should be noted that applying fertilizer at recommended rates as a split application improved PFP over F125 or PP125 fertilizer applications. Averaged across years, the PFP of TSp100 and Sp100 was 9% greater compared with F100 while the PFP of PP100 was 7% greater than F100. The annual PFP achieved for recommended rates averaged around 71 kg kg^{-1}, greater than the reported national average of 58 kg kg^{-1} but lower than the 83 kg kg^{-1} recorded for EOCD high yield irrigated maize in Nebraska (Wortmann et al., 2011). The PFP recorded by Sp75 and TSp75 treatments at Waseca did produce similar PFP's to the 83 kg kg^{-1} recorded by Wortmann et al. (2011) in Nebraska.

Across locations, the effect of N rate had the greatest influence on PFP. There was a clear trend of TSp50 consistently producing the greatest PFP with an average of 98 kg kg^{-}1 at Becker, 134 kg kg^{-1} at Lamberton and 80 kg kg^{-1} at Waseca (Table 1.3). This was followed by TSp75 and Sp75 at all three locations with an average of 75 kg kg-1 at Becker, 101 kg kg-1 at Lamberton and 65 kg kg-1 at Waseca, respectively (Table 1.3). At Becker, TSp100 and Sp100 consistently produced better PFP's compared with PP100 and F100 suggesting that improved NUE was achieved when N fertilizer was applied as a split application (Table 1.3). At Lamberton, there were no differences in PFP between treatments receiving similar rates (Table 1.3). At Waseca, applying N fertilizer at planting or as a split application tended to improve PFP compared with fall applications of N fertilizer (Table 1.3). Here, applying N fertilizer closer to times of rapid plant growth or plant uptake, either at planting or as split application, improved NUE.

Recovery efficiency

Recovery efficiency (RE) is one of the more complex NUE indicators and is defined as the difference in nutrient uptake in the aboveground parts of the maize plant between the fertilized and unfertilized treatments relative to the quantity of N fertilizer applied (Fixen et al., 2014). Typical first year RE response measures for cereal crops tend to range between 40-65%, however estimates on the average RE for maize is approximately 37%, but with a standard deviation of 30, respectively (Cassman et al., 2002; Fixen et al., 2014). Often lower RE suggest that changes in management could improve efficiency or that nutrients are accumulating in the soil (Fixen et al., 2014).

Unlike the other parameters there was no treatment x year x location interaction (Table 1.5). However, there was a treatment by location interaction, a year by treatment interaction, and a year by location interaction (Table 1.5).

Becker

The timing of fertilizer application had an impact on RE at Becker (Table 1.6). The TSp50 treatment recovered 60% of applied fertilizer, more than all other treatments except TSp100 (Table 1.6). There was no difference in RE between split treatments receiving at least 75% of recommended N fertilizer rates (Table 1.6). All TSp treatments, regardless of rate, were greater than the fall and preplant treatments while Sp75 and Sp100 were greater than the fall treatments (Table 1.6). Interestingly, the average RE increased by 6-8% annually from 24% in 2014 to 38% in 2016 (Table 1.7). This could be explained by the incorporation of the maize residue after each year, which likely improved the soil's organic matter content and soil N pool. The annual increase in RE at Backer along with increased fertilizer rate disagrees with the typical trend of RE to decrease as the amount of N-fertilizer applied increases (Cassman et al., 2002). However, it is possible that the returned crop residue improved N mineralization of soil OM content, improving the annual RE at Becker.

Lamberton

Over the three study years there was no difference in RE between treatments (Table 1.5). This could be due to high N mineralization in the Normania loam soil. The average RE was 78% in 2014, 68% in 2015 and 42% in 2016 (Table 1.5). This was similar to the RE of 67% recorded by Wortmann et al. (2010) using EONR in Nebraska. The RE of TSp50, TSp75 and Sp75 averaged 63% comparable to fall (64%) and preplant (60%) treatments (Table 1.5). These findings corroborate findings by Venterea et al. (2016) who also did not find any difference between split or single applied treatments in a rainfed system. However, the results could also demonstrate that slightly reducing the N rate and applying N as a split application could improve RE and NUE in continuous maize.

Waseca

Across 2014 to 2016, TSp100 had a recovery efficiency of 55%, greater than TSp50 (35%), F125 (36%), PP100 (31%), and PP125 (34%) (Table 1.5). The low average recovery efficiency of preplant applications could be due to leaching in 2015 when wet conditions in May, June and July likely leached applied fertilizer whereas the split applications benefited from N mineralization and a more established root structure to better facilitate N uptake. Split applications receiving at least 75% recommended rates recovered an average 51% of applied fertilizer compared with fall and preplant applications which averaged a RE of 36%. Although both recovery efficiencies are lower than Wortmann et al.’s (2010) reported RE of 75% for maize following soybean, the split application’s RE of 51% is comparable to the global compiled estimate of 55% RE (Fixen et al., 2014). Across treatments at Waseca there was an average RE of 42% which outperformed the average 37% RE reported by Cassman et al. (2002) for 55 on-farm experiments from 1995-1999 across the mid-west of the USA. The reduced RE of fall treatments compared with split applications across all three study years suggest that applying N fertilizer as a split application can improve nitrogen use efficiency.

Year x *Treatment*

In 2014, across locations there was no difference in RE between treatments (Table 1.7). In 2015, TSp75 had a RE of 71%, greater than all other treatments except TSp100 and Sp75 (Table 1.7). Preplant applications had an average RE of 36.5%, lower all other treatments except F125. It is likely that due to the wet May, June, and July in 2015, N fertilizer applied at preplant likely leached before it could be taken up, whereas the split applications, with an average RE of 62%, regardless of rate, likely benefited from N mineralization and a more established root structure to better facilitate N uptake (Table 1.1, Table 1.7). There was less difference between treatments in 2016 however TSp100 and TSp50 had y greater RE than F100, F125, and PP125 (Table 1.7). In particular, F100 had a lower RE than all treatments except F125 and PP125 (Table 1.7). Similar to 2015, the wet July and August recorded in 2016 likely improved N mineralization and maize growth resulting in improved production in split application treatments.

Conclusions

There is a clear need for site-specific N fertilizer management programs to maximize maize grain yields and improve NUE across different soils. On coarse-textured soils, split applications particularly TSp, clearly demonstrated that mid-season N applications improved maize grain yield, agronomic efficiency and PFP compared with single N fertilizer applications in the fall or just before planting. The poor yields and NUE of fall treatments, further confirms that coarse-textured soils have a high potential for leaching. The fact that the soil cannot retain N over the period of time in the year when high water infiltration rates are expected, highlights the importance of synchronizing N application with crop needs. The observed high yield, AE and PFP achieved using Sp100 and TSp100 in coarse textured soils demonstrated that split applications were better utilized in maize following maize systems. Future studies on whether there is an economic advantage to applying late season N applications on a commercial scale are now needed. Possible late season N application by large-scale growers could be applied through irrigation.

At Lamberton, the lack of differences in maize grain yield and NUE parameters between treatments applied at RN, suggests that high N mineralization rates are found on the Normania loam soil. However, the results from the split treatments indicate that there is a trend for increasing yields the longer the system was kept in a maize on maize cropping system. The high yields and NUE produced by TSp75 in 2015 and 2016 suggested that improved nutrient management was possible for managing N differently in Lamberton. It should be noted that increased precipitation, compared with the 35-year average, across the growing season in 2015 and 2016, likely improved N mineralization of the late season applied N for the TSp treatments. Future studies on the long-term yield trends comparing reduced N rate split applications to recommended N rate preplant or fall applications are needed. With TSp75 yielding approximately 5% less grain under continuous maize compared with treatments receiving recommended rates, studies on whether there is an economic advantage to applying a reduced recommended rate split application need to be carried out.

At Waseca, a Nicollet clay loam soil, the year-to-year trend suggested that split applications when applied at recommended rates tended to maximize grain yields. Furthermore, the lack of differences in maize grain yield in 2015 and 2016 between F and PP treatments, receiving recommended and 125% recommended rate, and Sp75 and TSp75 demonstrate the potential to better manage N fertilizer in Waseca. The yields achieved at 75% recommended rate using a split application indicate that in a SB-C-C-C system, applying N closer to rapid uptake of N in maize could improve NUE compared with a fall application. Similar to Lamberton, long-term yield trend studies comparing reduced recommended rate split applications to full fall or preplant applications are needed. Additionally, studies on whether there is an economic advantage to applying 75% recommended rate as a split application compared with recommended rates in the fall or preplant.

The benefit of applying N fertilizer as a TSp was demonstrated at Becker while the potential for better N management systems at Lamberton and Waseca was also established with evidence for shifting the application of N fertilizer closer to planting or as a split application. If the weather pattern observed during this study of warmer fall and wetter spring conditions continues, there may be more need to use a split application in fine textured soils in order to better manage soil N and maintain high maize yield.

Table 1.1. Summary of monthly and historical precipitation amounts for Becker, Lamberton and Waseca, MN.

		January	February	March	April	May	June	July	August	September	October	November	December	Annual sum
		Precipitation (mm)												
Becker														
35 year average		28	21	43	60	82	113	105	117	73	63	47	23	775
	2014	26	26	26	180	184	160	88	112	60	19	43	13	937
	2015	11	12	9	37	149	113	166	222	54	93	87	25	978
	2016	8	18	27	67	101	76	192	141	147	55	58	24	914
Lamberton														
35 year average		15	18	37	71	88	104	91	80	78	52	33	19	686
	2014	18	13	25	87	46	188	30	95	154	12	13	25	706
	2015	11	5	10	31	139	128	96	113	87	41	84	34	779
	2016	8	17	51	85	141	66	176	135	134	72	47	29	961
Waseca														
35 year average		32	25	63	82	100	119	112	121	93	68	55	38	908
	2014	36	40	35	142	73	329	30	81	59	35	28	18	906
	2015	19	19	29	70	121	194	188	152	149	31	101	88	1161
	2016	11	22	49	50	95	121	227	297	376	79	41	54	1422

Table 1.2. The effect of treatment x location x year on maize grain yield.

	Becker			Lamberton			Waseca		
Treatment †	2014	2015	2016	2014	2015	2016	2014	2015	2016
	$\mathrm{Mg\ ha^{-1}}$								
C	6.01 e¶	4.91 e	4.88 g	9.46 e	5.03 d	6.26 d	4.54 g	6.85 d	6.09 d
F100	5.70 e	6.77 d	7.95 f	13.81 abc	13.53 a	13.93 ab	8.56 de	15.32 a	11.29 bc
F125	5.82 e	8.19 d	10.21 e	14.51 ab	14.18 a	15.51 a	9.78 bc	14.15 ab	12.11 abc
PP100	7.09 d	10.10 c	12.62 cd	14.44 ab	13.56 a	14.48 ab	10.29 ab	13.50 b	13.36 a
PP125	7.56 d	11.74 b	14.35 ab	14.73 a	14.17 a	14.85 ab	11.23 a	14.03 ab	13.56 a
Sp75	8.92 c	12.03 b	13.60 bc	13.22 bcd	11.97 bc	13.99 ab	9.17 cd	13.96 ab	12.87 ab
Sp100	9.95 bc	15.17 a	14.66 ab	13.91 abc	13.64 a	14.87 ab	9.89 abc	15.30 a	13.03 a
TSp50	9.76 bc	9.86 c	11.07 de	12.21 d	11.03 c	11.32 c	6.64 f	11.56 c	10.81 c
TSp75	10.50 b	12.85 b	13.85 bc	12.75 cd	13.09 ab	13.50 b	7.88 ef	14.13 ab	12.69 ab
TSp100	12.65 a	14.52 a	15.73 a	13.20 bcd	13.25 ab	14.72 ab	9.51 bcd	15.45 a	13.33 a

† C, control no N applied; F100, N applied in the fall at 100% of the recommended rate; F125, N applied in the fall at 125% of the recommended rate; PP100, N applied at pre-plant in the spring at 100% of the recommended rate; PP125, N applied at pre-plant in the spring at 125% of the recommended rate; Sp75, half of the N applied at pre-plant in the spring and half at V6 at 75% of the recommended rate; Sp100, half of the N applied at pre-plant in the spring and half at V6 at 100% of the recommended rate; TSp50, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 50% of the recommended rate; TSp75, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 75% of the recommended rate; TSp100, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 100% of the recommended rate.

¶Means within the column followed by different low case letters are significantly different at $p \leq 0.05$.

Table 1.3. The effect of treatment x location x year on nitrogen agronomic efficiency (AE).

	Becker			Lamberton			Waseca		
Treatment †	2014	2015	2016	2014	2015	2016	2014	2015	2016
	kg kg^{-1}§								
C									
F100	-2 c¶	8 c	13 e	32 ab	42 c	38 d	30 c	42 ab	26 d
F125	-1 c	11 c	18 e	30 ab	36 c	37 d	31 c	29 c	24 d
PP100	6 c	22 b	33 cd	37 ab	42 c	41 cd	43 ab	33 bc	36 bc
PP125	7 c	23 b	32 d	31 ab	36 c	34 d	40 abc	29 c	30 cd
Sp75	23 b	40 a	49 ab	37 ab	46 bc	51 a	46 a	47 a	45 ab
Sp100	23 b	44 a	42 bc	33 ab	43 c	43 abcd	40 abc	42 ab	34 c
TSp50	45 a	42 a	53 a	41 a	59 a	50 ab	31 c	47 a	47 a
TSp75	36 a	45 a	51 a	33 ab	53 ab	48 abc	33 bc	48 a	44 ab
TSp100	39 a	41 a	46 ab	28 b	41 c	42 bcd	37 abc	43 ab	36 bc

† C, control no N applied; F100, N applied in the fall at 100% of the recommended rate; F125, N applied in the fall at 125% of the recommended rate; PP100, N applied at pre-plant in the spring at 100% of the recommended rate; PP125, N applied at pre-plant in the spring at 125% of the recommended rate; Sp75, half of the N applied at pre-plant in the spring and half at V6 at 75% of the recommended rate; Sp100, half of the N applied at pre-plant in the spring and half at V6 at 100% of the recommended rate; TSp50, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 50% of the recommended rate; TSp75, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 75% of the recommended rate; TSp100, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 100% of the recommended rate.

¶Means within the column followed by different low case letters are significantly different at $p \leq 0.05$.

§ Agronomic efficiency is the difference in grain yield at physiological maturity for a specific N fertilizer treatment and the grain yield at physiological maturity for the unfertilized treatment divided by the N rate.

Table 1.4. The effect of treatment x location x year on nitrogen partial factor productivity (PFP).

	Becker			Lamberton			Waseca		
Treatment †	2014	2015	2016	2014	2015	2016	2014	2015	2016
	kg kg^{-1}§								
C									
F100	34 ef¶	29 e	34 e	103 cd	67 c	69 cd	64 de	76 c	56 d
F125	28 f	28 e	35 e	86 e	56 d	61 de	58 e	56 e	48 d
PP100	42 e	43 d	54 d	107 c	67 c	72 c	77 b	67 d	66 c
PP125	36 ef	40 d	49 d	88 e	56 d	59 e	67 cde	56 e	54 d
Sp75	71 c	68 bc	77 b	131 b	79 b	92 b	91 a	92 b	85 b
Sp100	59 d	64 c	62 c	103 cd	68 c	74 c	74 bc	76 c	65 c
TSp50	116 a	84 a	94 a	181 a	109 a	112 a	99 a	115 a	107 a
TSp75	83 b	73 b	78 b	126 b	86 b	89 b	78 b	93 b	84 b
TSp100	75 bc	62 c	67 c	98 d	66 c	73 c	71 bcd	77 c	66 c

† C, control no N applied; F100, N applied in the fall at 100% of the recommended rate; F125, N applied in the fall at 125% of the recommended rate; PP100, N applied at pre-plant in the spring at 100% of the recommended rate; PP125, N applied at pre-plant in the spring at 125% of the recommended rate; Sp75, half of the N applied at pre-plant in the spring and half at V6 at 75% of the recommended rate; Sp100, half of the N applied at pre-plant in the spring and half at V6 at 100% of the recommended rate; TSp50, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 50% of the recommended rate; TSp75, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 75% of the recommended rate; TSp100, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 100% of the recommended rate.

¶Means within the column followed by different low case letters are significantly different at the $p \leq 0.05$.

§ Partial Factor Productivity is the grain yield produced per unit of N applied.

Table 1.5. Tests of fixed effects of urea fertilizer rate and timing of application on maize grain yield, recovery efficiency (RE), agronomic efficiency (AE) and partial factor productivity (PFP) from agricultural fields in Becker, Lamberton, and Waseca MN.

	Yield	RE	PFP	AE
Effect	---------------------------*P* > *F*---------------------------			
Treatment	<.0001	<.0001	<.0001	<.0001
Location	<.0001	<.0001	<.0001	<.0001
Location*Treatment	<.0001	0.0002	<.0001	<.0001
Year	<.0001	<.0001	<.0001	<.0001
Year*Treatment	<.0001	0.0071	<.0001	0.0317
Year*Location	<.0001	<.0001	<.0001	<.0001
Year*Location*Treatment	<.0001	0.5579	<.0001	<.0001

Table 1.6. The effect of location x treatment on nitrogen recovery efficiency (RE).

Treatment †	Becker		Lamberton		Waseca	
	kg kg^{-1}§					
C						
F100	0.068	d ¶	0.658	a	0.424	abcd
F125	0.112	d	0.614	a	0.358	bcd
PP100	0.209	cd	0.647	a	0.308	d
PP125	0.221	cd	0.557	a	0.344	cd
Sp75	0.357	bc	0.656	a	0.508	ab
Sp100	0.344	bc	0.636	a	0.493	abc
TSp50	0.601	a	0.619	a	0.353	bcd
TSp75	0.402	b	0.628	a	0.485	abc
TSp100	0.462	ab	0.632	a	0.548	a

† C, control no N applied; F100, N applied in the fall at 100% of the recommended rate; F125, N applied in the fall at 125% of the recommended rate; PP100, N applied at pre-plant in the spring at 100% of the recommended rate; PP125, N applied at pre-plant in the spring at 125% of the recommended rate; Sp75, half of the N applied at pre-plant in the spring and half at V6 at 75% of the recommended rate; Sp100, half of the N applied at pre-plant in the spring and half at V6 at 100% of the recommended rate; TSp50, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 50% of the recommended rate; TSp75, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 75% of the recommended rate; TSp100, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 100% of the recommended rate.

¶Means within the column followed by different low case letters are significantly different at the $p \leq 0.05$.

§ Recovery Efficiency is the difference in above ground N uptake between fertilized and unfertilized treatments relative to the amount of N fertilizer applied.

Table 1.7. The effect of year x treatment on nitrogen recovery efficiency (RE).

	2014		2015		2016	
	kg kg^{-1}§					
Treatment †						
C						
F100	0.433	a¶	0.504	bc	0.213	d
F125	0.403	a	0.423	cd	0.258	cd
PP100	0.383	a	0.363	d	0.418	abc
PP125	0.427	a	0.373	d	0.323	bcd
Sp75	0.388	a	0.644	ab	0.488	ab
Sp100	0.431	a	0.561	bc	0.482	ab
TSp50	0.468	a	0.543	bc	0.562	a
TSp75	0.386	a	0.707	a	0.423	abc
TSp100	0.496	a	0.643	ab	0.503	a

† C, control no N applied; F100, N applied in the fall at 100% of the recommended rate; F125, N applied in the fall at 125% of the recommended rate; PP100, N applied at pre-plant in the spring at 100% of the recommended rate; PP125, N applied at pre-plant in the spring at 125% of the recommended rate; Sp75, half of the N applied at pre-plant in the spring and half at V6 at 75% of the recommended rate; Sp100, half of the N applied at pre-plant in the spring and half at V6 at 100% of the recommended rate; TSp50, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 50% of the recommended rate; TSp75, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 75% of the recommended rate; TSp100, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 100% of the recommended rate.

¶Means within the column followed by different low case letters are significantly different at $p \leq 0.05$.

§ Recovery Efficiency is the difference in above ground N uptake between fertilized and unfertilized treatments relative to the amount of N fertilizer applied.

Chapter 2

Maize nutrient uptake influenced by timing and rate of N fertilizer application

Abstract

The timing and rate of nitrogen (N) fertilizer application has been shown to influence maize (Zea mays L.) nutrient uptake, but results have been inconsistent across locations and growing seasons. This study was conducted to determine whether fall, spring, and split applications of N fertilizer at varying rates influence biomass and nutrient uptake. Experiments were conducted from 2014 through 2016 to compare single (fall or spring applications) and split applications for differing N rates on an irrigated Hubbard-Mosford loamy sand complex at Becker and under rainfed conditions on a Normania loam soil at Lamberton and on a Nicollet clay loam soil at Waseca, MN. Nitrogen rates varied by locations and were always based on the University of Minnesota guidelines for each location. All three sites were planted to soybean in 2013 and to maize from 2014 to 2016. In coarse-textured soils applying N fertilizer in the fall reduced maize biomass and nutrient uptake compared with recommended rates of N fertilizer applied as a two-or three-way split application. In fine textured soils, not applying N fertilizer reduced maize tissue nutrient uptake compared with treatments receiving recommended rates of N fertilizer. Adhering to best management strategies for N management maximized maize nutrient uptake.

ABBREVIATIONS

SB-C-C-C, soybean-maize-maize-maize; F, fall application; N, nitrogen; PP, preplant application; R1, first reproductive stage of maize phenological development; Sp, two way split application; TSp, three way split N application. V4, four leaf-collar stage of maize phenological development; V6, six leaf-collar stage of maize phenological development; V8, eight leaf-collar stage of maize phenological development; V12, twelve leaf collar-stage of maize phenological development.

Introduction

Little attention has been paid to nutrient uptake in maize in response to different fertilization practices. Optimal maize production has been shown to be dependent on a season-long supply of P, S, Zn, and Cu, along with the acquisition of N, K, Mg, Mn, B, and Fe during the vegetative growth (Bender et al., 2013). Given that nutrient accumulation may vary considerably among soil microenvironments and agronomic management, there is a need to assess the impact of N fertilizer rate and timing of application on maize nutrient biomass uptake.

Although a two-year study on the impact of different N fertilizer rates on the uptake of N, P, K, and S in maize stover has been conducted in the upper Midwest, there has not been a recent study assessing the effect of N fertilizer rate and timing of application on nutrient uptake. In the earlier mentioned study, urea rates of 0-235 kg N ha^{-1} and 0-269 kg N ha^{-1} were applied at increasing rates of 34 and 45 kg increments, respectively (Sindelar et al., 2013). All fertilizer treatments were applied before planting in rainfed sites but in irrigated sites half of the urea fertilizer was applied at planting and the other half at the V4 maize growth stage (Sindelar et al., 2013). Supplemental P, K, S, and Zn were applied based on soil test results at recommended rates. Findings from fine textured soils demonstrated that stover N concentrations and removal increased from 0.08 to 0.18 kg N ha^{-1} for every kg N ha^{-1} increase in fertilizer while in coarse textured soils, stover N concentrations increased linearly by 0.15 kg N ha^{-1} for every kg N ha^{-1} (Sindelar et al., 2013). For P removal, there was a significant N rate by environment interaction with stover P removal varying across environments (Sindelar et al., 2013). When fertilizer N was applied at agronomically optimized rates (AONRs), stover P removal was estimated to be less than 10 kg P ha^{-1} (Sindelar et al., 2013). Similar to phosphorus, the removal of K by stover varied across environments. When using indigenous soil N, stover K removal ranged from 25 to 95 kg K ha^{-1} depending on location (Sindelar et al., 2013). The effect of location on stover K removal was further highlighted with stover K removal at AONRs ranging from 39 to 158 kg K ha^{-1}. Greater K uptake was demonstrated in irrigated environments compared with rainfed environments due to increased soil moisture (Sindelar et al., 2013). Across all environments, stover S removal at AONRs ranged from 2.8-4.8 kg S ha^{-1} in finer textured soils and was at least 5.0 kg S ha^{-1} in coarse textured soils (Sindelar et al., 2013). When relying solely on indigenous soil N, stover S removal across all environments ranged from 1.9 to 2.9 kg S ha^{-1} (Sindelar et al., 2013). Overall, nutrient removal by stover across all fertilizer N rates and environments within seven days of grain harvest was 46, 3.5, 76, and 3.7 kg ha^{-1} for

N, P, K, and S, respectively (Sindelar et al., 2013). When assessing the accumulation of macronutrients, Ciampitti et al. (2013) reported that plant nutrient contents at maturity responded predominantly to N rate, primarily reflecting biomass responses rather than nutrient ratios. Furthermore, relative nutrient contents at silking compared with maturity was 47% for P, 100% for K, and 58% for S (Ciampitti et al., 2013). Overall, improved biomass production and whole plant N uptake in nonlimiting P and K environments resulted in greater P, S, and K contents (Ciampitti et al., 2013). Due to the impact of environment on nutrient uptake more studies are needed to better understand whether nutrient uptake is impacted under different N rates and timing of application.

A long-term experiment in Eastern Europe exploring the effect of long term fertilizer applications in spring barley (*Hordeum vulgare* L.) nutrient concentrations reported that fertilizer application had a positive effect on N, P, and K concentration in the grain but there was no effect on Ca and Mg grain concentration (Hejcman et al., 2013). Among the micronutrients tested, only Fe increased in concentration in spring barley grain but this was attributed to low soil organic matter content and a low pH (Hejcman et al., 2013). This is in contrast to results from a long term fertilizer experiment in India (Shahid et al., 2016). In the study the authors reported that rice (*Oryza sativa* L.) Fe uptake varied from 765 to 1200 g ha^{-1} with significantly greater uptake recorded in plots receiving N, P, and manure compared with the control, N, and N+K treatments (Shahid et al., 2016). Treatments receiving manure also had significantly greater Zn uptake compared with the control treatment (Shahid et al., 2016). There was also a positive interaction between Mn and N application (Shahid et al., 2016). Rice Cu uptake ranged between 18.3 to 34.7 g ha^{-1} in the control and NPK+ manure treatments, respectively (Shahid et al., 2016). Application of just chemical fertilizers increased Cu uptake but a balanced application of NPK resulted in greater uptake (Shahid et al., 2016).

The effect of N fertilizer on maize micronutrient concentration has been variable. A long term fertilizer trail in China where 200 kg N ha^{-1}, 75 kg P_2O_5 ha^{-1}, and 150 kg P_2O ha^{-1} was applied to maize reported that not applying N or P fertilizer resulted in a low yield but high plant stalk micronutrient concentrations, except for Cu in maize stalks (Li et al., 2007). It was also reported that greater available soil P decreased the concentration of micronutrients in the crops (Li et al., 2007). These results contrast findings from West Africa where the application of nitrogen had no effect on the concentration of Zn, Cu, and Fe in maize ear leaf at full silk (Ogunlela et al., 1988). However, the application of 50, 100, and 150 kg N ha^{-1} did increase manganese concentrations by 9.9, 12.1, and 20%, respectively compared with the control (Ogunlela et al., 1988).

Given that maize grain yield and biomass production have increased over the last century due to improved breeding and agronomic management it is possible that the requirement and uptake of nutrients have increased (Bender et al., 2013). When looking at the accumulation of micronutrients throughout the growing season, Xue et al. (2014) reported that Zn accumulation in maize biomass increased throughout the growing season. By the silking stage (R1) more than three quarters of shoot Zn, Fe, and Cu had accumulated, while only half of the total Mn was taken up by maize (Xue et al., 2014). However, from the maize reproductive stage R3 to R6, there was a slight decrease in Fe and Cu shoot concentrations but a large decrease in Mn (Xue et al., 2014). Increases in yield and biomass production resulted in maize shoots containing more Fe, Mn and Cu at maturity but the pre-silking proportions of shoot Fe and Cu decreased (Xue et al., 2014). This indicates that with increasing yields, more Fe and Cu would be needed at both vegetative and reproductive stages (Xue et al., 2014). For improved Mn accumulatation, Xue et al. (2014) recommended applying Mn before silking, especially during V6 to V12 when the greatest acccumulation of shoot Mn generally occurred. However, optimized N supply achieved the greatest yield and increased grain concentrations of micronutrients compared with no or lower N supply (Xue et al., 2014). Excessive N supply did not increase micronutrient concentrations (Xue et al., 2014).. Given that optimized N fertilizer application does not seem to negatively impact macro and micro nutrient application, there is room to explore whether the time of appplication and varying rates of N fertilizer could impact macro and micro nutrient accumulation and maize biomass production. To date there has not been a study on the effect of time of N fertilizer application on macro and micro nutrient status in maize biomass. In order to better understand maize development, the objective of this study was to determine the impact that rate and timing of application of urea fertilizer has on the uptake of N, S, P, Cu, Fe, Mn, and Zn in maize tissue.

Materials and Methods

Site Description and Experimental Design

Field experiments were conducted at University of Minnesota Research and Outreach Centers near Lamberton, MN (44° 24’ N, 95° 30’ W), Waseca, MN (44° 07’, 93° 52’ W) and Becker, MN (45° 39’ N, 93° 89’ W) from 2014 to 2016. The rainfed sites at Lamberton had a Normania loam soil (Fine-loamy, mixed, superactive, mesic Aquic Hapludolls) and at Waseca a Nicollet clay loam soil (Fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquolls); while an irrigated Hubbard-Mosford loamy sand complex (Sandy, mixed, frigid Typic Hapludolls or frigid Entic Hapludolls) was used at Becker, MN. The experimental design was a randomized complete block design with four replications. Plot sizes at Becker and Waseca, MN were 4.6 m (6 rows) by 15 m and Lamberton was 6 m by 12 m. Plot size at the different locations differed due to the implements that each research center had for use in the research plots. All sites were planted to soybean [*Glycine max* L. (Merr.)] in 2013 and to maize from 2014 to 2016. For each year, the tillage system involved field cultivating in the spring prior to planting, and stalk chopping followed by disk ripping in the fall after maize harvest. Non-N starter fertilizer was applied in the spring following soil test and crop guidelines. Weed and pest management followed best management practices (BMP) and varied by location and year.

The selected recommended N rate (RN) in 2014 for maize following soybean at Becker, MN was 168 kg ha^{-1}, while at Lamberton and Waseca it was 135 kg ha^{-1}. For

2015 and 2016 the RN for maize following maize at Becker was 235 kg ha^{-1} while at Lamberton and Waseca, MN it was 202 kg ha^{-1}. All rates were based on University of Minnesota guidelines (Kaiser et al., 2012). The N source used for fall applications was SuperU® (46-0-0), a urea-based granule with urease and nitrification inhibitors, and for the pre-plant and in-season applications the N source was urea (46-0-0). All fertilizers were hand-applied and incorporated with tillage immediately after application in the fall and prior to planting. In-season applications were incorporated through irrigation at Becker. At Lamberton and Waseca, fertilizer was placed in 5 cm deep furrows in the inter-rows made using a hoe, after fertilizer application, furrow trenches were closed with soil. Treatments were applied to the same experimental plots in all years. The treatments tested in this study consisted of a non-fertilized control plus nine N management systems: F100, single SuperU application in the fall at 100% of RN; F125, single SuperU application in the fall at 125% of the RN; S100, single urea application applied at planting at 100% of the RN; S125, single urea application applied at planting at 125% of the RN; Sp75, split urea application at planting (one half) and V6 (one half) applied at 75% of the RN; Sp100, split urea application at planting (one half) and V6 (one half) applied at 100% of the RN; TSp50, split urea application at planting (one third), V6 (one third), and silking (one third) applied at 50% of the RN; TSp75, split urea application at planting (one third), V6 (one third), and silking (one third) applied at 75% of the RN; TSp100, split urea application at planting (one third), V6 (one third), and silking (one third) applied at 100% of the RN. The growth stages selected for N application in treatments TSp50, TSp75, and TSp100 were based on the nutrient requirement and time of maximum uptake by the crop (Abendroth, 2011). This strategy for split N application aimed to provide the crop with N at the times throughout the growing season when it was most needed.

Maize Tissue Sampling

In-season maize tissue samples were collected seven times during the growing season from each plot approximately one week before the V6 urea application (Pre-V6), one week after the V6 urea application (Post-V6), approximately 3 weeks after the V6 urea application (3wk-V6), one week before the R1 urea application (Pre-R1), one week after the R1 urea application (Post-R1), three weeks after the R1 urea application (3wk-R1) and at physiological maturity (PM) of the maize plant. A staggered sampling was used to minimize the potential interference of removing plants on the development of plants being used for yield estimate; in addition, tissue sampling was avoided in the rows that were going to be used for the estimation of yield. Three maize plants were sampled every 4 m from rows two and three plants from row five in six row plots, and from rows four and five in eight row plots. Stover samples were weighed wet in the field, samples collected after the V12 stage required the plants to be chopped in the field with a sub sample collected for moisture content. At Lamberton and Waseca, MN, grain yield was determined at harvest using a plot combine and a subsample of the grain was also collected for determination of total nutrient uptake at harvest. At Becker, 6 m from the center of rows three and four from each plot were hand harvested and a subsample of the grain was collected for determination of total nutrient uptake at harvest.

Stover samples from all sampling times and a grain subsample were dried in a forced-air dryer at 60ºC until constant mass and the dry weight was then determined for each subsample. The dry stover and grain samples were then ground to 1mm and sent to Lamberton, MN for chemical composition analysis. Samples were chemically analyzed for Cu, Fe, K, Mn, P, and Zn using inductively coupled plasma optical emission spectroscopy ICP-OES (PerkinElmer, Optima 8x00, Norwalk, CT) and from N and S using a CNS analyzer (Vario MACRO, Elementar, Langenselbold, Germany).

Statistical Analysis

Maize tissue nutrient biomass was analyzed by repeated measure ANOVA using the GLIMMIX Procedure in SAS at $P \leq 0.05$. Treatment, location, time of sampling, and year were considered fixed effects, while replication was considered random. The variable year was the repeated variable and the covariance structure that best fit the model for each nutrient was used. Tests for normality were performed prior to making inferences on main effects. When appropriate, pairwise mean comparisons (at $P \leq 0.05$) were made using the lines option in the GLIMMIX procedure of SAS. Because of funding limitations not all sampling times were analyzed for P, Cu, Fe, Mn, and Zn uptake in 2015 and 2016. Therefore, statistical analysis of the 2014 and 2015 data was performed separately from the 2016 data when appropriate.

Results and Discussion

Biomass Yield

Becker

There was a three-way interaction for treatment x year x time of sampling for maize biomass (Table 2.1). Differences in maize biomass began in most cases around the Pre-R1 stage and became more evident as the season progressed (Table 2.2). In the early developmental stages, 3wk-V6 to Pre-R1, the control plot had significantly less biomass than other treatments. In the later developmental stages from Post-R1 to PM, split applications applied with at least 75% recommended rate had greater biomass than fall-applied treatments at recommended or above-recommended rates. Biomass was maximized with split applications receiving recommended rates.

Lamberton

There was a two-way interaction of treatment x time of sampling, treatment x year, and year x time of sampling (Table 2.1).

Treatment x Time of sampling

Differences between treatments began around the 3wk-V6 stage and became more evident as the season progressed (Table 2.3). In the early developmental stages, 3wk-V6 to Pre-R1, the control and TSp50 had lower maize biomass than other treatments. In the later developmental stages, Post-R1 to PM, the application of N fertilizer at recommended rates in the fall, spring, or as a two-way split increased maize biomass compared with a three-way split.

Treatment x Year

In 2014, the application of at least recommended rates in the fall, spring or as a two-way split application averaged 10,955 to 11,495 kg ha^{-1}, greater than TSp100 (10,399 kg ha^{-1}). In 2015, Sp75 yielded 10,704 kg ha^{-1} biomass, similar to F100, PP100, PP125, Sp100, and TSp100 which yielded 10.66 to 10.88 Mg ha^{-1} (Table 2.3). Biomass yield was greatest under the F125 treatment, yielding an average of 11.48 Mg ha^{-1}, greater than all spring and split applications regardless of rate. In 2016, F125 yielded a biomass of 8.85 Mg ha^{-1}, greater than all other treatments except Sp100 (8.41 Mg ha^{-1}). Applying recommended rates in the spring (PP100) or as a two-way split (Sp100) yielded 8.16 Mg ha^{-1} and 8.41 Mg ha^{-1}, respectively, more than TSp100 (7.49 Mg ha^{-1}).

Year x Time of Sampling

Maize biomass increased each sampling time in 2014 and 2015 but plateaued after the 3wk-R1 sampling in 2016 (Table 2.3). Beginning around the 3wk-V6, the average biomass yield in 2016 was less than the biomass yield in 2014 and 2015. For example, at the Post-R1 sampling, maize biomass in 2014 and 2015 yielded 12,565 and 12,414 kg ha^{-1}, respectively, more than the maize biomass of 9,376 kg ha^{-1} at the same sampling in 2016.

Waseca

At Waseca there was a three-way interaction of treatment x year x time of sampling (Table 2.1). Differences in maize biomass began around the Pre-R1 stage and became more evident as the season progressed (Table 2.2). In 2014, there was little difference in biomass between treatments receiving the same rates although Sp75 did yield 15,746 kg ha^{-1} biomass and 20,166 kg ha^{-1} at 3wk-R1 and PM, respectively, greater or similar to biomass produced by treatments receiving recommended rates. In 2015, at 3wk-R1 there was no significant difference between split treatments receiving 75% recommended fertilizer rate and treatments receiving at least recommended rates, regardless of timing of application. However, by the PM sampling, F100 and TSp100 produced an estimate 25,368 and 25,433 kg ha^{-1}, respectively, greater than all treatments except Sp100 which produced a biomass of 24,920 kg ha^{-1}. In 2016 biomass was greatest under spring and split applications receiving recommend rates. At PM, these treatments yielded a biomass of 19,307 to 20,040 kg ha^{-1}, significantly greater than F100 and F125 which yielded 17, 414 and 17, 626 kg ha^{-1} biomass, respectively (Table 2.2).

In season maize nutrient uptake

Maize nitrogen uptake

Becker

There was a significant three-way interaction for treatment x year x time of sampling for maize tissue N uptake (Table 2.1). Differences in maize tissue N uptake in most cases started around the 3wk-V6 stage and become more evident as the season progressed (Table 2.4). At the early developmental stages 3wk-V6 to Pre-R1, treatments with N application split into two times (Sp treatments averaging 109 and 111 kg N ha^{-1} in 2015 and 2016, respectively) showed greater tissue N uptake than the other treatments (averaging less than 80 kg N ha^{-1} in 2015 and 2016) especially in 2015 and 2016. At the later developmental stage from Post-R1 to PM, N uptake was greater in plots receiving N application split into three times (Tsp treatments). It is also evident that there was a response to the N application rates. In general, the greater the N application rate the greater the N uptake by maize at Becker, for example in 2015 N uptake was 88, 114, and 135 kg for TSp50, TSp75, and TSp100, respectively. Application of N in the fall or spring had the lowest N uptake by maize, and in many instances uptake in those treatments was not different than the uptake observed in the control plots. This follows a similar trend to biomass yield with the fall and sometimes spring applications producing lower biomass than split applications receiving at least 75% recommended rate.

Lamberton

Treatment x Time of Sampling

At Lamberton, there were significant two-way interactions for treatment x time of sampling, year x treatment, and year x time of sampling (Table 2.1). Maize N uptake was affected by how N was applied prior to planting (Table 2.5). Differences between treatments began to at the Post-V6 sampling date where maize N uptake in the F125 treatment was greater than in the control and all split applications, regardless of rate until the final sampling. At the 3wk-V6 and Pre-R1 sampling dates, the F125 (135 and 180 kg

N ha^{-1} for 3wk-V6 and Pre-R1, respectively) treatment had taken up significantly more N than all treatments except PP100 (127 kg N ha^{-1}) and PP125 (121 kg N ha^{-1}). For the much of the later season, N uptake was greater when fertilizer was applied in the fall or spring prior to planting, and N uptake in split applications was intermediate. At the last sampling, PM, N uptake was similar among all treatments that received equivalent N rates averaging around 195 kg N ha^{-1}. In addition, there was a clear N uptake response to N rate starting at the Post-R1 and remaining consistent until PM for the split treatments.

Treatment x Year

In 2014, maize following soybean, plant N uptake in all fall and preplant treatments averaged between 153 to 157 kg N ha^{-1} which was greater than all other treatments (Table 2.5). In 2015, F125 again had greater plant N uptake than all treatments except PP125. In addition, in 2015 there was no difference in N uptake in treatments receiving similar N application rates. In 2016, fall and spring applied N were no longer the best treatments and the split applications had similar plant N uptake as fall and spring applications when equivalent N rates were applied. Although maize biomass in 2016 was lower than in 2014 and 2015, there were no significant difference in biomass between Sp100, F100, and PP100 across 2014-2016 (Table 2.3). In 2016 from May to September, Lamberton received 651mm of precipitation, compared with 512 mm in 2014 and 564 mm in 2015. In July and August 2016 Lamberton received 310 mm of precipitation, compared with 124 and 209 mm in 2014 and 2015, respectively. The wet summer in

2016 likely improved N uptake of split applications while possibly leaching some of the applied N from fall and spring applications.

Year x Time of Sampling

Maize N biomass increased every sampling date until 3wk-R1 where it plateaued in 2014 and declined in 2015 and 2016 (Table 2.5). It is likely that the decline in maize tissue N uptake observed in 2015 and 2016 at PM was due to a significant amount of N being translocated to the developing ear. After silking, N incorporated at the beginning of stalk elongation and stored in vegetative organs has been demonstrated through ^{15}N isotopes to almost totally remobilize either directly to the kernel or after transitory storage in the cob, husk, and shank (Cliquet, et al.,1990). The results reported in Table 2.5 also suggest that the maize plants started to translocate nutrients into the grain sooner in the growing cycle as the study went on. In 2014, the amount of N in the plant tissue was similar between 3wk-R1 and PM, in contrast it had decreased by 30 kg N ha^{-1} in 2015, and by 83 kg N ha^{-1} in 2016 (Table 2.5). Although the reasons for this is not yet understand it could be related to physiological changes due to monocropping or be weather related. Precipitation did increase in from May through September from 512 mm precipitation in 2014 to 651 mm in 2016. The increased precipitation might have impacted root growth, reducing the amount of N taken up, thus beginning the translocation of N to the cob earlier in the season.

Waseca

At Waseca tissue N uptake showed to be affected by the three-way interaction treatment x timing of sampling x year (Table 2.1). In 2014, differences started to take place at the 3wk-V6 sampling, where the control tended to have lower tissue N uptake than the treatments receiving N (Table 2.4). Meaningful differences between treatments started at the 3wk-R1 sampling and continued until the last sampling. During the 3wk-R1 to PM sampling, tissue N uptake was greater when 100% of the recommended rate was applied and decreased as N application rate decreased, especially at the PM sampling. In 2015, although differences started at the Post-V6 sampling, meaningful differences started at the Pre-R1 and remained until the PM sampling. There was a trend for tissue N uptake to be greater when N application was split into two or three applications during the growing season compared with single application. For example, tissue N uptake in the Sp100 and Tsp100 was greater than the PP100 from the Pre-R1 sampling to the last sampling at PM. There was also a clear response to N application rate, where tissue N uptake increased as N application rate increased in the split treatments. In 2016, differences started as soon as the Post-V6 sampling, which were in all cases due to the different rates applied and not due to the method of fertilizer application. The most meaningful differences in tissue N uptake started at the Post-R1 sampling. From the Post-R1 to PM sampling tissue N uptake tended to be greater in treatments where N application was split in two or three times and less in the fall treatments. For example, tissue N uptake in the TSp100 was 185 and 277 kg N ha^{-1} at Post-R1 and 3wk-R1, respectively, while tissue N uptake at F100 was 152 and 248 kg N ha^{-1} at Post-R1 and 3wk-R1, respectively (Table 2.4).

In general, there was a decrease in tissue N uptake at all locations from the 3wk-R1 to the PM sampling, which could be due to that by this stage a significant amount of N had been translocated from the plant tissue to the developing ear. The variation observed in tissue N uptake at Becker, Lamberton and Waseca confirms the significance of environment in maize productions as noted by Sindelar et al. (2013). At Becker and Waseca the influence of time of application on tissue N uptake is demonstrated with the control, spring, and fall applications having lower tissue N uptake than split applications applied at agronomically optimum N rates. In contrast, at Lamberton, tissue N uptake in the split application was as effective as fall and spring applications. The response of maize tissue N uptake to N rate, particularly at Lamberton and Waseca, supports conclusions that total aboveground maize N uptake responds to increasing rates of N fertilizer (Sindelar et al., 2015). Similar results were also reported by Halvorson & Jantalia, (2011) where stover yields increased with increasing N rates.

Maize sulfur uptake

Becker

There was a three-way interaction for year x time of sampling x treatment for maize S uptake (Table 2.1). Similar to maize N uptake, differences between treatments in maize tissue S biomass began around the 3wk-V6 stage and become more evident as the season progressed (Table 2.6). In general, at early developmental stages, 3wk-V6 to Pre-

R1, in all three years, treatments that supplied the recommended rate split into two and three applications (Sp100 and TSp100 treatments) showed greater tissue S uptake than the control plots, F100, and TSp50 treatments. In the later developmental stages from Post-R1 to PM, split treatments receiving at least 75% the recommended rate had greater maize tissue S uptake than the control plot and fall applied treatments. Similar to maize tissue N uptake, in general the greater the N application rate, the greater the S uptake by maize at Becker. Application of N in the fall had the lowest S uptake and in many instances uptake from those treatments were similar to uptake in the control.

Lamberton

At Lamberton there were significant two-way interactions for treatment x time of sampling and year x time of sampling (Table 2.1). Differences between treatments began at the Post-V6 sampling where plots receiving fall applied N, regardless of rate, had taken up more S than the non-N fertilized control and TSp50 treatments (Table 2.7). Differences between maize S tissue uptake in fall applied and TSp treatments became more pronounced throughout the growing season (Table 2.7). However, treatments applied all at pre-planting or as a two-way split (Sp) also took up greater amounts of S than the other treatments particularly later in the reproductive stages. In addition, N rate seemed to have a stronger effect on tissue S uptake than time of sampling as suggested by the fact that TSp50 (starting at Post-R1) and Sp75 (starting at 3wk-R1) usually had lower tissue S uptake than Sp100 and TSp100 (Table 2.7).

There was also a year x time of sampling interaction for tissue S uptake at Lamberton (Tables 2.1, Table 2.8). At all three years S uptake increased steadily until 3wk-R1. Although, S uptake continued to increase until the PM sampling in 2014, it decreased from 3wk-R1 to PM in 2015 and 2016 (Table 2.8). This different behavior in S uptake between 2014 compared with 2015 and 2016 is likely the cause for the significant interaction.

Waseca

At Waseca, S uptake by maize was affected by the two-way interactions of year x time of sampling and treatment by time of sampling (Table 2.1). In 2014 and 2015 maize tissue S uptake increased throughout the growing season reaching the maximum uptake value at the PM sampling (Table 2.8). In 2016 it peaked at 3wk-R1 before decreasing at PM (Table 2.8). The decrease in maize tissue S uptake from 3wk-R1 to the PM sampling in 2016 is the reason for the significant interaction.

Differences between treatments began at the Pre-R1 sampling where Sp100 had taken up more S than the non-N fertilized control, F100, PP100, and TSp50 treatments (Table 2.7). Treatments applied at recommended rates as a two-way split (Sp100) took up greater amounts of S than the reduced rate treatments particularly later in the reproductive stages (Table 2.8).

Sulfur uptake, particularly at Becker followed a similar pattern to whole-plant biomass N uptake. Particularly that in coarse textured soils maize tissue S uptake may be reduced if N is applied in the fall. Given that sulfur is known to leach in coarse-textured soils, it is likely that any mineralized or spring applied sulfur, particularly in low nutrient available plots, likely leached below the root zone. The uptake of S has been shown to respond to N rate and have a similar response to tissue accumulation and N content (Ciampitti et al., 2013). Although, the application of N can impact the uptake of S in the plant, fertilizer N has not been shown to affect basal stalk SO_4-S (Sutradhar, et al., 2017). The influence of N rate on S uptake at Lamberton supports similar conclusions by Ciampitti et al. (2013) who reported that S follows the N dilution model and can be influenced by N rate. The variation observed in tissue S uptake at Becker, Lamberton and Waseca confirms the significance of environment in maize productions and the difficulty of interpreting plant tissue data due to the impact of site-specific and management factors (Sindelar et al., 2013). Overall, this findings support findings demonstrating that the uptake of N and S are interrelated (Sutradhar, et al., 2017).

According to recommendations, sufficient levels of S in maize tissue is estimated to be between 0.15-0.40 g kg^{-1} S (Kaiser et al., 2011). At Becker, maize tissue S began to drop below 0.15 g kg^{-1} across all treatments at the 3wk-V6 stage each year except for Sp75, Sp100, and TSp100 at 3wk-V6 in 2015. For the remainder of the growing season maize tissue S was below 0.15 g kg^{-1} regardless of treatment. At Lamberton maize tissue S dropped below 0.15 g kg^{-1} for all treatments at the Pre-R1 stage in 2014 and 2015 and the Post-R1 sampling in 2016. At Waseca in 2014 and 2015, maize tissue S began to drop below 0.15 g kg^{-1} at 3wk-V6 except for fall and split treatments receiving at least 75% recommended rate. However, by Pre-R1 maize tissue S% had fallen below 0.15 g kg^{-1} across all treatments. In 2016, maize tissue S dropped below 0.15 g kg^{-1} at the Post-R1 sampling for all treatments except for F100, F125, PP125, TSp100, and TSp50 which all had 0.15 g kg^{-1} maize tissue S content.

Although maize tissue S levels can vary with maize growth stages, range broadly, and be affected by interactions with other nutrients, the decrease in maize tissue S throughout the growing season supports recommendations to apply S fertilizer on long-term continuous maize systems (Kaiser et al., 2011; Sutradhar, et al., 2017). Given that concentration can be affected by dilution and accumulation effects of plant mass, the use of nutrient uptake is likely a better measure to determine management strategies (Kaiser and Rubin, 2013).

Maize phosphorus uptake

Because of funding limitations not all sampling times were analyzed for P uptake in 2016. Therefore, statistical analysis of the 2014 and 2015 data were performed separately from the 2016 data (Table 2.1).

Becker

At Becker there was a treatment effect and a year x time of sampling interaction for 2014 and 2015 while in 2016 there was a treatment x time of sampling interaction (Table 2.1). Two and three-way split applied treatments applied at recommend N rates had greater maize tissue P uptake than fall and spring applied treatments, regardless of rate (Table 2.10). TSp75 also had greater P uptake than the fall and spring applied treatments (Table 2.10). For the year x time of sampling interaction, maize tissue P uptake increased until 3wk-R1 and decreased by the last sampling, suggesting that P had started to move from tissue into the developing cob (Table 2.9). For the treatment x time of sampling interaction, in 2016 differences between treatments became apparent at the Post-V6 sampling when the non-N fertilized control plot had lower maize tissue P uptake than all other treatments (Table 2.10). In addition, the spring applied N and split N applications tended to have greater P uptake than the fall and non-N fertilized control treatments when applied at respective rates at the Post-R1 and 3wk-R1 samplings (Table 2.10).

Lamberton

Year x Time of Sampling

At Lamberton there was an interaction for year x time of sampling for the 2014 and 2015 data and treatment x time of sampling for all three years (Table 2.1). Although there was a interaction, maize P uptake increased with each sampling date before peaking at 3wk-R1, which was followed by a decrease in P uptake at the last sampling (Table 2.9). The decrease in tissue P uptake in the last sampling is likely a result of P being translocated from the tissue into the developing ear.

Treatment x Time of Sampling

In 2014 and 2015 maize P uptake was greatest when all fertilizer was applied in the fall and lowest in the non-N fertilized control for the samplings taking place between 3wk-V6 to 3wk-R1 (Table 2.11). For the samples collected at the PM sampling date, tissue P uptake was greatest for the Sp100 and lowest for the F125, PP100 and Sp75 (Table 2.11). In 2016, application of at least 75% of the recommended rate in a two-way split or 100% the recommended rate in a three-way split had the same maize tissue P uptake compared with the fall and spring applied N (Table 2.11).

Waseca

At Waseca there was a treatment x year interaction in 2014 and 2015 and in 2016 there was a treatment x time of sampling interaction (Table 2.1). In 2014, N application did not affect maize tissue P uptake during the growing season as the greatest P uptake was observed in the non-N fertilized control (Table 2.10). In contrast, in 2015 all treatments receiving fertilizer had greater maize tissue P uptake than the non-N fertilized control plot (Table 2.10). In 2016, spring application of N tended to result in greater tissue P uptake for samples collected from Post-V6 to Post-R1 (Table 2.10). At PM all treatments receiving fertilizer had greater maize tissue P biomass than the non-N fertilized control (Table 2.10).

The data for P uptake at Lamberton suggests that N management is extremely important in a continuous maize cropping system. In the first two years of the study, split N application lead to equal or slightly greater P uptake at Lamberton, whereas in 2016, fall and spring application had the greatest tissue P uptake. This follows general consensus that N application can stimulate P uptake by plants (Ma and Zheng, 2016). It is possible that the residue N from the soybean was able to help with the uptake of nutrients such as P, and in 2016 when no N from soybean can be expected the fall and spring treatments were found to have better conditions for P uptake (Tables 14 and 15). At Waseca, no response to N was observed for P uptake suggesting that the residue from the soybean was able to supply the requirement amount of N to maximize P uptake. However, in 2015 and 2016 when little to no residue N should be expected from soybean, N application did influence P uptake. At Becker, spring or split application led to greater P uptake than the non-N fertilized control and fall applied N. A N rate and time of application interaction for biomass P accumulation has been observed in the upper Midwest (Sindelar et al. 2013). Given that all treatments were grown in a non-limiting P environment, the lack of differences of N rate on aboveground P uptake does not follow conclusions by Ziadi et al. (2007) that increasing N fertilization increases P concentrations in maize tissue uptake. However, Ciampitti et al. (2013) reported whole plant dilution functions of P were relatively unmodified by N rate.

In-season micronutrient uptake by maize

Due to funding limitations, not all sampling times were analyzed for Cu, Fe, Mn, and Zn uptake in 2016. Therefore, statistical analysis of the 2014 and 2015 data were performed separately from the 2016 data (Table 2.1).

Maize Cu uptake

Becker

There was a three-way interaction for treatment x year x time of sampling at Becker (Table 2.1). In 2014, differences between treatments began between Post-V6 and 3wk-V6 with split applications often having greater maize tissue Cu uptake than the control and fall treatments (Table 2.12). At the later sampling periods split treatments applied at recommended rates (Sp100 and TSp100) continued to have greater maize tissue Cu uptake than the non-N fertilized control, fall and preplant treatments (Table 2.12). In 2016 similar results were observed, with split applications having greater Cu tissue uptake than the fall and spring treatments (Table 2.12).

Lamberton

At Lamberton there was a treatment effect and a two-way year x time of sampling interaction (Table 2.1). Treatments applied in the spring had greater maize tissue Cu uptake than the non-N fertilized control and split treatments (Table 2.13). For the year x time of sampling interaction, maize tissue Cu uptake increased with each sampling date (Table 2.13).

Waseca

There was a three-way interaction for treatment x year x time of sampling at Waseca (Table 2.1). In 2014, differences between treatments began at Pre-R1 and with two-way split applications often having greater maize tissue Cu uptake than the non-N fertilized control and F100 (Table 2.12). At the later sampling periods, specifically PM, fall and spring application of 125% recommended rate (F125 and PP125) had greater Cu tissue uptake compared with other treatments (Table 2.12). There were no differences between treatments receiving recommended rates (F100, PP100, Sp100, and TSp100) (Table 2.12). In 2016, the application of fertilizer in the spring or as a split application at recommended rates (PP100, Sp100, and TSp100) increased Cu tissue uptake compared with the non-N fertilized control (Table 2.12).

As maize is only moderately sensitive to copper deficiency following best management practices should not result in a copper deficiency. The largest risk for copper deficiency is in coarse-textured soils however applying fertilizer as a two or three-way split application will improve maize tissue Cu uptake over the control and fall applications. Similarly, following recommended best management practices in finer-textured soils should not result in a copper deficiency. The sufficiency range for Cu from the maize ear leaf from silking to tassel is estimated to be between 3-15 mg kg^{-1} while stems have been estimated to contain 5.4 mg Cu kg^{-1} (Nan and Cheng, 2001). At Lamberton and Waseca, the maize tissue Cu content remained in the sufficiency range except for the control plot at PM when it dropped to 2 mg kg^{-1}. At Becker maize tissue Cu content also remained within sufficiency ranges until the Post-R1 to PM stage when the control and fall treatments dropped to 2 mg kg^{-1}. Unlike Losak et al. (2011), nitrogen management did have a significant effect on Cu uptake, particularly at the later stages of development. The later impact of nitrogen management on maize Cu uptake supports findings from Borges et al., (2009) that maize accumulates minimum amounts of Cu in the early developmental stages and only reaches maximum values near the end of the season. Following best management practices that maximize yield or biomass tended to increase the accumulation of Cu in the maize. Similar conclusions were drawn by Xue et al. (2014) when assessing micronutrient uptake in response to nitrogen supply. Overall, following best management practices should not result in Cu deficiency compared with no or lower N supply.

Maize Fe uptake

Becker

In 2014 and 2015 at Becker there was an interaction between year x time of sampling where maize tissue Fe uptake increased each sampling date (Table 2.1, Table 2.14). In 2016 there was a treatment effect where the control had less maize tissue Fe uptake than all other treatments (Table 2.1, Table 2.15). However, all treatments were comfortably in the nutrient sufficiency ranges for maize across the growing season (Schulte and Kelling, 1991).

Lamberton

At Lamberton, in 2014 and 2015 there was a year x time of sampling interaction while in 2016 there was a treatment x time of sampling interaction (Table 2.1). In 2014 maize tissue Fe uptake was greatest at Pre-R1 and 3wk-R1 while in 2015 maize tissue Fe uptake was greatest at PM (Table 2.14). In 2016 there were no differences in maize tissue Fe uptake at Post-V6, but at Post-R1 the application of at least 50% recommended rate of N fertilizer produced greater maize tissue Fe uptake than the control plot (Table 2.15). However, the application of 50% recommended rate as a three-way split (TSp50) also had lower maize tissue Fe uptake than the fall and two way split (Sp) treatments regardless of rate (Table 2.15). At 3wk-R1, applying recommended rates at preplant (PP100) produced more maize tissue Fe uptake than the non-N fertilized control plot and split treatments receiving less than recommended rates (Table 2.15). At Lamberton in 2016, the application of reduced rate split treatments resulted in lower tissue Fe uptake than fall and spring applications (Table 2.15). However, all treatments were within nutrient sufficiency levels throughout the season (Schulte and Kelling, 1991).

Waseca

At Waseca there was a treatment effect all three years and a two-way year x time of sampling interaction (Table 2.1). In 2014 and 2015 the control plot had less maize tissue Fe uptake than all other treatments regardless of time of application or rate (Table 2.15). In 2016 the control plot had less maize tissue Fe uptake than all other treatments except F125 (Table 2.15). Applying urea at recommended rates at preplant (PP100) produced greater maize tissue Fe uptake than the control and F125 treatment (Table 2.27). Similar to the other locations, maize Fe concentrations were within sufficiency levels (Schulte and Kelling, 1991).

The application of N fertilizer following best management practices tended to increase maize uptake of Fe compared with the non-N fertilized control plot. This is in contrast to findings from Ma and Zheng (2018) who reported that maize Fe uptake was not affected by N input. At Lamberton, increases in biomass production tended to increase the accumulation of Fe in aboveground biomass. Similar conclusions were also reported by Xue et al. (2014) when assessing the uptake requirement of Fe in response to nitrogen supply, biomass, and yield of maize. Given that treatment had a limited effect on maize Fe across all locations and that the uptake of Fe falls within nutrient sufficiency ranges, following best management practices to maximize grain and biomass yield should be advised.

Maize Mn uptake

Becker

Across all three years there was a three-way interaction between treatment x year x time of sampling (Table 2.1). Differences between treatments become apparent around the 3wk-V6 to Pre-R1 sampling where the non-N fertilized control treatment tended to have lower maize tissue Mn uptake than treatments applied as a two- or three-way split treatment at recommended rates (Table 2.16). As the maize developed, the differences in maize tissue Mn uptake between treatments applied in the fall and treatments applied at planting or as a two- or three-way split became greater. By PM in 2014, Sp100, TSp100, and TSp50 had greater maize tissue Mn uptake than the control and fall treatments. The results observed for Mn uptake in 2015 and 2016 were random and difficult to explain as there were no clear-cut trends.

Lamberton

At Lamberton in 2014 and 2015 there was a treatment effect and a two-way interaction for year x time of sampling (Table 2.1). For the treatment effect, across 2014 and 2015, single application of N had greater maize tissue Mn uptake than the split treatments (Table 2.18). For the year x time of sampling interaction, in 2014 maize tissue Mn uptake increased throughout the growing season before plateauing at 3wk-R1 (Table 2.17). However, in 2015, maize tissue Mn uptake increased until PM (Table 2.17). In 2016 there was a two-way interaction between treatment x time of sampling (Table 2.1).

In general, treatments receiving a single application of N had greater tissue Mn uptake then the application of split treatments (Table 2.18).

Waseca

At Waseca treatment, time of sampling and year were significant for 2014 and 2015 while there was a treatment x time of sampling interaction in 2016 (Table 2.1). Across 2014 and 2015, the TSp75 treatment had greater Mn tissue uptake than the control, fall, and PP100 treatments while Sp100 also had greater uptake than the control, F125, and PP100 (Table 2.18). The control treatment had lower maize tissue Mn uptake than all other treatments (Table 2.18). For time of sampling maize tissue Mn uptake increased every sampling date while the maize in 2015 had greater Mn uptake than 2014 (Table 2.17).

In 2016 there were differences between treatments at each sampling date (Table 2.1). Across all three samplings, treatments applied at preplant or as a two-way split (Sp) receiving at least recommended rates had greater maize tissue Mn biomass than the non-N fertilized non-N fertilized control plot and TSp50 (Table 2.18). Later in the growing season the control plot also had less maize tissue Mn uptake than all treatments receiving at least 75% recommended rates (Table 2.18).

Overall, applying N fertilizer at recommended rates and following best management strategies did not reduce maize tissue Mn uptake. These findings are similar to a two year field experiment by Losak et al. (2011) where the application of 120 and 240 kg N ha^{-1} also did not affect the contents of Mn in maize biomass. The application of inorganic and liquid dairy cattle (*Bos taurus*) manure on a calcareous soil has also been shown to increase Mn concentrations compared with the control plot (Nikoli and Matsi, 2011). The steady accumulation of Mn in maize tissue is consistent with findings from Borges et al. (2009). Similarly, there was little accumulation in the early stages of plant development but increased throughout the season (Borges et al., 2009). Given that maize tissue Mn concentrations, including the non-N fertilized control plots, were within nutrient sufficiency ranges of 20-150 mg kg^{-1}, following best management practices to maximize grain or biomass is recommended.

Maize Zn uptake

Becker

At Becker in 2014 and 2015 there was a two-way interaction for treatment x time of sampling and year x time of sampling while in 2016 there was a two-way treatment x time of sampling interaction (Table 2.1). In 2014, differences between treatments began at the Post-R1 stage with Sp75 having greater Zn uptake than the control, fall and spring applications (Table 2.21). Split applications continued to have greater maize tissue Zn uptake than fall and spring applications, regardless of rate, throughout the remainder of 2014 (Table 2.21). In 2015 differences began at the Pre-R1 stage with split applications receiving at least 75% recommended rate having greater Zn uptake than fall and spring applied treatments (Table 2.21).

Zinc uptake by maize seems to be slow in the early developmental stages and pick up around the 3wk-V6 stage. This is demonstrated by the rapid accumulation after the

Post-R1 stage (Table 2.19). Zinc also seems to move from the biomass into the developing ear very quickly as suggested by the relatively fast drop in Zn tissue levels by the PM sampling in 2014 (Table 2.19). In 2016, differences between treatments were apparent by the Post-R1 sampling with TSp100 having greater Zn tissue uptake than the non-N fertilized control and fall treatments (Table 2.20).

Lamberton

At Lamberton there was a two-way interaction for year x time of sampling for 2014 and 2015 (Table 2.1). Maize tissue Zn uptake increased with each sampling date and was greatest at 3wk-R1 in 2014 and at PM in 2015 (Table 2.19). In 2016 there was a treatment x time of sampling interaction (Table 2.1). At the Post-V6 sampling treatments applied in the fall and at preplant had greater maize tissue Zn uptake than the three-way split treatments (Table 2.20). The control treatment had lower maize tissue Zn uptake than all treatments receiving at least recommended rates (Table 2.20). Later in the maize development at Post-R1, the F125, PP125, and Sp100 treatments had greater maize tissue Zn uptake than the control plot and TSp50 and TSp100 treatments (Table 2.20). At PM, PP100 had greater maize tissue Zn uptake than PP125, Sp75 and the TSp treatments (Table 2.20).

Waseca

In 2014 and 2015 there was a two-way interaction of treatment x time of sampling and in 2016 there was a significant treatment effect (Table 2.1). In 2014 and 2015 differences between treatments began around the Pre-R1 sampling date (Table 2.21). In

2014 beginning at Pre-R1, the non-N fertilized control plot tended to have greater maize tissue Zn uptake than the TSp75 treatment, however in 2015 the TSp75 and TSp50 treatments often had the greatest maize tissue Zn uptake (Table 2.21). In 2016 there was a treatment effect where the non-N fertilized control plot had less maize tissue Zn uptake than all treatments except F125 (Table 2.20).

For 2014 and 2015 at Waseca, there seems to be a reversal in Zn accumulation compared maize biomass production particularly at PM. This seems to support findings by Xue et al. (2014) who reported that increasing grain yield reduced Zn reciprocal internal efficiencies suggesting that in the reproductive growth stages of maize there is a high Zn demand. At Waseca it is possible there was also a dilution effect on tissue Zn content as the N rate increased. Similar conclusions were reached by Ma and Zheng (2018) for when stover Zn content rapidly decreased as N rate increased under continuous maize. The differences in treatment effect at Lamberton and Waseca suggest that there is no clear pattern across locations that the timing or rate of N fertilizer applications negatively impacts maize tissue Zn uptake.

Conclusions

The results of this study demonstrate the importance of N management and following best management practices to avoid nutritional imbalances in the plant. In irrigated sandy soils the application of N fertilizer in the fall or in the spring resulted in less biomass and had less tissue nutrient compared with when N was applied as either a two or three-way split application. In fine textured soils contrasting results were observed. At Lamberton the application of N in the fall or spring in most cases resulted in greater nutrient removal, followed by the split applications. In Waseca the application of N fertilizer as split application in many cases resulted in greater nutrient uptake than fall or spring applications. Although nutrients concentrations were not limiting, in irrigated conditions late season N application has been demonstrated as a viable option. In rainfed fields the need for rainfall soon after fertilizer application will be critical to assure adequate moisture is available to dissolve and transport N into the root zone and allow plant uptake. These results demonstrate that optimizing N management to maximize biomass can increase crop nutrient uptake.

Table 2.1. Tests for fixed effects of urea fertilizer rate and timing of application on maize biomass and nutrient uptake of N, S, P, Cu, Fe, Mn, and Zn from maize tissue sampled throughout three growing seasons in Becker, Lamberton, and Waseca, MN.

Effects	Biomass			N			S			Cu		
	Becker	Lamberton	Waseca	Becker	Lamberton	Waseca	Becker	Lamberton	Waseca	Becker	Lamberton	Waseca
		2014-2016			2014-2016			2014-2016			2014-2016	
	Pr>F											
Treatment	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Time of Sampling	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Treatment x Time of Sampling	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.11	<.0001
Year	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.5726	0.0006	<.0001	<.0001
Year x Treatment	<.0001	<.0001	<.0001	<.0001	0.0004	<.0001	0.0003	0.485	0.2394	<.0001	0.7579	0.011
Year x Time of Sampling	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.014	0.0034
Treatment x Time of Sampling x Year	0.0001	0.59	0.0001	0.0001	0.1962	<.0001	<.0001	0.4542	0.8143	0.0424	0.8707	0.0114

Effects	P						Fe					
	Becker		Lamberton		Waseca		Becker		Lamberton		Waseca	
	2014-2015	2016	2014-2015	2016	2014-2015	2016	2014-2015	2016	2014-2015	2016	2014-2015	2016
	Pr>F											
Treatment	0.0244	<.0001	<.0001	<.0001	0.6344	0.0006	0.0189	0.0345	0.6958	0.0001	0.0037	0.053
Time of Sampling	<.0001	<.0001	<.0001	<.0001	0.0407	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Treatment x Time of Sampling	0.0862	0.0002	0.0441	<.0001	0.7856	0.0103	0.1788	0.3212	0.9341	0.0107	0.3469	0.6362
Year	0.0332	-	0.0014	-	0.005	-	0.0759	-	0.0015	-	0.0059	-
Year x Treatment	0.3646	-	0.1777	-	0.0228	-	0.3489	-	0.8371	-	0.1184	-

Year x Time of Sampling	0.006	-	<.0001	-	0.1337	-	0.0456	-	<.0001	-	<.0001	-
Treatment x Time of Sampling x Year	0.7441	-	0.9071	-	0.7137	-	0.8502	-	0.9466	-	0.1578	-

Effects	Mn					Zn					
	Becker	Lamberton		Waseca		Becker		Lamberton		Waseca	
	2014-2016	2014-2015	2016	2014-2015	2016	2014-2015	2016	2014-2015	2016	2014-2015	2016
	Pr>F										
Treatment	<.0001	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.2507	0.0153
Time of Sampling	<.0001	<.0001	<.0001	0.0306	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Treatment x Time of Sampling	<.0001	0.1608	<.0001	0.4156	0.0002	<.0001	0.0006	0.7307	<.0001	0.1033	0.4259
Year	<.0001	0.0068	-	<.0001	-	<.0141	-	0.0155	-	0.2864	-
Year x Treatment	<.0001	0.1625	-	0.0983	-	0.6097	-	0.623	-	0.2637	-
Year x Time of Sampling	<.0001	<.0001	-	0.063	-	0.0075	-	<.0001	-	<.0001	-
Treatment x Time of Sampling x Year	0.0203	0.6868	-	0.5292	-	1	-	0.4701	-	0.0494	-

Table 2.2. Maize biomass at Becker and Waseca 2014-2016.

Treatment¶	**Becker**	**Waseca**	**Becker**	**Waseca**	**Becker**	**Waseca**	**Becker**	**Waseca**	**Becker**	**Waseca**	**Becker**	**Waseca**	**Becker**	**Waseca**
	2014													
	Pre-V6†		Post-V6		3wk-V6		Pre-R1		Post-R1		3wk-R1		PM	
	kg ha^{-1}													
C	66	145	639	1435	1730	4043	2631 b*	5467 c	4691 d	7566 d	6739 e	10000 d	8710 d	18618 e
F100	82	145	732	1944	1957	5246	3288 ab	5534 bc	5060 cd	8030 cd	7077 e	12995 c	8406 d	22862 ab
F125	83	136	764	1402	1762	5176	3035 ab	6600 abc	4908 d	9727 ab	7916 e	14806 ab	9386 cd	23481 a
PP100	88	127	882	1864	2347	5281	3419 ab	7024 ab	5663 bcd	10814 a	7817 e	14318 abc	11108 c	20323 cd
PP125	100	133	968	1388	3101	4624	4151 ab	6406 abc	6975 ab	10517 ab	10492 d	15512 ab	14602 b	22932 a
Sp75	90	128	790	1560	2645	5314	4851 a	7076 a	8276 a	9283 bc	12342 cd	15746 a	14422 b	20166 cd
Sp100	82	127	750	1560	2775	5315	4831 a	7077 a	8518 a	10768 ab	15125 a	14113 bc	15768 b	21382 bc
TSp50	77	130	693	1508	2359	5141	4228 ab	6370 abc	6955 abc	10207 ab	11281 cd	13104 c	15423 b	18113 e
TSp75	83	145	701	1502	2624	5273	4154 ab	6891 abc	8230 a	9828 ab	13062 bc	12824 c	15406 b	19573 de
TSp100	97	140	818	1540	2734	5315	4660 a	6981 ab	8669 a	11018 a	14690 ab	13238 c	20848 a	20890 bc
	2015													
	Pre-V6		Post-V6		3wk-V6		Pre-R1		Post-R1		3wk-R1		PM	
	kg ha^{-1}													
C	14	69	466	522	1627 b	1982 b	3089 e	3751 c	4550 d	7450 f	6937 f	10421 d	9472 e	11246 f
F100	37	134	1080	1276	3671 a	4712 a	5289 bcd	7811 ab	8479 c	14180 ab	12405 e	19428 ab	14688 d	25368 a
F125	58	123	1346	1247	4174 a	4201 a	6890 abc	7785 ab	12042 a	12260 cde	16410 cd	19224 ab	19968 c	23109 cd
PP100	52	108	1450	1409	4405 a	3328 ab	7570 a	6906 b	10699 ab	10969 e	16768 bcd	18599 b	18287 c	19107 e
PP125	48	124	1246	1525	4035 a	4497 a	6368 abcd	8386 ab	10826 ab	14698 a	14912 d	19426 ab	19601 c	21914 d
Sp75	33	112	811	1047	3934 a	4463 a	5050 cd	8803 a	10107 bc	13143 bc	17786 abc	19039 ab	21962 b	22476 cd
Sp100	45	104	922	1308	4450 a	4714 a	6978 ab	8435 a	11799 ab	14020 ab	18605 ab	20183 a	22719 b	24920 ab
TSp50	28	114	594	1219	2977 ab	3558 a	4919 de	7392 ab	8522 c	11622 de	12479 e	16926 c	18286 c	19978 e

TSp75	36	112	878	1034	3968 a	4027 a	6200 abcd	8266 ab	10677 ab	13519 abc	18124 abc	19398 ab	22825 b	23806 bc
TSp100	35	90	869	1386	4072 a	4513 a	5879 abcd	8197 ab	11036 ab	13014 bcd	19255 a	19479 ab	26284 a	25433 a

2016

	Pre-V6		Post-V6		3wk-V6		Pre-R1		Post-R1		3wk-R1		PM	
							kg ha^{-1}							
C	42	57	643	1369	1897 c	1895 b	3229 c	4262 d	4468 f	7146 e	6870 f	10747 f	8945 e	10814 e
F100	124	86	1383	2117	3110 bc	2323 ab	6314 b	5974 bc	8559 e	9345 d	10625 e	18272 bcd	14279 d	17414 cd
F125	112	81	1484	2291	3788 ab	3175 ab	6499 b	5648 cd	8947 e	9775 cd	12840 d	16778 de	16893 c	17626 c
PP100	151	80	1638	2669	5133 a	3705 a	8444 a	7532 a	12799 a	12349 a	16370 ab	20206 a	22204 ab	20040 a
PP125	185	83	1815	2639	5208 a	3661 a	9168 a	7310 ab	11571 abc	11659 ab	16728 ab	18387 bc	23491 a	20013 a
Sp75	113	79	1424	2245	4430 ab	3112 ab	7479 ab	6342 abc	12447 ab	9454 d	14885 bc	16934 cde	21407 b	18397 bc
Sp100	98	74	1476	2452	5008 ab	3403 ab	7690 ab	6448 abc	10858 bcd	11062 abc	15118 bc	19088 ab	22690 ab	19508 ab
TSp50	93	50	1242	1961	4285 ab	2600 ab	6488 b	5746 cd	10358 cde	9745 cd	13266 cd	16375 e	17955 c	16075 d
TSp75	118	73	1266	2165	4395 ab	3002 ab	7512 ab	5421 cd	9655 de	10066 cd	14926 bc	17903 bcd	21294 b	18071 bc
TSp100	118	86	1638	2496	4840 ab	3460 a	8764 a	6598 abc	12524 ab	10608 bcd	17387 a	18947 ab	22677 ab	19307 ab

†Pre-V6, soil samples taken 1 week before V6 urea applications; Post-V6, soil samples taken 1 week after V6 urea applications; 3wk-V6, soil samples taken 3 weeks after V6 urea applications; Pre-R1, soil samples taken 1 week before R1 urea application; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications; PM, soil samples taken at physiological maturity.

¶C, control no N applied; F100, N applied in the fall at 100% of the recommended rate; F125, N applied in the fall at 125% of the recommended rate; PP100, N applied at pre-plant in the spring at 100% of the recommended rate; PP125, N applied at pre-plant in the spring at 125% of the recommended rate; Sp75, half of the N applied at pre-plant in the spring and half at V6 at 75% of the recommended rate; Sp100, half of the N applied at pre-plant in the spring and half at V6 at 100% of the recommended rate; TSp50, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 50% of the recommended rate; TSp75, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 75% of the recommended rate; TSp100, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 100% of the recommended rate.

*Means within the column followed by different lower case letters are significantly different at $p \leq 0.05$

Table 2.3. Treatment x year, treatment x time of sampling, and year x time of sampling for maize biomass at Lamberton 2014-2016.

Treatment	2014		2015		2016		Pre-V6†	Post-V6		3wk-V6		Pre-R1		Post-R1		3wk-R1		PM	
	kg ha^{-1}																		
C¶	7722	d*	6309	e	3966	g	72	1013	b	3040	c	5302	e	6974	e	12147	g	13446	f
F100	11428	a	11060	ab	8006	bc	154	2197	a	5869	a	8894	ab	12297	abc	18978	abc	22767	ab
F125	11495	a	11483	a	8852	a	152	2350	a	6042	a	9524	a	12919	ab	19689	a	23593	a
PP100	11434	a	10662	bc	8159	b	132	2024	ab	6015	a	8510	abc	12332	abc	18780	abc	22803	ab
PP125	11340	a	10794	bc	8150	b	131	2142	ab	5631	ab	8532	abc	13068	a	18019	bcd	23140	ab
Sp75	10297	b	10704	bc	7345	de	121	1724	ab	5147	ab	8442	abc	11769	bc	17609	de	21328	cd
Sp100	10955	a	10881	b	8409	ab	128	1926	ab	5497	ab	8361	bc	12657	ab	19105	ab	22897	ab
TSp50	9690	c	8229	d	6047	f	107	1436	ab	4554	b	7086	d	9430	d	14026	f	19281	e
TSp75	9863	bc	10320	c	6846	e	125	1683	ab	4901	ab	7461	cd	11192	c	16801	e	22234	bc
TSp100	10399	b	10733	bc	7491	cd	125	1788	ab	4999	ab	7913	bc	11879	bc	17847	cde	20903	d

Time of sampling	2014		2015		2016	
	kg ha^{-1}					
Pre-V6†	157	m	131	m	86	m
Post-V6	2579	k	1692	l	1214	l
3wk-V6	6806	h	4884	i	3819	j
Pre=R1	9188	g	9639	g	5181	i
Post-R1	12565	f	12414	f	9376	g
3wk-R1	16552	d	19312	c	16036	de
PM	25388	a	22751	b	15579	e

†Pre-V6, soil samples taken 1 week before V6 urea applications; Post-V6, soil samples taken 1 week after V6 urea applications; 3wk-V6, soil samples taken 3 weeks after V6 urea applications; Pre-R1, soil samples taken 1 week before R1 urea application; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications; PM, soil samples taken at physiological maturity.

¶C, control no N applied; F100, N applied in the fall at 100% of the recommended rate; F125, N applied in the fall at 125% of the recommended rate; PP100, N applied at pre-plant in the spring at 100% of the recommended rate; PP125, N applied at pre-plant in the spring at 125% of the recommended rate; Sp75, half of the N applied at pre-plant in the spring and half at V6 at 75% of the recommended rate; Sp100, half of the N applied at pre-plant in the spring and half at V6 at 100% of the recommended rate; TSp50, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 50% of the recommended rate; TSp75, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 75% of the recommended rate; TSp100, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 100% of the recommended rate.

*Means within the column followed by different lower case letters are significantly different at $p \leq 0.05$.

Table 2.4. Maize tissue N uptake at Becker and Waseca 2014-2016.

Treatment¶	**Becker**	**Waseca**	**Becker**	**Waseca**	**Becker**	**Waseca**	**Becker**	**Waseca**	**Becker**	**Waseca**	**Becker**	**Waseca**	**Becker**	**Waseca**
	2014													
	Pre-V6†		Post-V6		3wk-V6		Pre-R1		Post-R1		3wk-R1		PM	
	kg N ha^{-1}													
C	2	4.7	15	28	22 bc*	63 b	29 ef	69 b	38 d	68 c	53 f	83 d	46 d	142 d
F100	2.6	5.9	16	42	23 bc	93 a	34 def	70 b	41 d	92 abc	54 f	132 abc	45 d	180 abc
F125	2.5	5.2	16	32	21 c	89 ab	31 ef	85 ab	39 d	110 ab	67 ef	151 ab	46 d	210 a
PP100	3.1	4.8	21	45	31 bc	93 a	27 f	92 ab	49 d	121 ab	61 ef	141 abc	64 cd	160 bcd
PP125	3.7	5.6	25	35	44 abc	91 ab	47 cdef	84 ab	56 cd	132 a	84 de	161 a	82 bc	197 a
Sp75	2.5	5.4	22	33	44 abc	100 a	73 ab	96 ab	84 b	106 ab	100 cd	142 abc	68 bcd	162 bcd
Sp100	2.4	4.3	22	35	57 a	106 a	83 a	106 a	99 ab	122 ab	127 b	144 abc	86 bc	182 ab
TSp50	2.3	4.1	19	31	39 abc	83 ab	53 bcde	81 ab	77 bc	98 abc	124 bc	110 cd	88 b	148 cd
TSp75	2.4	4.7	19	31	40 abc	92 a	57 bcd	67 b	95 ab	91 bc	167 a	117 bcd	88 bcd	158 bcd
TSp100	2.8	4.9	22	33	45 ab	103 a	69 abc	88 ab	112 a	127 a	160 a	118 bcd	132 a	187 ab
	2015													
Treatment	Pre-V6		Post-V6		3wk-V6		Pre-R1		Post-R1		3wk-R1		PM	
	kg N ha^{-1}													
C	0.5	1.7	13 c	13 b	23 d	31 c	35 d	47 d	35 f	67 e	54 f	92 d	39 f	84 g
F100	1.7	5.4	33 abc	42 a	52 c	95 a	55 cd	129 abc	66 e	182 ab	92 e	223 ab	66 e	230 ab
F125	2.8	6.3	41 ab	40 a	79 b	86 ab	101 b	129 abc	115 bc	150 bcd	150 bc	214 abc	103 bcd	188 cd
PP100	2.6	3.3	49 a	42 a	84 b	62 b	111 b	110 bc	98 cd	116 d	135 cd	195 bc	79 de	156 ef
PP125	2.4	5.1	43 ab	51 a	77 b	93 a	99 b	137 ab	103 cd	186 a	129 cd	225 ab	91 cde	177 de
Sp75	1.4	3.9	28 abc	36 a	101 ab	94 a	102 b	148 a	114 bc	162 abc	173 b	208 bc	117 ab	198 bcd
Sp100	2.1	3.6	34 abc	47 a	116 a	109 a	139 a	153 a	163 a	189 a	210 a	253 a	121 ab	220 abc
TSp50	1.2	3.6	20 bc	38 a	51 c	60 b	66 c	103 c	80 de	130 cd	124 d	178 c	88 de	138 f

TSp75	1.6	3.5	31 abc	31 a	84 b	81 ab	102 b	128 abc	119 bc	169 ab	205 a	233 ab	114 abc	209 abcd
TSp100	1.5	3.2	32 abc	46 a	95 ab	106 a	109 b	141 ab	137 b	161 abc	220 a	254 a	135 a	236 a
							2016							
Treatment	Pre-V6		Post-V6		3wk-V6		Pre-R1		Post-R1		3wk-R1		PM	
							kg N ha^{-1}							
C	1.7	1.8	14 c	29 c	23 f	25 d	51 d	-	31 g	75 d	50 f	105 e	43 f	73 e
F100	4.9	3.2	33 bc	50 ab	44 ef	54 abc	113 c	-	65 f	152 bc	80 e	248 bcd	62 ef	126 cd
F125	4.5	3.1	39 ab	61 ab	59 de	55 abc	119 c	-	86 ef	151 c	102 e	216 d	76 e	134 cd
PP100	6.7	3.4	53 ab	82 a	95 abc	77 a	170 ab	-	149 ab	213 a	165 bc	265 abc	124 bcd	159 abc
PP125	8.8	3.5	61 a	77 a	108 ab	69 ab	178 ab	-	131 bc	194 a	176 abc	235 cd	133 abcd	157 abc
Sp75	4.3	3.2	45 ab	56 ab	105 ab	54 abc	158 b	-	148 ab	152 bc	153 cd	232 cd	121 cd	159 abc
Sp100	4.1	3.1	47 ab	66 ab	117 a	67 abc	180 ab	-	156 a	192 a	169 bc	296 a	145 ab	173 ab
TSp50	3.2	1.8	36 bc	44 bc	76 cd	40 cd	123 c	-	101 de	137 c	140 d	225 cd	144 abc	115 d
TSp75	4.3	2.8	39 ab	50 ab	85 bc	46 bcd	164 b	-	113 cd	154 bc	179 ab	235 cd	118 d	143 bcd
TSp100	5.1	3.4	53 ab	66 ab	104 ab	62 abc	189 a	-	171 a	185 ab	197 a	277 ab	150 a	185 a

†Pre-V6, soil samples taken 1 week before V6 urea applications; Post-V6, soil samples taken 1 week after V6 urea applications; 3wk-V6, soil samples taken 3 weeks after V6 urea applications; Pre-R1, soil samples taken 1 week before R1 urea application; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications; PM, soil samples taken at physiological maturity.

¶C, control no N applied; F100, N applied in the fall at 100% of the recommended rate; F125, N applied in the fall at 125% of the recommended rate; PP100, N applied at pre-plant in the spring at 100% of the recommended rate; PP125, N applied at pre-plant in the spring at 125% of the recommended rate; Sp75, half of the N applied at pre-plant in the spring and half at V6 at 75% of the recommended rate; Sp100, half of the N applied at pre-plant in the spring and half at V6 at 100% of the recommended rate; TSp50, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 50% of the recommended rate; TSp75, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 75% of the recommended rate; TSp100, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 100% of the recommended rate.

*Means within the column followed by different lower case letters are significantly different at $p \leq 0.05$.

Table 2.5. Treatment x year, treatment x time of sampling, and year x time of sampling for maize tissue N uptake at Lamberton 2014-2016.

Treatment	2014		2015		2016		Pre-V6†	Post-V6		3wk-V6		Pre-R1		Post-R1		3wk-R1		PM	
	kg ha^{-1}																		
C¶	71	e*	54	f	42	f	2.3	22	f	44	f	62	f	68	f	106	f	83	e
F100	153	a	144	bc	114	c	6.4	65	abc	118	bc	155	bc	175	bc	247	b	194	b
F125	155	a	163	a	139	a	6.6	74	a	135	a	180	a	186	ab	268	a	216	a
PP100	153	a	141	bc	129	ab	5.4	62	abcd	127	ab	171	ab	181	b	249	b	193	b
PP125	157	a	150	ab	120	bc	5.5	68	ab	121	ab	169	ab	200	a	231	bc	202	ab
Sp75	119	bc	130	cd	107	cd	4.5	47	de	101	cd	145	cd	159	cd	208	d	169	c
Sp100	134	b	143	bc	130	ab	5	56	bcd	115	bc	156	bc	183	ab	239	bc	195	b
TSp50	99	d	86	e	77	e	3.7	35	ef	78	e	99	e	117	e	144	e	137	d
TSp75	111	cd	125	d	94	d	5.4	45	de	89	de	112	e	151	d	202	d	167	c
TSp100	117	c	143	bc	111	c	4.8	50	cde	97	d	128	d	163	cd	226	c	197	b

Time of sampling	2014		2015		2016	
	kg ha^{-1}					
Pre-V6†	7	m	5	m	3	m
Post-V6	68	j	53	k	36	l
3wk-V6	119	g	105	h	83	i
Pre=R1	150	de	148	e	115	g
Post-R1	162	c	159	cd	154	cde
3wk-R1	190	b	228	a	218	a
PM	193	b	197	b	135	f

¶C, control no N applied; F100, N applied in the fall at 100% of the recommended rate; F125, N applied in the fall at 125% of the recommended rate; PP100, N applied at pre-plant in the spring at 100% of the recommended rate; PP125, N applied at pre-plant in the spring at 125% of the recommended rate; Sp75, half of the N applied at pre-plant in the spring and half at V6 at 75% of the recommended rate; Sp100, half of the N applied at pre-plant in the spring and half at V6 at 100% of the recommended rate; TSp50, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 50% of the recommended rate; TSp75,

a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 75% of the recommended rate; TSp100, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 100% of the recommended rate.

†Pre-V6, soil samples taken 1 week before V6 urea applications; Post-V6, soil samples taken 1 week after V6 urea applications; 3wk-V6, soil samples taken 3 weeks after V6 urea applications; Pre-R1, soil samples taken 1 week before R1 urea application; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications; PM, soil samples taken at physiological maturity.

*Means within the column followed by different lower case letters are significantly different at the $p \leq 0.05$.

Table 2.6. Maize tissue S uptake at Becker 2014-2016.

	2014												
	Pre-V6†	Post-V6		3wk-V6		Pre-R1		Post-R1		3wk-R1		PM	
	kg S ha^{-1}												
C¶	0.14	1.5		1.3	c*	2.9	c	4.9	c	6.6	d	8.3	e
F100	0.19	1.6		1.5	c	3.6	bc	5.2	c	7	cd	8.6	e
F125	0.18	2.1		2.4	abc	3.3	bc	4.5	c	7.9	cd	8.4	e
PP100	0.19	1.9		1.7	c	3.4	bc	5.5	c	7.3	cd	10	de
PP125	0.22	1.8		3.9	ab	5.2	ab	7.1	bc	9.6	bc	15	bc
Sp75	0.19	1.8		3.5	abc	6.5	a	8.9	ab	11	b	12	cd
Sp100	0.19	2.1		4.1	a	7.4	a	11	a	15	a	16	ab
TSp50	0.16	1.9		2.1	bc	5.3	ab	7.1	bc	12	ab	13	bcd
TSp75	0.17	1.7		2.8	abc	5.1	ab	8.9	ab	15	a	13	bcd
TSp100	0.21	1.9		2.3	abc	6.2	a	10	a	15	a	18	a
	2015												
	Pre-V6	Post-V6		3wk-V6		Pre-R1		Post-R1		3wk-R1		PM	
	kg S ha^{-1}												
C	0.05	1.1	b	1.9	d	3.4	c	3.1	d	4.9	f	7.6	c
F100	0.13	2.6	a	4.1	bc	5.7	b	5.4	cd	7.5	ef	9.9	bc
F125	0.21	3	a	5.6	abc	8.6	a	8.2	abc	12	bc	12	ab
PP100	0.18	3.5	a	5.9	ab	9.1	a	6.8	bc	11	cd	10	bc
PP125	0.17	3	a	5.5	abc	8.2	ab	7.8	bc	10	cde	13	ab
Sp75	0.11	1.8	a	6.3	ab	7.8	ab	7.7	bc	13	bc	13	a
Sp100	0.15	2	a	6.7	a	10	a	11	a	16	a	13	ab
TSp50	0.1	1.4	a	3.8	c	5.6	b	5.5	cd	8.7	de	10	bc
TSp75	0.12	2	a	5.5	abc	7.9	ab	7.6	bc	14	ab	12	ab
TSp100	0.13	2	a	6.2	ab	8.5	a	8.3	ab	14	ab	13	a
	2016												
	Pre-V6	Post-V6		3wk-V6		Pre-R1		Post-R1		3wk-R1		PM	
	kg S ha^{-1}												
C	0.13	1.5	b	2.2	c	3.6	e	5	d	6.5	f	7.9	d

F100	0.35	3.4	ab	4.1	bc	6.8	d	8.1	c	10	e	11	c
F125	0.3	3.6	a	5.3	ab	7.7	bc	9.1	c	12	de	11	c
PP100	0.41	3.9	a	7	a	10	ab	13	ab	17	ab	17	a
PP125	0.48	4.4	a	7.4	a	10	ab	14	a	18	a	16	ab
Sp75	0.29	3.8	a	6.8	a	9.1	abc	15	a	16	abc	15	ab
Sp100	0.24	4.3	a	7	a	9.7	ab	13	ab	16	abc	16	ab
TSp50	0.25	3.1	ab	5.9	a	7	cd	9.8	c	13	cd	12	c
TSp75	0.31	3	ab	6.3	a	9.1	abc	11	bc	15	bcd	14	bc
TSp100	0.35	4	a	6.8	a	11	a	13	ab	17	ab	16	ab

†Pre-V6, soil samples taken 1 week before V6 urea applications; Post-V6, soil samples taken 1 week after V6 urea applications; 3wk-V6, soil samples taken 3 weeks after V6 urea applications; Pre-R1, soil samples taken 1 week before R1 urea application; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications; PM, soil samples taken at physiological maturity.

¶C, control no N applied; F100, N applied in the fall at 100% of the recommended rate; F125, N applied in the fall at 125% of the recommended rate; PP100, N applied at pre-plant in the spring at 100% of the recommended rate; PP125, N applied at pre-plant in the spring at 125% of the recommended rate; Sp75, half of the N applied at pre-plant in the spring and half at V6 at 75% of the recommended rate; Sp100, half of the N applied at pre-plant in the spring and half at V6 at 100% of the recommended rate; TSp50, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 50% of the recommended rate; TSp75, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 75% of the recommended rate; TSp100, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 100% of the recommended rate.

*Means within the column followed by different lower case letters are significantly different at $p \leq 0.05$.

Table 2.7. The effect of treatment x time of sampling on maize tissue S uptake at Lamberton and Waseca 2014-2016

Lamberton

Treatment	Pre-V6†	Post-V6		3wk-V6		Pre-R1		Post-R1		3wk-R1		PM	
	kg S ha^{-1}												
C¶	0.25	2.7	c*	4.8	d	6.7	d	7.3	d	13	e	15	d
F100	0.46	5.6	a	11	a	12	a	14	ab	21	ab	21	a
F125	0.45	5.8	a	9.7	ab	13	a	14	ab	21	a	20	ab
PP100	0.39	5.2	ab	9.9	ab	13	a	14	ab	21	ab	20	ab
PP125	0.37	5.4	ab	9	ab	12	ab	15	a	19	bc	19	abc
Sp75	0.38	4.6	ab	9	ab	12	ab	13	ab	18	cd	19	bc
Sp100	0.4	5.2	ab	9.4	ab	12	a	15	a	21	ab	21	a
TSp50	0.35	3.8	bc	7.2	c	8.7	c	9.8	c	14	e	16	d
TSp75	0.39	4.7	ab	8.2	bc	9.4	c	12	b	18	d	18	c
TSp100	0.4	4.9	ab	8.4	bc	10	bc	13	b	19	cd	19	bc

Waseca

Treatment	Pre-V6†	Post-V6		3wk-V6		Pre-R1		Post-R1		3wk-R1		PM	
	kg S ha^{-1}												
C¶	0.25	3		4.1	b	5	d	7.6	c	9.3	d	12	d
F100	0.34	4		6.7	a	7.1	c	12	b	17	a	15	ab
F125	0.31	4		6.4	a	7.7	abc	12	b	17	ab	15	a
PP100	0.25	4		6.4	a	7.5	bc	13	ab	15	c	13	cd
PP125	0.31	4		6.6	a	8.2	abc	14	a	17	ab	15	ab
Sp75	0.32	4		6.5	a	9	ab	12	b	17	a	14	abc
Sp100	0.28	4		7.3	a	9.4	a	14	a	18	a	16	a
TSp50	0.27	3		5.7	ab	7.5	bc	12	ab	15	bc	12	d
TSp75	0.29	3		6.3	a	7.9	abc	12	b	16	abc	14	bcd
TSp100	0.28	4		7.1	a	8.4	abc	13	ab	16	abc	15	ab

†Pre-V6, soil samples taken 1 week before V6 urea applications; Post-V6, soil samples taken 1 week after V6 urea applications; 3wk-V6, soil samples taken 3 weeks after V6 urea applications; Pre-R1, soil samples taken 1 week before R1 urea application; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications; PM, soil samples taken at physiological maturity.

¶C, control no N applied; F100, N applied in the fall at 100% of the recommended rate; F125, N applied in the fall at 125% of the recommended rate; PP100, N applied at pre-plant in the spring at 100% of the recommended rate; PP125, N applied at pre-plant in the spring at 125% of the recommended rate; Sp75, half of the N applied at pre-plant in the spring and half at V6 at 75% of the recommended rate; Sp100, half of the N applied at pre-plant in the spring and half at V6 at

100% of the recommended rate; TSp50, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 50% of the recommended rate; TSp75, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 75% of the recommended rate; TSp100, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 100% of the recommended rate.

*Means within the column followed by different lower case letters are significantly different at $p \leq 0.05$.

Table 2.8. The effect of Year x Time of sampling on maize tissue S uptake at Lamberton and Waseca from 2014-2016

	Lamberton						Waseca					
Time of Sampling	2014		2015		2016		2014		2015		2016	
	kg S ha^{-1}											
Pre-V6†	0.51	k*	0.44	k	0.2	k	0.35	f*	0.31	g	0.21	e
Post-V6	7.1	h	4.6	i	2.7	j	2.7	e	2.7	f	5.8	c
3wk-V6	10	f	8.4	g	7.1	h	7.3	d	6	e	5.6	d
Pre-R1	13	e	11	f	9	g	7.1	d	8.4	d	-	-
Post-R1	15	d	11	f	12	e	11	c	11	c	15	b
3wk-R1	17	c	20	b	17	c	13	b	15	b	19	a
PM	24	a	18	c	14	d	20	a	17	a	5.8	c

†Pre-V6, soil samples taken 1 week before V6 urea applications; Post-V6, soil samples taken 1 week after V6 urea applications; 3wk-V6, soil samples taken 3 weeks after V6 urea applications; Pre-R1, soil samples taken 1 week before R1 urea application; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications; PM, soil samples taken at physiological maturity.

*Means within the column followed by different lower case letters are significantly different at $p \leq 0.05$.

Table 2.9. The effect of year x time of sampling on maize tissue P uptake at Becker and Lamberton 2014-2016.

	Becker			Lamberton		
Time of sampling	2014	2015	2016	2014	2015	2016
			kg P ha^{-1}			
Pre-V6†	0.39 h*	0.18 i	- -	0.58 j	0.35 j	- -
Post-V6	3.1 g	3.9 f	6.2 c	9 h	5.9 i	4.8 c
3wk-V6	7 e	11 cd	- -	18 f	13 g	- -
Pre-R1	11 d	15 c	- -	24 d	27 d	- -
Post-R1	16 c	18 c	25 b	21 e	32 c	27 b
3wk-R1	30 a	26 b	35 a	41 b	49 a	48 a
PM	13 c	- -	- -	14 g	43 b	- -

†Pre-V6, soil samples taken 1 week before V6 urea applications; Post-V6, soil samples taken 1 week after V6 urea applications; 3wk-V6, soil samples taken 3 weeks after V6 urea applications; Pre-R1, soil samples taken 1 week before R1 urea application; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications; PM, soil samples taken at physiological maturity.

*Means within the column followed by different lower case letters are significantly different at $p \leq 0.05$.

Table 2.10. The effect of treatment for Becker 2014-2015, treatment x year for Waseca 2014-2015, and treatment x time of sampling for Becker and Waseca on maize tissue P uptake.

	Becker	Waseca		Becker			Waseca		
				Post-V6†	Post-R1	3wk-R1	Post-V6†	Post-R1	3wk-R1
Treatment	2014-2015	2014	2015	2016					
	Kg P ha^{-1}								
C¶	9.0 g*	24 a	21 b	3.6 b	14 d	22 d	7.1 b	27 d	35 b
F100	10.6 f	20 bc	30 a	6.2 a	20 c	29 cd	8.1 ab	32 bcd	57 a
F125	11.5 def	20 bc	28 a	6.3 a	23 bc	30 bcd	9.3 ab	32 bcd	54 a
PP100	11.7 def	20 abc	27 a	6.8 a	29 ab	37 ab	9.6 a	41 a	65 a
PP125	12.2 cde	18 c	27 a	7.2 a	26 ab	39 a	10 a	40 ab	64 a
Sp75	12.4 bcd	20 abc	28 a	7.5 a	32 a	36 abc	8.7 ab	31 cd	60 a
Sp100	13.8 a	18 c	30 a	6.5 a	28 ab	37 abc	9 ab	39 abc	61 a
TSp50	11.0 ef	24 ab	30 a	6.2 a	24 bc	36 abc	8.5 ab	33 abcd	54 a
TSp75	13.7 ab	20 abc	28 a	5.9 a	24 bc	40 a	8.4 ab	32 bcd	61 a
TSp100	13.4 abc	21 abc	28 a	6.6 a	30 ab	41 a	9.2 ab	35 abcd	67 a

† Post-V6, soil samples taken 1 week after V6 urea applications; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications.

¶C, control no N applied; F100, N applied in the fall at 100% of the recommended rate; F125, N applied in the fall at 125% of the recommended rate; PP100, N applied at pre-plant in the spring at 100% of the recommended rate; PP125, N applied at pre-plant in the spring at 125% of the recommended rate; Sp75, half of the N applied at pre-plant in the spring and half at V6 at 75% of the recommended rate; Sp100, half of the N applied at pre-plant in the spring and half at V6 at

100% of the recommended rate; TSp50, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 50% of the recommended rate; TSp75, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 75% of the recommended rate; TSp100, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 100% of the recommended rate.

*Means within the column followed by different lower case letters are significantly different at $p \leq 0.05$.

Table 2.11. The effect of treatment x time of sampling on maize tissue P uptake at Lamberton 2014-2016.

	Pre-V6†	Post-V6		3wk-V6	Pre-R1	Post-R1		3wk-R1		PM
	2014-2015	2014-2015	2016	2014-2015	2014-2015	2014-2015	2016	2014-2015	2016	2014-2015
Treatment	kg P ha^{-1}									
C¶	0.32	4.3	2.7 d*	11 b	18 d	19 c	15 d	37 e	30 d	28 abc
F100	0.31	8.9	5.6 ab	18 a	29 ab	28 a	29 ab	53 ab	54 ab	30 abc
F125	0.64	9.9	6.8 a	18 a	30 a	29 a	30 a	53 a	56 a	27 bc
PP100	0.49	7.6	6.2 a	15 ab	27 abc	28 a	27 ab	47 c	57 a	25 c
PP125	0.55	8.4	5.6 ab	17 ab	27 abc	30 a	31 a	48 abc	49 abc	30 abc
Sp75	0.53	7.6	3.8 cd	13 ab	28 abc	28 a	28 ab	44 cd	48 abc	27 bc
Sp100	0.54	7.3	6.1 a	16 ab	27 abc	29 a	31 a	47 bc	57 a	33 a
TSp50	0.46	6.1	3.6 cd	14 ab	21 d	20 bc	22 c	37 e	40 cd	25 bc
TSp75	0.53	7.4	3.9 cd	14 ab	23 cd	25 ab	27 ab	39 de	45 bc	30 ab
TSp100	0.27	6.8	4.3 bc	14 ab	24 bcd	28 a	25 bc	45 cd	49 abc	30 abc

†Pre-V6, soil samples taken 1 week before V6 urea applications; Post-V6, soil samples taken 1 week after V6 urea applications; 3wk-V6, soil samples taken 3 weeks after V6 urea applications; Pre-R1, soil samples taken 1 week before R1 urea application; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications; PM, soil samples taken at physiological maturity.

¶C, control no N applied; F100, N applied in the fall at 100% of the recommended rate; F125, N applied in the fall at 125% of the recommended rate; PP100, N applied at pre-plant in the spring at 100% of the recommended rate; PP125, N applied at pre-plant in the spring at 125% of the recommended rate; Sp75, half of the N applied at pre-plant in the spring and half at V6 at 75% of the recommended rate; Sp100, half of the N applied at pre-plant in the spring and half at V6 at 100% of the recommended rate; TSp50, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 50% of the recommended rate; TSp75,

a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 75% of the recommended rate; TSp100, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 100% of the recommended rate.
*Means within the column followed by different lower case letters are significantly different at $p \leq 0.05$.

Table 2.12. Maize tissue Cu uptake at Becker and Waseca 2014-2016

	Becker	**Waseca**	**Becker**	**Waseca**	**Becker**	**Waseca**	**Becker**	**Waseca**	**Becker**	**Waseca**	**Becker**	**Waseca**	**Becker**	**Waseca**
	2014													
Treatment¶	Pre-V6†		Post-V6		3wk-V6		Pre-R1		Post-R1		3wk-R1		PM	
	kg Cu ha^{-1}													
C	0.0005	0.001	0.004	0.007	0.008 b*	0.015 b	0.011 d	0.024 bc	0.012 d	0.022 f	0.02 e	0.026 e	0.02 e	0.038 e
F100	0.001	0.001	0.005	0.011	0.008 b	0.024 ab	0.013 cd	0.022 c	0.015 cd	0.027 ef	0.021 e	0.053 bc	0.02 e	0.061 b
F125	0.001	0.001	0.005	0.008	0.008 b	0.022 ab	0.012 cd	0.027 abc	0.017 cd	0.033 cde	0.023 e	0.058 b	0.018 e	0.075 a
PP100	0.001	0.001	0.006	0.011	0.011 ab	0.023 ab	0.014 cd	0.032 ab	0.015 cd	0.041 abc	0.026 e	0.057 b	0.026 de	0.059 bc
PP125	0.001	0.001	0.007	0.009	0.015 ab	0.022 ab	0.016 bcd	0.03 abc	0.019 bcd	0.042 ab	0.035 d	0.071 a	0.034 cd	0.078 a
Sp75	0.001	0.001	0.005	0.008	0.015 ab	0.023 ab	0.023 ab	0.034 a	0.03 a	0.035 bcde	0.039 d	0.057 b	0.034 cd	0.056 bc
Sp100	0.001	0.001	0.005	0.009	0.017 a	0.024 ab	0.027 a	0.036 a	0.034 a	0.044 a	0.057 bc	0.038 d	0.044 b	0.064 b
TSp50	0.001	0.001	0.005	0.008	0.012 ab	0.02 ab	0.018 bcd	0.028 abc	0.021 bc	0.031 de	0.049 c	0.043 d	0.04 bc	0.044 de
TSp75	0.001	0.001	0.005	0.008	0.013 ab	0.021 ab	0.019 bc	0.031 ab	0.027 ab	0.032 de	0.06 b	0.043 d	0.043 b	0.052 cd
TSp100	0.001	0.001	0.006	0.008	0.015 ab	0.025 a	0.023 ab	0.031 ab	0.034 a	0.039 abcd	0.07 a	0.047 cd	0.07 a	0.057 bc
	2015													
Treatment	Pre-V6†		Post-V6		3wk-V6		Pre-R1		Post-R1		3wk-R1		PM	
	kg Cu ha^{-1}													
C	0	0.001	0.003	0.003	0.007 c	0.007 b	-	-	-	-	-	-	-	-
F100	0	0.001	0.008	0.007	0.016 b	0.021 a	-	-	-	-	-	-	-	-
F125	0.0008	0.001	0.009	0.007	0.019 b	0.019 a	-	-	-	-	-	-	-	-
PP100	0.0008	0.001	0.009	0.009	0.019 b	0.014 ab	-	-	-	-	-	-	-	-
PP125	0.0003	0.001	0.009	0.01	0.02 ab	0.021 a	-	-	-	-	-	-	-	-
Sp75	0	0.001	0.006	0.005	0.022 ab	0.019 a	-	-	-	-	-	-	-	-
Sp100	0.0003	0.001	0.006	0.007	0.027 a	0.021 a	-	-	-	-	-	-	-	-
TSp50	0	0.001	0.004	0.007	0.015 bc	0.013 ab	-	-	-	-	-	-	-	-
TSp75	0.0003	0.001	0.006	0.005	0.02 ab	0.016 ab	-	-	-	-	-	-	-	-
TSp100	0.0003	0.001	0.006	0.008	0.022 ab	0.021 a	-	-	-	-	-	-	-	-
	2016													
Treatment	Pre-V6†		Post-V6		3wk-V6		Pre-R1		Post-R1		3wk-R1		PM	
	kg Cu ha^{-1}													
C	-	-	0.005 b	0.008 b	-	-	-	-	0.007 d	0.02 e	0.021 g	0.02 f	-	-
F100	-	-	0.009 ab	0.013 ab	-	-	-	-	0.018 c	0.044 cd	0.032 f	0.054 d	-	-
F125	-	-	0.008 ab	0.016 ab	-	-	-	-	0.023 bc	0.046 bcd	0.039 f	0.058 bcd	-	-
PP100	-	-	0.014 a	0.02 a	-	-	-	-	0.04 a	0.069 a	0.069 cd	0.067 ab	-	-
PP125	-	-	0.013 a	0.019 a	-	-	-	-	0.04 a	0.054 b	0.079 b	0.057 cd	-	-
Sp75	-	-	0.011 ab	0.015 ab	-	-	-	-	0.041 a	0.041 cd	0.066 de	0.063 abcd	-	-

Sp100	-	-	0.01 ab	0.016 ab	-	-	-	-	0.044 a	0.055 b	0.077 b	0.068 a	-	-
TSp50	-	-	0.008 ab	0.012 ab	-	-	-	-	0.025 bc	0.038 d	0.06 e	0.045 e	-	-
TSp75	-	-	0.008 ab	0.013 ab	-	-	-	-	0.027 b	0.043 cd	0.077 bc	0.055 d	-	-
TSp100	-	-	0.012 ab	0.017 a	-	-	-	-	0.04 a	0.049 bc	0.091 a	0.065 abc	-	-

†Pre-V6, soil samples taken 1 week before V6 urea applications; Post-V6, soil samples taken 1 week after V6 urea applications; 3wk-V6, soil samples taken 3 weeks after V6 urea applications; Pre-R1, soil samples taken 1 week before R1 urea application; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications; PM, soil samples taken at physiological maturity.

¶C, control no N applied; F100, N applied in the fall at 100% of the recommended rate; F125, N applied in the fall at 125% of the recommended rate; PP100, N applied at pre-plant in the spring at 100% of the recommended rate; PP125, N applied at pre-plant in the spring at 125% of the recommended rate; Sp75, half of the N applied at pre-plant in the spring and half at V6 at 75% of the recommended rate; Sp100, half of the N applied at pre-plant in the spring and half at V6 at 100% of the recommended rate; TSp50, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 50% of the recommended rate; TSp75, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 75% of the recommended rate; TSp100, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 100% of the recommended rate.

*Means within the column followed by different lower case letters are significantly different at $p \leq 0.05$.

Table 2.13. The effect of treatment and year x time of sampling on maize tissue Cu uptake at Lamberton 2014-2016.

Treatment	2014-2016		Time of sampling	2014		2015		2016	
			kg Cu ha^{-1}						
C	0.023	g*	Pre-V6†	0.001	k*	0.0009	k	-	-
F100	0.056	b	Post-V6	0.019	h	0.013	i	0.008	j
F125	0.060	ab	3wk-V6	0.039	f	0.028	g	-	-
PP100	0.059	ab	Pre-R1	0.056	d	-	-	-	-
PP125	0.060	a	Post-R1	0.053	de	-	-	0.05	e
Sp75	0.046	d	3wk-R1	0.088	b	-	-	0.07	c
Sp100	0.050	c	PM	0.096	a	-	-	-	-
TSp50	0.034	f							
TSp75	0.040	e							
TS100	0.043	de							

†Pre-V6, soil samples taken 1 week before V6 urea applications; Post-V6, soil samples taken 1 week after V6 urea applications; 3wk-V6, soil samples taken 3 weeks after V6 urea applications; Pre-R1, soil samples taken 1 week before R1 urea application; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications; PM, soil samples taken at physiological maturity.

*Means within the column followed by different lower case letters are significantly different at $p \leq 0.05$.

Table 2.14. The effect of year x time of sampling on maize tissue Fe uptake at Becker, Lamberton, and Waseca for 2014-2015

	Becker				Lamberton				Waseca			
	2014		2015		2014		2015		2014		2015	
Time of sampling	kg Fe ha^{-1}											
Pre-V6†	0.03	g*	0.02	g	0.06	e*	0.05	g	0.11	hi	0.09	i
Post-V6	0.18	f	0.22	f	0.86	d	0.57	fg	0.47	fg	0.35	gh
3wk-V6	0.32	e	0.51	d	1.82	c	1.30	de	0.87	de	0.69	ef
Pre-R1	0.86	c	0.93	bc	2.39	ab	0.85	ef	0.72	e	0.63	ef
Post-R1	0.91	bc	1.15	ab	1.87	bc	3.76	b	0.99	d	1.12	bcd
3wk-R1	1.10	b	1.10	bc	2.73	a	2.05	c	1.27	bc	1.02	cd
PM	1.32	a	-	-	1.96	bc	5.43	a	1.34	b	4.01	a

†Pre-V6, soil samples taken 1 week before V6 urea applications; Post-V6, soil samples taken 1 week after V6 urea applications; 3wk-V6, soil samples taken 3 weeks after V6 urea applications; Pre-R1, soil samples taken 1 week before R1 urea application; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications; PM, soil samples taken at physiological maturity.

*Means within the column followed by different lower case letters are significantly different at $p \leq 0.05$.

Table 2.15. The effect of treatment at Becker 2016, treatment x year at Waseca 2014-2016, and treatment x time of sampling at Lamberton 2016 for maize tissue Fe uptake

	Becker		Waseca				Lamberton					
Treatment	2016		2014 & 2015		2016		Post-V6†		Post-R1		3wk-R1	
							kg Fe ha^{-1}					
C¶	0.65	b*	0.63	b	0.58	c	0.11	b*	0.29	d	1.03	c
F100	0.95	a	1.04	a	0.83	ab	0.23	a	0.72	ab	1.43	abc
F125	0.95	a	1.05	a	0.71	bc	0.27	a	0.72	ab	1.73	a
PP100	1.15	a	0.92	a	0.93	a	0.26	a	0.65	abc	1.7	a
PP125	1.1	a	1	a	0.89	ab	0.26	a	0.78	a	1.42	abc
Sp75	1.04	a	1.1	a	0.82	ab	0.18	a	0.71	ab	1.23	bc
Sp100	1.01	a	1.06	a	0.85	ab	0.27	a	0.71	ab	1.68	ab
TSp50	1.14	a	91	a	0.77	ab	0.16	ab	0.49	c	1.07	c
TSp75	0.99	a	1.01	a	0.82	ab	0.18	ab	0.61	abc	1.1	c
TSp100	1.17	a	1.13	a	0.86	ab	0.2	a	0.59	bc	1.36	abc

¶C, control no N applied; F100, N applied in the fall at 100% of the recommended rate; F125, N applied in the fall at 125% of the recommended rate; PP100, N applied at pre-plant in the spring at 100% of the recommended rate; PP125, N applied at pre-plant in the spring at 125% of the recommended rate; Sp75, half of the N applied at pre-plant in the spring and half at V6 at 75% of the recommended rate; Sp100, half of the N applied at pre-plant in the spring and half at V6 at 100% of the recommended rate; TSp50, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 50% of the recommended rate; TSp75, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 75% of the recommended rate; TSp100, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 100% of the recommended rate.

† Post-V6, soil samples taken 1 week after V6 urea applications; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications.

*Means within the column followed by different lower case letters are significantly different at $p \leq 0.05$

Table 2.16. Maize tissue Mn uptake at Becker 2014-2016

				2014			
Treatment	Pre-V6	Post-V6	3wk-V6	Pre-R1	Post-R1	3wk-R1	PM
				kg Mn ha^{-1}			
C	0.008	0.038	0.09	0.13 b*	0.15 d	0.27 e	0.31 e
F100	0.01	0.053	0.11	0.17 ab	0.2 cd	0.32 de	0.37 de
F125	0.01	0.06	0.1	0.19 ab	0.25 bc	0.37 cd	0.4 cde
PP100	0.01	0.063	0.13	0.17 ab	0.22 bcd	0.37 cd	0.44 bcd
PP125	0.013	0.078	0.17	0.21 ab	0.32 ab	0.47 b	0.65 a
Sp75	0.01	0.048	0.12	0.22 ab	0.3 ab	0.43 bc	0.46 bcd
Sp100	0.01	0.048	0.15	0.26 a	0.36 a	0.62 a	0.66 a
TSp50	0.01	0.045	0.13	0.19 ab	0.24 bcd	0.42 bc	0.5 b
TSp75	0.01	0.045	0.12	0.18 ab	0.27 abc	0.49 b	0.48 bc
TSp100	0.01	0.05	0.13	0.22 ab	0.31 ab	0.59 a	0.68 a
				2015			
Treatment	Pre-V6	Post-V6	3wk-V6	Pre-R1	Post-R1	3wk-R1	PM
				kg Mn ha^{-1}			
C	-2.23E-15	0.028	0.083 b	0.12 f	0.12 d	-	-
F100	0.003	0.08	0.2 a	0.22 de	0.41 ab	-	-
F125	0.01	0.11	0.24 a	0.34 bc	0.31 c	-	-
PP100	0.008	0.1	0.25 a	0.46 a	0.38 bc	-	-
PP125	0.01	0.1	0.22 a	0.36 bc	0.41 ab	-	-
Sp75	0.003	0.045	0.18 a	0.32 bc	0.32 bc	-	-
Sp100	0.005	0.058	0.25 a	0.4 ab	0.47 a	-	-
TSp50	-1.47E-15	0.038	0.17 ab	0.21 ef	0.2 d	-	-
TSp75	0.003	0.056	0.18 a	0.29 cde	0.3 c	-	-
TSp100	0.003	0.053	0.19 a	0.3 cd	0.34 bc	-	-
				2016			
Treatment	Pre-V6	Post-V6	3wk-V6	Pre-R1	Post-R1	3wk-R1	PM
				kg Mn ha^{-1}			
C	-	0.04 c	-	-	0.11 f	0.24 e	-
F100	-	0.1 abc	-	-	0.35 de	0.47 cd	-

F125	-	0.1 abc	-	-	0.39 cd	0.62 b	-
PP100	-	0.14 ab	-	-	0.62 a	0.69 b	-
PP125	-	0.19 a	-	-	0.7 a	0.94 a	-
Sp75	-	0.09 bc	-	-	0.48 bc	0.53 c	-
Sp100	-	0.1 abc	-	-	0.51 b	0.7 b	-
TSp50	-	0.07 bc	-	-	0.28 e	0.43 d	-
TSp75	-	0.07 bc	-	-	0.32 de	0.46 cd	-
TSp100	-	0.09 bc	-	-	0.49 b	0.68 b	-

†Pre-V6, soil samples taken 1 week before V6 urea applications; Post-V6, soil samples taken 1 week after V6 urea applications; 3wk-V6, soil samples taken 3 weeks after V6 urea applications; Pre-R1, soil samples taken 1 week before R1 urea application; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications; PM, soil samples taken at physiological maturity.

¶C, control no N applied; F100, N applied in the fall at 100% of the recommended rate; F125, N applied in the fall at 125% of the recommended rate; PP100, N applied at pre-plant in the spring at 100% of the recommended rate; PP125, N applied at pre-plant in the spring at 125% of the recommended rate; Sp75, half of the N applied at pre-plant in the spring and half at V6 at 75% of the recommended rate; Sp100, half of the N applied at pre-plant in the spring and half at V6 at 100% of the recommended rate; TSp50, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 50% of the recommended rate; TSp75, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 75% of the recommended rate; TSp100, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 100% of the recommended rate.

*Means within the column followed by different lower case letters are significantly different at $p \leq 0.05$.

Table 2.17. The effect of year x time of sampling at Lamberton and time of sampling at Waseca on maize tissue Mn uptake 2014-2015.

	Lamberton				Waseca	
Time of Sampling	2014		2015		2014-2015	
			kg Mn ha^{-1}			
Pre-V6†	0.01	gh*	0.004	h	0.01	g
Post-V6	0.2	fg	0.13	gh	0.08	f
3wk-V6	0.48	e	0.34	ef	0.2	e
Pre-R1	0.73	d	0.66	de	0.31	d
Post-R1	0.72	de	2.14	b	0.71	c
3wk-R1	1.32	c	0.75	d	0.93	b
PM	1.32	c	3.07	a	1.12	a

†Pre-V6, soil samples taken 1 week before V6 urea applications; Post-V6, soil samples taken 1 week after V6 urea applications; 3wk-V6, soil samples taken 3 weeks after V6 urea applications; Pre-R1, soil samples taken 1 week before R1 urea application; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications; PM, soil samples taken at physiological maturity.

*Means within the column followed by different lower case letters are significantly different at $p \leq 0.05$.

Table 2.18. The effect of treatment in 2014-2015 and treatment x time of sampling in 2016 at Lamberton and Waseca on maize tissue Mn uptake.

	Lamberton	Waseca	Lamberton			Waseca		
	2014-2015	2014-2015	2016			2016		
Treatment			Post-V6†	Post-R1	3wk-R1	Post-V6	Post-R1	3wk-R1
					kg Mn ha^{-1}			
C¶	0.62 e*	0.32 d	0.05 f	0.36 c	0.59 e	0.06 c	0.19 d	0.25 d
F100	1.03 abc	0.46 bc	0.13 bcde	0.74 a	1.29 abc	0.11 abc	0.32 c	0.56 bc
F125	1.15 ab	0.46 c	0.19 a	0.78 a	1.46 a	0.12 ab	0.32 c	0.53 bc
PP100	1.01 abc	0.44 c	0.16 abc	0.77 a	1.46 a	0.15 a	0.44 ab	0.55 bc
PP125	1.25 a	0.52 abc	0.16 ab	0.84 a	1.25 abc	0.16 a	0.44 ab	0.6 b
Sp75	0.97 bcd	0.52 abc	0.095 def	0.71 a	1.16 bcd	0.12 ab	0.36 bc	0.62 b
Sp100	1.09 abc	0.55 ab	0.14 abcd	0.79 a	1.42 ab	0.16 a	0.53 a	0.79 a
TSp50	0.74 de	0.47 abc	0.075 ef	0.53 bc	0.87 de	0.09 bc	0.28 cd	0.42 c
TSp75	0.89 cd	0.56 a	0.088 ef	0.68 ab	1.05 cd	0.12 ab	0.38 bc	0.65 ab
TSp100	1.06 abc	0.50 abc	0.11 cde	0.67 ab	1.19 abc	0.13 ab	0.39 abc	0.61 b

† Post-V6, soil samples taken 1 week after V6 urea applications; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications.

¶C, control no N applied; F100, N applied in the fall at 100% of the recommended rate; F125, N applied in the fall at 125% of the recommended rate; PP100, N applied at pre-plant in the spring at 100% of the recommended rate; PP125, N applied at pre-plant in the spring at 125% of the recommended rate; Sp75, half of the N applied at pre-plant in the spring and half at V6 at 75% of the recommended rate; Sp100, half of the N applied at pre-plant in the spring and half at V6 at 100% of the recommended rate; TSp50, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 50% of the recommended rate; TSp75,

a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 75% of the recommended rate; TSp100, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 100% of the recommended rate.

*Means within the column followed by different lower case letters are significantly different at $p \leq 0.05$.

Table 2.19. The effect of year x time of sampling at Becker and Lamberton on maize tissue Zn uptake in 2014-2015.

	Becker				Lamberton			
Time of sampling	2014		2015		2014		2015	
	kg Zn ha^{-1}							
Pre-V6†	0.003	g*	0.001	g	0.005	h	0.004	h
Post-V6	0.02	fg	0.03	fg	0.08	g	0.06	g
3wk-V6	0.055	ef	0.09	e	0.19	e	0.13	f
Pre-R1	0.09	e	0.16	d	0.26	d	0.18	e
Post-R1	0.21	c	0.27	b	0.19	e	0.24	d
3wk-R1	0.56	a	-	-	0.51	b	-	-
PM	0.25	b	-	-	0.31	c	0.73	a

†Pre-V6, soil samples taken 1 week before V6 urea applications; Post-V6, soil samples taken 1 week after V6 urea applications; 3wk-V6, soil samples taken 3 weeks after V6 urea applications; Pre-R1, soil samples taken 1 week before R1 urea application; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications; PM, soil samples taken at physiological maturity.

*Means within the column followed by different lower case letters are significantly different at $p \leq 0.05$.

Table 2.20. The effect of treatment x time of sampling at Becker and Lamberton, and treatment at Waseca on maize tissue Zn uptake in 2016.

	Becker					Lamberton						Waseca	
Treatment	Post-V6†	Post-R1		3wk-R1		Post-V6		Post-R1		3wk-R1			
	kg Zn ha^{-1}												
C¶	0.018	0.096	c*	0.18	e	0.018	d	0.14	d	0.3	e	0.18	c
F100	0.031	0.15	bc	0.2	de	0.4	bc	0.2	abc	0.44	abc	0.23	ab
F125	0.031	0.16	b	0.22	cde	0.055	a	0.23	a	0.46	ab	0.22	bc
PP100	0.037	0.2	ab	0.27	abc	0.049	ab	0.2	abc	0.51	a	0.25	ab
PP125	0.041	0.18	ab	0.28	abc	0.043	b	0.23	a	0.41	bcd	0.27	a
Sp75	0.043	0.23	a	0.25	bcd	0.26	cd	0.21	ab	0.41	bcd	0.23	ab
Sp100	0.039	0.19	ab	0.27	abc	0.044	b	0.22	a	0.47	ab	0.26	ab
TSp50	0.033	0.17	ab	0.26	bcd	0.025	cd	0.17	c	0.35	de	0.24	ab
TSp75	0.03	0.15	bc	0.28	ab	0.027	cd	0.2	abc	0.37	cde	0.24	ab
TSp100	0.036	0.22	a	0.32	a	0.03	c	0.18	bc	0.4	bcd	0.25	ab

†Post-V6, soil samples taken 1 week after V6 urea applications; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications.

¶C, control no N applied; F100, N applied in the fall at 100% of the recommended rate; F125, N applied in the fall at 125% of the recommended rate; PP100, N applied at pre-plant in the spring at 100% of the recommended rate; PP125, N applied at pre-plant in the spring at 125% of the recommended rate; Sp75, half of the N applied at pre-plant in the spring and half at V6 at 75% of the recommended rate; Sp100, half of the N applied at pre-plant in the spring and half at V6 at 100% of the recommended rate; TSp50, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 50% of the recommended rate; TSp75, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 75% of the recommended rate; TSp100, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 100% of the recommended rate.

*Means within the column followed by different lower case letters are significantly different at $p \leq 0.05$.

Table 2.21. The effect of treatment x time of sampling on maize tissue Zn uptake at Becker and Waseca 2014 & 2015.

Treatment	Becker	Waseca	Becker	Waseca	Becker	Waseca	Becker	Waseca	Becker	Waseca	Becker	Waseca	Becker	Waseca
	2014													
	Pre-V6†		Post-V6		3wk-V6		Pre-R1		Post-R1		3wk-R1		PM	
	kg Zn ha^{-1}													
C¶	0.002	0.005	0.02	0.05	0.04	0.08	0.06	0.3 a*	0.17 b	0.24	0.37 d	0.45 a	0.21 b	0.54 a
F100	0.003	0.004	0.02	0.03	0.04	0.07	0.08	0.2 ab	0.15 b	0.15	0.32 d	0.4 a	0.21 b	0.4 bc
F125	0.003	0.003	0.02	0.02	0.05	0.05	0.08	0.21 ab	0.15 b	0.18	0.38 d	0.38 ab	0.19 b	0.42 b
PP100	0.003	0.003	0.02	0.04	0.05	0.03	0.08	0.21 ab	0.15 b	0.21	0.33 d	0.4 a	0.21 b	0.31 cde
PP125	0.003	0.003	0.02	0.03	0.06	0.03	0.08	0.15 b	0.21 ab	0.17	0.61 c	0.39 ab	0.28 ab	0.29 de
Sp75	0.003	0.004	0.02	0.02	0.06	0.07	0.1	0.25 ab	0.32 a	0.22	0.93 a	0.46 a	0.22 b	0.28 e
Sp100	0.003	0.003	0.02	0.03	0.07	0.08	0.11	0.23 ab	0.24 ab	0.21	0.78 b	0.28 b	0.28 ab	0.27 e
TSp50	0.003	0.004	0.02	0.04	0.06	0.13	0.09	0.28 a	0.24 ab	0.24	0.56 c	0.48 a	0.27 ab	0.43 b
TSp75	0.003	0.004	0.02	0.03	0.06	0.11	0.11	0.26 ab	0.2 ab	0.16	0.67 bc	0.38 ab	0.25 b	0.39 bcd
TSp100	0.003	0.004	0.02	0.04	0.06	0.06	0.09	0.2 ab	0.23 ab	0.22	0.67 bc	0.41 a	0.4 a	0.38 bcde
	2015													
	Pre-V6†		Post-V6		3wk-V6		Pre-R1		Post-R1		3wk-R1		PM	
	kg Zn ha^{-1}													
C	0	0.002	0.01	0.02	0.04 b	0.02	0.1 c	0.1 b	0.15 e	0.2	-	-	-	0.47 cde
F100	0.001	0.003	0.03	0.02	0.09 a	0.1	0.13 bc	0.15 ab	0.21 d	0.28	-	-	-	0.52 bcd
F125	0.002	0.003	0.03	0.01	0.11 a	0.02	0.19 a	0.15 b	0.33 a	0.23	-	-	-	0.59 ab
PP100	0.002	0.003	0.04	0.03	0.1 a	0.07	0.19 a	0.13 b	0.27 bc	0.2	-	-	-	0.62 ab
PP125	0.001	0.003	0.03	0.03	0.1 a	0.06	0.13 bc	0.15 ab	0.22 cd	0.26	-	-	-	0.41 e
Sp75	0.001	0.003	0.02	0.02	0.08 ab	0.07	0.17 ab	0.15 ab	0.34 a	0.23	-	-	-	0.58 abc
Sp100	0.001	0.003	0.03	0.02	0.11 a	0.07	0.2 a	0.16 ab	0.33 a	0.27	-	-	-	0.47 de
TSp50	0.001	0.003	0.02	0.03	0.07 ab	0.03	0.13 bc	0.14 b	0.22 cd	0.23	-	-	-	0.68 a

TSp75	0.002	0.003	0.03	0.02	0.09 a	0.05	0.18 a	0.26 a	0.31 ab	0.26	-	-	-	0.55 bcd
TSp100	0.001	0.002	0.02	0.04	0.09 a	0.08	0.17 ab	0.14 b	0.33 a	0.22	-	-	-	0.47 de

†Pre-V6, soil samples taken 1 week before V6 urea applications; Post-V6, soil samples taken 1 week after V6 urea applications; 3wk-V6, soil samples taken 3 weeks after V6 urea applications; Pre-R1, soil samples taken 1 week before R1 urea application; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications; PM, soil samples taken at physiological maturity.

¶C, control no N applied; F100, N applied in the fall at 100% of the recommended rate; F125, N applied in the fall at 125% of the recommended rate; PP100, N applied at pre-plant in the spring at 100% of the recommended rate; PP125, N applied at pre-plant in the spring at 125% of the recommended rate; Sp75, half of the N applied at pre-plant in the spring and half at V6 at 75% of the recommended rate; Sp100, half of the N applied at pre-plant in the spring and half at V6 at 100% of the recommended rate; TSp50, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 50% of the recommended rate; TSp75, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 75% of the recommended rate; TSp100, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 100% of the recommended rate.

*Means within the column followed by different lower case letters are significantly different at $p \leq 0.05$.

Chapter 3

Time and rate of urea influence soil enzyme activity

Abstract

The timing and rate of nitrogen (N) fertilizer application has been shown to influence soil enzyme activity, but results have been inconsistent across locations and growing seasons. This study was conducted to determine whether fall, spring, and split applications of N fertilizer at varying rates influence fluorescein diacetate (FDA) hydrolysis, glucosidase, phosphatase, and sulfatase activity. Experiments were conducted from 2014 through 2016 to compare single (fall or spring applications) and split applications for differing N rates on an irrigated Hubbard-Mosford loamy sand complex at Becker and under rainfed conditions on a Normania loam soil at Lamberton and on a Nicollet clay loam soil at Waseca, MN. Nitrogen rates varied by locations and were always based on the University of Minnesota guidelines for each location. All three sites were planted to soybean in 2013 and to maize from 2014 to 2016. Soil samples were collected seven times each season. At all three locations, enzyme activity fluctuated across the growing season. Fertilizer N application did not impact FDA, glucosidase or phosphatase activity. However, there was a negative correlation of r = -0.0749, -0.0704, and -0.08810 for year and FDA activity at Becker, Lamberton, and Waseca, respectively. In course textured soils applying N fertilizer as a split application increased sulfatase activity 13-21 mg of p-nitrophenyl

released kg^{-1} soil hr^{-1}. The decline in enzyme activity suggests that intensive management of corn negatively affects soil microbial activity.

ABBREVIATIONS

SB-M-M-M, soybean-maize-maize-maize; F, fall application; FDA, fluorescein diacetate hydrolysis; N, nitrogen; PP, preplant application; R1, first reproductive stage of maize phenotypical development; Sp, two way split N application; TSp, three way split N application; V6, six leaf-collar stage of maize phenological development.

Introduction

Soil organisms can play a pivotal role in nutrient cycling and availability for crop production. Soil microflora (bacteria and fungi), and soil fauna (protozoa, and invertebrates such as nematodes, mites, and earthworms) can influence the availability of nutrients for crop production through the decomposition of crop residues, mineralization and immobilization of nutrients, biological nitrogen fixation, and bioturbation (Giller, et al., 1997; Bünemann et al., 2006). In particular, the soil microbial biomass, made up by soil bacteria and fungi, are considered a labile pool of nutrients in particular C, N, P, and S (Bünemann et al., 2006). The mineralization or immobilization of nutrients by soil microbial biomass can better determine the temporal pattern of nutrient availability, soil nutrient status, and the overall net productivity of the agroecosystem (Wardle et al., 1999). Furthermore, soil microbial biomass is regarded as an early indicator of changes in soil fertility and agroecosystem properties (Moore et al., 2000). However, the impact of applying N fertilizer on soil microbial biomass and activity is still not fully understood. A study assessing the impact of crop rotations and N fertilization on microbial biomass C and N in two long-term field experiments in Iowa determined that microbial C and N were affected by crop rotation and plant cover but not by N fertilization (Moore et al., 2000). This corroborates an earlier greenhouse study where soil microbial biomass and enzyme activity were also correlated with total C inputs (Fauci & Dick, 1994). The authors reported that long-term N application was found to decrease organic matter and soil biological activity; while short-term N application had a limited effect on microbial biomass C and soil enzyme activities (Fauci & Dick, 1994). The adverse effects of long-term N fertilizer application on soil enzyme activity was also reported from a 20 year fertilization regime in a vegetable greenhouse production system (Zhang et al., 2015). They found the application of 300 kg N ha^{-1} and 600 kg N ha^{-1} as urea decreased soil organic C, and α-glucosidase and β-glucosidase activity compared with the application of 75 Mg ha^{-1} of horse manure compost at planting (Zhang et al., 2015). However, a meta-analysis based on 107 datasets from 64 long-term trials from around the world concluded that the application of mineral fertilizer application increased microbial biomass by 15.1 % compared with unfertilized control treatments (Geisseler & Scow, 2014). The addition of mineral fertilizer also increased soil organic carbon content by 12.1% compared with unfertilized control plots and was found to be a major factor contributing to the overall increase in microbial biomass with mineral fertilization (Geisseler & Scow, 2014). It should be noted that the duration of the trial also affected the response of soil microbial biomass, with the increase in microbial activity being highest in studies that had been in place for at least 20 years (Geisseler & Scow, 2014). However, a different meta-analysis of 82 field studies found that microbial biomass declined 15% on average under N fertilization but fungi and bacteria were not affected (Treseder, 2008). In particular, Treseder (2008) noted that as N load increased the decline in microbial biomass became more negative.

Extracellular enzymes involved in the degradation on soil organic matter are often of interest because their activity often responds faster to changes in soil management and can provide an early indication of changes in soil health (Bandick and Dick, 1999; Turner et al., 2002; Das and Varma, 2010). β-glucosidase activity is a useful enzyme for soil quality monitoring because of its central role in the enzymatic degradation of cellulose, the main component of plant polysaccharides, and soil organic matter cycling (Turner et al., 2002). β-glucosidase catalyzes the hydrolysis process by cleaving cellobiose to release two moles of glucose per mole of cellobiose thus regulating the supply of energy for microorganisms unable to directly take up cellobiose (Turner et al., 2002). As β-glucosidase has a central role in soil organic matter cycling, its activity is useful for monitoring soil quality as it can provide a reflection of past biological activity and the capacity of soil to stabilize the soil organic matter (Sherene, 2017). To our knowledge there has been limited or no studies that compared the effect of fertilizer rate and timing of application in field trials on β-glucosidase in maize grown consecutive years. Studies have primarily compared the effect of tillage, crop rotation, or the application of organic materials on β-glucosidase activity. A study comparing the effect of tillage and crop rotation on soil enzyme activity in a clay-loam textured Typic Hapludoll soil reported that the activities of β-glucosidase was 18% greater in no-till soils than ridge till soils (Zhang et al., 2014). Crop rotation did not impact enzyme activities with an exception that the β-glucosidase activity was greater in ridge till soils under monoculture maize than under maize-soybean rotation (Zhang et al., 2014). The application of organic materials also tends to improve β-glucosidase activity. A greenhouse vegetable trial reported that β-glucosidase activity increased when 75 Mg ha^{-1} of horse manure compost was applied at planting followed by a side dress of either 300 or 600 kg N ha^{-1} of urea due to a greater turnover rate of soil C (Zhang et al., 2015). This supports the findings of the meta-analysis by Geisseler and Scow (2014) and Jian et al. (2016) where the application of N fertilizer was found to increase β-glucosidase activity by 11 to 15 %. Understanding that microbial activity is influenced by changes in soil organic carbon, increased productivity should increase the amount of plant residue that is returned to the soil, thus increasing soil organic matter content over the years (Geisseler & Scow, 2014). Due to the role of soil organic carbon in β-glucosidase activity, increased biomass production over time could increase enzyme activity. However, an incubation study using soil from 28 different ecosystems across North America where no C inputs were added concluded that β-glucosidase activity decreased by 12% while soil microbial biomass decreased by an average of 35% when N was added (Ramirez et al., 2012). Because no C inputs were added to the soils during the year long incubation study, it was concluded that adding N depressed soil microbial activity by shifting the metabolic capabilities of soil bacterial communities (Ramirez et al., 2012). By yielding communities that are less capable of decomposing more recalcitrant soil carbon pools, the addition of N lead to a potential increase in soil carbon sequestration rates (Ramirez et al., 2012).

Similar to β-glucosidase activity, soil phosphatase tends to increase with the addition of organic matter. In a rice-wheat rotation system on a sandy loam calcareous alluvisol in China, the application of 100% recommended rate of urea, 80% recommended rate of urea, and a mixture of 30% recommended rate of urea and 50% recommended rate of organic N increased phosphatase activity by 8 to 71% compared with the control (Guan et al., 2011). In addition, by reducing traditional N fertilizer doses by 20% and replacing 50% of N fertilizer by organic matter, phosphatase activity increased by 35 to 74% compared with traditional N fertilizer doses (Guan et al., 2011).

The effects of N fertilizer application on phosphatase activity were also investigated in a pasture enriched grazed-pasture system. Pots received either 100% N recommendation in a split application, 50% N recommendation in a single application, or 0% of N recommendation for Triticale (x*Triticosecale rimpaui Wittm*. L.). Cattle grazed the plots the following spring through May and in the summer the plots were over-seeded with cowpea (*Vigna unguiculate* L.), fertilized at the same rates by reference to N recommendations for Bermuda grass (*Cynodon dactylon* L.), and grazed by cattle until September (Dillard et al., 2015). Nitrogen fertilization was reported to have had no effect on soil phosphatase activity, electrical conductivity, or concentrations of water-soluble P (Dillard et al., 2015). Similarly, a field experiment assessing the effect of applying different rates of urea-N and broiler litter-N on phosphatase activity reported that alkaline phosphatase activity increased with increasing rates of broiler litter while it did not increase with increasing rates of urea (Fereidooni et al., 2013).

Although field studies report that the application of urea does not affect soil phosphatase activity, incubation studies report an increase in soil phosphatase activity with the application of N fertilizer. This was demonstrated by an incubation experiment where increasing doses of P, NO_3^--N, or NH_4^+-N were applied to evaluate the fertilizer effect on soil phosphatase activity and microbial biomass carbon in the bulk soil (Paredes et al., 2011). In this study, N was applied at 0, 50, 100, 150, and 300 mg N kg^{-1} soil as either nitrate (NO_3^--N) or ammonium (NH_4^+-N) to soil samples that were previously fertilized with 300 mg P kg^{-1} (Paredes et al., 2011). Findings showed that bulk soil phosphatase activity progressively increased in response to NO_3-N application and plateaued at 100 mg N kg^{-1}; however, increasing the application of NH_4^+-N had no effect on phosphatase activity (Paredes et al., 2011). The lack of increased phosphatase activity when ammonium was applied could be attributed to a microbial reallocation of C to biomass or enzyme production (Schimel and Weintraub, 2003). This supports findings from a 28 day soil incubation study which investigated the responses of extracellular enzymes to simple and complex nutrient inputs reporting no increases in enzymatic activity (Allison and Vitousek, 2005). This suggests that either microbes are mineralizing less C from protein sources due to reduced protease production, or that ammonium has a direct negative effect on microbial respiration rates (Allison and Vitousek, 2005). More specifically, results from the study of Allison and Vitousek (2005) showed that β-glucosidase and acid phosphatase activities increased in treatments where only carbon and nitrogen were added while glycine aminopeptidase and acid phosphatase activities declined in response to ammonium and phosphate additions. Overall, enzyme activity tended to increase when its target nutrient was present in complex but not simple form, and carbon and nitrogen was available (Allison and Vitousek, 2005).

Given that arylsulfatase is secreted to release soil available S from organic S (mainly ester sulfates) for plant growth, it can be an useful indicator of soil health (Wang et al., 2016). Similar to the activities of glucosidase and phosphatase, arylsulfatase has been shown to be highly correlated with soil organic C content (Deng and Tabatabai, 1997a). In a study comparing the application of different rates of ammonium sulfate and organic fertilizer after cultivating 24 crops of vegetables for three consecutive years in a greenhouse, greater arylsulfatase activity was found in the soil treated with at least 540 kg N ha^{-1} yr^{-1} of compost (Chang, et al., 2007). Arylsulfatase was also significantly and linearly correlated with the organic matter content of the soils (Chang et al., 2007). Only a limited number of studies have researched the effect of N fertilizer on arylsulfatase. In the semi-arid grasslands of Inner Mongolia, a 9-year study was undertaken on the effect of urea and water addition on P and S concentrations in three soil aggregate fractions (Wang et al., 2016). Microaggregates (<0.25mm) were found to retain the highest total P and S concentrations due to microbial decomposition (Wang et al., 2016). However, the addition of N and water increased available soil S by up to 150% across the three soil aggregate fractions (Wang et al., 2016). However, soil acidification due to N addition decreased aryl-sulfatase activity by 40%, while the addition of water increased activity (Wang et al., 2016). The impact of N and water addition on aryl-sulfatase activity highlights the complex relationship of soil enzyme activity with fertilizer and water. In a different long-term fertilizer experiment, the activity of sulfatase was also found to be greatest in the 0.1-2 μm fraction (Zhang et al., 2015). However, in the 200 to 2000 μm fraction, sulfatase activity was greatest under the NPK (300 -150 -150 kg ha^{-1}) treatment unlike most other enzymes, which recorded greatest activity under NPKM (swine (*Sus* L.) manure (69% H_2O), 15 - 21-14 g kg^{-1}) treatments (Zhang et al., 2015). The application of crop residue can also increase the activity of arylsulfatase, particularly in soils with greater organic carbon content (Perucci and Scarponi, 1983; Falih and Wainwright, 1996). This corroborates other research where increases in enzyme activity were correlated to increases in soil organic C content (Fauci and Dick, 1994).

Over the years correlations have been established on the effect of tillage, pH, and residue management on soil enzymes involved in C, N, P and S cycling in soils (Deng and Tabatabai, 1997; Brockett et al., 2012). However, more information is needed to better understand the complex relationship between soil enzyme activity and N fertilizer management. In particular, little research has explored the effect of different N rates and timing of application in maize following maize systems on soil enzymes. Due to the variability in findings between incubation and field trials, there is more need to research the complex relationship between soil enzyme activity and N fertilizer application and timing of application. This study was designed to provide an opportunity to assess how the application of different N rates and timing of application impacted soil enzyme activity under a SB-M-M-M production system. Given the role of soil organic matter in soil enzyme activity, it is likely that the fertilizer management systems that increases soil organic matter through greater residue return should increase soil enzyme activity.

Materials and Methods

Site Description and Experimental Design

Field experiments were conducted at University of Minnesota Research and Outreach Centers near Lamberton, MN (44° 24’ N, 95° 30’ W), Waseca, MN (44° 07’, 93° 52’ W) and Becker, MN (45° 39’ N, 93° 89’ W) from 2014 to 2016. The rainfed sites at Lamberton had a Normania loam soil (Fine-loamy, mixed, superactive, mesic Aquic Hapludolls) and at Waseca a Nicollet clay loam soil (Fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquolls); while an irrigated Hubbard-Mosford loamy sand complex (Sandy, mixed, frigid Typic Hapludolls or frigid Entic Hapludolls) was used at Becker, MN. The experimental design was a randomized complete block design with four replications. Plot sizes at Becker and Waseca, MN were 4.6 m (6 rows) by 15 m and Lamberton was 6 m by 12 m, the differences in plot sizes were due to the differences in implements available at each location for tillage and planting operations. All sites were planted to soybean [*Glycine max* L. (Merr.)] in 2013 and to maize from 2014 to 2016. For each year, the tillage system involved field cultivating in the spring prior to planting, and stalk chopping followed by disk ripping in the fall after maize harvest. Non-N starter fertilizer was applied in the spring following soil test and crop guidelines. Pest management followed best management practices (BMP) and varied by location and year.

The selected recommended N rate (RN) in 2014 for maize following soybean at Becker, MN was 168 kg ha^{-1}, while at Lamberton and Waseca it was 135 kg ha^{-1}. For 2015 and 2016 the RN for maize following maize at Becker was 235 kg ha^{-1} while at Lamberton and Waseca, MN it was 202 kg ha^{-1}. All rates were based on University of Minnesota guidelines (Kaiser et al., 2012). The N source used for fall applications was SuperU® (46-0-0), a urea based granule with urease and nitrification inhibitors, and for the pre-plant and in-season applications the N source was urea (46-0-0). All fertilizers were hand-applied and incorporated with tillage immediately after application in the fall and prior to planting. In-season applications were incorporated through irrigation at Becker. At Lamberton and Waseca, fertilizer was placed in 5 cm deep furrows in the inter-rows made using a hoe, after fertilizer application, furrow trenches were closed with soil. Treatments were applied to the same experimental plots in all years. The treatments tested in this study consisted of a non-fertilized control plus nine N management systems: F100, single SuperU® application in the fall at 100% of RN; F125, single SuperU® application in the fall at 125% of the RN; S100, single urea application applied at planting at 100% of the RN; S125, single urea application applied at planting at 125% of the RN; Sp75, split urea application at planting (one half) and V6 (one half) applied at 75% of the RN; Sp100, split urea application at planting (one half) and V6 (one half) applied at 100% of the RN; TSp50, split urea application at planting (one third), V6 (one third), and silking (one third) applied at 50% of the RN; TSp75, split urea application at planting (one third), V6 (one third), and silking (one third) applied at 75% of the RN; TSp100, split urea application at planting (one third), V6 (one third), and silking (one third) applied at 100% of the RN. The growth stages selected for N application in treatments TSp50, TSp75, and TSp100 were based on the literature information regarding nutrient requirement and time of maximum uptake of the crop (Hanway, 1962; Jokela and Randall, 1989; Abendroth et al., 2011). This strategy for split N application aimed to provide the crop with N at the times throughout the growing season when it was most needed.

Soil sampling and analysis

Each growing season soil cores were collected from each plot approximately one week before the V6 urea application (Pre-V6), one week after the V6 urea application (Post-V6), approximately 3 weeks after the V6 urea application (3wk-V6), one week before the R1 urea application (Pre-R1), one week after the R1 urea application (Post-R1), three weeks after the R1 urea application (3wk-R1) and at physiological maturity (PM) of the maize plant.

At each sampling event 6 soil cores representing the plot area were collected from the 0-15cm depth. In each plot 3 cores were collected from between rows two and three, and 3 cores from between rows four and five. For each set of 3 cores, one core was collected in the row, one core approximately a quarter into the row and one core in the middle of the row. Care was given not to sample within 1.5 m of the row edges. The six soil cores for each plot were bulked and dried in a low-temperature drier (35 ºC) for a week before being shipped to Lamberton, MN for analysis.

Fluoroscein Diacetate (FDA) Hydrolysis

Adapted by Adam and Duncan (2001), 1.0 g of air-dried soil (<2mm) was mixed with 7.5mL of potassium phosphate buffer, then in a fume hood 0.10mL of FDA was added to the soil sample, mixed and incubated at 37ºC for 1 hour. After incubation, 5.0 mL of chloroform:methanol solution was added, capped tightly, shaken, and centrifuged for 5 minutes at 3500 epm. 250 µL of the filtrated samples were measured using a spectrophotometer (Epoch Biotek, Winooski, VT) at a 490nm wavelength.

Glucosidase activity

Adapted from Dick (2011), 1.0 g of air-dried soil (<2mm) was mixed with 4.0mL of modified universal buffer (pH 6.0) and 1mL of p-Nitrophenyl-β-D-glucosidase (PNG) (50 mM), capped and then incubated at no less than 40ºC for 1 hour. After incubation, 1 mL of 0.5 $CaCl_2$ and 4.0mL of 0.1 M Tris(hydroxymethyl) aminomethane(THAM) buffer (100 mM, pH 12) were added and the solution was centrifuged for 5 min. Taking care not to transfer any organic matter or soil particles, 1mL of each sample was transferred into cluster tubes and then 250 µL of the filtrated samples were transferred into a 96 cell plate and measured using a spectrophotometer (Epoch Biotek, Winooski, VT) at a 400nm wavelength. Enzyme activity per kg of soil per hour was expressed as a production of p-nitrophenol (PN).

$$PN\ \frac{\mu g}{1\ L} \times \frac{0.005\ L}{0.001\ kg\ soil} \times \frac{1}{hr} = \text{PN}\ \frac{\mu g}{kg\ soil\ hr} \qquad [1]$$

Phosphomonoesterases activity

Adapted from Dick (2011), the replicate soil sample (1.0 g) was incubated with 4.0mL of buffer pH 6.5 and 1 mL of *p*-nitrophenyl phosphate solution (0.05 m) at 37ºC for 1 hour. After incubation, 1 mL of $CaCl_2$ (0.5 M) and 4.0mL NaOH (0.5 M) was added, mixed, and centrifuged for 5 minutes. Taking care not to transfer any organic matter or soil particles, 1mL of each sample was transferred into cluster tubes and then 250 µL of the filtrated samples were transferred into a 96 cell plate and measured using a spectrophotometer (Epoch Biotek, Winooski, VT) at a 410nm wavelength. Enzyme activity per kg of soil per hour was expressed as a production of p-nitrophenol (PN).

$$PN\ \frac{\mu g}{1\ L} \times \frac{0.005\ L}{0.001\ kg\ soil} \times \frac{1}{hr} = \text{PN}\ \frac{\mu g}{kg\ soil\ hr} \qquad [2]$$

Arylsulfatase activity

Adapted from Dick et al. (2001), 1.0 g of air-dried sieved (<2mm) soil and 4.0mL of acetate buffer and 1mL of p-nitrophenyl sulfate solution (0.05 m) was added, and then incubated at 37ºC for 1 hour. After incubation 1 mL of $CaCl_2$ (0.5 M) and 4.0mL of Tris(hydroxymethyl) aminomethane(THAM) buffer (100 mM, pH 12) were added and the solution was centrifuged for 5 minutes at 3500 epms. Taking care not to transfer any organic matter or soil particles, 1mL of each sample was transferred into cluster tubes and then 250 µL of the filtrated samples were transferred into a 96 cell plate and measured using a spectrophotometer (Epoch Biotek, Winooski, VT) at a 420nm wavelength. Enzyme activity per kg of soil per hour (h) is expressed as production of PN according to:

$$PN\ \frac{\mu g}{1\ L} \times \frac{0.005\ L}{0.001\ kg\ soil} \times \frac{1}{hr} = \mathrm{PN}\ \frac{\mu g}{kg\ soil\ hr} \qquad [3]$$

Statistical Analysis

Soil enzyme activity was analyzed by repeated measure ANOVA using the GLIMMIX Procedure in SAS at $P \leq 0.05$. Treatment, location, time of sampling, and year were considered fixed effects, while replication was considered random. The variable year was the repeated variable and the covariance structure that best fit the model for fluoroscein diacetate hydrolysis was CSH for Becker, Lamberton and Waseca. The covariance structure that best fit the model for glucosidase activity was ARH (1) for Becker, and CSH for Lamberton and Waseca. The covariance structure that best fit the model for phosphatase activity was CSH for Becker and Lamberton, and UN for Waseca. The covariance structure that best fit the model for sulfatase activity was CS for Becker, UN for Lamberton and CSH for Waseca. Tests for normality were performed prior to making inferences on main effects. When appropriate, pairwise mean comparisons (at $P \leq 0.05$) were made using the lines option in the GLIMMIX procedure of SAS. Linear associations were assed with Pearson's correlation coefficient using the CORR procedure of SAS.

Results

Fluorescein Hydrolytic Activity (FDA)

Becker

There was no treatment or interactive effects on FDA activity at Becker however time of soil sampling and year were significant (Table 3.1). Over three growing seasons, microbial activity as determined by FDA hydrolysis was greatest at Becker when sampled approximately 3 weeks after the V6 urea application (3wk-V6) with an average for all three years of 931mg of fluorescein released kg^{-1} soil hr^{-1} (Table 3.2). This was followed by microbial activity in soil samples taken one week after the V6 urea application (Post-V6) and one week after the R1 urea application (Post-R1) (Table 3.2). Soil samples taken one week before the V6 urea application (Pre-V6) and one week before the R1 urea application (Pre-R1) had a recorded FDA hydrolysis of 703 mg of fluorescein released kg^{-1} soil hr^{-1} and was only greater than samples taken approximately 3 weeks after the R1 urea application (3wk-R1) and soil samples taken at physiological maturity (PM) (Table 3.2). The increase in microbial activity as measured by FDA hydrolysis at Post-V6 and Post-R1 suggests that the application of urea increased microbial activity leading to the greater FDA hydrolysis values at Becker.

In 2014, annual soil FDA hydrolysis at Becker averaged 885 mg of fluorescein kg^{-1} soil hr^{-1}, significantly greater than in 2015 and 2016 (Table 3.3). Although there was no difference in enzyme activity between 2015 and 2016, there was a significant negative correlation (r = -0.749, P = 0.005) between FDA hydrolysis and year suggesting that maize following maize decreased microbial activity as measured by FDA at this location (Table 3.3).

Lamberton

At Lamberton there was a year by time of sampling interaction for FDA hydrolysis (Table 3.1). In 2014, soil samples taken Pre-V6 recorded 931 mg of fluorescein kg^{-1} soil hr^{-1}, greater than other sampling times at Lamberton regardless of treatment and year (Table 3.4). In 2014, the application of urea fertilizer decreased FDA hydrolysis compared with pre-urea application concentrations. FDA hydrolysis at Pre-V6 and Pre-R1 was greater than the concentrations in samples collected in the Post-V6 and Post-R1 sampling time (Table 3.4). In 2015, the effects of time of sampling where less evident than those observed for 2014; however, FDA hydrolysis at Pre-V6 (656 mg of fluorescein kg^{-1} soil hr^{-1}) was significantly larger than those observed at PM (487 mg of fluorescein kg^{-1} soil hr^{-1}). In 2016, there were no differences in fluorescein kg^{-1} soil hr^{-1} between sampling times (Table 3.4). Similar to Becker, there was a negative correlation between FDA hydrolysis and year suggesting that there was a decreasing trend in enzyme activity from 2014 to 2016 (r = -0.704, P = 0.011). Although there was a spike in activity in the middle of the season, around the Pre-R1 sampling time, microbial activity decreased as a function of time in 2014 (Table 3.4).

Waseca

At Waseca there was a treatment and year by time of sampling interaction (Table 3.1). Sp100 had significantly greater FDA hydrolysis than all treatments except Sp75 and the control plot (Table 3.5). Sp75 also had significantly greater activity than spring applied treatments, regardless of rate and TSp75 (Table 3.5). In 2014, FDA hydrolysis was greatest at Pre-V6 with 543 mg of fluorescein kg^{-1} soil hr^{-1} and decreased until the sampling taking place at 3 wks-V6 (Table 3.1). Microbial activity in soil samples taken after 3wk-V6 increased slightly compared with samples collected at 3wk-V6 but were still similar to those levels observed for samples collected at Post-V6 (Table 3.4). In 2015, FDA hydrolysis was greater in samples taken Pre-V6 and decreased as the season progressed (Table 3.4). The only exception was a spike in FDA hydrolysis detected at the Post-R1 sampling time (Table 3.4). In 2016, soil samples taken Post-V6 and 3wk-V6 recorded 410 and 438 mg of fluorescein kg^{-1} soil hr^{-1}, greater than all later sampling dates (Table 3.4). Similar to the other locations, FDA hydrolysis at Waseca was greater at the beginning of each season and decreased over the coarse of the growing season (r = -0.8810, P = 0.0002).

Over the coarse of the study correlation trends across all three locations suggested that microbial activity declined the longer the cropping system stays in maize. With a correlation of r = -0.749 (P = 0.005), microbial activity at Becker in 2014 averaged 885 mg of fluorescein kg^{-1} soil hr^{-1} while in 2016 it had decreased to 651 mg of fluorescein kg^{-1} soil hr^{-1} (Table 3.3). Similar trends were also found from 2014 to 2016 at Lamberton (r = -0.704, P = 0.011) and Waseca (r = -0.881, P = 0.0002) where enzyme activity declined by 156 and 77 mg of fluorescein kg^{-1} soil hr^{-1}, respectively (Table 3.4).

Glucosidase Activity

Becker

At Becker there was a year by time of sampling interaction for glucosidase activity (Table 3.1). In 2014, samples taken Pre-V6 had the greatest and samples taken at Post-V6 had the lowest concentrations of p-nitrophenyl released kg^{-1} soil hr^{-1} (Table 3.6). Glucosidase activity increased after the Pre-V6 sampling time (Table 3.6). In 2015, glucosidase activity fluctuated during the growing season; it was greater at the beginning of the season, decreased after the Post-V6 sampling, and had a slightly though significant increase after the Pre-R1 sampling (Table 3.6). In 2016, glucosidase activity behaved much differently than in 2014 and 2015, it was greater in the beginning and at the end of the season than during the growing season (Table 3.6).

Lamberton

At Lamberton there was a year by time of sampling interaction for glucosidase activity (Table 3.1). In 2014, the greatest glucosidase activity, 930 mg of p-nitrophenyl released kg^{-1} soil hr^{-1}, was observed for soil samples taken at Pre-V6 and the lowest for samples collected at Post-V6, 421 mg of p-nitrophenyl released kg^{-1} soil hr^{-1} (Table 3.6). After the Post-V6 sampling, glucosidase activity started to slowly increase and reached a maximum 687 mg of p-nitrophenyl released kg^{-1} soil hr^{-1} by PM (Table 3.6). In 2015, there were fewer differences in glucosidase activity between soil sampling times. However, soil samples collected at 3wk-R1 were found to have 895 mg of p-nitrophenyl released kg^{-1} soil hr^{-1}, more than all other samples taken that year (Table 3.6). In 2016, glucosidase activity in soil samples taken at Post-V6 and Post-R1 were greater than those of samples collected at Pre-V6, Pre-R1, 3wk-V6 and 3wk-R1 (Table 3.6).

Waseca

At Waseca there was a year by time of sampling interaction for glucosidase activity (Table 3.1). In 2014, glucosidase activity at the beginning of the season (Pre-V6) was 993 mg of p-nitrophenyl kg^{-1} soil hr^{-1} which was greater than all other sampling times during the 2014 season (Table 3.6). Soil glucosidase activity decreased sharply by the next sampling at Post-V6 to 611 mg of p-nitrophenyl released kg^{-1} soil hr^{-1}, showing a slow increase during the season and by the end of the season glucosidase activity at PM, 817 mg of p-nitrophenyl released kg^{-1} soil hr^{-1}, was almost as high as at the beginning of the season (Table 3.6). In 2015, there were almost no changes in glucosidase activity throughout the growing season with the exception of soil samples collected at Post-R1 being lower than those for samples collected at PM, with 822 and 929 mg of p-nitrophenyl released kg^{-1} soil hr^{-1}, respectively (Table 3.6). In 2016, glucosidase activity fluctuated throughout the season starting with the lowest concentration observed at Pre-V6, 722 mg of p-nitrophenyl released kg^{-1} soil hr^{-1}, (Table 3.6). However, at the next sampling (Post-V6) glucosidase activity had already reached the second highest level for the season (830 mg of p-nitrophenyl released kg^{-1} soil hr^{-1}) (Table 3.6). Glucosidase activity in soil samples taken after Post-V6 decreased and remined low until the soil samples collected at 3wk-R1 when glucosidase activity increased again to levels similar to those observed at Post-V6 sampling (Table 3.6).

Acid Phosphatase Activity

Becker

No differences were observed for acid phosphatase activity at Becker (Table 3.1). However, there was a negative correlation between year and acid phosphatase activity. Acid phosphatase activity declined from 644 mg of p-nitrophenyl released kg^{-1} soil hr^{-1} in 2014 to 450 mg of p-nitrophenyl released kg^{-1} soil hr^{-1} in 2016 ($r = -0.716$, $P = 0.0088$) (Table 3.6). These results suggest that acid phosphatase activity could be impacted in coarse-textured soils under maize following maize.

Lamberton

At Lamberton there was a year by time of sampling interaction for phosphatase activity (Table 3.1). In 2014, acid phosphatase activity was greater in soil samples collected prior to the in-season fertilizer applications (Pre-V6 and Pre-R1) averaging 2,146 and 2,105 mg of p-nitrophenyl released kg^{-1} soil hr^{-1}, respectively (Table 3.6). In 2014, acid phosphatase activity decreased after fertilizer application (Table 3.7). In 2015 the application of fertilizer also decreased acid phosphatase activity but only for the N applied around the R1 stage (Table 3.7). The results showed that acid phosphatase activity at Pre-V6, Post-V6, and Pre-R1 ranged between 2,159 to 2,175 mg of p-nitrophenyl released hr^{-1} soil kg^{-1}, greater than all other sampling times (Table 3.7). In 2016, the application of fertilizer also decreased acid phosphatase activity around the R1 growth stage, but levels increased by the 3wk-R1 sampling (Table 3.7). Acid phosphatase activity for the 3wk-V6, 3wk-R1 and PM ranged from 1,903 to 1,926 mg of p-nitrophenyl released hr^{-1} soil kg^{-1}, greater than other sampling times in 2016 (Table 3.7).

Waseca

At Waseca there was a year by time of sampling interaction for phosphatase activity (Table 3.1). At the Waseca location, there were differences due to time of sampling only in 2014 (Table 3.7). Here, soil samples taken at Pre-V6 (1,644 mg of p-nitrophenyl released kg^{-1} soil hr^{-1}) were greater than soil samples taken Post-V6, 3wk-V6, 3wk-R1, and PM (Table 3.7). In 2014, acid phosphatase activity also decreased, though not always significantly, after the application of fertilizer at V6 the R1 maize growth stage (Table 3.7).

Sulfatase Activity

Becker

At Becker treatment had an effect on sulfatase activity (Table 3.1). Sulfatase activity in the control treatment averaged 93 mg of p-nitrophenyl released kg^{-1} soil hr^{-1} which was greater than the F100, F125, PP100, PP125, and Sp100 treatments which ranged from 71 to 81 mg of p-nitrophenyl released kg^{-1} soil hr^{-1} (Table 3.8). The treatments that received the largest N application rate (F125 and PP125) had the lowest enzyme activity recorded averaging 71 mg of p-nitrophenyl released kg^{-1} soil hr^{-1} (Table 3.8).

There was also an interactive effect between year and time of soil sampling (Table 3.1). In 2014, sulfatase activity in Pre-V6 soil samples had 138 mg of p-nitrophenyl released kg^{-1} soil hr^{-1}, larger than all other sampling times which ranged between 86 to 91 mg of p-nitrophenyl released kg^{-1} soil hr^{-1}(Table 3.9). In 2015, an opposite trend was observed and sulfatase activity was greater later in the season, particularly in samples collected at 3wk-R1 and lowest for the first four sampling times

(Table 3.9). In 2016, sulfatase activity had more variability throughout the season being greater in the first and last sampling times than compared with the mid-season sampling (Table 3.9). Over the coarse of the investigation there was also a negative correlation between sulfatase activity and year (r = -0.709, P = 0.0099).

Lamberton

At Lamberton there was a treatment effect (P = 0.0648) on sulfatase activity (Table 3.1). Sulfatase activity in the non-N fertilized control treatment and TSp50 were greater than PP125 and Sp75 while sulfatase activity in F100 was also greater than Sp75 (Table 3.8).

There was also an interactive effect ($P \leq 0.05$) between year and time of soil sampling (Table 3.1). In 2014, sulfatase activity was greater at Pre-V6 with 224 mg of p-nitrophenyl released kg^{-1} soil hr^{-1}, more than any other sampling time taken that growing season (Table 3.9). For the remainder of the 2014 season sulfatase activity was stable with the exception of a spike in enzyme activity observed at the sampling taken on 3wk-R1 (Table 3.9). The spike in activity could be due to the cumulative 14 mm of rain Lamberton received two to five days before the sampling date. The increased moisture likely stimulated sulfatase activity possibly resulting in the enzyme activity spike. In 2015, a similar behavior was observed compared with 2014; sulfatase activity of samples collected early in the season (Pre-V6 179 mg of p-nitrophenyl released kg^{-1} soil hr^{-1}) was greater than samples collected during the growing season with the exception for a spike in sulfatase activity in samples collected at 3wk-R1 (210 mg of p-nitrophenyl released kg^{-1} soil hr^{-1}) (Table 3.9). Three to six days before the 3wk-R1 sampling date, Lamberton received 8.1mm of precipitation which might account for the spike in sulfatase activity. In 2016, the results were similar to those observed for 2014 and 2015, where sulfatase activity was highest in the first sampling (Pre-V6 160 mg of p-nitrophenyl released kg^{-1} soil hr^{-1}) followed with a decrease as the season progressed. However, in 2016 there also a spike in sulfatase activity later in the season which was observed for samples collected at the Post-R1 (162 mg of p-nitrophenyl released kg^{-1} soil hr^{-1}) sampling. In this case Lamberton received 1.8 mm of precipitation 48 hours before soil sampling date. Like Becker there was a negative correlation in sulfatase activity and year (r = -0.757, P = 0.0044).

Waseca

At Waseca there was an interactive effect between year and time of soil sampling (Table 3.1). In 2014, sulfatase activity taken in soil samples at Pre-V6 had 678 mg of p-nitrophenyl released hr^{-1} soil kg^{-1}, greater than Pre-R1 and Post-R1 sampling times which ranged from 365 to 375 mg of p-nitrophenyl released kg^{-1} soil hr^{-1} (Table 3.9). In 2015, there were no differences in sulfatase activity between sampling times with samples ranging from 335 to 581 mg of p-nitrophenyl released kg^{-1} soil hr^{-1} (Table 3.9). In 2016, there was a large spike in sulfatase activity at 3wk-V6 (887 mg of p-nitrophenyl released kg^{-1} soil hr^{-1}) which was greater than all other sampling times which ranged between 263 to 464 mg of p-nitrophenyl released kg^{-1} soil hr^{-1} (Table 3.9). Given that Waseca received a cumulative 130 mm of rain over the week leading up to the day of sampling 3wk-V6, the moisture could account for the spike in sulfatase activity.

Across years, sulfatase activity declined annually at Becker (*r* = -0.709, P = *0.0099*) and Lamberton (*r* = -0.757, *P* = 0.0044). In 2014, sulfatase activity at Becker averaged 95 mg of p-nitrophenyl released kg^{-1} soil hr^{-1}, but declined to 63 mg of p-nitrophenyl released kg^{-1} soil hr^{-1} by 2016, respectively (Table 3.9). At Lamberton sulfatase activity in 2014 averaged 178 mg of p-nitrophenyl released kg^{-1} soil hr^{-1} but average concentrations declined to 140 mg of p-nitrophenyl released kg^{-1} soil hr^{-1} in 2016 (Table 3.9).

Discussion

Fluoroscein Diacetate (FDA) Hydrolysis

Although N rate and time of urea application did not have a significant effect on fluorescein hydrolytic activity at all locations, findings demonstrate that its activity can fluctuate across a growing season (Table 3.2, Table 3.3, Table 3.4). As only half of the treatments received a V6 urea application and a third of the treatments a R1 urea application, it is unclear why FDA activity across all treatments at Becker was greater one and three weeks after the respective urea applications. However, the increased microbial activity after N application could be due to an indirect effect. Given that added N will increase root growth and thus C inputs it is possible that the application of N had an indirect effect of stimulating soil C inputs through root and shoot biomass and exudates (Fauci and Dick, 1994). Given the low soil organic matter content at Becker any increase in soil C input would likely improve soil microbial activity unlike at Lamberton and Waseca where enzyme activity was primarily greater earlier in the growing season (Table 3.3, Table 3.4). Another possibility for the lack of clear difference due to rate or time of application at all three locations could be due to the maize roots keeping inorganic N in the soil solution low, along with N leaching, thus diminishing any immediate or direct effects of N application on soil enzyme activity (Fauci and Dick, 1994).

The negative correlation between FDA hydrolysis and year across all three locations suggests that overall microbial activity is declining the longer the soil is under continuous maize. This is similar to findings from two long term field experiments in Iowa which reported that microbial C and N were lower in fields under continuous maize than in multi-cropping systems, and not influenced by fertilization (Moore et al., 2000). The decline in microbial activity under continuous maize compared with a more diverse crop rotation may be due to factors such as a reduction in easily decomposable organic compounds returned to the soil, less root density, a less stable microclimate, and poorer soil structure (Moore et al., 2000). Soil enzyme activty was also reported to be lower under continous maize than under a forage system while microbial biomass C was particultarly lower in maize treated with 180 kg N ha^{-1} of NH_4NO_3 than with 360 kg N ha^{-1} as liquid hog manure (Lalande et al., 2005). However, if maize is added to a soybean rotation then enzyme activity is comparable to soil under continous soybean (Vargas Gil et al., 2009). Future long term studies on how continuous maize and N rates affect enzyme activity could provide more insight into which factors are influencing the decline in microbial activity.

Glucosidase Activity

The lack of any clear effect of N fertilizer rate or timing of application on glucosidase activity supports similar findings from Iowa where glycosidase activity was found to not be affected by the application of 180 kg N ha^{-1} before maize (Dodor and Tabatabai, 2005). However, the lack of effect of N fertilizer rate on glycosidase activity is contrary to two meta-analyses that reported that N fertilizer can increase glycosidase activity (Geisseler and Scow, 2014; Jian et al., 2016). The lack of significant correlations between year and glucosidase activity at all three locations is supported by findings from a 2-year tillage and crop rotation experiment on a clay loam soil (Zhang et al.,2014). They found no differences in glucosidase activity between continuous maize and a maize-soybean rotation (Zhang et al., 2014). However, glucosidase activity was reported to be affected by crop rotation, being greater under a four year rotation than under continuous maize (Dodor and Tabatabai, 2005). Here the lack of diversity of residue, plant cover, and root density were hypothesized as likely causes for the reduction of glucosidase activity under continuous maize compared with a more diverse crop rotation (Dodor and Tabatabai, 2005). Given that cropping systems have been shown to influence glycosidase activity, it is possible that glycosidase activity could decrease under a longer continuos maize system (Moore et al., 2000; Dodor and Tabatabai, 2005). Although other studies have reported variable findings on the effect of N fertilizer and continuous maize on glycosidase activity, it is difficult to compare the results among the studies due to the sensitivity of its activity to different conditions such as soil moisture or soil organic carbon stocks.

Acid phosphatase activity

As starter phosphorous fertilizer was annually applied at University of Minnesota recommended rates, the lack of effect by varying N fertilizer rates and timing of application suggests that in coarse textured soils phosphatase activity was not affected by N fertilizer rates or timing of application. Although there was a sampling time by year interaction at Lamberton and Waseca, the lack of any differences between sampling times in 2015 and 2016 at Waseca further corresponds to findings from other field trials where the application of N fertilizer was not shown to affect soil phosphatase activity (Dillard et al., 2015; Fereidooni et al., 2013; Lalande et al., 2005). At Lamberton, soil samples taken Pre-V6 and Pre-R1 in 2014 and 2015 had greater acid phosphatase activity than other sampling dates. However, with no N fertilizer treatment effects it is difficult to contribute the findings directly to the application of N fertilizer. The significance of sampling date could have been due to influences of environmental conditions such as moisture and temperature (Lalande et al., 2005). The dominant influence of sampling date on soil properties, including alkaline phosphomonoesterase activity, was also reported by Shi et al. (2013) on an 18 year maize-soybean rotation in eastern Canada fertilized with 0, 17.5, and 35 kg P ha^{-1}, respectively. The impact of temporal variations of phosphatase activity could also be affected by a combination of environmental conditions such as soil pH and P availability (Dick et al., 2000; Shi et al., 2013). The negative correlation between year and acid phosphatase activity at Becker but not Lamberton or Waseca could be due to the lower soil organic matter content at Becker. Acid phosphatase activity has been shown to be correlated with organic C and organic C could have an important role in protecting and maintaining acid phosphatase activity (Deng and Tabatabai, 1997b). Given that coarse textured soils tend to have lower soil organic matter and a greater leaching and erosion potential it is possible that intensive cultivation of continuous maize on coarse textured soils may reduce phosphatase activity.

Sulfatase activity

The treatment effects at Becker suggest that in coarse-textured soils, the application of N fertilizer increased sulfatase activity (Table 3.8). Given that sulfatase activity is often correlated with soil organic matter content it is possible that the application of N fertilizer helped stimulate the breakdown of soil organic matter with the arylsulfatase enzyme converting organic sulfur to sulfate sulfur. In the cases of plots receiving no urea but having greater sulfatase activity, the lack of inputs could possibly stabilized sulfatase activity. Given that soil acidification due to N addition has been shown to decrease arylsulfatase activity by 39.6% (Wang et al., 2016c), and that sulfate sulfur can be leached, is possible that single large applications of urea in the fall and at planting could both have repressed sulfatase activity over time. Given that the addition of N in incubated soils has been shown to increase S mineralization, it is possible that the application of urea at F125 and PP125 increased S mineralization earlier in the season allowing more opportunity for it to leave the soil system (Wang et al., 2016; Ghani, et al., 1992). Due to linear relationships between soil organic C, total N, and total S in soils (Gharmakher et al., 2009), the low N holding capacity and soil organic matter content at

Becker suggests that without organic amendments, growing continuous maize will continue to reduce sulfatase activity over time.

Although not as drastic as Becker, the increased sulfatase activity in the control plots and TSp50 treatments in Lamberton could be due to the soil organic sulfur being mineralized earlier in the season after larger applications of urea (Wang et al., 2016). A 31 month study comparing the application of 100 Mg ha^{-1} of poultry manure, sewage sludge, barley straw (*Hordeum vulgare* L.) or fresh alfalfa (*Medicago sativa* L.) reported that sulfatase activity substantially increased after the application of these organic amendments (Martens, et al., 1992). Furthermore, the first application of 25 Mg ha^{-1} for each amendment resulted in the greatest increase in activity compared with the other application rates (Martens, et al., 1992). This spike in activity may be not clearly demonstrated by time of sampling at Becker, Lamberton or Waseca but it is possible that earlier applications of urea at planting could have increased sulfatase activity.

At Lamberton and Waseca soil moisture seemed to affect sulfatase activity. A spike in sulfatase activity was often preceded by precipitation in the days leading up to the sampling date. In particular, the significantly large spike of sulfatase activity after 130 mm of rain over six days at Waseca is the likely reason for the increase in sulfatase activity. Increased precipitation has been found to significantly increase labile SOC in the 0-10cm depth (Zhou et al., 2013). Macro and microclimatic changes have been shown to influence microbial activity particularly when soil pore space is half to two thirds saturated (Fekete et al., 2007). However, in anaerobic or near anaerobic conditions microbial activity will decrease (Fekete et al., 2007). In forest soils arylsulfatase activity has been found to be greater in the spring after snowmelt than from July to August when conditions are drier (Fekete et al., 2007).

The significant negative correlation of year and sulfatase activity at Becker and Lamberton suggests that soil sulfatase activity is declining under a continuous maize system. Given that sulfatase activity has been demonstrated to be responsive to the application of organic C, the decline in activity may be due to a reduction in SOC under maize following maize (Deng and Tabatabai, 1997; Falih and Wainwright, 1996; Martens, et al., 1992).

Conclusions

Microbial activity fluctuates across the growing season and can change over time due to cropping system, particularly in coarse textured soils. In this study the decline in FDA hydrolysis at all three locations suggests that intensive management of continuous maize negatively affects soil microbial activity. Although enzyme activity declined at all three sites, there was no correlation between glucosidase activity and year. Given that maize residue was returned and ploughed in every year, it is likely that the residue was an adequate source of energy for glucosidase enzymes. The decline in acid phosphatase activity in coarse- but not fine-textured soils, suggests that soil organic carbon stocks influenced acid phosphatase activity. The effect of treatment on sulfatase activity at Becker and Lamberton suggests that the application of urea in the fall or at planting resulted in a decline in activity later in the growing season. Additionally, the spikes in sulfatase activity after precipitation events hint at the importance of soil moisture in enzyme activity. A greater number of long-term field trials on the effect of continuous maize, N application, and moisture are needed to better understand the complex interactions influencing soil enzyme activity.

Table 3.1. Tests for fixed effects of urea fertilizer rate and timing of application on FDA hydrolysis, glucosidase activity, phosphatase activity, and sulfatase activity from agricultural soils sampled throughout three growing seasons in Becker, Lamberton, and Waseca, MN

	FDA hydrolysis			Glucosidase activity			Phosphatase activity			Sulfatase activity		
Effect	Becker	Lamberton	Waseca	Becker	Lamberton	Waseca	Becker	Lamberton	Waseca	Becker	Lamberton	Waseca
	Pr>F											
Treatment	0.8636	0.5958	0.0295	0.6986	0.0887	0.1479	0.751	0.8031	0.5592	0.0012	0.0648	0.9747
Time of Sampling	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.1302	<.0001	<.0001	<.0001	<.0001	<.0001
Time of Sampling x Treatment	0.7288	0.4229	0.8624	0.7539	0.7765	0.3367	0.676	0.9266	0.9342	0.4555	0.999	0.7029
Year	0.0407	0.0497	<.0001	0.016	0.0009	0.0263	0.0678	<.0001	0.49	0.0189	0.0132	0.8352
Year x Treatment	1	0.9988	0.9428	0.9957	0.8306	0.9989	1	0.9966	1	0.9155	0.9933	1
Year xTime of sampling	0.0697	<.0001	<.0001	<.0001	<.0001	<.0001	0.1286	<.0001	<.0001	<.0001	<.0001	<.0001
Year x Time of sampling x Treatment	0.7289	0.9856	0.9999	0.9918	0.7919	0.6177	0.6547	0.9161	0.6289	0.1574	0.9796	0.6902

Table 3.2. The effect of Time of sampling on FDA Hydrolysis at Becker

Time of sampling †	FDA Hydrolysis mg of fluorescein released kg^{-1} soil hr^{-1}	
Pre-V6	703	c
Post-V6	769	b
3 wks-V6	931	a
Pre-R1	703	c
Post-R1	780	b
3 wks-R1	668	d
PM	641	e

†Pre-V6, soil samples taken 1 week before V6 urea applications; Post-V6, soil samples taken 1 week after V6 urea applications; 3wk-V6, soil samples taken 3 weeks after V6 urea applications; Pre-R1, soil samples taken 1 week before R1 urea application; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications; PM, soil samples taken at physiological maturity.

¶Means within the column followed by different lower case letters are significantly different at the p-level 0.05

Table 3.3. The effect of year on FDA hydrolysis at Becker

Year	FDA Hydrolysis mg of fluorescein released kg^{-1} soil hr^{-1}	
2014	885	a*
2015	695	b
2016	651	b

*Means within the column followed by different lower case letters are significantly different at the p-level 0.05.

Table 3.4. The effect of year x time of sampling on FDA Hydrolysis at Lamberton and Waseca

	Lamberton						Waseca					
Time of sampling †	2014		2015		2016		2014		2015		2016	
	mg of fluorescein released kg^{-1} soil hr^{-1}											
Pre-V6	931	a*	656	cde	540	efg	543	b	618	a	392	fgh
Post-V6	636	cdef	557	efg	524	fg	434	def	443	cde	410	ef
3 wks-V6	521	fg	534	efg	605	cdefg	401	efg	433	def	438	cdef
Pre-R1	865	b	593	defg	615	cdef	441	cde	391	fgh	-	-
Post-R1	720	c	534	efg	603	cdefg	479	cd	482	c	335	ij
3 wks-R1	692	cd	534	efg	520	fg	459	cd	355	hij	360	ghi
PM	657	cde	487	g	520	fg	461	cd	322	j	364	ghi

†Pre-V6, soil samples taken 1 week before V6 urea applications; Post-V6, soil samples taken 1 week after V6 urea applications; 3wk-V6, soil samples taken 3 weeks after V6 urea applications; Pre-R1, soil samples taken 1 week before R1 urea application; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications; PM, soil samples taken at physiological maturity.

*Means within the column followed by different lower case letters are significantly different at the p-level 0.05.

Table 3.5. The effect of treatment on FDA hydrolysis at Waseca

Treatment	mg of fluorescein released kg^{-1} soil hr^{-}	
C†	433	abc*
F100	420	bc
F125	425	bc
PP100	402	c
PP125	410	c
Sp75	456	ab
Sp100	467	a
TSp50	421	bc
TSp75	419	c
TSp100	422	bc

†C, control no N applied; F100, N applied in the fall at 100% of the recommended rate; F125, N applied in the fall at 125% of the recommended rate; PP100, N applied at pre-plant in the spring at 100% of the recommended rate; PP125, N applied at pre-plant in the spring at 125% of the recommended rate; Sp75, half of the N applied at pre-plant in the spring and half at V6 at 75% of the recommended rate; Sp100, half of the N applied at pre-plant in the spring and half at V6 at 100% of the recommended rate; TSp50, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 50% of the recommended rate; TSp75, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 75% of the recommended rate; TSp100, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 100% of the recommended rate

*Means within the column followed by different lower case letters are significantly different at the p-level 0.05.

Table 3.6. The effect of year x time of sampling on glucosidase activity at Becker, Lamberton, and Waseca

	Becker						Lamberton						Waseca					
Time of sampling †	2014		2015		2016		2014		2015		2016		2014		2015		2016	
	mg of p-nitrophenyl released kg^{-1} soil hr^{-1}																	
Pre-V6	281	ab*	246	bc	190	efgh	930	a	682	bcde	581	g	993	a	910	abc	722	gh
Post-V6	158	hi	306	a	195	defgh	421	i	693	bc	694	bc	611	i	894	abcd	830	bcde
3 wks-V6	180	fgh	217	def	123	i	494	h	666	bcdef	589	g	667	h	845	bcde	822	cdef
Pre-R1	198	defg	198	defg	172	gh	520	h	706	b	628	ef	673	h	896	abcd		
Post-R1	217	cdef	225	cde	172	gh	649	cdef	640	def	688	bcd	672	h	822	cdef	733	fgh
3 wks-R1	223	cde	249	bc	138	i	625	fg	895	a	632	ef	756	efg	902	abcd	804	defg
PM	201	defg	234	cd	221	cdef	687	bcd	695	bc	657	bcdef	817	cdefg	929	ab	883	bcd

†Pre-V6, soil samples taken 1 week before V6 urea applications; Post-V6, soil samples taken 1 week after V6 urea applications; 3wk-V6, soil samples taken 3 weeks after V6 urea applications; Pre-R1, soil samples taken 1 week before R1 urea application; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications; PM, soil samples taken at physiological maturity.

*Means within the column followed by different lower case letters are significantly different at the p-level 0.05.

Table 3.7. The effect of year x time of sampling on acid phosphatase activity at Lamberton and Waseca

	Lamberton						Waseca					
Time of sampling †	2014		2015		2016		2014		2015		2016	
	mg of p-nitrophenyl released kg^{-1} soil hr^{-1}											
Pre-V6	2146	a*	2159	a	1736	c	1644	ab	1709	ab	1504	abc
Post-V6	1448	e	2175	a	1713	cd	1232	c	1739	ab	1468	abc
3 wks-V6	1721	cd	1879	b	1926	b	1123	c	1533	abc	1499	abc
Pre-R1	2105	a	2166	a	1631	d	1573	abc	1597	abc	-	-
Post-R1	1892	b	1903	b	1406	e	1338	abc	1484	abc	1293	bc
3 wks-R1	1508	e	1917	b	1903	b	1171	c	1771	a	1437	abc
PM	1417	e	1869	b	1907	b	1158	c	1400	abc	1314	abc

†Pre-V6, soil samples taken 1 week before V6 urea applications; Post-V6, soil samples taken 1 week after V6 urea applications; 3wk-V6, soil samples taken 3 weeks after V6 urea applications; Pre-R1, soil samples taken 1 week before R1 urea application; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications; PM, soil samples taken at physiological maturity.

*Means within the column followed by different lower case letters are significantly different at the p-level 0.05

Table 3.8. The effect of treatment on sulfatase activity at Becker and Lamberton

Treatment †	Becker		Lamberton	
	mg of p-nitrophenyl released kg^{-1} soil hr^{-1}			
C	93	a**	175	a*
F100	81	bcd	167	abc
F125	72	d	156	abcd
PP100	76	cd	163	abcd
PP125	71	d	147	cd
Sp75	89	ab	147	d
Sp100	80	bcd	166	abcd
TSp50	92	ab	171	ab
TSp75	85	abc	157	abcd
TSp100	89	ab	153	bcd

†C, control no N applied; F100, N applied in the fall at 100% of the recommended rate; F125, N applied in the fall at 125% of the recommended rate; PP100, N applied at pre-plant in the spring at 100% of the recommended rate; PP125, N applied at pre-plant in the spring at 125% of the recommended rate; Sp75, half of the N applied at pre-plant in the spring and half at V6 at 75% of the recommended rate; Sp100, half of the N applied at pre-plant in the spring and half at V6 at 100% of the recommended rate; TSp50, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 50% of the recommended rate; TSp75, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 75% of the recommended rate; TSp100, a third of the N applied at pre-plant in the spring, a third at V6 and a third at R1 at 100% of the recommended rate.

**Means within the column followed by different lower case letters are significantly different at the p-level 0.05.

* Means within the column followed by different lower case letters are significantly different at the p-level 0.10.

Table 3.9. The effect of year x time of sampling on sulfatase activity at Becker, Lamberton, and Waseca

	Becker						Lamberton						Waseca					
Time of sampling †	2014		2015		2016		2014		2015		2016		2014		2015		2016	
	mg of p-nitrophenyl released kg^{-1} soil hr^{-1}																	
Pre-V6	138	a*	70	cdefgh	70	cdefg	224	a	179	cd	160	defg	678	ab	581	abcd	346	cde
Post-V6	91	bc	83	cdefg	59	h	173	de	155	efgh	136	ghi	611	abc	528	bcde	296	de
3 wks-V6	86	cdef	67	efgh	61	gh	166	de	132	i	127	i	472	bcde	478	bcde	887	a
Pre-R1	86	cdef	68	defgh	64	fgh	168	de	161	defg	133	hi	446	bcde	429	bcde	-	-
Post-R1	86	cdef	91	bc	58	h	160	defg	119	i	162	def	365	cde	335	cde	263	e
3 wks-R1	90	bcd	143	a	55	h	195	bc	210	ab	140	fghi	375	cde	546	bcde	265	e
PM	89	cde	112	b	74	cdefg	163	def	177	cd	124	i	455	bcde	574	abcd	464	bcde

†Pre-V6, soil samples taken 1 week before V6 urea applications; Post-V6, soil samples taken 1 week after V6 urea applications; 3wk-V6, soil samples taken 3 weeks after V6 urea applications; Pre-R1, soil samples taken 1 week before R1 urea application; Post-R1, soil samples taken 1 week after R1 urea applications; 3 wks-R1, soil samples taken 3 weeks after R1 urea applications; PM, soil samples taken at physiological maturity.

*Means within the column followed by different lower case letters are significantly different at the p-level 0.05.

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