Fate of Pre-Plant and Split-Applied 15Nitrogen Enriched Urea in Corn

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Jared Arthur Spackman

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Fabián Fernández

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This dissertation is dedicated to my wife Kathryn and our sons in the hopes that they will continuously seek knowledge and wisdom throughout their lives.

ABSTRACT

Nitrogen (N) is essential to produce high yielding corn (*Zea mays* L.), but excess fertilization before rapid corn N uptake may result in N loss from the soil-corn system that reduces fertilizer N use efficiency, causes an economic loss for producers, and negatively impacts the environment. Urea fertilizer applications at planting are common, but recent years of wet springs in the U.S. upper Midwest has fostered a greater interest in split-applications that may avoid early spring N loss and improve fertilizer N supply to the crop. Fertilizer N rate, application timing, soil physical and chemical properties, and weather patterns can modify fertilizer-derived N distribution (FDN) in the soil profile, uptake by the crop, and potential for N loss in the year of application and subsequent years. Thus, the objectives of this study were to investigate the effects of fertilizer N rate and application timing on 1) FDN distribution and form in the soil, 2) FDN and soil-derived N (SDN) uptake and partitioning by the corn crop, and 3) fertilizer recovery and reuse in the soil-corn system over two consecutive growing seasons.

Three studies were initiated in both 2014 (Becker14, Clara City14, Waseca14) and 2015 (Becker15, Lamberton15, Waseca15) in Minnesota, at sites that represented agronomically important soils. Urea fertilizer (46% N) was applied at planting at 45 kg N ha^{-1} increments from 0 to 270 or 315 kg N ha^{-1}. An additional treatment was split-applied as 45 kg N ha^{-1} at planting and 90 kg N ha^{-1} when the corn had four fully developed leaves (V4). Labeled ^{15}N urea (5 atom %) fertilizer was applied to microplots in the 45, 135, and 225 kg N ha^{-1} treatments, as well as the 45/90 kg N ha^{-1} split-application treatment. Soil samples were collected in the first growing season within eight days of ^{15}N urea fertilizer application (PA), when the corn had eight fully developed leaves (V8),

at tasseling (R1), and post-harvest (PHY1). In the second growing season, soil samples were collected at pre-plant (PPY2) and post-harvest (PHY2). Aboveground plant samples were collected at V8, R1, and physiological maturity (R6) in the first growing season, and R6 in the second growing season.

At PA, 63 to 112% of FDN was recovered from the top 60 cm of the soil profile across all sites, except Becker14 and Clara City14 where 55 and 43% of the applied FDN were recovered by corn averaged across all treatments, respectively. Low recovery of FDN at Becker14 was likely due to N leaching through the loamy sand soil profile following greater-than-normal April through June precipitation. At Clara City14, low recovery of FDN was due to NH_3 volatilization from inadequate incorporation of urea into the soil profile. Of the total FDN in the soil at PA, 72 to 90% was in the soil organic N fraction and temporarily protected from loss. The majority of soil FDN was in the top 15 cm of the soil profile, but FDN was observed in all soil sampling depths indicating that leaching of NO_3^- and soluble organic FDN is rapid irrespective of the soil texture. The soil inorganic FDN concentration was greatest immediately after fertilization but decreased to background levels (<10% of the applied N rate) by V8 or R1. Likewise, the concentration of FDN in corn biomass was greatest early in the season but decreased as soil FDN concentration decreased and as the corn increasingly assimilated inorganic SDN.

At the end of the first growing season, approximately 20 to 47% of FDN was recovered in the soil-crop system. Aboveground corn FDN recovery ranged from low values of 2.8 to 4.4% at Becker15 (loamy sand) to higher values of 34.0 to 41.9% at Lamberton15 (loam) that reflected the N loss potential of each soil. Very low FDN recovery at Becker15 indicates that urea fertilizer applied at planting should not be done at sites with coarse-textured soils. Averaged across sites and treatments, 28% of the aboveground FDN was in the stover, 69% was in the grain, and 4% was in the cobs. Because only a small portion of FDN was returned to the soil-crop system from the first year corn residue and because soil organic FDN was fairly stable, ≤ 7.4 kg FDN ha^{-1} was assimilated in the aboveground biomass across all sites at the end of the second year, indicating residual FDN does not supply an agronomically important amount of N to the succeeding crop.

The 45/90 kg N ha^{-1} split-application improved corn grain yield over the 135 kg N ha^{-1} single application at planting on coarse-textured soils by 3.3 Mg ha^{-1} on average but there was no yield improvement on fine-textured soils. Likewise, the split-application had greater FDN uptake values than the 135 kg N ha^{-1} treatment during the early vegetative development stages but did not differ from the 135 kg N ha^{-1} treatment at R6 in either aboveground FDN recovery or the soil FDN content. Partitioning of FDN to plant parts were similar between the split- and the single application. These results indicate that split-applications will likely have the greatest improvement in fertilizer use efficiency and grain production on coarse-textured soils that are prone to significant N loss.

Overall, this study illustrated that a single or split-application of urea may be an acceptable management strategy when the soil and environmental conditions do not favor N loss. However, if spring conditions continue to be wetter-than-normal, urea applications done at planting or as early split-applications are poorly retained in the soil resulting in poor recovery efficiency by the corn crop and recycling for future crops. Other N sources may need to be considered, such as polymer-coated urea or anhydrous ammonia, that delay nitrification until later in the growing season for decreased risk of leaching and denitrification. Delaying the split-application a few weeks longer may be another way to avoid spring leaching events for improved FDN recovery. While fertilizer N significantly increased yield at 11 of 12 site-years in this study, ultimately, SDN accounted for most of the total N uptake and yield potential of the crop. This result re-emphasizes the importance of maintaining soil health and its N supplying capacity because its depletion will result in increasingly expensive input costs to achieve similar production levels.

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List of Abbreviations

ANI	Added nitrogen interaction
FDN	Fertilizer-derived nitrogen
FD_{NH4-N}	Fertilizer-derived ammonium–N
FD_{NO3-N}	Fertilizer-derived nitrate–N
FD_{ON}	Fertilizer-derived organic nitrogen
FD_{TIN}	Fertilizer-derived total inorganic nitrogen
FD_{TN}	Fertilizer-derived total nitrogen
$FNUE_{15N}$	Fertilizer nitrogen use efficiency using the isotopic method
$FNUE_{diff}$	Fertilizer nitrogen use efficiency using the difference method
PA	Post-^{15}N enriched urea application at planting
PHY1	Post-harvest in the year of ^{15}N application
PHY2	Post-harvest in the year after ^{15}N application
PPY2	Pre-plant in the year after ^{15}N application
R1; R6	Reproductive development stage one or six
SDI	Shannon diversity index
SDN	Soil derived nitrogen
TIN	Total inorganic nitrogen–N
V4; V8	Vegetative phenological development stage four or eight

CHAPTER 1: SOIL- AND FERTILIZER-DERIVED NITROGEN RECOVERY IN CORN AFTER TWO YEARS OF ^{15}NITROGEN ENRICHED UREA FERTILIZATION

SYNOPSIS

Nitrogen is an essential component of today's agriculture system to produce high-yielding corn (*Zea mays L.*), but over-application of fertilizer N or application before rapid corn N uptake may result in N loss that negatively impacts the environment and reduces fertilizer use efficiency. To better understand how fertilizer rate and application timing impact corn grain yield and fertilizer recovery in the soil-corn system, six two-year field studies were conducted at Minnesota field sites. Three studies were initiated in 2014 (Becker14, Clara City14, Waseca14) and three studies in 2015 (Becker15, Lamberton15, Waseca15). Urea fertilizer was applied at planting at 45 kg N ha^{-1} increments from 0 to 270 or 315 kg N ha^{-1}. An additional treatment was split-applied as 45 kg N ha^{-1} at planting and 90 kg N ha^{-1} at the V4 corn development stage to coincide with the timing of the pre-sidedress nitrate test. Labeled ^{15}N urea fertilizer was applied to microplots in the 45, 135, and 225 kg N ha^{-1} treatments as well as the split-application. Aboveground corn samples were collected at R6 and soil samples were collected post-harvest at the end of the first (PHY1) and second (PHY2) years. At eight of twelve site-years, corn grain yield increased linearly with N rate, indicative of significant N loss following spring precipitation. Split-applications improved corn grain yield over a single application at planting on coarse-textured soils by 3.3 Mg ha^{-1} but there was no yield improvement on fine-textured soils. Following wet spring conditions, fertilizer loss at Becker14, Becker15, Clara City14, and Waseca14 resulted in no significant treatment difference in aboveground soil-derived N (SDN) uptake, which represented 58 to 96% of the total N taken up. Aboveground corn fertilizer-derived N (FDN) uptake increased with increasing application rate at all sites but FDN recovery was variable ranging from low values of 2.8 to 4.4% at Becker15 and high values of 34.0 to 41.9% at Lamberton15. Total FDN (soil plus aboveground plant) recovery ranged from 20 to 47% at PHY1 and 16 to 27% at PHY2. In sites with high N loss, fertilizer N use efficiency estimates using the difference method ($FNUE_{diff}$) were generally larger and more variable than estimates using the isotope method ($FNUE_{15N}$), but the estimates were similar when N loss was low. This study illustrates that urea fertilizer applied at planting may be an effective N management strategy when the potential for N loss is low, but under wet spring conditions, urea is poorly retained in the soil resulting in poor recovery efficiency and recycling for future crops.

INTRODUCTION

Agricultural grain production has managed to keep pace with population growth due in part to nitrogen (N) fertilizer use, but nitrogenous fertilizers are a significant source of reactive N that negatively impacts air and water quality and modifies natural ecosystems (Cassman et al., 2003). The anthropogenic footprint associated with N fertilizer use may be reduced by improving fertilizer use efficiency within the soil-crop system. As fertilizer N recovery is maximized, a larger portion of N ends up in the marketable product [i.e., corn grain] or applied N is stabilized into soil organic matter (Allen et al., 1973; Recous et al., 1988a; González-Prieto et al., 1997). Fertilizer-derived N (FDN) stabilized as organic N compounds in soil organic matter may increase soil fertility if there is a net increase in total soil N (Cassman et al., 2003). Nitrogen use efficiency is influenced by soil physical and chemical characteristics including soil texture, pH, and organic matter; field management practices; fertilizer rate, source, and timing; and external factors such as precipitation patterns, disease, or pest pressure (Van Cleemput et al., 2008; Raun and Schepers, 2008).

A crop's fertilizer recovery efficiency is estimated using one of two methods. In the traditional, non-^{15}N approach, fertilizer N use efficiency is calculated using the difference method ($FNUE_{diff}$).

$$FNUE_{diff}(\%) = \frac{N\ uptake_{fertilized\ treatment} - N\ uptake_{unfertilized\ treatment}}{fertilizer\ N\ applied} \times 100 \qquad [1.1]$$

This method measures the net effect of fertilization on N recovery in the crop and assumes that N fertilization has no impact on microbial activity (especially mineralization-immobilization transformations), or root growth and N uptake patterns between the fertilized and non-fertilized plots (Sørensen, 1982; Jokela and Randall, 1997; Schindler and Knighton, 1999; Cassman et al., 2002; Stevens et al., 2005a). The second method uses fertilizer labeled with stable isotope ^{15}N as a tracer. Labeled fertilizer is the only practical way to differentiate between FDN and soil-derived N (SDN) and to assess total fertilizer N recovery and loss to the environment under field conditions (Sanchez and Blackmer, 1988). Fertilizer N use efficiency using the isotopic method ($FNUE_{15N}$) is calculated as the quotient of the mass of FDN recovered in the plant and the applied fertilizer N rate multiplied by 100 to convert to a percentage. The $FNUE_{15N}$ method assumes that plant N uptake is not influenced by isotopic substitution or biological interchange where ^{15}N labeled ions replace non-labeled ions through mineralization-immobilization processes (Schindler and Knighton, 1999; Harmsen, 2003). Because each method has potential shortcomings, Jansson (1958) recommended calculating fertilizer N recovery using both methods.

Labeled ^{15}N budgets have been successfully used to quantify FDN recovered in aboveground biomass, in the soil, and as environmental loss in a variety of cropping systems including barley (*Hordeum vulgare* L.) (Dev and Rennie, 1979; Chantigny et al., 2004), wheat (*Triticum aestivum* L.) (Recous et al., 1988b; a; Tran and Tremblay, 2000; Liang et al., 2013; Wang et al., 2016), sorghum (*Sorghum bicolor* L. Moench) (Harmsen and Moraghan, 1988), sudangrass (*Sorghum sudanense* L.) (Westerman et al., 1972; Allen et al., 1973), and corn (Sanchez and Blackmer, 1988; Timmons and Baker, 1992; Reddy and Reddy, 1993; Jokela and Randall, 1997; Tran and Giroux, 1998; Stevens et al., 2005a; b). An additional advantage provided by labeled ^{15}N budgets is that fertilizer N can be quantified over multiple growing seasons providing additional information regarding the long-term recovery and stability of FDN in the soil.

Past research has shown that N fertilizer use efficiency is reduced and fertilizer loss increases with increasing fertilizer application rates, especially when rates exceed crop demand (Hart et al., 1986; Francis et al., 1993; van Groenigen et al., 2010; Burzaco et al., 2013; Struffert et al., 2016). Despite decades of N fertilizer research, selecting the optimal N fertilizer rate that minimizes fertilizer loss and optimizes grain yield is challenging because of uncertain future weather patterns and because the soil naturally supplies plant-available N through mineralization in response to soil moisture and temperature conditions and field management practices (Fernandez et al., 2017). Additionally, the timing of fertilizer application modifies when fertilizer N is available for crop uptake, soil immobilization-mineralization processes, or N loss mechanisms.

The objective of this study was to utilize ^{15}N labeled urea fertilizer to investigate the effect of fertilizer rate and application timing on SDN and FDN recovery in the soil-corn system at the end of the first and second growing seasons. An additional objective was to compare the $FNUE_{diff}$ and $FNUE_{15N}$ methods.

MATERIALS AND METHODS

Site Description

Field experiments were conducted at six field sites over two consecutive growing seasons in Minnesota, USA. For the 2014 to 2015 growing seasons, field sites were established at the University of Minnesota Sand Plain Research Farm at Becker (Becker14), on a cooperator farmer field at Clara City (Clara City14), and at the University of Minnesota Research and Outreach Center at Waseca (Waseca14). For the 2015 to 2016 growing seasons, field sites were established at the University of Minnesota Sand Plain Research Farm at Becker (Becker15) and the University of Minnesota Research and Outreach Centers at Lamberton (Lamberton15) and Waseca (Wasesca15). The soil series were Hubbard loamy sand at Becker sites, Bearden-Quam silty clay loam complex at Clara City14, Normania loam at Lamberton15, and Nicollet-Webster clay loam complex at Waseca sites. Additional soil physical and chemical properties were reported in Chapter 3. All field sites were planted to corn the year before and during the study and were rain-fed except the two field sites at Becker that received 266, 188, and 203 mm of supplemental irrigation throughout the growing season in 2014, 2015, and 2016, respectively as determined by the water-balance approach (Steele et al., 2010). Air temperature and precipitation data were obtained for each site from the nearest National

Weather Service station (NOAA, 2020). Total monthly and 30-yr normal precipitation values were tabulated individually by site while the three 2014 to 2015 or three 2015 to 2016 sites' 30-yr normal monthly minimum and maximum air temperatures were averaged. Individual site 30-yr normal monthly minimum and maximum temperature values were within ±1.0 °C of the reported averaged 30-yr normal except at Becker in June, July, and August in 2014 and 2016 that were 1.1 to 1.4 °C warmer

Experimental Design and Treatment Details

Treatments were arranged in a randomized complete block design with four replications. Treatment plots were 21.3 m x 3.0 m at Becker and Lamberton sites, 12.2 m x 3.4 m at Clara City14, and 15.2 m by 4.6 m at Waseca sites (Figure 1.1). Nitrogen fertilizer treatments consisted of urea (46–0–0, $N–P_2O_5–K_2O$) applied within 7 d of planting at 45 kg N ha^{-1} rate increments from 0 to 270 kg N ha^{-1} at all sites, except Becker sites that received an additional rate of 315 kg N ha^{-1}. Urea was incorporated into the soil profile within 24 h of fertilization using 6 mm of irrigation or a field cultivator to minimize ammonia volatilization loss potential. An additional fertilizer treatment (45/90) consisted of 45 kg N ha^{-1} as urea ammonium nitrate (28–0–0, $N–P_2O_5–K_2O$) dribbled over the seed row as a starter application and top-dressed at the V4 corn phenological development stage (Abendroth et al., 2011) with 90 kg N ha^{-1} of granular urea impregnated with the urease inhibitor N-(n-butyl) thiophosphoric triamide, Agrotain (Koch Fertilizer LLC, Wichita, KS).

Within select treatments (45, 135, 225 kg N ha^{-1} rates and the 45/90 kg N ha^{-1} split-application), unconfined microplots were established with dimensions of 4.0 m x 2.3 m at

Becker and Lamberton sites, 3.4 m x 2.8 m at Clara City14, and 2.4 m x 3.8 m at Waseca sites (Figure 1.1). The microplots were centered on the width dimension of the treatment plot and placed 1.5 m from one end of the treatment plot to minimize edge effects on plants within the microplot.

In the first year, unlabeled fertilizer was applied to the treatment plot, except for the microplot area that was covered with plastic sheeting during fertilization to prevent contamination. Within seven days of unlabeled fertilizer N application, the microplots were fertilized at the appropriate rate with 5 atom % ^{15}N enriched urea dissolved in 1.7 L of deionized water and broadcast sprayed over the entire microplot area using a CO_2 pressurized hand-sprayer for the 45, 135, and 225 kg N ha^{-1} treatments. For the 45/90 kg N ha^{-1} treatment, 45 kg urea-N ha^{-1} was banded over the seed row at planting and 90 kg ha^{-1} of ^{15}N enriched urea was uniformly applied to the soil surface at V4 being careful to avoid vegetative tissues. Because the ^{15}N enriched fertilizer did not include a urease inhibitor, the fertilizer was immediately incorporated into the soil profile with 6 mm of irrigation at Becker sites, 6 mm of water sprinkled over the soil surface with a water tank at Waseca and Lamberton sites, or with a hand rake at Clara City14. In the second year, unlabeled fertilizer was applied to the entire treatment plot including the microplot area.

Agronomic Practices and Sample Collection and Analysis

Other than N, each site was fertilized and limed according to University guidelines to maximize corn grain yield (Kaiser et al., 2018). Corn was planted during April or May in a 76 cm row spacing at all sites except Clara City that was planted in a 56 cm row spacing. DEKALB DKC44-13 RIB AR was planted at Clara City and Waseca sites while

Becker and Lamberton sites were planted with Pioneer P9917AMX with a final stand count of 77,000 to 87,000 plants ha^{-1}.

Treatment plots containing microplots were delineated into a ^{15}N enriched and non-enriched sampling and harvest areas (Figure 1.1). Within these areas, plant samples were collected at physiological maturity (R6) and soil samples were collected post-harvest. To minimize the risk of sample cross-contamination, the non-^{15}N enriched soil and plant samples were collected and processed first using dedicated equipment for that purpose. Also, to avoid contamination, ^{15}N enriched microplot samples were processed from the lowest to the highest applied N rate with a thorough cleaning of equipment between samples.

A six-aboveground plant composite sample was collected from both the non-^{15}N enriched sampling area and the ^{15}N enriched microplot area. Because of frost and hail damage to Waseca14 plots in 2014, R6 corn biomass was not sampled from the non-^{15}N enriched sampling area. Plant tissue samples were chipped, dried to constant mass in a forced-air oven (60 °C), ground, and analyzed for total N concentration by combustion analysis (Horneck and Miller, 1998) and ^{15}N concentration (UC Davis, 2020). The mass of N in the plant tissue was determined as the product of N concentration and dry matter mass.

Grain yield was measured in October by harvesting two rows from each plot and adjusting grain moisture content to 155 g kg^{-1} (Figure 1). First, all ears were hand-harvested from the microplot area and the remaining aboveground corn biomass was cut at ground level, bundled, labeled with the plot number, and removed from the microplot

(Spackman and Fernández, 2020). Following bulk plot harvest to clear the field, non-^{15}N enriched biomass was raked off the microplot area and microplot biomass was chipped and returned to the appropriate microplot. Frozen soil conditions at Waseca14 delayed the return of ^{15}N enriched biomass to the microplots and post-harvest soil sampling until early April 2015.

Post-harvest soil samples were collected each year from within the non-^{15}N enriched sampling area and the center of the ^{15}N enriched microplot area at depths of 0- to 30-, 30- to 60-, and 60- to 90-cm depths. A two-core (5-cm diameter) composite soil sample was collected using a hydraulic probe in the non-^{15}N enriched sampling area where one core was collected from within the corn row and the second core was collected between corn rows. In the ^{15}N enriched microplot, a three-core composite soil sample was collected using a hydraulic probe where one core was collected from within the corn row and two cores were collected between the corn rows. The greater number of cores collected from within the microplot was to increase the precision of sample estimates of the ^{15}N enrichment of the soil (Gomez and Gomez, 1984). Soil samples were dried in a forced-air oven at 35 °C until constant mass, ground to pass through a 2-mm sieve, and analyzed for nitrate-N (Gelderman and Beegle, 2012) and ammonium-N (Bremner and Mulvaney, 1982). Soil samples from the ^{15}N-enriched microplot and non-^{15}N enriched sampling area were analyzed for total inorganic ^{15}N concentration using the diffusion protocol described by Khan et al. (1998). Acidified paper disks from the diffusion procedure and soil samples were analyzed for total nitrogen and ^{15}N concentration using an Elementar Vario EL Cube or Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to either an Isoprime VisION IRMS (Elementar UK Ltd, Cheadle, UK) or a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) (UC Davis, 2020).

Following soil sampling, fall and spring tillage was performed parallel to the length dimension of the plot. To minimize lateral soil movement into or out of the microplot, tillage was performed at reduced speeds traveling from the non-^{15}N enriched area of the plot towards the ^{15}N-enriched microplot in the fall and the opposite direction in the spring.

Data Analysis

Each site's data was analyzed individually at $P \leq 0.05$ using SAS (v. 9.4, SAS Institute, 2012). The responses of corn grain yield and R6 aboveground corn N uptake to N fertilizer rate were assessed using the REG and NLIN procedures of SAS. Selected models produced the largest correlation coefficient and met the assumptions of normally distributed residuals (Kutner et al., 2004). Because the response of aboveground plant N uptake to fertilizer N was linear for all sites, the GLM procedure of SAS was used to compare regression line slopes and intercepts between sites (SAS Institute, 2004).

Comparisons between the 0, 45, 135, 225, and 45/90 kg N ha^{-1} treatments were made using the GLIMMIX procedure of SAS. Nitrogen treatment was considered a fixed effect while block was considered a random effect. The RESIDUALPANEL option was selected to visually verify the residuals were normally distributed and treatment variances were homogeneous. The PDIFF option was utilized to make pairwise mean comparisons ($P \leq 0.05$).

The TTEST procedure was used to test if the difference between response variable means for the ^{15}N-enriched and non-^{15}N enriched sampling areas was different from zero ($P \leq 0.05$) for each treatment within site and year of sampling.

The fraction of ^{15}N (N_f) in the plant or soil sample was calculated as

$$N_f = \frac{(A_{microplot} - A_{non-15N\ enriched\ sampling\ area})}{(A_{fertilizer} - A_{non-15N\ enriched\ sampling\ area})} \qquad [1.2]$$

where A is the measured atom % ^{15}N enrichment of the plant or soil sample collected from within the ^{15}N enriched microplot ($A_{microplot}$), the non-^{15}N enriched sampling area ($A_{non\text{-}15N\ enriched\ sampling\ area}$), or the ^{15}N enrichment of the fertilizer applied to the plot ($A_{fertilizer}$). The mass of FDN and SDN in the plant and soil samples were calculated using estimates from the ^{15}N enriched microplot as:

$$FDN_{Plant\ or\ Soil}(kg\ ha^{-1}) = N_f \times total\ mass\ of\ N_{Plant\ or\ Soil}\ (kg\ ha^{-1}) \qquad [1.3]$$

$$SDN_{Plant\ or\ Soil}(kg\ ha^{-1}) = total\ mass\ of\ N_{Plant\ or\ Soil}\ (kg\ ha^{-1}) - FDN_{Plant\ or\ Soil} \qquad [1.4]$$

RESULTS AND DISCUSSION

Weather Conditions

April through June was typically cooler- and wetter-than-normal in 2014, especially for Becker14 and Waseca14 (Fig. 1.2A). Greater-than-normal precipitation likely favored leaching loss at Becker14 while standing water was present for several days following June precipitation events at Waseca14 and Clara City14 that may have favored denitrification and leaching loss through tile drainage (Coyne, 2008). Drier-than-normal conditions in July resulted in water stress for corn plants (Chapter 2), except at Becker14 that had 121 mm of supplemental irrigation in July. An early frost on 13 Sept. 2014 killed the top half of the corn canopy at Waseca14 and was followed by a hail event on 20 Sept. 2014.

In 2015, drier-than-normal conditions early April facilitated planting in late April to early May (although Lamberton15 was planted 21 May 2015). May through August precipitation was similar to or greater-than-normal reducing the risk of drought stress (Figure 1.2A, 1.2B). Air temperatures were normal throughout the growing season at all sites and were warmer-than-normal from September through December. Similarly, in 2016, monthly average maximum temperatures were normal but monthly average minimum temperatures were warmer-than-normal (Figure 1.2B). In 2016, monthly precipitation at all sites was near normal from January through June but was wetter-than-normal from July through September (Figure 1.2B).

Cumulative growing season (April through September) and annual precipitation were similar to or greater-than-normal at all sites and years (Table 1.1). This result is in agreement with the broader pattern of annual precipitation increasing (by up to 20%) across the U.S. Midwest over the past century (Melillo et al., 2014). It is expected that annual precipitation will continue to increase where spring months experience greater-than-normal precipitation (USGCRP, 2017), similar to 2014 and 2015 in this study. Additionally, summer months may have longer dry periods interrupted with large precipitation events. This was observed in 2016 at Waseca15 that received a single 104 mm precipitation event in August and a 194 mm event over 48 h in September. It is expected that these “mega-events” will become more frequent in the U.S. Midwest

(Seeley, 2015) and may significantly affect nutrient cycling processes including increased risk of nutrient loss to the environment from agricultural systems.

Non-^{15}Nitrogen Enriched Grain Yield and R6 Plant Nitrogen Uptake

Grain yield responded linearly to urea fertilizer applied at planting in eight of twelve site-years (Table 1.2). Since the highest rates were at least 1.5-fold greater than current guidelines (Kaiser et al., 2018), linear responses are indicative of N loss from the soil-crop system. In the non-fertilized check plot at Becker sites that had coarse-textured soils, corn grain yield was on average 27% less than the same treatment on finer-textured sites (Table 1.2). The lower yield is likely related to less N mineralization and nutrient retention because, in the top 15 cm of the soil, Becker sites had only a 26% (15.5 g kg^{-1} on average) of the soil organic matter and 15% (4.4 $cmol_c$ kg^{-1} on average) of the cation exchange capacity of sites with fine-textured soils on average.

The yield regression slope was greater for Becker14 ($P < 0.001$) and Becker15 ($P = 0.001$) in the year following ^{15}N labeled urea application, indicating a greater response of yield to N rate. In contrast, Waseca14 had equal slopes ($P = 0.102$) both years but a smaller intercept ($P < 0.001$) in 2014. Lower yield in 2014 is associated with greater-than-normal precipitation in April and June that favored NO_3^- leaching and denitrification before corn establishment and rapid corn N uptake (Figure 1.2; Spackman et al., 2019). Water deficiency during the early reproductive development stages from July through the second week of August in 2014 reduced water and nutrient absorption further stressing corn plants on non-irrigated sites (Stasovski and Peterson, 1991) and contributed to reduced grain yield potential (Abendroth et al., 2011). Kernel weight was further reduced for Waseca14 in 2014 following the frost and hail event in September when corn grain maturity was at the early R5 development stage. In 2015, greater-than-normal precipitation in May and June likely favored N leaching and denitrification loss at Becker15 and Waseca15. Lamberton15 in 2015 was non-responsive to fertilizer N and produced an average yield of 12.6 Mg ha^{-1}. This likely reflects the little potential for N loss and adequate amounts of water given such that monthly precipitation was like the 30-yr normal. Also, pre-plant total inorganic N (TIN) (0–60 cm depth) was 11.6 mg kg^{-1} (98 kg TIN ha^{-1}) which contributed to the overall N availability to the crop. During the 2016 growing season, Lamberton15 had a quadratic response to N fertilizer and produced 10.9 Mg grain ha^{-1} at the agronomic optimum N rate of 242 kg N ha^{-1}. The general trend of improved yield potential from 2014 < 2015 < 2016 was also reflected in Minnesota's average corn grain yield that was 9.8 Mg ha^{-1} in 2014, 11.8 Mg ha^{-1} in 2015, and 12.1 Mg ha^{-1} in 2016 (USDA NASS, 2020). These results illustrate that in the U.S. upper Midwest, soil N availability and corn grain yield potential is closely related to the weather during the growing season, especially the timing and amount of precipitation.

Plant N uptake increased linearly with N fertilizer rate for all sites (Table 1.2). All sites had similar slopes (0.00036; P = 0.122) except Becker15 and Waseca15 in 2016 where the slopes were similar (0.00056; P =0.391) and greater than the other site-years. For the group of sites with a slope of 0.00036, the intercept was 0.043 Mg ha^{-1} on average for Becker sites, 0.077 Mg ha^{-1} on average for Clara City14 in 2014 and Waseca14 in 2014 and 2015, 0.124 Mg ha^{-1} on average for Clara City14 in 2015 and Lamberton15 in 2016, and 0.203 Mg ha^{-1} for Lamberton15 in 2015. Nitrogen uptake results for the different sites were similar to differences observed for corn grain yield and illustrate the dependency of grain production on plant N uptake (Cassman et al., 2002). When growing season conditions favor biomass production, the demand for and uptake of mineral N increases (Karlen et al., 1988; Bender et al., 2013). It is probable that the warmer-than-normal minimum temperatures and wetter-than-normal conditions in July and August in 2016 (Figure 1.2B) favored greater biomass production (data not shown) and was partially responsible for the greater slope associated with Becker15 and Waseca15 relative to the other sites.

Only coarse-textured soils exhibited differences in grain yield between the single 135 kg N ha^{-1} application of urea and the 45/90 kg N ha^{-1} split-applied at the V4 corn development stage (Table 1.2). The split-application improved corn grain yield 1.7-fold on average relative to the application at planting for Becker14 and Becker15 in 2015 and Becker15 in 2016. At these sites, an additional 35 to 164 kg urea-N ha^{-1} was required to achieve a similar yield as the V4 split illustrating the economic and environmental protection advantages of split-applications for coarse-textured soils (Rubin et al., 2016; Spackman et al., 2019). There was no significant difference in corn grain yield or plant N uptake on fine-textured sites, except Waseca15 in 2016 where the single application improved N uptake by 0.026 Mg ha^{-1} (Table 1.2). In a related study, soil inorganic N content in the non-fertilized checkplot was greater and maintained at higher concentrations throughout the growing season on fine-textured soils than coarse-textured soils (Spackman, 2018) and likely indicates the influence of soil texture on mineralization potential and N loss susceptibility. In fine-textured soils, large aggregates and small micropores may protect inorganic and soluble organic N from loss, especially from leaching that occurs predominantly through macropores following large precipitation events (Balasubramanian et al., 1973). These results suggest that the fine-textured soils conserved sufficient plant-available N from the application at planting through the V4 development stage such that there was no difference in the overall plant-available N between the 135 and 45/90 kg N ha^{-1} treatments. This may indicate that there is a large window of time when supplemental fertilization may be applied without impacting the corn grain yield potential associated with that N rate.

Soil- and Fertilizer-Derived Nitrogen in the First Year

There was no difference in the amount of SDN accumulated in aboveground biomass between the non-fertilized and fertilized treatments at Becker14, Becker15, Clara City14, and Waseca14, except at Becker15 where SDN uptake in the 45/90 kg N ha^{-1} treatment was 11.7 kg ha^{-1} less than the non-fertilized check (Table 1.4). This exception should be regarded with caution as it likely does not reflect SDN uptake in the non-^{15}N enriched treatment area due to different amounts of total N uptake between the ^{15}N enriched and non-^{15}N enriched sampling areas and will be discussed in greater detail later (Table 1.3). At Waseca15, there was a trend of increasing SDN uptake with increasing fertilizer rate but only the 225 kg N ha^{-1} treatment was greater than the non-fertilized check (Table 1.4). At Lamberton15, there was a trend of decreasing SDN uptake with increasing fertilizer rate, but only the 225 kg N ha^{-1} treatment was significantly less than the non-fertilized check.

The lack of response, increase, and decrease of SDN uptake to fertilizer application rate reflect three possible responses following N fertilization and are often referred to as a "priming effect" or an "added N interaction" (ANI). Jenkinson et al. (1985) defined an

ANI as “any effect that the addition of N to a soil may have on the N already present”. Concerning crop uptake, it is the difference of plant SDN uptake in a non-fertilized and fertilized treatment (Harmsen, 2003). A lack of response (an ANI value of zero where there is no difference in SDN uptake) occurs when differences in soil N are due to the quantity of inorganic N lost from the soil rather than plant or microbial demand for N, such as leaching or volatilization loss (Jenkinson et al., 1985). Because Becker sites had coarse-textured soils and low cation exchange capacity for inorganic N retention, leaching loss was rapid soon after fertilization (Chapter 3) forcing the crop to rely on soil organic matter mineralization for most of its N uptake. Likewise, NH_3 volatilization at Clara City14 was likely high as only 42% of FDN (averaged across N treatments) was in the top 60 cm of the soil profile within eight days of application (Chapter 3). Despite the low FDN recovery, total N uptake in the ^{15}N enriched area was ≥73% of the ^{15}N non-enriched area and not statistically different ($P \geq 0.075$) from each other (Table 1.3). Other studies similarly reported a lack of ANI attributing it to coarse-textured soils and low soil organic matter or low mineralization-immobilization turnover rates (Recous et al., 1988b; Torbert et al., 1992; Schindler and Knighton, 1999).

An increase of SDN uptake in response to fertilizer rate, or positive ANI, may be “real” or “apparent”. Real ANIs may occur when fertilization allows roots to explore a greater volume of the soil and intercept more SDN (Sørensen, 1982). Apparent ANIs may occur following pool substitution where 1) FDN is concurrently immobilized with SDN mineralization resulting in greater SDN availability and uptake or 2) denitrification of labeled inorganic N favors uptake of SDN (Jenkinson et al., 1985; Azam, 2002). At Waseca15, the positive ANI was likely due to pool substitution where unlabeled N was substituted for labeled N as 31 to 68% at V8 and 25 to 44% at R1 of the applied FDN, was in the top 60 cm of the soil, primarily in the organic N fraction (Chapter 3). Further, inorganic SDN (0–60 cm depth) increased with increasing N rate ($P \leq 0.004$) at V8 and R1 (data not shown).

A decrease of SDN uptake in response to fertilizer rate, or negative ANI, at Lamberton15 was likely due to several factors including 1) a high N supplying capacity of the loam soil throughout the growing season (189 kg SDN ha^{-1} taken up in the non-fertilized checkplot); 2) fertilizer N rates over the amount needed to supplement the soil N supply that resulted in increasing inorganic SDN content with increasing N rate at PHY1 (22.1 to 39.4 kg N ha^{-1}; 0- to 90-cm depth); and 3) rapid biomass production and accumulation of plant N by V8 accounting for 78 to 103% of the PHY1 plant FDN uptake and 56 to 62% of the total plant N uptake (Chapter 2). These conditions favored pool substitution where labeled N substituted for unlabeled N during plant uptake and was similar to a non-responsive site reported by Jokela and Randall (1997). Campbell and Paul (1978) and Bigeriego et al. (1979) similarly reported negative ANIs attributing the result to the excessive use of fertilizer and replacement of nutrient losses from soil organic matter. Jokela and Randall (1997) also observed that fertilizer applied at planting is more likely to be immobilized producing a positive ANI while in-season applications are more likely to be immediately assimilated by the crop, producing a more negative ANI due to less soil N being taken up. Added N interaction values may change throughout a study and may initially be negative due to the high accessibility of FDN to crop roots but become positive as inorganic FDN in the soil is depleted or crop roots explore the soil profile assimilating unlabeled N (Garabet et al., 1998a; b; Harmsen, 2003). If immobilization of labeled N following fertilization is rapid, an ANI may be positive but as immobilized labeled N is re-mineralized, it may become zero or negative, depending on the amount of labeled N taken up (Jenkinson et al., 1985).

Aboveground corn FDN uptake increased with increasing N rate at all sites (Table 1.4). At Becker14 and Becker15, the 45/90 kg N ha^{-1} treatment had the greatest FDN uptake of all treatments and was 2.1- and 4.1-fold greater than the 135 kg N ha^{-1} treatment. At Clara City14 and Waseca sites, the 45/90 kg N ha^{-1} treatment was similar to the 225 kg N ha^{-1} treatment and greater than the 135 kg N ha^{-1} treatment. At Lamberton15, the 45/90 kg N ha^{-1} treatment was not different from the 135 kg N ha^{-1} treatment. At most sites, corn FDN uptake was improved because 90 kg FDN ha^{-1} was not subject to N loss or immobilization for 21 to 34 days relative to fertilizer applied at planting. Further, because urea is nonionic, it may enter the root by simple diffusion being absorbed faster than NH_4^+ or NO_3^- (Viets, 1965). These data support the concept that in-season fertilizer applications improve plant FDN uptake relative to a single application at planting (Bigeriego et al., 1979; Jokela and Randall, 1997), but the benefit is greatest on soils that are prone to N loss or have low mineralization potential. For soils with low N loss potential or high mineralization rates, split-applications may delay the amount of time that fertilizer N is available to the crop and may increase post-harvest N loss if it is not utilized before harvest or if dry conditions prevent its incorporation into the rooting zone (Jokela and Randall, 1997; Spackman et al., 2019).

The mass of FDN taken up by the crop generally increased in the order Becker15 < Becker14 < Clara City14 < Waseca14 < Waseca15 < Lamberton15. Yet, despite the difference in FDN uptake magnitudes, the percent FDN of the total N taken up by the crop was similar within rate and across sites and averaged 7, 21, 31, and 34% for the 45, 135, 225, and 45/90 kg N ha^{-1} treatments, respectively. This may indicate that for these studies, fertilizer uptake is proportional to the N rate applied at planting while split-applications improve fertilizer uptake due to improved synchrony to corn demand. While N fertilizer represents an expensive and important component of corn grain production, this result also re-emphasizes the importance of maintaining soil productivity and its N supplying capacity as 66 to 93% of the total corn N uptake was derived from the soil. These values are similar to estimates typically reported (50 to 80%) (Kimble et al., 2007) and long-term N rate studies (54 to 83%) that have approached a quasi-stable steady-state (Stevens et al., 2005a). While soil-derived inorganic N is free, the short-term exploitation of soil N reserves and failure to protect the soil from depletion will result in increasingly expensive inputs costs to achieve similar production levels. Long-term studies that utilize the same N management strategy over time are critical for determining if a particular management strategy builds or mines soil N stores.

Like corn FDN uptake, residual soil FDN content increased with increasing N rate. However, the percentage of soil FDN relative to the applied N rate decreased with increasing N rate at PHY1 (Table 1.4). Allen et al. (1973) proposed that each soil has a threshold amount of N required by soil microorganisms to meet their metabolic needs. When fertilizer N exceeds that demand, inorganic N availability increases with increasing N rate favoring crop uptake and inorganic N loss mechanisms. This theory is supported by the fact that despite the 45/90 kg N ha^{-1} treatment receiving N as two applications, there was no difference in the total amount of FDN remaining in the soil between the 135 and 45/90 kg N ha^{-1} treatment at PHY1 at any of the sites. The only exception occurred at

Lamberton15 where the 45/90 kg N ha^{-1} treatment had 13.9 kg FDN ha^{-1} less than the 135 kg N ha^{-1} treatment. This difference was likely due to the soil threshold level having already been met since, at the time of the split at V4, there was 82 kg inorganic SDN ha^{-1} in the top 60 cm of the non-fertilized checkplot (data are not shown).

Across all sites, total FDN (aboveground plant plus soil to 90 cm) and unaccounted FDN increased with increasing N rate (Table 1.4) consistent with others (Allen et al., 1973; Olson, 1980; Sanchez and Blackmer, 1988). For Becker sites, the mass of FDN was greatest for the 45/90 kg N ha^{-1} treatment while at all other sites the 135 and 45/90 kg N ha^{-1} treatments were not different and were typically less than the 225 kg N ha^{-1} rate (Table 1.4). Averaged across all N treatments, the percentage of the applied FDN rate recovered in the soil-corn system (± standard error of the mean) was 25 ± 5%, 20 ± 3%, 29 ± 1%, 50 ± 3%, 36 ± 3%, and 47 ± 3% for Becker14, Becker15, Clara City14, Lamberton15, Waseca14, and Waseca15, respectively and is similar to values observed by Timmons and Cruze (1990) (17 to 47%) and less than others reports of 72 to 89% (Olson, 1980; Reddy and Reddy, 1993; Jokela and Randall, 1997; Schindler and Knighton, 1999). Unaccounted FDN ranged from 50 to 79% of the applied rate and did not differ between the 45, 135, and 225 kg N ha^{-1} treatments within each site (Table 1.4). This contrasts the results of Reddy and Reddy (1993), who observed unaccounted FDN was 3-fold greater when the N application rate increased from 100 to 200 kg N ha^{-1}. The greatest values of unaccounted FDN occurred at Becker and Clara City sites due to their high potential for N loss from leaching and volatilization following fertilization (Chapter 3). Following above-average precipitation after fertilization, Sanchez and Blackmer (1988) likewise reported that more than half of the applied FDN was lost from the rooting zone by processes other than plant harvest. Unaccounted FDN represents a large reactive N load potentially contaminating ground- and surface-waters or increasing greenhouse gas emissions. It also represents a significant loss on investment of up to \$196 ha^{-1} in this study (\$1.10 kg^{-1} N). If future spring conditions continue to be wet favoring N loss, corn producers may need to shift away from urea applied at planting to other N sources, application timings, or management practices to avoid these environmental and economic losses. However, these management practices may have other risks that may also favor N loss or poor N utilization. For example, delaying a split-application too late into the growing season may result in inadequate incorporation of top-dressed urea by precipitation into the root zone on rain-fed systems (Spackman et al., 2019) while anhydrous NH_3 applications may increase nitrous oxide emission that is a potent greenhouse gas (Venterea et al., 2005). Each of these N management practices should be carefully examined to determine what field and environmental conditions produce the best FDN uptake and recovery.

Soil- and Fertilizer-Derived Nitrogen in the Second Year

Like this study, Westerman and Kurtz (1972) and Chichester and Smith (1978) sought to quantify FDN in the soil over multiple growing seasons but they did not apply additional unlabeled N at the beginning of the second year to avoid diluting the ^{15}N signal. However, this practice does not reflect production agriculture practices that often require 150 kg N ha^{-1} of supplemental fertilizer to achieve economically optimal yields (Kaiser et al., 2018). Olson (1980) applied labeled ^{15}N fertilizer to the entire microplot area both years, but because of residual labeled fertilizer N, it was difficult to differentiate first-year residual FDN from second-year FDN. Because this study applied unlabeled fertilizer in the second year, it was difficult to differentiate second-year corn SDN uptake from unlabeled fertilizer N applied the second year. Second-year FDN uptake includes first-year residual labeled N from mineralized crop residue or soil organic matter.

At Becker14, Becker15, Clara City14, and Lamberton15, there was no difference in SDN uptake when fertilizer treatments were ≤135 kg N ha^{-1} except at the Becker sites where the 45/90 kg N ha^{-1} treatment significantly improved SDN uptake. At Waseca14 and Waseca15, there was no difference in SDN uptake between the 0 and 45 kg N ha^{-1} treatments while all other treatments had greater SDN uptake values. Greater SDN uptake values at larger N application rates is likely primarily due to applying unlabeled fertilizer N in the second year. Added N interactions may have also occurred, but are impossible to discern since labeled N fertilizer was not used. Establishing an additional ^{15}N enriched microplot during the second year would have allowed for quantification of SDN in the second year similar to others (Jokela and Randall, 1997; Schindler and Knighton, 1999).

Like first-year, second-year corn FDN uptake and residual soil FDN increased with increasing N rate at all sites where the 45/90 kg N ha^{-1} treatment was similar to the 225 kg N ha^{-1} treatment at Becker sites and similar to the 135 kg N ha^{-1} treatment at the other sites (Table 1.4). This result is likely an artifact from the first year where FDN in the corn biomass increased with increasing N rate or split. Following first-year residue incorporation, some of the corn residue and immobilized FDN was re-mineralized and became available to the second-year corn. Although the amount of FDN recovered in the second year corn crop increased with greater N rates or split-applications, ≤ 7.4 kg N ha^{-1} was taken up across all sites, which represents a negligible (0.8 to 4.1%) amount of the total N taken up by the second year corn crop (Table 1.4). This result is similar to a long-term N rate study that found 2 to 8 kg FDN ha^{-1} was assimilated in the second year corn biomass (Stevens et al., 2005a). The majority (76 to 92% across sites and treatments) of the residual FDN in the soil-crop system was in the soil (Table 1.4), primarily in the organic N fraction (Chapter 3). From PHY1 to PHY2, soil FDN (averaged across treatments) increased by 7.0 kg ha^{-1} at Becker14 and decreased by 6.6, 1.9, 3.2, 4.8, and 8.6 kg ha^{-1} at Becker15, Clara City14, Lamberton15, Waseca14, and Waseca15, respectively. Over time and following additional mineralization-immobilization reactions, this organic FDN fraction likely became an increasingly recalcitrant part of the soil (Allen et al., 1973; Kelley and Stevenson, 1987; González-Prieto et al., 1997; Stevens et al., 2005b).

The majority of FDN losses from the soil-corn system occurred during the first year as there were no differences of unaccounted FDN for any of the treatments between PHY1 and PHY2 at Becker14, Becker15, and Clara City14 ($P > 0.059$). At Lamberton15, Waseca14, and Waseca15 unaccounted FDN increased from PHY1 to PHY2, except at Waseca14 where the 45 and 135 kg N ha^{-1} treatments were not different ($P > 0.101$). At these sites, the difference of unaccounted FDN from PHY1 to PHY2 increased with increasing N rate and was likely due to mineralization of first-year corn residue and previously immobilized FDN that mineralized and then either leached or denitrified from the system during the second year growing season. Others have observed significant leaching loss during the fallow fall-to-spring period (Cameron et al., 1978; Bauder and Montgomery, 1979; Sanchez and Blackmer, 1988; Randall and Vetsch, 2005; Lawlor et al., 2008) but the majority of labeled N loss occurred during the growing season in this study (Chapter 3). Overall, averaged across all N treatments, the percentage of the applied FDN rate accounted for in the soil-corn system at the end of the second year (± standard error of the mean) was 27 ± 5%, 23 ± 3%, 23 ± 1%, 17 ± 3%, 23 ± 3%, and 16 ± 3% for Becker14, Becker15, Clara City14, Lamberton15, Waseca14, and Waseca15. Despite differences in soil texture, soil organic matter, regions of Minnesota, and fertilizer treatment, by PHY2 the percent of the originally applied FDN remaining in the soil-corn system was similar. This highlights the complexity to manage N to improve its retention in fields with annual cropping systems. Future work should further examine the mechanisms that drive FDN retention in soil organic matter and why FDN recovered was proportional to the applied N rate. This could involve investigations into which soil organic N pools the FDN is stored in and if microbial populations and soil physical and chemical properties including soil organic matter, clay content, soil aggregate size, and cation exchange capacity affect the soil's ability to immobilize FDN.

Microplot Considerations

For conclusions drawn from the ^{15}N enriched sampling area to be successfully extrapolated to the rest of the treatment, both the ^{15}N enriched and non-^{15}N enriched sampling areas' fertilizer source, application form, plot management, and crop responses should be similar (Khanif et al., 1984; Hauck et al., 1994; Van Cleemput et al., 2008). Across sites and years, there was generally no difference in R6 aboveground plant N uptake between the ^{15}N enriched and non-^{15}N enriched sampling areas at $P \leq 0.1$ significance level (Table 1.3). However, there were some exceptions. Corn N uptake at Becker15 in 2015 within the ^{15}N enriched microplot was 52% and 50% of the non-enriched sampling area for the 225 and 45/90 kg N ha^{-1} treatments, respectively. Other pairwise comparisons were significantly different at other sites, but the difference of N uptake between the ^{15}N enriched and non-enriched sampling areas was less than ±25% of the non-enriched sampling area.

Fertilizer form and distribution at the time of application may influence rates of urea hydrolysis, ammonification, nitrification, and mineralization-immobilization by soil microbes (Touchton and Hargrove, 1982; Recous et al., 1988a; Christianson et al., 1993; Reddy and Reddy, 1993; Kissel et al., 2015). Urea granules were broadcast applied and incorporated on the non-^{15}N enriched sampling area producing localized concentrations of N (Christianson et al., 1993) whereas the ^{15}N enriched microplot fertilizer was dissolved in water and then uniformly sprayed onto the soil surface, increasing the potential for chemical interactions with soil enzymes and microorganisms (Touchton and Hargrove, 1982). Faster rates of hydrolysis and nitrification or immobilization may be expected with the broadcast spray application that may partially account for the observed differences in N uptake. However, in this study, differences due to N form and distribution were reduced by incorporating the fertilizer application with light tillage or irrigation within 24 h of fertilization.

One potential issue inherent with plant and soil sampling is ensuring that the composite sample adequately represents the treatment. Randomly selected samples whose measured values are smaller or larger than the true treatment mean can result in significant differences between the ^{15}N enriched and non-enriched sampling areas. Other researchers have collected a composite sample of three (Olson, 1980; Timmons and Baker, 1992; de Oliveira Silva et al., 2017), four (Sanchez and Blackmer, 1988; de Oliveira Silva et al., 2017), five (Jokela and Randall, 1997), seven (Jokela and Randall, 1997), and nine (Olson, 1980) corn plants for determining plant N uptake. In this study, six plants per composite sample were selected from pre-determined locations to allow for repeated sampling events, to minimize worker sample collection bias, to provide a more representative sample, and to reduce sampling error (Gomez and Gomez, 1984; Spackman and Fernández, 2020).

Because the microplot was unconfined, soil water and plant roots could move laterally across the microplot boundary assimilating non-^{15}N enriched N from outside the microplot and diluting the ^{15}N signal (Follett et al., 1991; Van Cleemput et al., 2008). To avoid this potential issue, post-harvest soil and plant samples were not collected from a border area within the outside edge of the ^{15}N enriched sampling area (Chapter 2, Spackman and Fernández, 2020). Sanchez et al. (1987) reported that a 2 by 2 m unconfined microplot area is adequate to minimize errors from the lateral movement of FDN and is sufficiently large to measure the ^{15}N signal in subsequent years. Likewise, ^{15}N dilution is minimal when plant samples are collected ≥38 cm from the edge of the microplot and not different from plant samples collected from the center of the microplot (Jokela and Randall, 1987).

Given the considerations above and that the majority of the treatments had no difference in total N uptake between the ^{15}N enriched and non-enriched sampling area, the results from the ^{15}N enriched microplot can likely be extrapolated to the rest of the treatment plot with a high degree of confidence. However, conclusions drawn from within the ^{15}N enriched sampling area in Becker15 in 2015 may not accurately reflect N cycling dynamics in the non-^{15}N enriched sampling area for the 225 and 45/90 kg N ha^{-1} treatments and should be regarded with caution.

Comparison of the Fertilizer Nitrogen Use Efficiency Methods

The $FNUE_{15N}$ and $FNUE_{diff}$ method each have benefits and shortcomings as previously described. Although some researchers seek to determine which measure is best for determining fertilizer use efficiency (Campbell and Paul, 1978; Olson, 1980; Schindler and Knighton, 1999; Stevens et al., 2005a), the two methods do not necessarily measure the same N processes (Harmsen, 2003; Meisinger et al., 2008). Because the $FNUE_{diff}$ method does not have a labeled N component, it cannot differentiate between FDN and SDN (Azam, 2002), and is better for examining the combined SDN and FDN use efficiency or recovery for crop production (Harmsen, 2003; Meisinger et al., 2008). Further, because the $FNUE_{diff}$ method uses a non-fertilized checkplot, it is possible to obtain negative values if a fertilized treatment assimilates less N than the non-fertilized control treatment, as might occur on soil with high inorganic SDN availability (Moraghan et al., 1984a). The $FNUE_{15N}$ method is best suited for tracking the fate of applied fertilizer N through N cycling processes or for quantifying labeled fertilizer N in the soil-crop system (Harmsen, 2003; Meisinger et al., 2008).

At Becker14 and Clara City14, $FNUE_{diff}$ values were similar across fertilizer treatments and averaged 25.4 and 19.6%, respectively but $FNUE_{diff}$ was -3.8% for the 45 kg N ha^{-1} treatment at Becker15 and 33.8% on average across the other treatments (Table 1.5). There was no difference in $FNUE_{15N}$ values observed between the 45, 135, and 225 kg N ha^{-1} treatments at Becker14, Becker15, Clara City14, and Waseca14 with average values of 10.3, 3.7, 15.3%, and 11.2%, respectively. However, all fertilizer treatment $FNUE_{15N}$ values were less than the 45/90 kg N ha^{-1} treatment (except at Clara City14 that was not different). Using $FNUE_{diff}$ would show that fertilizer recovery was proportional to the applied N rate for all treatments at Becker14 and treatments > 45 kg N ha^{-1} at Becker15. However, by examining $FNUE_{15N}$ as well, it is revealed that the split-application significantly improved labeled fertilizer N recovery in the crop by 2.6-fold at Becker14, 4.4-fold at Becker15, and 2.3-fold at Waseca14 averaged across the 45, 135, and 225 kg N ha^{-1} treatments, respectively.

At Lamberton15 and Waseca15, the 45 kg N ha^{-1} treatment had the greatest $FNUE_{diff}$ value at 68.1 and 70.9%, respectively while the other treatments were not different and averaged 18.5 and 33.4%, respectively. Based on the $FNUE_{diff}$ patterns observed at Lamberton15, fertilizer recovery was reduced at greater N application rates, potentially due to N loss (Hart et al., 1986). However, when $FNUE_{15N}$ is examined, it becomes obvious that there was a trend of increasing FDN recovery with greater N rates that accounted for 34.0 to 41.9% of the applied N rate at Lamberton15 and 19.7 to 37.2% at Waseca15. Thus, the reduced $FNUE_{diff}$ values were due to the increasing value in the denominator of Equation 1 with each additional unit of fertilizer N applied. The results across the sites in this study demonstrate the importance of examining both $FNUE_{15N}$ and $FNUE_{diff}$ to obtain a more holistic understanding of the impact an N fertilizer treatment has on total N and FDN recovery.

Across all sites, $FNUE_{diff}$ typically had larger standard error of the mean values than $FNUE_{15N}$ and, at Becker sites, $FNUE_{diff}$ values were typically larger than $FNUE_{15N}$ (Table 1.5). The variance of the $FNUE_{diff}$ estimate is the sum of the variance associated with the non-fertilized check and the fertilized treatment (Moraghan et al., 1984b). Thus, in most comparisons between $FNUE_{diff}$ and $FNUE_{15N}$, the $FNUE_{diff}$ estimate will be larger and more variable. The $FNUE_{diff}$ method generally gives greater recovery values than $FNUE_{15N}$ due to ANIs following denitrification or immobilization of labeled N, resulting in overestimates of fertilizer use efficiency by the crop (Harmsen and Moraghan, 1988; Azam, 2002). This response is common and was reported by other researchers (Westerman et al., 1972; Torbert et al., 1992; Jokela and Randall, 1997). However, Schindler and Knighton (1999) reported $FNUE_{diff}$ values less than $FNUE_{15N}$ due to high soil N availability that resulted in similar N uptake in the fertilized and non-fertilized plots. Because the assumptions associated with $FNUE_{diff}$ and $FNUE_{15N}$ may never be fully satisfied in agricultural field settings, the two estimates should be compared and it should be recognized that the true fertilizer use efficiency estimate probably lies somewhere between the two values (Jenkinson et al., 1985).

CONCLUSIONS

Current fertilizer guidelines suggest that urea applied at planting is an acceptable N management strategy for most U.S. upper Midwest corn cropping systems. However, recent years have received greater-than-normal precipitation from April through June resulting in significant leaching loss on coarse-textured soils and leaching and denitrification losses on fine-textured soils. This study demonstrated that under wet spring conditions, urea fertilizer recovery in the soil and plant was less than 36% of the applied N rate with the majority of the FDN lost early in the growing season. These losses represent a substantial economic loss and potential harm to the environment. Split-applications provided significant improvements in yield and $FNUE_{15N}$ over a single application done at planting, but the improvement was greatest on coarse-textured soils suggesting that urea applications done at planting should be avoided in favor of split-applications.

The soil texture was a major component of N fertility and strongly determined the likelihood that N would be available to the crop throughout the growing season. Coarse-textured soils had the lowest yield and residual soil FDN while a loam soil had the greatest yield and residual soil FDN at the end of the first growing season. However, by the end of the second growing season, total FDN remaining in the soil-crop system was similar across all soil textures potentially indicating that the long-term retention or immobilization of N is constrained less by soil texture and more by the stability of the organic fraction that the FDN is bound to.

Fertilizer-derived N from the first-year crop residue and soil was recovered in the second year crop but was less than 5% of the total N taken up. This indicates that producers should not rely on residual FDN to supply an agronomically important amount of N for the subsequent crop following wet years. Although not observed in this study, if the previous summer and fall conditions were dry, residual FDN in the soil may be substantial and not warrant additional fertilizer.

Irrespective of the fertilizer recovery efficiency method used, most fertilizer efficiency estimates were less than 40% and were typically closer to 20% (Table 1.5). Increasing fertilizer use efficiency is essential because it represents a reduction of reactive N in the environment and a greater return on a producer's fertilizer investment. Split-applications significantly improved $FNUE_{15N}$ estimates at four of the six sites likely because the split was done when the crop had an established root system and because there was less time for the FDN to mix with the soil before it was assimilated (Harmsen and Moraghan, 1988). Fertilizer recovery and grain yield may be further improved if delayed until V6 or V8 as the crop is rapidly accumulating biomass and assimilating N

(Spackman et al., 2019). However, with later applications of top-dressed urea is the risk of inadequate precipitation to incorporate the fertilizer into the root zone. Because May and June months will likely continue to be wetter-than-normal (USGCRP, 2017), other fertilizer sources, such as polymer-coated urea or anhydrous ammonia, applied at planting may be better suited to preserve fertilizer N in the NH_4^+ form. However, these fertilizer sources may have other risks such as safety concerns or greater N_2O emissions relative to other N sources (Venterea et al., 2005). Other N management strategies that may further improve N use efficiency include variable rate applications (Randall et al., 2008), crop sensing technologies (Paiao et al., 2020) paired with (Paiao, 2017) or without soil sampling (Andraski and Bundy, 2002; Walker et al., 2018), and precision agriculture technologies that combine crop models and soil property and yield maps (Meisinger et al., 2008). The goal of each of these strategies is to avoid N applications above the crop demand to maximize fertilizer recovery. While most sites had inadequate N to maximize corn grain yield, Lamberton15 was non-responsive. At this site, the only way to improve fertilizer N use efficiency was by applying little (<45 kg N ha^{-1}) to no additional fertilizer N. When making fertilizer applications at planting, the challenge is correctly predicting how much fertilizer N will be needed to supplement the soil N supply.

Table 1.1 Cumulative growing season and annual precipitation for the 2014, 2015, and 2016 growing seasons and the 30-year normal from 1981 to 2010.

Year	Becker		Clara City		Lamberton		Waseca	
	Apr. – Sept.	Annual	Apr. – Sept.	Annual	Apr. – Sept.	Annual	Apr. – Sept.	Annual
	Precipitation (mm)							
2014	693	991	521	747			571	903
2015	607	877	527	713	564	780	804	1160
2016	541	836			651	960	1115	1428
30-Year Normal	473	764	445	706	460	708	546	903

Table 1.2 Regression parameter estimates of corn grain yield (Mg ha^{-1}) and N uptake (kg N ha^{-1}) by fertilizer application rate for the equation $y=a+bx+cx^2$. Grain yield and standard error of the means (SE) are presented for the single 135 kg N ha^{-1} (Single) and 45/90 kg N ha^{-1} V4 split-applied (Split) treatments. Within site, treatment means followed by the same letter are not significantly different between Single and Split at $P \leq$ 0.05.

Site	Year	Parameter estimates					Treatment Differences			
		a	b	c	$P > F$	Adj. R^2	Single	Split	SE	$P > F$
							Mg ha^{-1}			
						Grain yield				
Becker14	2014	2.31	0.0123		<0.001	0.39	4.1a	6.5a	1.1	0.216
Becker14	2015	0.78	0.0286		<0.001	0.80	3.3b	8.0a	0.8	0.026
Becker15	2015	1.51	0.0214		<0.001	0.68	4.0b	7.9a	0.8	0.013
Becker15	2016	1.80	0.0358		<0.001	0.82	6.7b	7.9a	1.1	0.010
Clara City14	2014	4.99	0.0231		<0.001	0.65	6.4a	9.1a	0.7	0.070
Clara City14	2015	6.48	0.0529	-0.00011	<0.001	0.71	11.7a	12.4a	0.6	0.353
Lamberton15	2015	12.6			0.199	-	12.4a	12.0a	0.3	0.262
Lamberton15	2016	7.28	0.0301	-0.00006	0.0025	0.38	10.2a	10.8a	1.0	0.672
Waseca14	2014	3.22	0.0211		<0.001	0.60	5.6a	6.4a	0.7	0.053
Waseca14	2015	3.53	0.0278		<0.001	0.86	9.7a	10.1a	0.5	0.211
Waseca15	2015	4.75	0.0261		<0.001	0.79	7.5a	10.4a	0.6	0.094
Waseca15	2016	5.39	0.0479	-0.00007	<0.001	0.92	10.7a	10.3a	0.5	0.186
						N uptake at R6				
Becker14	2014	0.048	0.00023		<0.001	0.518	0.083a	0.104a	0.010	0.235
Becker14	2015	0.037	0.00035		<0.001	0.637	0.065b	0.116a	0.011	0.043
Becker15	2015	0.045	0.00030		<0.001	0.634	0.088a	0.131a	0.018	0.066
Becker15	2016	0.039	0.00059		<0.001	0.708	0.106a	0.136a	0.018	0.239
Clara City14	2014	0.082	0.00030		<0.001	0.510	0.097a	0.135a	0.018	0.245
Clara City14	2015	0.122	0.00042		<0.001	0.671	0.163a	0.178a	0.015	0.514
Lamberton15	2015	0.203	0.00021		<0.001	0.544	0.225a	0.217a	0.010	0.586
Lamberton15	2016	0.126	0.00037		0.001	0.335	0.204a	0.196a	0.028	0.792
Waseca14	2014	-	-		-	-	-	-	-	-
Waseca14	2015	0.073	0.00038		<0.001	0.741	0.120a	0.140a	0.007	0.095
Waseca15	2015	0.077	0.00036		<0.001	0.650	0.109a	0.138a	0.011	0.147
Waseca15	2016	0.091	0.00051		<0.001	0.830	0.175a	0.149b	0.007	0.005

Table 1.3 Treatment means and standard error of the means (SE) for total aboveground corn N uptake for the ^{15}N enriched and non-enriched sampling areas at physiological maturity (R6) in the first and second year after ^{15}N enriched fertilizer application. The probability that the difference (Diff) between the paired sampling area means is different from 0 is also given.

Site	Treatment	^{15}N Enriched		^{15}N Non-Enriched		Diff	^{15}N Enriched		^{15}N Non-Enriched		Diff
		Mean	SE	Mean	SE	*P* > *t*	Mean	SE	Mean	SE	*P* > *t*
		First Year					Second Year				
		kg N ha^{-1}					kg N ha^{-1}				
Becker14	45	56.8	2.2	57.8	5.2	0.809	50.5	4.7	55.4	3.5	0.513
	135	72.4	11.5	80.5	6.6	0.328	66.1	9.2	59.9	3.1	0.459
	225	71.7	4.4	96.6	9.5	0.105	98.6	11.1	95.3	26.6	0.977
	45/90	85.6	3.7	97.6	9.4	0.355	103.4	13.2	101.3	11.6	0.931
Becker15	45	46.7	3.0	47.1	3.4	0.949	38.7	4.3	63.1	9.2	0.150
	135	52.9	4.2	80.1	18.9	0.276	69.2	12.7	105.1	20.8	0.291
	225	57.0	4.7	109.9	15.0	0.052	123.3	19.2	163.3	16.3	0.296
	45/90	59.0	3.5	117.8	9.4	0.010	119.7	2.3	127.1	6.9	0.241
Clara City14	45	75.3	9.0	91.9	12.6	0.337	118.1	5.3	123.3	14.4	0.693
	135	88.0	7.0	97.0	9.7	0.622	176.8	35.5	156.1	13.0	0.605
	225	120.3	16.7	163.7	4.9	0.075	246.1	13.1	225.1	9.5	0.411
	45/90	108.5	8.9	123.3	17.6	0.433	183.8	29.1	174.4	16.1	0.754
Lamberton15	45	197.9	14.7	219.7	9.3	0.075	108.8	18.2	147.4	18.0	0.159
	135	227.9	10.7	223.8	9.4	0.724	123.8	18.1	188.7	30.4	0.177
	225	236.4	12.9	241.1	15.3	0.956	181.6	28.9	210.7	36.9	0.578
	45/90	214.2	11.1	197.8	15.5	0.457	163.7	8.1	198.6	15.7	0.158
Waseca14	45	53.9	7.1	-	-	-	69.9	1.9	92.4	5.3	0.025
	135	70.3	5.4	-	-	-	137.2	16.2	125.1	15.3	0.063
	225	96.6	12.0	-	-	-	148.3	14.4	148.5	15.9	0.970
	45/90	96.2	12.3	-	-	-	118.0	6.5	134.0	7.4	0.050
Waseca15	45	99.1	11.0	102.5	7.1	0.493	91.9	7.0	102.4	4.9	0.071
	135	121.3	14.9	106.7	8.5	0.364	139.2	13.0	172.7	7.4	0.172
	225	168.4	20.0	141.0	12.9	0.099	190.9	14.8	198.5	5.9	0.696
	45/90	142.3	9.9	127.4	6.6	0.157	130.5	14.8	149.2	5.3	0.379

Table 1.4 A budget of soil-derived N (SDN) and fertilizer-derived N (FDN) in the aboveground corn biomass at physiological maturity (Plant), FDN in the soil (inorganic plus organic; 0–90 cm) following harvest, total plant and soil FDN, and unaccounted for FDN from the soil-corn system in the first and second years following ^{15}N fertilizer application. Second-year SDN includes N from soil organic matter mineralization and unlabeled urea fertilizer applied the second year. The standard error of the means (SE) are also given. Within site and column, treatment means followed by the same letter are not significantly different ($P \le 0.05$).

Site	N rate	First Year					Second Year				
		Plant		Soil	Total	Unaccounted†	Plant		Soil	Total	Unaccounted†
		SDN	FDN				SDN	FDN			
		kg N ha^{-1}									
Becker14	0	48.7a	-	-	-	-	45.7b	-	-	-	-
	45	53.0a	3.8c	6.2b	10.0c	34.8d	49.3b	1.1c	6.0c	7.2c	35.2d
	135	55.7a	16.8b	11.4ab	28.2b	106.3b	64.0b	2.1b	18.0b	20.1b	103.5b
	225	49.0a	22.8b	14.3a	37.0b	187.1a	95.4a	3.2a	24.8ab	28.1ab	180.7a
	45/90	49.6a	36.0a	18.8a	54.8a	79.7c	100.3a	3.1a	29.9a	33.0a	77.2c
	SE	3.9	3.8	2.6	3.7	3.7	8.9	0.2	2.7	2.8	4.3
	$P > F$	0.608	<0.001	0.037	<0.001	<0.001	0.002	<0.001	0.001	<0.001	<0.001
Becker15	0	48.8a	-	-	-	-	51.2b	-	-	-	-
	45	44.8ab	1.9c	8.6b	10.5d	34.3d	38.1b	0.6b	4.0b	4.7b	35.0d
	135	47.5ab	5.4b	15.5b	20.8c	113.7b	68.1b	1.1b	9.6ab	10.7ab	107.9b
	225	50.8a	6.2b	25.7a	31.9b	192.3a	121.0a	2.3a	11.9a	14.2a	179.4a
	45/90	37.1b	21.9a	15.6b	37.5a	97.0c	117.6a	2.2a	13.4a	15.6a	92.6c
	SE	3.5	1.3	2.2	1.8	1.8	10.5	0.2	2.4	2.4	4.6
	$P > F$	0.111	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	0.037	0.013	<0.001
Clara City14	0	87.2a	-	-	-	-	116.0b	-	-	-	-
	45	68.9a	6.5c	6.4c	12.9c	32.0c	117.1b	1.0c	4.3c	5.4c	37.1d
	135	68.1a	20.0b	16.2b	36.2b	98.3b	174.0ab	2.9b	11.5b	14.4b	111.8b
	225	82.3a	38.0a	27.4a	65.4a	158.8a	238.7a	7.4a	23.1a	30.5a	170.6a
	45/90	76.6a	32.0a	11.1bc	43.1b	91.4b	181.4ab	2.4bc	14.6b	17.0b	92.2c
	SE	8.1	0.6	2.7	7.2	7.2	22.2	0.62	2.5	2.5	3.2
	$P > F$	0.619	<0.001	0.001	<0.001	<0.001	0.011	<0.001	0.002	<0.001	<0.001

† Unaccounted for FDN was calculated as the applied N rate minus total FDN for the first year while the second year was calculated as the applied N rate minus second-year total FDN minus FDN exported in grain from the first year.

Table 1.4 Continued.

Site	N rate	First Year					Second Year				
		Plant		Soil	Total	Unaccounted	Plant		Soil	Total	Unaccounted
		SDN	FDN	FDN	FDN	FDN	SDN	FDN	FDN	FDN	FDN
		kg N ha^{-1}									
Lamberton15	0	189.1a	-	-	-	-	118.5b	-	-	-	-
	45	182.6ab	15.3c	7.9c	23.1c	21.7c	107.5b	1.4c	6.0c	7.4c	36.4c
	135	169.4abc	58.5b	19.6b	78.1ab	56.4bc	120.1ab	3.8b	12.3b	16.0b	115.5b
	225	149.9c	94.1a	37.7a	108.1a	116.1a	174.2a	7.4a	26.2a	33.6a	184.8a
	45/90	163.3ab	51.0b	5.7c	56.7bc	77.8ab	160.0ab	3.7b	13.6b	17.3b	114.3b
	SE	12.3	5.5	3.0	14.4	14.4	18.0	0.7	2.0	1.9	2.0
	P > *F*	0.115	0.001	<0.001	0.014	0.008	0.086	0.001	<0.001	<0.001	<0.001
Waseca14	0	49.6a	-	-	-	-	69.8b	-	-	-	-
	45	49.5a	4.4c	13.5b	17.9c	26.9c	69.0b	0.9c	9.9c	10.8c	33.4c
	135	57.4a	12.8b	25.9b	38.7b	95.8b	133.3a	3.9b	23.9b	27.8b	103.8b
	225	64.0a	32.6a	54.0a	86.6a	137.6a	142.7a	5.6a	37.5a	43.1a	177.3a
	45/90	62.0a	34.2a	17.9b	52.1b	82.4b	114.4a	3.6b	20.6bc	24.2b	107.8b
	SE	6.9	2.6	5.1	5.1	5.1	10.6	0.3	4.3	4.3	4.3
	P > *F*	0.402	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	0.005	0.001	<0.001
Waseca15	0	70.6b	-	-	-	-	86.1c	-	-	-	-
	45	90.2ab	8.9c	12.7b	21.6c	23.3c	90.8c	1.1c	6.7b	7.8c	36.2c
	135	89.0ab	32.3b	23.0b	55.3b	79.2b	137.0b	2.2b	15.1b	17.3b	115.6b
	225	105.9a	62.5a	38.4a	100.9a	123.3a	186.7a	4.2a	28.5a	32.7a	188.3a
	45/90	92.2ab	50.2a	24.7ab	74.9b	59.6b	128.9b	1.6bc	14.3b	15.9bc	117.4b
	SE	10.7	4.7	4.7	8.0	8.0	11.5	0.3	2.8	2.8	2.7
	P > *F*	0.406	<0.001	0.017	0.001	<0.001	0.001	<0.001	0.003	0.001	<0.001

Table 1.5 Fertilizer N use efficiency estimated using the difference method ($FNUE_{diff}$) and isotopic method ($FNUE_{15N}$). The standard error of the means (SE) are also presented.

Site	N Rate	$FNUE_{diff}$	$FNUE_{15N}$
	kg N ha^{-1}	%	
Becker14	45	20.3a†	8.5b
	135	23.6a	12.4b
	225	21.3a	10.1b
	45/90	36.3a	26.7a
	SE	7.5	2.5
	P > F	0.429	0.002
Becker15	45	-3.8b	4.2b
	135	23.2ab	4.0b
	225	27.2a	2.8b
	45/90	51.1a	16.2a
	SE	9.3	1.0
	P > F	0.017	<0.001
Clara City14	45	10.5a	14.3a
	135	7.2a	14.8a
	225	34.0a	16.9a
	45/90	26.8a	23.6a
	SE	15.9	3.8
	P > F	0.600	0.166
Lamberton15	45	68.1a	34.0b
	135	25.8b	43.3a
	225	23.1b	41.9ab
	45/90	6.5b	37.8ab
	SE	12.8	3.3
	P > F	0.028	0.089
Waseca14	45	-	9.7b
	135	-	9.5b
	225	-	14.5b
	45/90	-	25.3a
	SE	-	1.7
	P > F	-	<0.001
Waseca15	45	70.9a	19.7b
	135	26.8b	23.9b
	225	31.3b	27.8b
	45/90	42.1ab	37.2a
	SE	9.3	3.0
	P > F	0.034	0.005

† Within column and site, means followed by the same lowercase letter are not significantly different.

Figure 1.1 Diagrams illustrating the dimensions and placement of the ^{15}N enriched microplot, non-^{15}N enriched sampling area, and harvest area for each site.

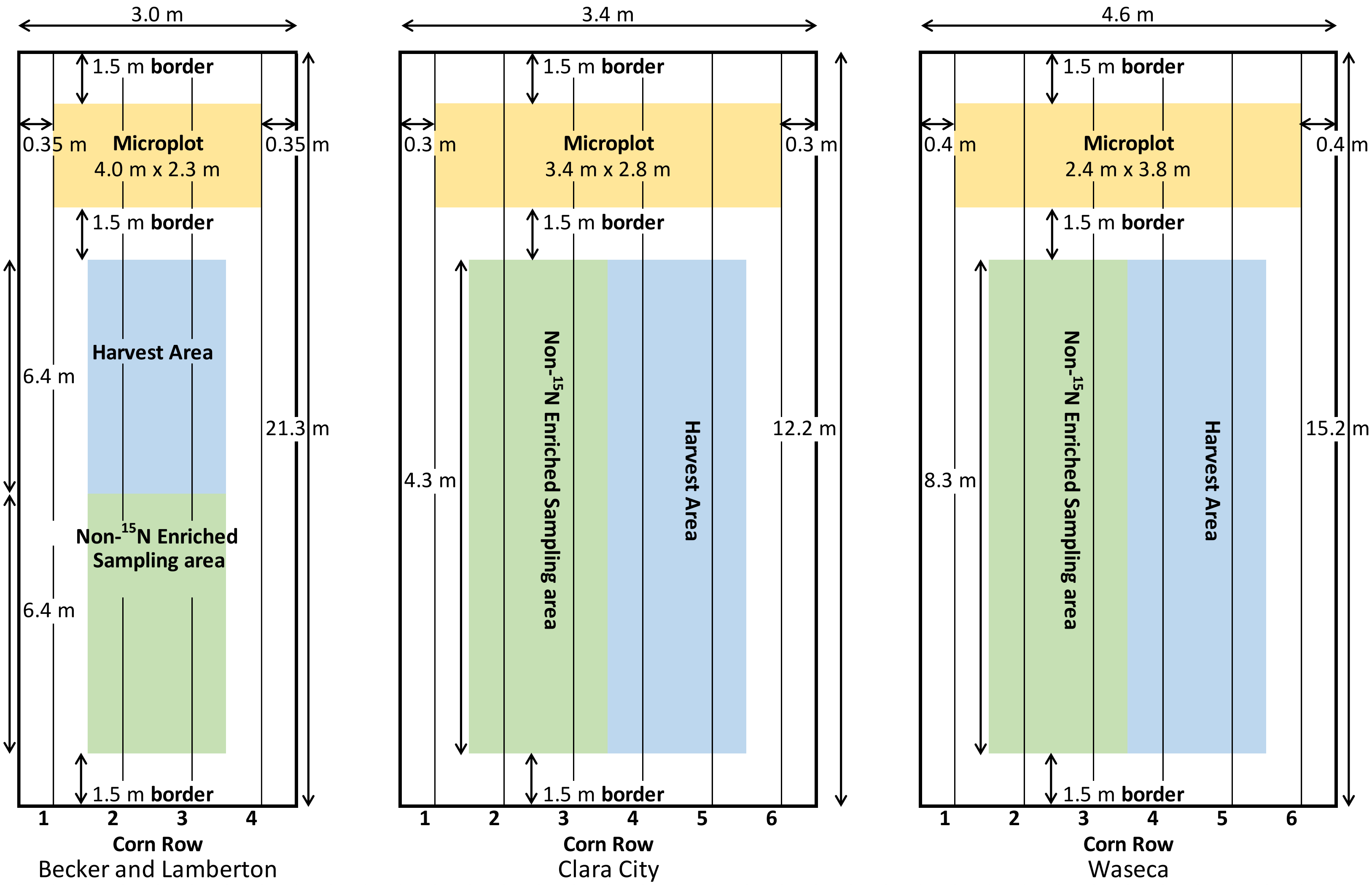
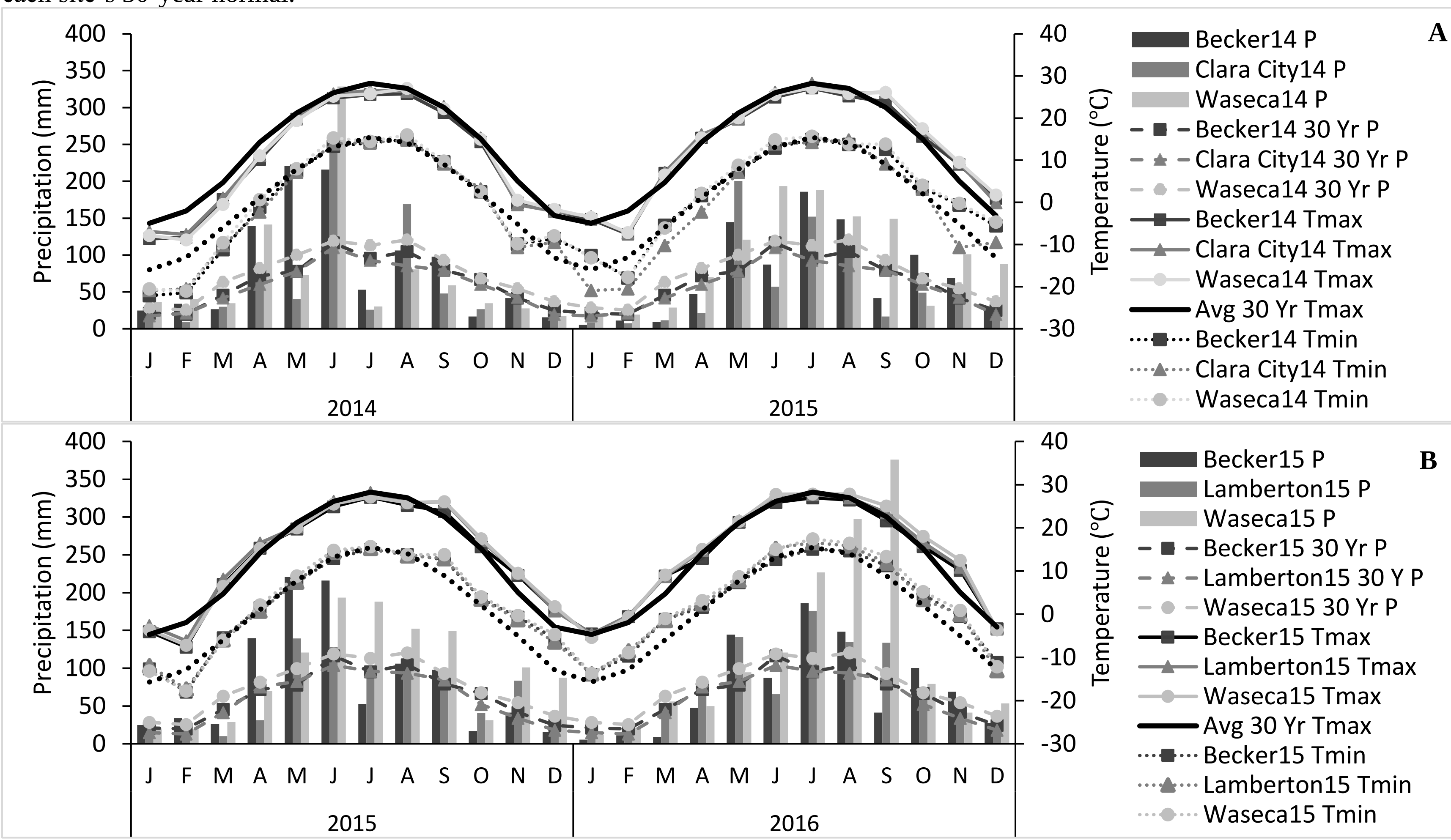


Figure 1.2 Monthly total precipitation (P) and average minimum (Tmin) and maximum (Tmax) monthly temperatures for each site. The 30-year normal precipitation (30 Yr P) is presented for each site individually while the 30-year Tmin and Tmax are the average of the three 2014–2015 sites (A) and three 2015–2016 sites (B). The averaged 30-year Tmin and Tmax values were within ±1.4 ℃ of each site's 30-year normal.



CHAPTER 2: ^{15}NITROGEN UPTAKE AND PARTITIONING IN CORN IN RESPONSE TO FERTILIZER APPLICATION RATE AND TIMING

SYNOPSIS

Urea fertilizer applications at planting are becoming increasingly important for U.S. upper Midwest corn (*Zea mays* L.) production but wet spring conditions may result in significant nitrogen (N) fertilizer loss. Split-applications may avoid wet conditions and improve fertilizer uptake and use efficiency. Field studies were performed on six field sites to determine the effect of urea fertilizer rate and application timing on fertilizer-derived N (FDN) and soil-derived N (SDN) plant uptake over two consecutive growing seasons. Fertilizer treatments included a non-fertilized check plot, 45, 135, 225 kg urea-N ha^{-1} applied at planting, and a split-application of 45 kg N ha^{-1} at planting and 90 kg N ha^{-1} at the four collared leaf stage (V4). Labeled ^{15}N urea (5 atom %) was applied to microplots within each fertilized treatment. Aboveground plant samples were collected at V8, R1, and R6 in the first year and R6 the second year. Wet spring conditions at four sites resulted in significant N loss that limited plant uptake of FDN and SDN. The percentage of total N uptake as FDN was greatest closest to the time of fertilizer application but decreased over time as SDN increasingly became the dominant N source. The split-application significantly improved FDN uptake over the 135 kg N ha^{-1} treatment but did not improve total N uptake in the first year at any site illustrating the importance of SDN for corn production. Fertilizer N use efficiency using the isotopic method ($FNUE_{15N}$) was 2.8 to 43.3% across all sites where 0.8 to 10.4% was in the stover, 1.8 to 31.4% was in the grain, and 0.1 to 0.7% was in the cob at the end of the first year. At the end of the second year, approximately 0.5% of the originally applied FDN was in the

stover, 1.5% was in the grain, and 0.2% was in the cob. Partitioning of FDN into the stover, grain, and cob fractions was similar in both years between the 135 kg N ha^{-1} single application at planting and the 45/90 kg N ha^{-1} split where approximately 26% of the FDN taken up was in the stover, 72% was in the grain, and <7% was in the cob. This study illustrates the importance of ensuring adequate N availability through fertilization, but ultimately, the soil-crop system should be managed for SDN uptake as >61% of the total N uptake was from the soil.

INTRODUCTION

Since the advent and widespread adoption of synthetic nitrogen (N) fertilizer, the United States' mean annual corn grain yield has increased from approximately 3.4 Mg ha^{-1} in 1960 to 10.5 Mg ha^{-1} in 2019 (USDA NASS, 2020). Fertilizer N allows producers to supplement the native soil N supply to ensure adequate availability, but application rates over the agronomic demand are known to increase the risk of N loss to the environment (Olson, 1980; Francis et al., 1993; Randall and Vetsch, 2005; Stevens et al., 2005b; Struffert et al., 2016; Fernandez et al., 2017). Current strategies to predict the appropriate N application rate are moderately successful and were summarized by (Morris et al., 2018) to include yield goal with or without crop credits, the maximum return to N, soil inorganic N tests (i.e., pre-sidedress nitrate test), plant sensing technologies, plant tissue tests, and computer simulation models. Irrespective of the N rate management strategy, uncontrollable factors, including precipitation patterns, temperature, and soil N transformations, make N management challenging.

In the U.S. upper Midwest, approximately 59% of corn producers apply fertilizer N in the spring before planting (Bierman et al., 2012). While this management strategy may favor farmers' logistical constraints, N loss can be significant due to upper Midwest fields receiving one-third of annual precipitation from April through June (Randall and Vetsch, 2005; Spackman et al., 2019) and corn's low demand for N during the same period (Bender et al., 2013). As predicted for the upper Midwest, climate change is resulting in wetter springs (USGCRP, 2017). This change along with an increase in the use of urea as the major N source in the upper Midwest instead of anhydrous ammonia (Bierman et al., 2012; MDA, 2020), which is initially more resilient to nitrification after application, can have important impacts on improving N use efficiency. Other factors, including soil texture, may further impact the suitability of pre-plant applications for economic and environmental reasons. Split-applying N fertilizer is an alternative practice that can improve the synchrony of N fertilizer availability with corn N demand for improved N use efficiency (Dinnes et al., 2002; Mueller et al., 2017; Morris et al., 2018; Spackman et al., 2019) but selecting the gross N application rate is still a significant challenge.

Without the use of the ^{15}N labeled fertilizers, it is difficult to quantify the impact of fertilizer N on corn N uptake because soil organic matter mineralization can supply a part to all of the total corn N demand (Yost et al., 2012; Spackman, 2018). Utilizing ^{15}N enriched or depleted fertilizers, researchers can track and quantify the fate of labeled fertilizer N into the plant over time and into individual plant tissues (Hauck et al., 1994) providing valuable information about the timing of fertilizer N uptake and utilization that can be used to optimize producers' return on their fertilizer investment. Extensive labeled ^{15}N research has been performed using a variety of cropping systems (Dev and Rennie, 1979; Harmsen and Moraghan, 1988; Recous et al., 1988a) and labeled fertilizer N sources including anhydrous ammonia, ammonium sulfate, urea, oxamide, urea ammonium nitrate, and ammonium nitrate (Allen et al., 1973; Kowalenko, 1978; Sanchez and Blackmer, 1988; Timmons and Baker, 1991; Reddy and Reddy, 1993; Jokela and Randall, 1997) that has established many of the ^{15}N tracer experimental techniques used today (Hauck et al., 1994; Van Cleemput et al., 2008). Work in corn has shown total plant recovery to be 21 to 60% of the applied FDN (Olson, 1980; Reddy and Reddy, 1993; Jokela and Randall, 1997) with as little as 6 to 9% (Reddy and Reddy, 1993) and as much as 38% (Jokela and Randall, 1997) of the applied FDN exported in the grain. The second-year recovery of FDN in the grain was only 0.6 to 6.6% of the original FDN rate (Jokela and Randall, 1997).

Along with changes in N source and climate patterns mentioned earlier, corn breeding advancements have improved grain yield (Bittman and Kowalenko, 2004). All these variables can have important impacts on N use efficiency, but relatively little has been done in recent years to truly quantify fertilizer N use efficiency or SDN contributions under these new conditions. The objective of the study was to better understand how fertilizer uptake and utilization patterns are affected by the rate and timing of fertilizer application and to quantify FDN and SDN assimilation in a continuous corn-corn cropping system over two consecutive growing seasons.

MATERIALS AND METHODS

Site Description

Field experiments were established at the University of Minnesota Research and Outreach Centers in Minnesota at Becker in 2014 and 2015 (Becker14 and Becker15), Waseca in 2014 and 2015 (Waseca14 and Waseca15), and Lamberton in 2015 and 2016 (Lamberton15 and Lamberton16), and a cooperator farmer field at Clara City in 2014 (Clara City14). Corn was grown the year before site establishment and in the two consecutive years that the study was performed. Sites were rain-fed except Becker sites that were irrigated using the water balance method (Steele et al., 2010). Irrigation was applied at 266 mm in 2014, 188 mm in 2015, and 203 mm in 2016. Treatment N rates were not adjusted for irrigation water N content because the nitrate-N concentration in irrigation water was at background levels (< 10 mg kg^{-1}; Lamb et al., 2015).

Daily precipitation data were obtained for each site from the nearest National Weather Service station (NOAA, 2020). Each year was divided into several periods representative of distinct crop N use patterns or N cycling processes. The periods represented harvest from the prior year to early spring thaw (1 November – 30 April), early corn growth and spring N fertilizer applications when inorganic N content is high and uptake is low (1 May – 30 June), rapid vegetative growth before reproductive development stages characteristic of high N demand (1 July – 30 July), and reproductive development and senescence stages (1 August – 30 October). The daily abundance and evenness of precipitation distribution during each period was calculated using the Shannon diversity index (SDI):

$$D = \frac{-\sum(pi * \ln(pi))}{\ln(n)} \qquad [2.1]$$

where *pi* is the fraction of daily precipitation relative to the total precipitation in a given period, ln is the natural logarithm, and *n* is the number of days in the given period (Tremblay et al., 2012). An SDI value close to one indicates that daily precipitation was evenly distributed across the period while a value of zero indicates that all precipitation occurred on a single day as a single event (Tremblay et al., 2012).

Soil chemical and physical properties differed by location and were described in Chapter 3. The predominant soil texture in the top 90 cm was Hubbard loamy sand at Becker, Bearden-Quam silty clay loam complex at Clara City, Normania loam at Lamberton, and Nicollet-Webster clay loam complex at Waseca.

Experimental Design and Plant Sample Collection

Treatment plots were arranged in a randomized complete block design replicated four times with plot dimensions of 21.3 m x 3.0 m at Becker and Lamberton, 12.2 m x 3.4 m at Clara City, and 15.2 m by 4.6 m at Waseca. Treatment plots were delineated into a non-^{15}N enriched sampling area and harvest area, and a ^{15}N enriched microplot area (Figure 1.1). Unconfined microplots were placed 1.5 m from one end of the treatment plot and had dimensions of 4.0 m x 2.3 m at Becker and Lamberton sites, 3.4 m x 2.8 m at Clara City, and 2.4 m x 3.8 m at Waseca sites (Figure 2.1; 2.2).

During N treatment fertilization in the first year, the ^{15}N enriched microplot area was covered with plastic sheeting to prevent fertilization with unlabeled urea. Treatments consisted of unlabeled urea fertilizer (46-0-0, N–P_2O_5–K_2O) broadcast at rates of 0, 45, 135, and 225 kg N ha^{-1} and incorporated with either irrigation or tillage moving from the ^{15}N enriched microplot area to the non-^{15}N enriched sampling area (Table 2.1). An additional application was split-applied as 45 kg N ha^{-1} as urea ammonium nitrate (28–0–0, N–P_2O_5–K_2O) band applied over the seed row as a starter application and top-dressed at the V4 corn phenological development stage (Abendroth et al., 2011) as a broadcast application of 90 kg N ha^{-1} granular urea impregnated with the urease inhibitor N-(n-butyl) thiophosphoric triamide, Agrotain (Koch Fertilizer LLC, Wichita, KS).

During the establishment year (^{15}N application year), each microplot was fertilized at the appropriate rate with 5 atom % excess ^{15}N urea dissolved in 1.7 L of water and applied using a CO_2 pressurized hand-sprayer (0.14 MPa). The 45, 90, and 225 kg N ha^{-1} treatments were uniformly applied to the microplot area while the 45/90 kg N ha^{-1} treatment was band applied as starter and uniformly applied at V4 (without Agrotain) being careful to avoid application on vegetative tissues. Within 24 h of application, microplot fertilizer was incorporated into the soil profile using irrigation or light tillage to minimize volatilization loss (Table 2.1). During the second year, microplots were fertilized with unlabeled fertilizer as the rest of the treatment plot. Other than N, each site's soil fertility was managed according to the University of Minnesota's guidelines for corn production (Kaiser et al., 2018).

Becker and Lamberton sites were planted with Pioneer P9917AMX on 76 cm row spacings while Clara City and Waseca sites were planted with DEKALB DKC44-13 RIB AR on 56 cm row spacings at Clara City and 76 cm row spacings at Waseca. Both hybrids possessed herbicide tolerance (glyphosate and glufosinate) and contained *Bacillus thuringiensis* resistance traits (Cry34Ab1, Cry35Ab1). Separate aboveground plant samples were collected from within the non-^{15}N enriched and ^{15}N enriched microplot sampling areas at the V8, R1, and R6 corn phenological development stages in the first year and at R6 in the second year (Abendroth et al., 2011). The study was designed to minimize the impact of the sampling strategy on soil N availability and corn physiological development of plants within the microplot. Plant samples were taken from the outer edges of the microplot gradually moving towards the center of the plot with each successive sampling event (Figure 2.1; 2.2; Spackman and Fernández, 2020). At each sampling event, a six-plant composite sample was collected leaving at least two plants between sampled plants (Figure 2.1; 2.2). Plant tissue samples collected at R6 were partitioned into grain and stover (stalks, leaves, husks, and cobs). In 2015, stover samples were partitioned into cobs and the rest of the sample. Plant tissue samples were chipped, dried to constant mass in a forced-air oven (60 ℃), a representative subsample was ground to a pass through a 0.177-mm sieve using a roller mill, and analyzed for total N concentration by combustion analysis (Horneck and Miller, 1998) and ^{15}N concentration (UC Davis, 2020). Following R6 sample collection in the ^{15}N application year, all remaining microplot biomass was removed, the remaining treatment plot area was harvested, the microplot area was raked free of residue, and the microplot biomass (excluding the grain and cob) was chipped and re-applied to the microplot (Spackman and Fernandez, 2020). All V8, R1, and R6 sampled biomass (except ≤100 g) was likewise returned to the ^{15}N enriched sampling area. Corn residue was incorporated with fall and/or spring tillage performed parallel to the length dimension of the plot in opposite directions at reduced speeds to minimize lateral soil movement into or out of the microplot area (Table 2.1). In all procedures, the non-^{15}N enriched plant samples were collected and processed before the ^{15}N enriched samples to minimize the risk of cross-contamination.

Data Analysis

Tissue sample N mass was calculated as the product of tissue N concentration and dry matter mass. Total aboveground corn N uptake was the sum of the mass of N in the corn stover, grain, and cob fractions. Following sample analysis for ^{15}N concentration, the fraction of ^{15}N (N_f) in the plant sample was calculated as

$$N_f = \frac{(A_{microplot} - A_{non-15N\ enriched\ sampling\ area})}{(A_{fertilizer} - A_{non-15N\ enriched\ sampling\ area})} \quad [2.2]$$

where A is the measured atom % ^{15}N enrichment of the plant sample collected from within the ^{15}N enriched microplot ($A_{microplot}$), the non-^{15}N enriched sampling area ($A_{non\text{-}15N\ enriched\ sampling\ area}$), or the ^{15}N enrichment of the fertilizer applied to the plot ($A_{fertilizer}$). The mass of fertilizer-derived N (FDN) and soil-derived N (SDN) in the plant samples were calculated as

$$FDN\ (kg\ ha^{-1}) = N_f \times total\ mass\ of\ N\ (kg\ ha^{-1}) \quad [2.3]$$

$$SDN\ (kg\ ha^{-1}) = total\ mass\ of\ N\ (kg\ ha^{-1}) - FDN \quad [2.4]$$

Fertilizer use efficiency using the isotopic dilution method ($FNUE_{15N}$) was calculated as the quotient of FDN and the applied fertilizer N rate multiplied by 100 to convert to a percent (Stevens et al., 2005a).

Linear and quadratic relationships of FDN, SDN, and total N uptake to the applied fertilizer rate (0, 45, 135, and 225 kg N ha^{-1} treatments) were assessed using the REG procedure of SAS. Selected models produced the largest correlation coefficient and had normally distributed residuals (Kutner et al., 2004). When neither model was significant, the means of the 0, 45, 135, and 225 kg N ha^{-1} treatments were reported along with the linear model *P*-value.

Each field experiment was analyzed individually and within the sampling event at $P \leq 0.05$ using SAS (v. 9.4, SAS Institute, 2012). The GLIMMIX procedure of SAS was used to make N treatment mean comparisons where N fertilizer treatment was considered a fixed effect and replication was considered a random effect. Assumptions of normally distributed residuals and homogeneous treatment variances were visually inspected using the RESIDUALPANEL option. The PDIFF option was utilized to make pairwise mean comparisons ($P \leq 0.05$).

RESULTS AND DISCUSSION

Shannon Diversity Index

Monthly total precipitation and air-temperatures were previously described in Chapter 1. Briefly, 2014 growing season conditions were cooler- and wetter-than-normal during early vegetative growth at all field experiments while the weeks just before and following tasseling were drier-than-normal, resulting in drought stress and reduced grain yield. Air temperatures and precipitation were similar to the 30-year normal in 2015 and during vegetative growth in 2016, but conditions were wetter-than-normal from corn tasseling through harvest in 2016.

In this study, an SDI value within ±0.05 of the 30-year normal was considered normal and cumulative precipitation within ±30 mm of the 30-year normal was considered normal. May through June SDI values and cumulative precipitation were typically similar to or greater-than-normal at all sites in all years with an overall average SDI value of 0.63 (Table 2.2). Larger SDI values indicate a more even distribution of precipitation during the period of interest. While evenly distributed precipitation ensures adequate soil moisture for the developing corn crop, greater-than-normal precipitation may increase the risk of N loss through leaching and denitrification processes, drowned-out corn in low-lying areas, and limit field accessibility for weed, disease, and nutrient management. In June 2014, greater-than-normal precipitation at Clara City and Waseca resulted in several days of standing water that likely reduced inorganic N availability to the crop, but did not result in stand differences across the field sites. During routine soil sampling events in 2014, the water table was approximately 30- to 60-cm below the soil surface at Clara City and Waseca from mid-June through mid-July and likely reduced root exploration of the soil profile, although rooting depth was not systematically verified at any time during the growing season. From 1 July through 30 July 2014, SDI and cumulative precipitation values were less-than-normal at all sites, except at Waseca14 where only cumulative precipitation was less-than-normal. A shallow root system combined with drier-than-normal conditions immediately before tasseling may have reduced yield potential at these sites (Abendroth et al., 2011; Bender et al., 2013; Chapter 1).

Shannon diversity index values ≤0.37 from 1 July through 30 July at Clara City and Lamberton in 2015 and Lamberton in 2016 were due to the majority of the period's precipitation occurring as one to three large (> 50 mm in 24 h) events (Chapter 3). Large precipitation events were observed from May through September at Becker one time in 2014, four times in 2015, and one time in 2016; at Clara City one time in 2014 and five times in 2015; at Lamberton one time in 2015 and one time in 2016; and at Waseca four times in 2014, two times in 2015, and five times in 2016. One extreme event occurred on 21 September 2016 at Waseca when 258 mm of precipitation fell over 48 h. Climate projections for the U.S. Midwest Corn Belt predict fewer, larger precipitation events during the summer months resulting in surface ponding and runoff on fine-textured soils and leaching in all soil types, which favors soil erosion and N losses from the soil-crop system (USGCRP, 2017). Given these predictions, it may be expected that future SDI values will trend towards 0 during the summer months.

August through October cumulative precipitation and SDI values were normal or greater-than-normal at all sites and years except at Waseca in 2014 where cumulative precipitation was less-than-normal and at Clara City in 2015 where cumulative precipitation and SDI was less-than-normal (Table 2.2). Adequate soil moisture availability during the reproductive stages is essential for ovule fertilization, final kernel count, and grain fill whereas water stress will reduce yield (Abendroth et al., 2011). During this time, corn assimilates the last quarter to one-third of its total plant N (Karlen et al., 1988; Bender et al., 2013) of which the majority is translocated to the grain (de Oliveira Silva et al., 2017). Because soil inorganic N concentration is typically low during the reproductive development stages (Spackman et al., 2019; Spackman, 2018), adequate soil moisture is essential for the continued supply of soil N via mineralization (Griffin, 2008) and uptake with soil moisture.

Plant N Uptake in Response to Nitrogen Rate

First Year

Total aboveground N and FDN typically increased linearly with increasing N fertilizer rate at all sites and sampling events (V8, R1, and R6) during the first year of ^{15}N application (Figure 2.3; Table 2.3). Soil derived N was often non-responsive to N fertilizer rate at Becker and Clara City sites, increased linearly with the increasing N rates at Waseca sites, and decreased with increasing N fertilizer rates at Lamberton15 (Figure 2.3; Table 2.3). The goal of N fertilization is to supplement soil N to adequately meet corn N demand. As noted in Chapter 1, soil inorganic N availability was insufficient at all N fertilizer rates to optimize corn grain production at Becker, Clara City, and Waseca sites during the first year following N loss. The lack of response of SDN to fertilizer rate at Becker and Clara City sites indicates that fertilization did not affect SDN plant uptake (Table 2.3; Figure 2.3). The lack of an added N interaction (ANI) is probably due to significant N loss from the soil system (both FDN and SDN) via leaching and volatilization (at Clara City) soon after fertilization (Chapter 3). By V8, only 20 to 37% of the applied FDN was in the soil (0 to 60 cm) across N rates at Becker14, Becker15, and Clara City14 (Chapter 3). Further, the 45, 135, and 225 kg N ha^{-1} treatments had similar total inorganic N (TIN) (0 to 60 cm) content as the non-fertilized check at V8 at Becker15 (P = 0.254), while at Clara City14 TIN increased with N rate but the 45 kg N ha^{-1} treatment was similar to the non-fertilized check (P < 0.001; data not shown). Because of significant N loss before V8, total N uptake had a quadratic response to N rate at Clara City14 at V8 and R6 where the functions' vertices occurred at a fertilizer N rate of 51 and 67 kg ha^{-1}, respectively (Table 2.3; Figure 2.3). This may indicate that at least 50 to 70 kg N ha^{-1} was lost from the soil during the growing season representing a significant N load to the environment. This estimate is reasonable as Lawlor et al. (2008) observed annual tile-drainage NO_3–N losses within a range of 0 to 109 kg N ha^{-1} over 15 consecutive years in Iowa.

The positive ANI at Waseca14 and Waseca15 sites (Table 2.3; Figure 2.3) may be due to pool substitution where SDN was taken up following denitrification of labeled fertilizer N or due to fertilization stimulating root development and exploration of the soil profile (Jenkinson et al., 1985; Harmsen and Moraghan, 1988). Pool substitution following denitrification was the likely explanation as standing water was observed for several days at Waseca14 in June 2014. At Waseca15, well-distributed precipitation throughout the growing season (Table 2.2) may have favored denitrification from within saturated soil pores in an unsaturated soil profile (Coyne, 2008). Leaching was also likely significant following large precipitation events as 29 to 52% of FDN was in the top 60 cm of the soil profile at V8 at Waseca14 and TIN was similar (13.2 kg N ha^{-1}) between the 45 kg N ha^{-1} and non-fertilized check (P<0.001). Because fertilizer loss was significant, $FNUE_{15N}$ was ≤14.5% of the applied fertilizer N by R6 (Table 2.5). In a long-term N rate study, Stevens et al. (2005) also noted that fertilization promoted the uptake of SDN, indicating that soil N availability was limited by the need for additional mineralization, though in their study the function was quadratic rather than linear.

In contrast to the previously described sites, Lamberton15 had a negative ANI (Figure 2.3; Table 2.3) that was likely due to Lamberton15's low potential for soil N loss, high concentration of inorganic FDN in the soil following fertilization, and rapid accumulation of plant biomass and FDN by V8. At V8, the proportion of plant FDN uptake:total N uptake was similar to inorganic soil FDN:total inorganic FDN at each N rate (data not shown). This likely occurred because plants do not discriminate between ^{14}N and ^{15}N during N assimilation (Foster et al., 1985) and young corn plants are capable of rapidly assimilating and storing high concentrations of N as amino acids and other organic compounds for later use (Viets, 1965). At Lamberton15, the N rate at which the relative contribution of FDN and SDN to total N uptake was equal occurred at approximately the 225 kg ha^{-1} treatment at V8, similar to Clara City14, Waseca14, and Waseca15 (Figure 2.3). At R1, a similar observation was made at Lamberton15 and Waseca15, likely due to retention of FDN at these sites, but SDN was the main contributor to total N at Waseca14 at all N rates (Figure 2.3). At Becker14, FDN and SDN equally contributed to total plant N uptake at an N rate of 131 kg ha^{-1} at V8 and 171 kg ha^{-1} at R1 (Table 2.3). Because Becker14 had low inorganic SDN content and a low mineralization capacity relative to the other sites, it took a smaller rate of fertilizer to achieve equal FDN and SDN uptake in the crop. These results show that the percent of total N uptake as FDN uptake is greatest early in the growing season, close to the time of application but over time, uptake of SDN increasingly becomes the dominant N source of total plant N uptake. By R6, and similar to other studies, 61 to 96% of the total N uptake across sites was SDN (Stevens et al., 2005, Bigeriego et al., 1979; Olson, 1980; Torbert et al., 1993; Jokela and Randall, 1997; Omay et al., 1998). These results indicate that even at the highest N rate, SDN was a greater contributor to plant N assimilation than FDN. Though outside the scope of this study, the results illustrate that field management that maintains or improves soil health and mineralization potential should be considered as part of a field's nutrient and economic management plan. Providing soil cover during fallow periods with a cover crop or living mulch may be one way to prevent soil erosion and to prevent residual inorganic N from being lost before the next growing season (Alexander et al., 2019).

Across the N rates of the study (45, 135, and 225 kg N ha^{-1}), 48 to 56% of the total FDN accumulated in aboveground plant biomass at R6 was assimilated by V8 at Becker14; 11 to 61% at Becker15; 22 to 47% at Clara City14, Waseca14, and Waseca15;

and 76 to 104% at Lamberton15 (Table 2.4). By R1, 59 to 107% of the total FDN was assimilated in aboveground plant tissue for Becker14, Clara14, Lamberton15, and Waseca15 while 29 to 62% was assimilated for Becker15 and Waseca14. Generally, the percentage of the total FDN recovered increased with increasing N rate. These results suggest that the majority of FDN applied at planting is assimilated in aboveground plant biomass by R1 in corn cropping systems (Bender et al., 2013). Some additional FDN is assimilated into corn biomass after R1, but it was likely only available in small quantities as FDN was mineralized from organic forms (Chapter 3).

Second Year

Across all sites, 0.8 to 3.3% of FDN was recovered in the second year corn biomass (Table 2.5) representing 0.6 to 7.4 kg N ha^{-1}, assuming there was no added N interaction from the unlabeled fertilizer applied in the second year (Table 2.4). Fertilizer-derived N increased with increasing N rate at all sites and was likely primarily due to a greater amount of FDN returned in the corn biomass from the previous year. Others have observed similar findings (Westerman and Kurtz, 1972; Stevens et al., 2005a). Re-mineralization of FDN in soil organic matter may have also contributed to second-year FDN uptake, but the amount was likely only a small percentage as FDN in the organic N fraction was likely more recalcitrant than FDN in corn residue. This is supported by the fact that FDN in the organic N fraction decreased by only 30% on average from harvest the first year to harvest the second year (Chapter 3). These results indicate that fertilizer rate and timing can continue to influence FDN uptake in residual years, but the magnitude was small and likely not of agronomic importance to justify management adjustments based on previous year's N management.

Because FDN accounted for only a small portion of the total plant N uptake, SDN (that included unlabeled fertilizer applied at the beginning of the second year) and total N had similar responses to fertilizer rate (Table 2.3). Total plant N uptake increased linearly with increasing N rate at all sites except Becker15 and Lamberton15 where the non-fertilized treatment was only different from the 225 kg N ha^{-1} treatment at both sites. The quadratic response may be indicative of significant fertilizer loss, similar as was previously described for Clara City14.

Plant Nitrogen Uptake in Response to Nitrogen Fertilizer Timing

Split-applications are often cited as one fertilizer management technique to improve plant N uptake and avoid fertilizer N leaching and denitrification loss associated with spring precipitation (Morris et al., 2018). Plant FDN uptake was improved by the 45/90 kg N ha^{-1} split-application relative to the single 135 kg N ha^{-1} application at all sites throughout the first growing season sampling events except at Clara City14 where only the R6 event was improved and never at Lamberton15 (Table 2.4). Because the single 135 kg N ha^{-1} application was done at planting, several weeks elapsed when FDN was subject to immobilization or loss mechanisms. Conversely, the split-application was done when the crop had an established root system allowing fertilizer N to be rapidly assimilated into the aboveground biomass (Bigeriego et al., 1979). This was especially evident for the coarse-textured soils at Becker14 and Becker15 where the split-application improved FDN uptake by 2.8- and 10.6-fold, respectively (Table 2.4). While the split-application improved FDN uptake, across sites and sampling events there was generally no difference in total or SDN uptake between the split-application or the 135 kg N ha^{-1} single application except at Becker14 and Becker15 for total N and Becker15 for

SDN uptake (Table 2.4). At V8, the split-application increased total N uptake 2.2-, 3.5- and 1.6-fold over the 135 kg N ha^{-1} treatment at Becker14, Becker15, and Waseca15, respectively but by R6 in the first year, there was no difference between either treatment at any site. The lack of differences of total N uptake by the end of the season was likely due to the greater proportion of total N as SDN that effectively masked the fertilizer effect. Thus, while the split-application improved labeled ^{15}N uptake at most sites, the effect of fertilizer timing was not sufficiently large to significantly improve total plant N uptake. Based on this result alone, one might conclude that split-applications provide no benefit over a single application at planting, but as was previously reported in Chapter 1, split-applications significantly improved grain yield on coarse-textured soils where N loss was significant. These results also suggest that fertilizer applications done at planting may perform as well as split-applications when the risk of N loss is low.

In the second year, only Becker sites had significant differences in FDN, SDN, and total N uptake between the 135 and 45/90 kg N ha^{-1} treatments. Averaged across the Becker sites, the split-application improved FDN uptake by 1.7-fold, SDN uptake by 1.6-fold, and total N uptake by 1.6-fold (Table 2.4). These results contrasted the first year, but greater SDN and total N uptake were likely due to more favorable growing season conditions for corn in the second year of each study (Chapter 1). Greater FDN uptake in the 45/90 kg N ha^{-1} treatment in the second year was likely due to the greater mass of FDN returned to the soil from the first-year residue as was previously discussed for the N rate treatments. Based on the first- and second-year results, split-applications will improve FDN uptake on most soils but will likely have the greatest impact on coarse-textured soils that have low mineralization capacity and poor inorganic N retention.

Fertilizer Nitrogen Use Efficiency in the Plant

Although FDN uptake increased with increasing fertilizer rate, there was generally no difference in $FNUE_{15N}$ at V8, R1, or R6 in the first year between the 45, 135, and 225 kg N ha^{-1} treatments (Table 2.5). A similar observation was made for R6 samples by Stevens et al. (2005) but contrasted others (Sanchez and Blackmer, 1988; Reddy and Reddy, 1993, Jokela and Randall, 1997) where $FNUE_{15N}$ decreased with increasing rate. The only exceptions were Waseca14 and Waseca15 at V8 and Waseca14 at R1 where $FNUE_{15N}$ values increased with increasing N rate and Lamberton15 at R6 where the $FNUE^{15}N$ value for the 135 kg N ha^{-1} treatment was greater than the 45 kg N ha^{-1} treatment (Table 2.5).

At V8, $FNUE_{15N}$ ranged from 2.2 to 7.9% across the N rate treatments (45, 135, and 225 kg N ha^{-1}) across all sites except Becker15 where $FNUE_{15N}$ was 0.5 to 3.5% and Lamberton15 where $FNUE_{15N}$ was 31.7 to 36.0%. At all sites except Clara City14 and Lamberton15, $FNUE_{15N}$ was greater for the 45/90 kg N ha^{-1} treatment than the other N rate treatments and ranged from 10.3 to 17.9% at V8 (Table 2.5). Lamberton15's greater $FNUE_{15N}$ values compared to the other sites correspond to this site accumulating 56 to 62% of the final R6 aboveground dry biomass by V8. In comparison, the other sites had accumulated only 6 to 29% of the final R6 biomass by V8 (data not shown). Lamberton15's rapid biomass accumulation was likely in response to favorable soil and weather conditions producing 12.6 Mg grain ha^{-1} irrespective of the N fertilizer rate (Chapter 1). Corn breeding strategies have focused on improving yield, increasing pest and disease resistance, improving drought tolerance, or adaptation to cooler climates (Duvick and Cassman, 1999; Bittman and Kowalenko, 2004) but selective breeding that accelerates N uptake earlier in the vegetative stages may be one way to improve fertilizer use efficiency and minimize FDN and SDN loss during the wet spring months. This likely would require increasing the rate of biomass production during the early vegetative development stages to provide an adequate sink for assimilated N. Increasing the root density in the soil may be another strategy to improve N assimilation because most inorganic N is intercepted via soil water mass flow.

Fertilizer N use efficiency increased by -0.1 to 14.2 percentage points from V8 to R1 and -6.9 to 10.5 percentage points from R1 to R6 across all sites and fertilizer treatments (Table 2.5). From V8 to R1, the greatest increase occurred at Clara City14 and Waseca15 and the smallest increase occurred at Becker15 (Table 2.5). From R1 to R6, Waseca14 and Waseca15 had the greatest increase in $FNUE_{15N}$ values. The 45/90 kg N ha^{-1} treatment had the greatest $FNUE_{15N}$ values at R6 at all sites except Clara City14 and Lamberton15 with values that ranged from 16.2 to 37.2%.

Although Lamberton15 had the largest $FNUE_{15N}$ values of all the sites, $FNUE_{15N}$ only increased from V8 to R6 for the 225 kg N ha^{-1} treatment. Likewise, there was little increase of $FNUE_{15N}$ at most sites from R1 to R6 except in the 45/90 kg N ha^{-1} split application. As described in Chapter 3, fertilizer N is rapidly incorporated into the soil organic N fraction that is several orders of magnitude greater than the applied fertilizer rate. Re-mineralization occurs at a slower rate and is diluted into a larger inorganic SDN pool. This result explains why there was only a small increase of $FNUE_{15N}$ values from R1 to R6 at Becker14, Becker15, Clara City14, and Waseca15 where approximately 6 to 19% of the applied FDN was potentially mineralized during that period (Chapter 3, the difference between FDN_{TN} at R1 and R6). However, during this same period, 55 to 66%

was potentially mineralized at Lamberton15. While a large amount of potentially mineralized FDN at Lamberton15 would be expected to increase FDN uptake in the crop, inorganic FDN was only 8, 31, 45, and 22% of the total inorganic N at R1 for the 45, 135, 225, and 45/90 kg N ha^{-1} treatments, respectively and 6% of the total inorganic N at R6 averaged across treatments (data not shown). Thus, dilution of the ^{15}N signal in the larger inorganic SDN pool may explain the lack of $FNUE_{15N}$ improvement at Lamberton15.

At R6 in the second year, $FNUE_{15N}$ was ≤3.3% across all sites and treatments. While significant differences did occur, they did not appear to follow a pattern except at Becker14 where the 45 and 45/90 kg N ha^{-1} treatments improved $FNUE_{15N}$ by 0.95 percentage points relative to the other treatments. Although the 45/90 kg N ha^{-1} treatments often had greater FDN uptake and $FNUE_{15N}$ values at R6 in the first year, it did not correspond to larger recoveries in the second year.

Fertilizer-Derived Nitrogen Partitioning at R6

Like total aboveground FDN uptake, the mass of FDN in the stover (leaves, stalks, and cobs in 2014 and 2016), grain, and cobs (in 2015 only) generally increased with increasing N rate across all sites in both the first and second years (Table 2.6). Fertilizer-derived N uptake in the 45/90 kg N ha^{-1} treatment was greater than the 225 kg N ha^{-1} treatment at Becker and Waseca sites and not different from the 135 kg N ha^{-1} treatment at Lamberton15 and Clara City14 in the first year. In the second year, FDN uptake was not different between the 45/90 and 135 kg N ha^{-1} treatments at all sites except at Becker sites where the 45/90 and 225 kg N ha^{-1} treatments were similar.

Of the total FDN recovered in the plant at the end of the first year, 18 to 50% (average of 28% across all sites and treatments) was present in the aboveground stover (stalk, leaves, and cobs in 2014 or 2016), 46 to 82% (average of 69%) was present in the grain, and 2 to 7% (average of 4%) was in the cobs (except at the 45 kg N ha^{-1} treatment at Becker15 that was 28%). Similar results have been reported (Jokela and Randall, 1997; Stevens et al., 2005a). At the end of the second year, 19 to 33% (average of 24%) was in stover, 58 to 81% (average of 73%) was in the grain, and 3 to 22% (average of 7%) was in the cob. Although split-applications resulted in greater FDN uptake in the grain and stover, partitioning of FDN to the different plant tissues occurred at similar percentages within sites across treatments. The V4 split-application was likely done early enough in the crop's vegetative development that FDN was not preferentially translocated to the grain as might have happened with a later split-application (Mueller et al., 2017).

The $FNUE_{15N}$ values at the end of the first year were 0.8 to 10.4% (average of 5.2% across all sites and treatments) in the aboveground stover (stalk, leaves, and cobs in 2014 or 2016), 1.8 to 31.4% (average of 14.2%) in the grain, and 0.1 to 1.5% (average of 1.0%) in the cobs. At the end of the second year, 0.2 to 0.8% (average of 0.5%) was in stover, 0.6 to 2.5% (average of 1.5%) was in the grain, and 0.1 to 0.7% (average of 0.2%) was in the cob. At the end of the first growing season after labeled fertilizer application, others have reported $FNUE_{15N}$ values of 7 to 35% in the stover (Bigeriego et al., 1979; Olson, 1980; Sanchez and Blackmer, 1988; Reddy and Reddy, 1993; Schindler and Knighton, 1999) and 6 to 38% in the grain (Olson, 1980; Sanchez and Blackmer, 1988; Reddy and Reddy, 1993; Jokela and Randall, 1997; Schindler and Knighton, 1999), and 0.7 to 2% in the cob (Schindler and Knighton, 1999). At the end of the second or third years, FDN in the grain had been reported at 0.3 to 6.6% of the applied N rate (Sanchez and Blackmer, 1988; Jokela and Randall, 1997). Combined over the two years of this study, only 2 to 34% (average of 14%) of the applied FDN was removed in the grain across the 45, 135, and 225 kg N ha^{-1} treatments and across all sites, while 12 to 29% (average of 21%) was removed in the 45/90 kg N ha^{-1} treatment. The $FNUE_{15N}$ values in this study tended to be at the lower end of reported recoveries and highlight the fact that this study had overall conditions conducive to N loss, but Lamberton15 values were similar to those reported in other studies. Similar low recoveries should be expected if spring conditions continue to be wetter-than-normal and may require using other N sources that do not nitrify as quickly as urea, later application timings, or field management practices to minimize N loss to the environment.

CONCLUSIONS

Recent years have received greater-than-normal precipitation in May and June that saturate the soil profile. Under these conditions, urea applications done at planting are at an elevated risk of leaching and denitrification loss, especially on coarse-textured soils. Because these FDN losses typically occur before rapid corn N uptake and the N supplying capacity of the soil is typically less than corn N demand, $FNUE_{15N}$ is low and corn grain yield is not maximized. However, when conditions do not favor significant N loss early in the season, urea applied at planting may be an effective N management strategy.

This study demonstrated that the ratio of FDN:total N uptake was greatest closest to the time of fertilization and decreased with time as plant available FDN content in the soil decreased and SDN was increasingly assimilated. This indicates that to improve

$FNUE_{15N}$, fertilizer applications should be delayed until closer to the time of rapid corn N uptake. While FDN recovery was increased by delayed application, there was no difference in SDN or total N uptake between a single or split-application at R6 in the first year potentially suggesting that overall, the split did not reduce the total potential N loss to the environment. Additionally, fertilizer rate and timing management strategies can do little to prevent SDN loss during wet spring months when the corn is small and has low N demand. During this period, other soil N management strategies, such as cover crops, may be required to retain soil N until the corn has an established root system. If cover crops are used, the challenge would be to ensure that cover crop residue mineralization coincides with corn N demand. Otherwise, re-mineralized FDN and SDN loss may simply be delayed until later in the growing season.

Table 2.1 Field study management practices and activities for each site over two consecutive growing seasons.

Year	Activity†	Becker14	Clara City14	Waseca14	Becker15	Lamberton15	Waseca15
		Day of the Year					
1	Pre-plant soil sampling	125	143	126	90	124	112
	Spring tillage‡	127 FC	148 FC	139 FC	91 FC	141 FC	118 FC
	Non-^{15}N enriched urea fertilization§	141 I	148 FC	142 FC	111 FC	141 FC	125 FC
	Plant	134	150	143	117	141	125
	Non-^{15}N enriched urea ammonium nitrate fertilization	141	154	143	121	147	125
	^{15}N enriched urea fertilization§	142 I	154 HR	143 I	118 I	142 I	132 I
	Post-^{15}N enriched fertilization soil sampling	150	161	149	125	148	140
	V4 Non-^{15}N enriched urea fertilization	163	176	177	152	166	161
	V4 ^{15}N enriched urea fertilization§	163 I	176 HR	177 I	152 I	166 I	161 I
	V8 plant and soil sampling	182	191	188	173	189	177
	R1 plant and soil sampling	204	217	217	215	217	204
	R6 plant and soil sampling	274	288	273	260	268	265
	Harvest	286	296	294	286	287	282
	Post-harvest soil sampling	308	310	-	293	307	294
	Reapply ^{15}N enriched biomass	311	310	97	304	314	295
	Fall tillage‡	311 D,C	313 C	-	-	315 D,C	295 D,C
2	Pre-plant soil sampling	93	104	96	82	106	105
	Spring tillage‡	91 FC	108 FC	98 D	118 D	127 FC	125 FC
	Non-^{15}N enriched urea fertilization§	111 I	107 FC	118 FC	118 I	127 FC	125 FC
	Plant	117	120	120	119	128	126
	Non-^{15}N enriched urea ammonium nitrate fertilization	121	125	120	123	128	127
	V4 Non-^{15}N enriched urea fertilization	152	160	156	158	165	159
	R6 plant and soil sampling	260	274	265	264	272	263
	Harvest	286	279	285	288	293	293
	Post-harvest soil sampling	292	300	295	302	315	298

† V, vegetative stage of corn phenological development; R, reproductive stage of corn phenological development.

‡ Tillage method: D, disk; C, chisel/ripper; FC, field cultivator.

§ Method of fertilizer incorporation: FC, field cultivator; I, ≥ 6 mm irrigation or water tank; HR, hand rake.

¶ ^{15}N enriched microplot biomass was returned at the beginning of the second year.

Table 2.2 Shannon diversity index values illustrating the abundance and evenness of precipitation during the periods representing harvest from the prior year to early spring thaw (1 Nov. – 30 Apr.), early corn growth and N fertilizer applications (1 May – 30 June), rapid vegetative growth and development before reproduction (1 July – 30 July), and reproductive development and senescence (1 Aug. – 30 Oct.). A value of 1 indicates an equal amount of precipitation fell each day during the period while a value of 0 indicates all precipitation occurred on a single day during the period. Shannon diversity index values do not include irrigation events at Becker.

Site	Period	Shannon Diversity Index				Cumulative Precipitation (mm)			
		2014	2015	2016	30–Year Normal	2014	2015	2016	30–Year Normal
Becker	1 Nov. – 30 Apr.	0.90	0.47	0.69	0.56	280	130	249	222
	1 May – 30 June	0.63	0.63	0.72	0.59	436	232	131	194
	1 July – 30 July	0.38	0.47	0.49	0.48	53	186	165	95
	1 Aug. – 30 Oct.	0.50	0.60	0.67	0.55	221	290	293	251
Clara City	1 Nov. – 30 Apr.	0.66	0.44	-	0.54	148	119	-	202
	1 May – 30 June	0.67	0.58	-	0.59	279	257	-	188
	1 July – 30 July	0.25	0.36	-	0.46	26	152	-	93
	1 Aug. – 30 Oct.	0.54	0.48	-	0.55	243	167	-	225
Lamberton	1 Nov. – 30 Apr.	-	0.38	0.75	0.60	-	95	279	198
	1 May – 30 June	-	0.65	0.62	0.60	-	267	207	186
	1 July – 30 July	-	0.37	0.34	0.47	-	96	176	96
	1 Aug. – 30 Oct.	-	0.56	0.65	0.56	-	241	340	230
Waseca	1 Nov. – 30 Apr.	0.91	0.50	0.70	0.66	301	182	328	290
	1 May – 30 June	0.60	0.70	0.54	0.64	401	314	215	219
	1 July – 30 July	0.61	0.49	0.59	0.52	30	188	227	113
	1 Aug. – 30 Oct.	0.61	0.63	0.61	0.58	174	333	752	282

Table 2.3 Regression parameter estimates of total aboveground corn biomass N (TN) and, uptake of fertilizer-derived N (FDN) and soil-derived N (SDN) by fertilizer application rate for the equation $Uptake=a+bx+cx^2$.

Site	Time of Sampling	Plant Uptake	Parameter Estimates			Adj. R^2†	$P>F$
			a	b	c		
Becker14	V8	FDN	0.2	0.05085		0.68	<0.001
		SDN	6.9				0.838
		TN	7.0	0.05193		0.50	0.001
	R1	FDN	-0.7	0.10673		0.46	0.003
		SDN	17.6				0.381
		TN	15.6	0.12022		0.33	0.015
	R6 Y1	FDN	0.1	0.10602		.66	<0.001
		SDN	51.6				0.961
		TN	51.6	0.10714		0.33	0.012
	R6 Y2	FDN	0.3	0.01348		0.88	<0.001
		SDN	41.3	0.21990		0.63	<0.001
		TN	41.6	0.23338		0.65	<0.001
Becker15	V8	FDN	-0.3	0.01713		0.81	<0.001
		SDN	2.0	0.01600		0.80	<0.001
		TN	1.6	0.03313		0.85	<0.001
	R1	FDN	-0.1	0.01498		0.94	<0.001
		SDN	16.5	-0.03010	0.00020	0.35	0.024
		TN	16.6	-0.02340	0.00024	0.62	0.001
	R6 Y1	FDN	0.5	0.02820		0.68	<0.001
		SDN	48.0				0.466
		TN	47.0				0.054
	R6 Y2	FDN	0.0	0.00969		0.82	<0.001
		SDN	48.1	-0.19630	0.00234	0.65	<0.001
		TN	48.2	-0.18970	0.00235	0.66	<0.001

† Adj. R^2: Adjusted R^2

Table 2.3 Continued.

Site	Time of Sampling	Plant Uptake	Parameter Estimates			Adj. R^2†	*P>F*
			a	b	c		
Clara City14	V8	FDN	-1.4	0.07833		0.56	0.001
		SDN	13.9				0.286
		TN	14.4	-0.08180	0.00080	0.39	0.015
	R1	FDN	-2.2	0.16800		0.72	<0.001
		SDN	40.0				0.949
		TN	38.3	0.16978		0.48	0.003
	R6 Y1	FDN	-0.9	0.16764		0.73	<0.001
		SDN	76.6				0.888
		TN	85.5	-0.22910	0.00172	0.37	0.020
	R6 Y2	FDN	-0.4	0.03212		0.83	<0.001
		SDN	103.6	0.57180		0.63	<0.001
		TN	103.1	0.60392		0.65	<0.001
Lamberton15	V8	FDN	1.5	0.32022		0.93	<0.001
		SDN	65.2	0.54647	-0.00225	0.43	0.010
		TN	64.5	0.96089	-0.00266	0.86	<0.001
	R1	FDN	-0.6	0.38789		0.98	<0.001
		SDN	138.8	-0.12750		0.20	0.046
		TN	138.2	0.26037		0.57	<0.001
	R6 Y1	FDN	-1.3	0.42726		0.93	<0.001
		SDN	191.3	-0.19850		0.33	0.014
		TN	190.0	0.22872		0.36	0.011
	R6 Y2	FDN	-0.1	0.03240		0.83	<0.001
		SDN	118.2	-0.34630	0.00265	0.27	0.051
		TN	118.3	-0.32480	0.00270	0.31	0.037

† Adj. R^2: Adjusted R^2

Table 2.3 Continued.

Site	Time of Sampling	Plant Uptake	Parameter Estimates				
			a	b	c	Adj. R^2†	$P>F$
Waseca14	V8	FDN	0.13	0.00213	0.00028	0.86	<0.001
		SDN	5.4	0.04061		0.55	<0.001
		TN	6.2	0.01359	0.00041	0.75	<0.001
	R1	FDN	-1.4	0.08387		0.88	<0.001
		SDN	30.0				0.540
		TN	29.9	0.06999		0.27	0.023
	R6 Y1	FDN	0.7	0.03033	0.00049	0.92	<0.001
		SDN	48.2	0.06844		0.17	0.060
		TN	46.4	0.20976		0.61	<0.001
	R6 Y2	FDN	0.0	0.02599		0.94	<0.001
		SDN	65.7	0.37513		0.64	<0.001
		TN	65.7	0.40112		0.67	<0.001
Waseca15	V8	FDN	-0.7	0.07691		0.96	<0.001
		SDN	8.5	0.03081		0.45	0.003
		TN	7.9	0.10773		0.90	<0.001
	R1	FDN	-1.6	0.21650		0.94	<0.001
		SDN	32.0	0.09752		0.53	<0.001
		TN	30.4	0.31402		0.86	<0.001
	R6 Y1	FDN	-2.3	0.27870		0.90	<0.001
		SDN	75.8	0.13002		0.21	<0.044
		TN	73.5	0.40872		0.64	<0.001
	R6 Y2	FDN	0.1	0.01783		0.91	<0.001
		SDN	78.0	0.46554		0.80	<0.001
		TN	78.1	0.48336		0.81	<0.001

† Adj. R^2: Adjusted R^2

Table 2.4 Plant N uptake means measured as fertilizer-derived N (FDN), soil-derived N (SDN), and total aboveground plant N (TN) with their associated standard errors (SE) at the V8, R1, and R6 corn physiological development stages in the first year (R6 Y1) and at R6 the second year (R6 Y2).

Site†	N rate	V8			R1			R6 Y1			R6 Y2		
		FDN	SDN	TN	FDN	SDN	TN	FDN	SDN	TN	FDN	SDN‡	TN
		kg ha^{-1}											
Becker14	0		6.6a			15.0a			48.7a			45.7b	
	45	2.1c†	6.8a	8.9c	2.4b	18.0a	20.4b	3.8c	53.0a	56.8b	1.2c	49.3b	50.5b
	135	8.4b	7.4a	15.9b	15.2ab	19.1a	34.3ab	16.8b	55.7a	72.4ab	2.1b	64.0b	66.1b
	225	10.9b	6.8a	17.7b	22.0a	18.2a	39.9ab	22.8b	49.0a	71.7ab	3.2a	95.4a	98.6a
	45/90	24.2a	8.5a	32.7a	30.0a	20.4a	50.3a	36.1a	49.6a	85.6a	3.1a	100.3a	103.4a
	SE	1.8	0.9	2.5	5.2	2.3	7.5	3.8	3.9	6.5	0.2	8.9	10.1
	P > *F*	<0.001	0.532	<0.001	<0.001	0.579	0.083	0.001	0.655	0.073	0.001	0.002	0.012
Becker15	0		1.9c			16.2ab			48.8a			51.2b	
	45	0.2c	2.7c	2.9d	0.6b	16.3ab	16.9b	1.9c	44.8ab	46.7a	0.6b	38.1b	38.7b
	135	1.6c	4.5ab	6.0c	1.5b	15.6b	17.2b	5.4bc	47.5ab	52.9a	1.1b	68.1b	69.2b
	225	3.8b	5.4a	9.2b	3.5b	20.0ab	23.5b	6.2b	50.8a	57.0a	2.3a	121.0a	123.3a
	45/90	17.0a	4.0b	21.0a	25.8a	23.9a	49.7a	21.9a	37.1b	59.0a	2.2a	117.6a	119.7a
	SE	0.7	0.3	0.8	2.2	2.6	4.3	1.3	3.5	3.9	0.2	10.5	11.8
	P > *F*	<0.001	<0.001	<0.001	<0.001	0.178	0.001	<0.001	0.111	0.195	<0.001	<0.001	0.002
Clara City14	0		14.3a			37.3a			87.2a			116.0b	
	45	1.4b	11.2a	12.6b	3.8c	42.1a	45.9b	6.5c	68.9a	75.3c	1.0c	117.1b	118.1b
	135	6.9b	10.9a	17.8b	19.6b	39.2a	58.8ab	20.0b	68.1a	88.0bc	2.9b	174.0ab	176.8ab
	225	17.7a	19.1a	36.7a	36.5a	41.4a	77.9a	37.9a	82.3a	120.3a	7.4a	238.7a	246.1a
	45/90	6.9b	15.1a	22.0b	21.9b	44.3a	66.2ab	31.9a	76.6a	108.5ab	2.4bc	181.4ab	183.8ab
	SE	3.2	3.8	6.7	4.9	6.0	8.9	4.9	8.1	11.0	0.6	22.2	24.0
	P > *F*	0.009	0.248	0.019	0.004	0.855	0.042	0.001	0.417	0.029	<0.001	0.011	0.030

† Within column and site, means followed by the same lowercase letter are not significantly different.

‡ The R6 Y2 SDN value includes fertilizer N applied during the second year in addition to mineralized non-labeled SDN.

Table 2.4 Continued.

Site†	N rate	V8			R1			R6 Y1			R6 Y2		
		FDN	SDN	TN	FDN	SDN	TN	FDN	SDN	TN	FDN	SDN‡	TN
		kg ha^{-1}											
Lamberton15	0		59.8c			129.5a			189.1a			118.5b	
	45	15.9c	95.2a	111.1b	16.3c	141.9a	158.2a	15.3c	182.6ab	197.9a	1.4c	107.5b	108.8b
	135	48.5b	91.3ab	139.9a	51.5b	127.1a	178.6a	58.5b	169.4abc	227.9a	3.8b	120.1ab	123.8ab
	225	71.3a	76.4bc	147.7a	87.0a	105.0a	192.0a	94.1a	149.9c	237.6a	7.4a	174.2a	181.6a
	45/90	49.1b	81.0ab	130.0ab	60.3b	140.9a	201.2a	51.0b	163.3bc	214.2a	3.7b	160.0ab	163.7ab
	SE	4.4	5.9	6.2	6.1	13.9	19.3	5.5	12.3	12.1	0.7	18.0	19.7
	P > F	<0.001	0.006	0.013	<0.001	0.365	0.461	<0.001	0.047	0.201	0.001	0.086	0.091
Waseca14	0		5.7b			34.7a			49.6a			69.8b	
	45	1.0b	7.5b	8.6c	1.5b	26.2a	27.7b	4.4c	49.5a	53.9c	0.9c	69.0b	69.9b
	135	5.3b	9.5b	14.8bc	7.9b	30.3a	38.2ab	12.8b	57.4a	70.3bc	3.9b	133.3a	137.2a
	225	14.7a	15.3a	30.0a	18.8a	28.7a	47.4a	32.6a	64.0a	96.6a	5.6a	142.7a	148.3a
	45/90	13.9a	10.2b	24.1ab	19.9a	30.6a	50.6a	34.2a	62.0a	96.2ab	3.6b	114.4a	118.0a
	SE	1.5	1.7	3.2	2.2	3.9	5.6	2.6	6.9	9.7	0.3	10.6	11.4
	P > F	<0.001	0.009	0.003	0.001	0.648	0.067	<0.001	0.271	0.013	<0.001	<0.001	0.002
Waseca15	0		7.5c			29.0b			70.6b			86.1c	
	45	2.2d	11.4abc	13.6c	5.8c	40.0ab	45.8c	8.9c	90.2ab	99.1c	1.1c	90.8c	91.9c
	135	9.4c	12.1ab	21.6b	28.4b	45.5a	73.8b	32.3b	89.0ab	121.3bc	2.2b	137.0b	139.2b
	225	17.0b	15.4a	32.4a	47.2a	53.1a	100.2a	62.5a	105.9a	168.4a	4.2a	186.7a	190.9a
	45/90	23.5a	10.8bc	34.3a	42.7a	46.6a	89.3ab	50.2a	92.2ab	142.3ab	1.6bc	128.9b	130.5b
	SE	1.2	1.5	2.3	4.0	4.5	8.2	4.7	10.0	14.5	0.3	11.5	12.8
	P > F	<0.001	0.016	<0.001	<0.001	0.027	0.006	<0.001	0.057	0.008	<0.001	<0.001	0.001

† Within column and site, means followed by the same lowercase letter are not significantly different.

‡ The R6 Y2 SDN value includes fertilizer N applied during the second year in addition to mineralized non-labeled SDN.

Table 2.5 Fertilizer-derived N (FDN) recovery efficiency measured using the isotopic method ($FNUE_{15N}$) and the associated standard error (SE) at the V8, R1, and R6 corn physiological development stages in the first year (R6 Y1) and at R6 the second year (R6 Y2).

Site	N rate	V8	R1	R6 Y1	R6 Y2
		$FNUE_{15N}$† (%)			
Becker14	45	4.7b‡	5.4b	8.5b	2.6a
	135	6.2b	11.2b	12.4b	1.6b
	225	4.9b	9.5b	10.1b	1.4b
	45/90	17.9a	22.2a	26.7a	2.3a
	SE	1.1	2.8	2.5	0.2
	P > *F*	<0.001	0.008	0.002	0.002
Becker15	45	0.5b	1.4b	4.2b	1.3ab
	135	1.2b	1.1b	4.0b	0.8c
	225	1.7b	1.5b	2.8b	1.0bc
	45/90	12.6a	19.1a	16.2a	1.6a
	SE	0.5	1.6	1.0	0.1
	P > *F*	<0.001	<0.001	<0.001	0.010
Clara City14	45	3.2a	8.5a	14.3a	2.3b
	135	5.1a	14.5a	14.8a	2.1b
	225	7.9a	16.2a	16.9a	3.3a
	45/90	5.1a	16.2a	23.6a	1.8b
	SE	2.1	3.2	3.8	0.4
	P > *F*	0.301	0.164	0.166	0.015
Lamberton15	45	35.3a	36.2a	34.0b	3.0a
	135	36.0a	38.2a	43.3a	2.8a
	225	31.7a	38.7a	41.9ab	3.3a
	45/90	36.3a	44.7a	37.8ab	2.8a
	SE	3.3	4.8	3.3	0.5
	P > *F*	0.732	0.621	0.089	0.820
Waseca14	45	2.3c	3.3c	9.7b	2.0b
	135	3.9bc	5.9bc	9.5b	2.9a
	225	6.5b	8.3b	14.5b	2.5ab
	45/90	10.3a	14.8a	25.3a	2.7ab
	SE	1.0	1.5	1.7	0.2
	P > *F*	0.001	0.003	<0.001	0.084
Waseca15	45	4.8c	12.9b	19.7b	2.4a
	135	7.0bc	21.0b	23.9b	1.6bc
	225	7.5b	21.0b	27.8b	1.9ab
	45/90	17.4a	31.6a	37.2a	1.2c
	SE	0.8	3.2	3.0	0.2
	P > *F*	<0.001	0.017	0.005	0.008

† Calculated as the quotient of FDN taken up in aboveground plant biomass and the applied fertilizer N rate in the first year.

‡ Within column and site, means followed by the same lowercase letter are not significantly different.

Table 2.6 Fertilizer-derived N (FDN) and soil-derived N (SDN) partitioned into stover (stalk, leaves, husks, and cobs in 2014 and 2016), grain, and cob portions of aboveground corn plant biomass at R6 during the first (R6 Y1) and second (R6 Y2) growing seasons after ^{15}N enriched fertilizer application.

Site†	N rate	R6 Y1						R6 Y2					
		Stover		Grain		Cob		Stover		Grain		Cob	
		FDN	SDN	FDN	SDN	FDN	SDN	FDN	SDN	FDN	SDN	FDN	SDN
		kg ha^{-1}											
Becker14	0	.	15.2a	.	33.4a	.	.	.	12.7b	.	30.5b	.	2.6b
	45	1.3c	13.4a	2.5c	39.6a	.	.	0.3c	13.9b	0.8c	32.6b	0.1c	2.7b
	135	5.9b	14.8a	10.9bc	40.9a	.	.	0.5b	17.0b	1.5b	43.6b	0.1b	3.4b
	225	7.3b	12.2a	15.4b	36.8a	.	.	0.7a	22.4a	2.3a	67.5a	0.2a	5.5a
	45/90	11.7a	13.2a	24.3a	36.4a	.	.	0.7a	25.1a	2.2a	70.6a	0.1b	4.6a
	SE	1.1	1.9	2.8	2.8	.	.	0.04	1.5	0.2	7.4	0.01	0.4
	P>F	<0.001	0.791	0.002	0.634	.	.	<0.001	<0.001	0.001	0.004	0.002	<0.001
Becker15	0	.	12.7a	.	33.7ab	.	2.4ab	.	13.8b	.	34.2b	.	.
	45	0.4b	12.2a	0.9c	29.6ab	0.5b	3.0a	0.2b	13.1b	0.4b	25.0b	.	.
	135	2.7ab	13.5a	2.5bc	31.7ab	0.2b	2.3ab	0.3b	18.2b	0.8b	49.9b	.	.
	225	1.8b	12.8a	4.1b	35.2a	0.4b	2.7a	0.5a	28.0a	1.8a	93.0a	.	.
	45/90	5.4a	9.2b	15.0a	26.1b	1.5a	1.8b	0.5a	29.5a	1.7a	88.1a	.	.
	SE	1.1	1.0	0.6	2.5	0.2	0.3	0.1	2.6	0.2	9.2	.	.
	P>F	0.050	0.065	<0.001	0.151	0.010	0.127	0.006	0.002	<0.001	<0.001	.	.
Clara City14	0	.	16.1a	.	71.1a	.	.	.	26.3	.	85.7c	.	4.0b
	45	1.3c	13.0a	5.1c	55.9a	.	.	0.2b	26.3	0.8b	86.2bc	0.04a	4.6ab
	135	4.1b	13.2a	15.9b	54.8a	.	.	0.6b	36.9	2.1b	130.5abc	0.1a	6.5ab
	225	7.5a	14.6a	30.5a	67.7a	.	.	1.5a	51.1	4.3a	170.4a	1.6a	17.2a
	45/90	5.6ab	13.7a	26.3a	62.9a	.	.	0.5b	41.4	1.8b	133.6ab	0.1a	6.3ab
	SE	1.0	1.7	4.0	6.6a	.	.	0.1	6.0	0.5	15.5	0.7	4.2
	P>F	0.001	0.712	0.001	0.376	.	.	<0.001	0.060	0.005	0.011	0.343	0.223

† Within column and site, means followed by the same lowercase letter are not significantly different.

Table 2.6 Continued.

Site†	N rate	R6 Y1						R6 Y2					
		Stover		Grain		Cob		Grain		Stover		Cob	
		FDN	SDN	FDN	SDN	FDN	SDN	FDN	SDN	FDN	SDN	FDN	SDN
Lamberton15	0	.	44.8a	.	137.9a	.	6.4a	.	25.4b		87.0bc	.	.
	45	3.6c	40.4ab	11.1c	135.3ab	0.6b	6.9a	0.3c	27.8b	1.0c	79.7c	.	.
	135	14.1b	41.8ab	42.4b	121.6bc	2.0a	6.0a	0.7bc	25.3b	3.0b	94.7abc	.	.
	225	22.4a	31.3b	68.4a	108.8c	2.3a	3.8b	1.7a	42.4a	5.7a	131.8a	.	.
	45/90	12.7b	39.2ab	36.4b	118.1c	1.8ab	6.0a	0.8b	24.5ab	2.9b	125.5ab	.	.
	SE	1.4	4.7	4.2	8.0	0.4	0.8	0.1	4.1	0.6	14.0	.	.
	P>*F*	<0.001	0.285	<0.001	0.017	0.057	0.062	<0.001	0.055	0.002	0.075	.	.
Waseca14	0	.	12.8a	.	49.6a	.	.	.	17.2b	.	49.5b	.	3.0c
	45	2.1c	15.3a	2.3b	49.5a	.	.	0.2c	16.2b	0.6c	49.5b	0.1c	3.3bc
	135	4.5b	15.3a	8.4b	57.4a	.	.	0.9b	31.5a	2.9b	96.8a	0.2b	5.0a
	225	9.5a	16.4a	23.1a	64.0a	.	.	1.5a	34.6a	3.8a	103.0a	0.3a	5.0a
	45/90	8.9a	14.1a	25.3a	62.0a	.	.	1.0b	27.7a	2.5b	82.4a	0.2b	4.2ab
	SE	0.7	1.7	2.1	6.9	.	.	0.1	3.0	0.3	7.6	0.02	0.5
	P>*F*	0.001	0.553	<0.001	0.271	.	.	<0.001	0.001	0.001	<0.001	<0.001	0.001
Waseca15	0	.	18.6b	.	49.3b	.	2.7b	.	24.6c	.	61.5c	.	.
	45	2.4c	22.5ab	6.1c	64.2ab	0.4c	3.5ab	0.3c	23.3c	0.8c	67.5c	.	.
	135	8.6b	21.0ab	22.1b	64.3ab	1.6b	3.7ab	0.6b	33.9b	1.6b	103.1b	.	.
	225	14.9a	23.3a	45.2a	78.7a	2.4a	3.8a	1.0a	45.2a	3.2a	141.5a	.	.
	45/90	13.2a	20.4ab	35.0a	68.6a	2.0ab	3.1ab	0.4bc	29.6bc	1.2bc	99.3b	.	.
	SE	0.7	2.0	4.1	8.3	0.2	0.3	0.1	3.3	0.3	8.4	.	.
	P>*F*	<0.001	0.210	0.003	0.068	<0.001	0.212	<0.001	0.001	0.001	<0.001	.	.

† Within column and site, means followed by the same lowercase letter are not significantly different.

Figure 2.1 Diagram of the microplot at Becker and Lamberton sites illustrating the relative positions of soil and plant sampling events taken over two consecutive growing seasons.

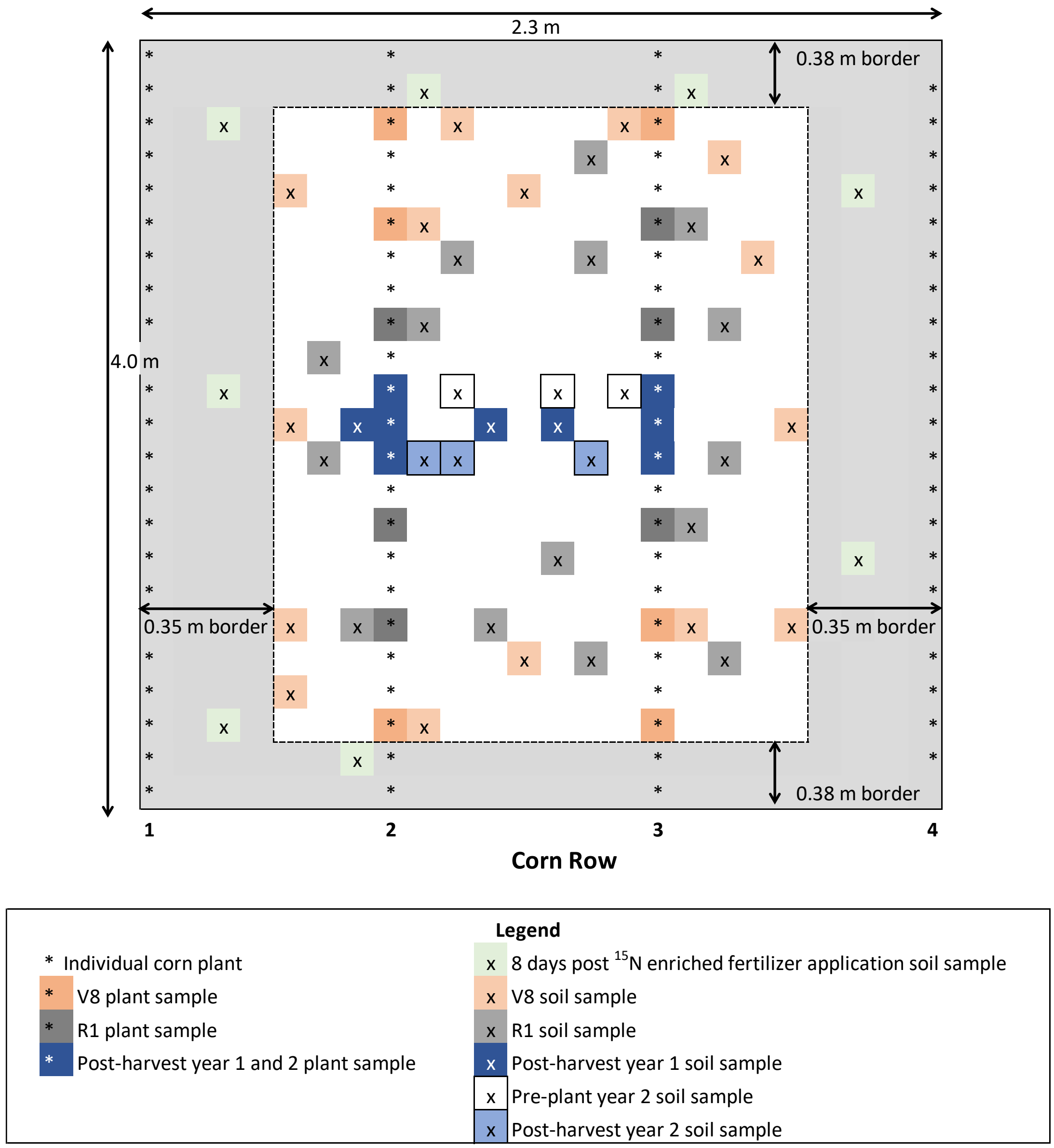


Figure 2.2 Diagram of the microplot at Clara City illustrating the relative positions of soil and plant sampling events taken over two consecutive growing seasons.

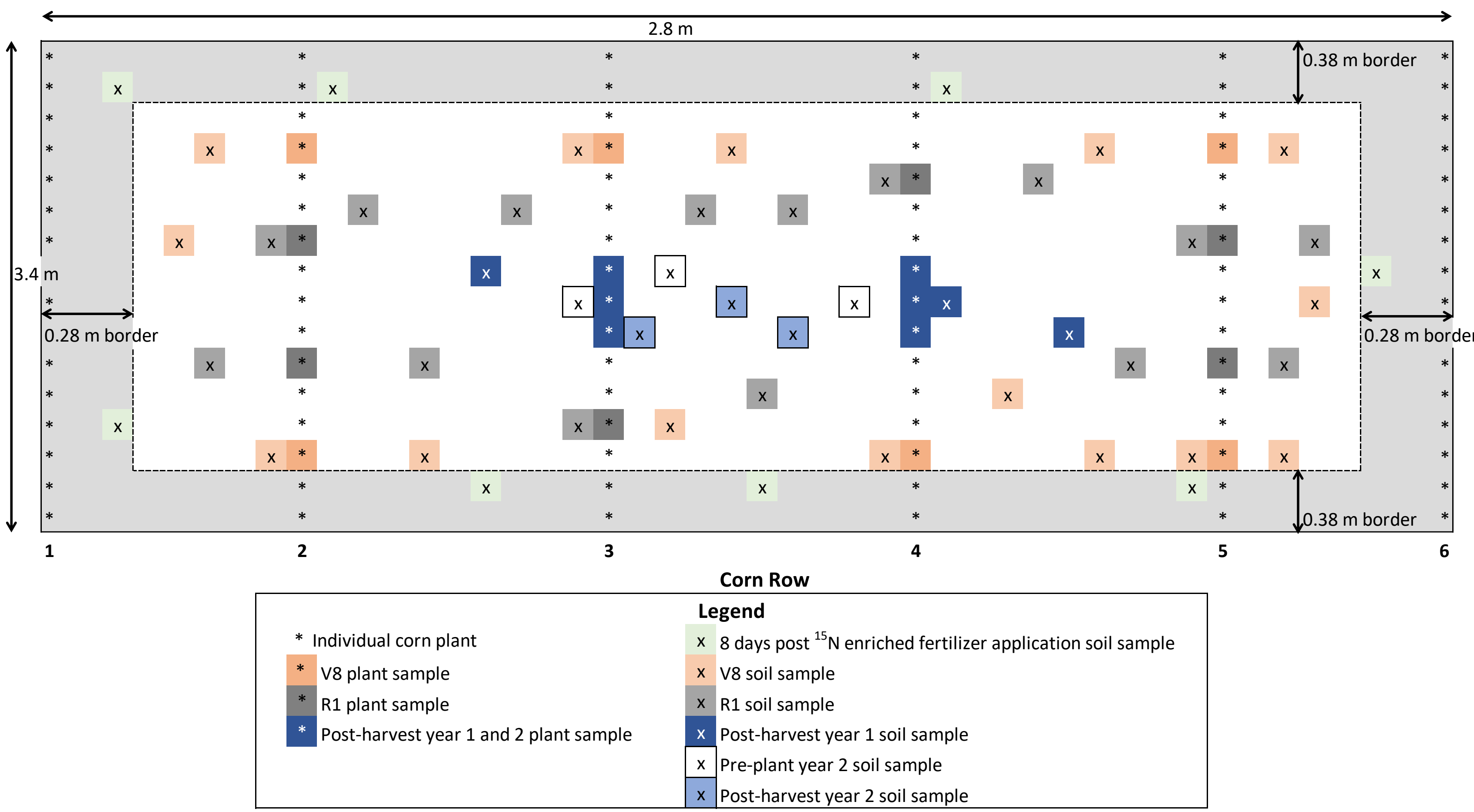


Figure 2.3 The response of aboveground plant N uptake to the applied fertilizer N rate partitioned into fertilizer-derived N (FDN), soil-derived N (SDN), and total N. Treatment means are shown where solid symbols represent single fertilizer applications and symbols with open centers represent the 45/90 kg N ha^{-1} split-application. Within N variable and plant sample timing, treatment means followed by the same letter are not significantly different ($P \leq 0.05$) where lower-case letters to the left of the triangle symbols correspond to total N, lower-case letters to the right of the square symbols correspond to SDN, and upper-case letters above the circle symbols correspond to FDN.

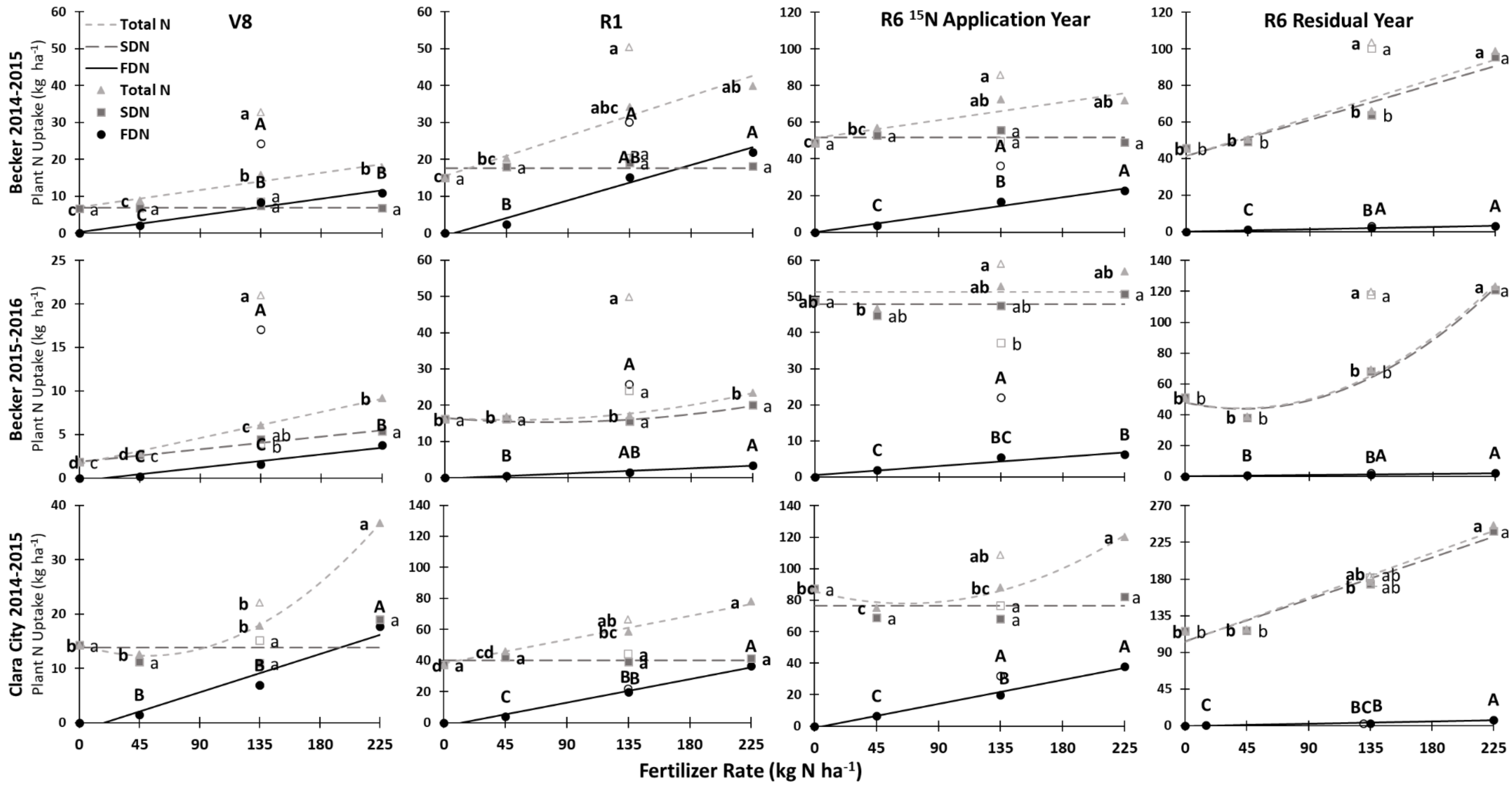
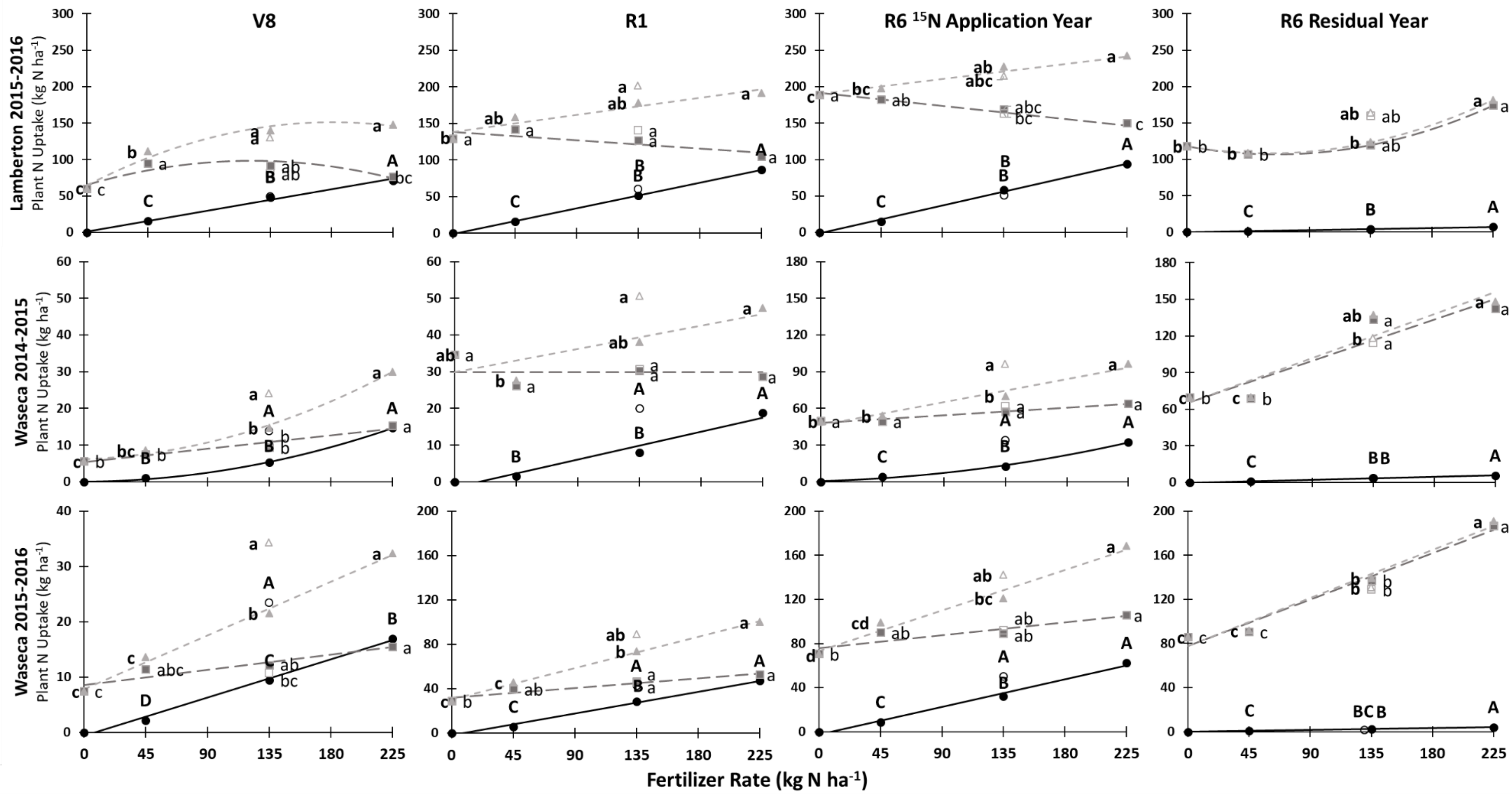


Figure 2.3 Continued.



CHAPTER 3: CHAPTER 3: ^{15}NITROGEN DISTRIBUTION AND RECOVERY IN THE SOIL IN RESPONSE TO FERTILIZER APPLICATION RATE AND TIMING

SYNOPSIS

The fate of nitrogen (N) fertilizer in the soil profile is influenced by application rate and timing, soil physical and chemical properties, and weather patterns after the application. Six field studies were conducted at four sites in Minnesota to examine how ^{15}N labeled urea fertilizer rate and application timing impacted fertilizer-derived N (FDN) distribution and form through the soil profile over two consecutive growing seasons of continuous corn (*Zea mays* L.). Three N fertilizer rates (45, 135, and 225 kg ha^{-1}) were applied at planting and one split-application was done as 45 kg N ha^{-1} at planting and 90 kg N ha^{-1} at the four collared leaf stage (V4). Soil samples were collected four times during the first growing season and at pre-plant (PPY2) and post-harvest (PHY2) the second growing season. Within eight days of the planting application (PA), 38 to 100% of the applied FDN was accounted for in the top 60 cm of the soil profile. Low recoveries were likely due to leaching loss on loamy sand soils and volatilization loss from inadequate fertilizer incorporation using hand-rakes on a silty clay loam soil. In the sampled profile, inorganic FDN concentration was greatest immediately following fertilization but was less than 10% of the applied N rate from corn tasseling through the end of the study. Likely due to rapid soil microbial immobilization or clay-fixation following application, the majority of the FDN present in the soil was fertilizer-derived organic N (FD_{ON}) that was located in the top 15 to 30 cm of the soil profile from PA to PHY2. There was no difference in FDN in the soil between the single 135 kg N ha^{-1} or split 45/90 kg N ha^{-1} applications from PHY1 through PHY2. Across sites and fertilizer treatments, 6 to 30% of the applied FDN remained in the soil at post-harvest in

the first year (PHY1) while 5 to 22% remained at PHY2 indicating increased FD_{ON} stabilization and resistance to weathering over time.

INTRODUCTION

Inorganic fertilizer represents an expensive input cost to producers but can return significant dividends when appropriately managed. Decades of N fertilizer research have identified best management practices to reduce environmental loss, improve fertilizer use efficiency, and maximize agronomic returns. However, the majority of these practices are based on the net effect of fertilization on a given metric, such as grain yield, total plant N uptake, or residual inorganic N at harvest. While useful, such studies only provide a limited understanding of N cycling and fate of N fertilizer. Because the soil naturally supplies plant-available N through soil organic matter mineralization, without stable isotope-labeled fertilizer it is difficult to precisely determine how much fertilizer N was recovered by the crop, remained in the soil, or was lost from the soil-crop system. Nitrogen management guidelines may be improved by quantifying FDN in the soil pools post-fertilization and throughout the growing season.

Following fertilizer application, multiple ^{15}N studies have shown that fertilizer N may be found in a variety of plant available and unavailable forms. Eight days after labeled urea–N fertilization, fertilizer N was found in amino sugars (2%), extractable inorganic NH_4–N (5%), organic NH_3–N (7%), amino acids (22%), nonhydrolyzable organic N (31%), and unidentified acid-soluble N (33%) (González-Prieto et al., 1997). Others have found 16 to 47% of the applied FDN remains in the soil at PHY1 (Allen et al., 1973; Timmons and Cruse, 1990; Tran and Giroux, 1998) and 15 to 26% of the originally applied FDN rate was still accounted for two or more years after application (Allen et al., 1973; Timmons and Baker, 1992). Of the residual

FDN, 65 to 95% was in the semi-stabilized organic N fraction in the top 15 cm of the soil profile (Olson, 1980; Timmons and Cruse, 1990).

In recent years, the U.S. upper Midwest has experienced a shift from anhydrous ammonia to urea as the major N source (Bierman et al., 2012; MDA, 2020). This shift is occurring as changes in climate are resulting in wetter springs (Seeley, 2015) that favor N loss before plant N uptake. These conditions combined with the need to improve profitability and tighten scrutiny on the use of N fertilizer to mitigate negative effects on the environment are resulting in unprecedented changes in N management. Urea applications are often done before planting, but increasingly, in-season split-applications are becoming commonplace. Variations of fertilizer rate, application timing, soil physical and chemical properties, and weather patterns can significantly affect FDN distribution in the soil profile, availability to the crop, and potential for N loss in the year of application and subsequent years (Jokela and Randall, 1997). Studies that evaluate FDN across growing seasons for pre-plant urea and in-season split-applications are lacking. Moreover, evaluation of N cycling in the context of climate change is needed to improve N management. For these reasons, the objectives of this study are to investigate the effects of fertilizer rate and timing on fertilizer distribution through the soil profile and cycling of fertilizer N between plant available and non-available forms.

MATERIALS AND METHODS

Six field sites were used for two consecutive growing seasons in Minnesota, USA. Three sites were utilized during the 2014 to 2015 growing seasons at the University of Minnesota Research and Outreach centers at Becker (Becker14) and Waseca (Waseca14) and a grower cooperator field at Clara City (Clara City14). Three additional sites were utilized during the 2015 to 2016 growing seasons at the University of Minnesota Research and Outreach centers at

Becker (Becker15), Lamberton (Lamberton15), and Waseca (Waseca15). Site locations and soil taxonomic descriptions are presented in Table 3.1. Each study site was divided into quarters and a 10-core (1.8 cm diameter; 15 cm depth) composite soil sample was collected from each quarter and analyzed for soil texture (hydrometer method; Gee and Bauder, 1986), pH (1:1 soil/water; Peters et al., 2012), soil organic matter (Combs and Nathan, 2012), cation exchange capacity and ammonium-acetate exchangeable K, Ca, and Mg (Warncke and Brown, 2012), Olson-P for Clara City14 (pH >7.2), and Bray-P1 for all other sites (Frank et al., 2012). The average of the four quarters is presented in Table 3.2. An additional 10-core (1.8 cm diameter) composite soil sample was collected from each quarter at the 0- to 30- and 30- to 60-cm depths and analyzed for NO_3–N (NO_3–N + NO_2–N) (Gelderman and Beegle, 2012) and NH_4–N (Bremner and Mulvaney, 1982) and are presented in Table 3.3. Soil bulk density was measured at the center of the 0- to 15-, 15- to 30-, 30- to 60-, and 60- to 120-cm depths from eight 5-cm deep samples per site using the intact soil core method (Blake and Hartge, 1986) (Table 3.4).

Experimental Design

The study was set up in a randomized complete block design with four replications in a continuous corn cropping system. Treatment plot dimensions, corn row spacings, and corn hybrid differed between sites as described in detail in Chapter 2. Briefly, each site had designated areas for end-of-season grain yield measurement (harvest area), in-season non-^{15}N labeled plant and soil sample collection, and a ^{15}N enriched microplot area for plant and soil sample collection (Figure 1.1). The non-confined microplot area was placed 1.5 m from one end of the treatment plot and centered on the width dimension. A 1.5 m border area was designated between the microplot and the non-^{15}N enriched sampling or harvest areas where no plant tissue or soil samples were collected to minimize the risk of sample contamination or creating an edge effect. During the site establishment year (first year), N fertilizer treatments were applied to the entire treatment plot area except for the microplot that was protected with plastic sheeting during plot fertilization. Treatments consisted of 45, 135, and 225 kg N ha^{-1} of unlabeled urea (46-0-0, $N–P_2O_5–K_2O$) broadcast applied and incorporated within 7 d of planting (Table 2.1). An additional treatment was split-applied as 45 kg N ha^{-1} of unlabeled urea ammonium nitrate (28–0–0, $N–P_2O_5–K_2O$) banded over the seed row within 6 d of planting and top-dressed with 90 kg N ha^{-1} granular urea impregnated with the urease inhibitor N-(n-butyl) thiophosphoric triamide, Agrotain (Koch Fertilizer LLC, Wichita, KS) at the V4 corn phenological development stage (Abendroth et al., 2011). The microplots, which were covered with plastic sheeting during application of the unlabeled fertilizer, were fertilized with 5 atom % excess ^{15}N urea dissolved in 1.7 L water at the same N rate and within 7 d of the non-labeled fertilizer application (Table 2.1). A pressurized hand-sprayer was used to broadcast apply the 45, 135, and 225 kg N ha^{-1} treatments to the entire microplot area while the 45/90 kg N ha^{-1} treatment was band applied over the seed row at planting and evenly applied over the entire microplot area at V4 (Table 2.1). Following each ^{15}N enriched fertilizer application, the fertilizer was incorporated into the soil profile using a hand rake at Clara City, ≥6 mm of water from irrigation at Becker, or a water tank at Lamberton and Waseca. Second-year treatment plots were the same as the first year plots except that only unlabeled N fertilizer was applied to the entire treatment plot including the microplot. Other than N management, each site was managed to produce high yielding corn according to the University of Minnesota guidelines (Kaiser et al., 2018).

Volumetric water content was measured hourly using 5TM soil moisture and temperature sensors and Em50 digital data loggers (METER, Pullman, WA). Sensors were installed in each replication of the 225 kg N ha^{-1} treatment at the 0- to 5-, 5- to 10-, 10- to 15-, 30- to 35-, and 40-

to 45-cm depths at each site, except Becker and Waseca sites during the 2015 growing season where soil moisture sensors were placed in the 2015 to 2016 study only. Web Soil Survey was used to estimate the depth of each soil horizon and each depths' respective permanent wilting point (Soil Survey Staff, 2020). Water field capacity within each soil horizon was estimated as the volumetric water content measured with the 5TM sensors approximately 24 h following ≥ 0.75 cm precipitation or irrigation event averaged across the four replications. The total available water capacity within each soil horizon was the difference between field capacity and permanent wilting point. The soil-water deficit was estimated using the checkbook method (Steele et al., 2010) and was corrected with soil moisture data every seven days using values estimated from the soil moisture sensors. Soil-water deficit estimates in the root zone were calculated as the quotient of the difference between water volume at field capacity and measured water volume and the total available water and multiplied by 100 to convert to percent. Water loss (percolation below the root zone plus runoff) was estimated as the volume of water applied to the soil exceeding field capacity (Steele et al., 2010).

At Becker, irrigation was applied 17 times in 2014 (266 mm), 13 times in 2015 (188 mm), and 15 times in 2016 (203 mm) as determined using the checkbook method (Steele et al., 2010) or for fertilizer incorporation. Because fertilizer guidelines in Minnesota are not adjusted when NO_3–N concentration in irrigation water is ≤ 10 mg kg^{-1} (Lamb et al., 2015) and concentrations in this study were approximately 10 mg kg^{-1}, treatment N rates were not adjusted for irrigation water N content. The overall N load from irrigation water was ≤ 27 kg ha^{-1} per growing season. Daily precipitation was recorded for each site from the nearest National Weather Service Station (NOAA, 2020).

Soil and plant samples were collected from within the ^{15}N enriched microplot and non-^{15}N enriched sampling areas. Plant sample collection was described in Chapter 2. In the first year, soil samples were collected at PA, at the V8 and R1 corn phenological development stages, and PHY1 (Table 2.1). Due to frozen soil conditions following harvest, Waseca14 PHY1 soil samples were not collected until the following spring. Second-year soil samples were collected at PPY2 and PHY2. In the non-^{15}N enriched sampling area, a four-core (1.8-cm diameter) composite soil sample was collected at PA, V8, and R1 using a hand probe while a two-core (5-cm diameter) composite soil sample was collected at PHY1, PPY2, and PHY2 using a hydraulic probe. Within the microplot, an eight-core (1.8 cm diameter) composite soil sample was collected at PA while a 15-core (1.8 cm diameter) composite soil sample was collected at V8 and R1 at a ratio of one core collected within the corn row for every three cores collected between the corn rows. A three-core composite soil sample was collected at PHY1, PPY2, and PHY2 using a hydraulic probe where one core was collected from within the corn row and two cores were collected between the corn rows. The greater number of cores collected per sampling event in the ^{15}N enriched microplot was to improve the precision of the microplot soil sample ^{15}N enrichment estimates (Gomez and Gomez, 1984) because it was expected that the ^{15}N concentration of soil samples collected from within the microplot would be more variable than samples collected from the non-^{15}N enriched sampling area. The soil samples were collected at the 0- to 15-, 15- to 30-, and 30- to 60-cm depths at PA, V8, and R1 in the first year, at the 0- to 30-, 30- to 60-, and 60- to 90-cm depths at PHY1 and PHY2, and the 0- to 15-, 15- to 30-, 30- to 60-cm, and 60- to 120-cm depths at PPY2. Because the microplot had unconfined borders, the ^{15}N concentration could be diluted as soil water moved laterally across the microplot border (Sanchez et al., 1987). To avoid this potential error, soil samples were collected $\geq$ 0.28 m (Clara

City) or ≥ 0.35 m (Becker, Lamberton, and Waseca) within the outside edge of the microplot except for the PA sampling event where the risk of lateral movement of ^{15}N enriched fertilizer before sampling was deemed minimal (Figure 2.1; 2.2; Spackman and Fernández, 2020). As described in Spackman and Fernández (2020), early-season soil and plant samples were collected near the outside edges of the microplot and each subsequent sampling event moved closer to the center of the microplot to avoid previously sampled areas (Figure 2.1; 2.2). Following harvest in the first year, tillage was performed in the fall and/or spring to incorporate corn residue and prepare the seedbed (Table 2.1). Second-year soil and plant samples were collected from the center of the microplot to avoid ^{15}N dilution from the lateral movement of soil following tillage events. All soil samples were dried in a forced-air oven (35 °C), ground to pass through a 2-mm sieve, and analyzed for NO_3–N (Gelderman and Beegle, 2012) and NH_4–N (Bremner and Mulvaney, 1982).

Inorganic ^{15}N concentration was determined using the heated diffusion protocol with suspended, acidified filter paper NH_3 traps described by Khan et al. (1998). The PA and V8 soil samples were analyzed for NH_4–N and total inorganic N (TIN) (NH_4–N + NO_3–N +NO_2–N) using separate diffusions and all other samples were analyzed for TIN. A subsample of each soil sample was finely ground to pass through a 0.177-mm sieve using a roller mill and prepared for total N and ^{15}N concentration analysis as described in Spackman and Fernández (2020). The acidified paper disks from the diffusion protocol and the soil samples were analyzed for N content and ^{15}N concentration using an Elementar Vario EL Cube or Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to either an Isoprime VisION IRMS (Elementar UK Ltd, Cheadle, UK) or a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) (UC Davis, 2020). In all sample collection and handling processes, non-^{15}N enriched samples were collected and processed using designated equipment before ^{15}N enriched samples to minimize the risk of ^{15}N contamination. Additionally, ^{15}N enriched samples were processed in order of the least to greatest applied N rate with a thorough cleaning of equipment between samples. The soil samples from the fourth replicate of the Waseca14 PA 45/90 kg N ha^{-1} treatment were removed from the analysis due to likely sample contamination during sample processing.

Data Analysis

Following acidified filter paper analysis for ^{15}N concentration and N content, the ^{15}N content of NO_3–N was calculated using the isotope-dilution equation (Saghir et al., 1993):

$$A_{NN} = (N_{TIN}A_{TIN} - N_{AN}A_{AN})/N_{NN} \quad [3.1]$$

where *A* is the measured atom % ^{15}N, *N* is the micrograms of N measured in the sample before diffusion, and the subscripts represent NH_4–N (*AN*), NO_3–N (*NN*), or TIN. The fraction of ^{15}N derived from fertilizer (*Nf*) was calculated individually for each form of N quantified (NH_4–N, NO_3–N, TIN, or total N) as:

$$Nf = \frac{(A_{microplot} - A_{non-15N\ enriched\ sampling\ area})}{(A_{fertilizer} - A_{non-15N\ enriched\ sampling\ area})} \quad [3.2]$$

where *A* is the measured atom % ^{15}N enrichment of the soil sample collected from within the ^{15}N enriched microplot ($A_{microplot}$), the non-^{15}N enriched sampling area ($A_{non-15N\ enriched\ sampling\ area}$), or the ^{15}N enrichment of the labeled fertilizer applied to the plot ($A_{fertilizer}$). Fertilizer-derived N (FDN) was calculated for each measured N form as:

$$FDN\ (kg\ ha^{-1}) = Nf \times N \times D \times Bd \times 10 \quad [3.3]$$

where *N* is the mass of the N form in the sample (mg kg^{-1}), *D* is the soil sample depth (m), and *Bd* is the bulk density of the soil at the measured depth (Mg m^{-3}). Fertilizer-derived soil organic N (FD_{ON}) was considered the difference between total soil FDN (FD_{TN}) and TIN FDN (FD_{TIN}). Fertilizer recovery efficiency (FR) was calculated as the quotient of FDN measured in a specific N form (i.e. NH_4–N, NO_3–N, organic, etc.) and the applied fertilizer N rate multiplied by 100.

Each field experiment was analyzed individually at $P \leq 0.05$ using SAS (v. 9.4, SAS Institute, 2012). Repeated measures analysis was performed using the MIXED procedure of SAS and the first-order autoregressive covariance structure where N fertilizer treatment, the timing of soil sampling, and soil sampling depth were considered fixed effects while block and interactions of fixed effects with block were considered random effects. When significant fixed effects or interactions of fixed effects were obtained, treatment means were assessed using the SLICE and PDIFF options of the MIXED procedure of SAS. To meet assumptions of normally distributed residuals, soil NO_3–N, NH_4–N, and TIN values were natural log-transformed, analyzed, and then back-transformed for presentation.

RESULTS AND DISCUSSION

Soil Water in the First Year

The soil moisture sensors were placed in the highest applied N rate that was expected to have the greatest demand for soil water and produce the greatest amount of corn biomass. While soil moisture deficit and cumulative water loss in this treatment may not be the same for other N treatments, it was assumed that the overall seasonal soil-water patterns were similar across all N fertilizer treatments. This assumption is supported by (Lawlor et al., 2008) who observed no differences in cumulative subsurface drainage losses between N rate treatments in 13 of 15 years on a field site in Iowa from 1990 to 2004.

During the 2014 and 2015 growing seasons, irrigation and precipitation events were frequent in May and June and resulted in the rooting zone soil water deficit being typically less than 20% (Figure 3.1). Because the soil water deficit was low from the time of N fertilization at planting through the end of June, precipitation often exceeded field capacity and resulted in cumulative water loss of 200 to 243 mm at sites established in 2014 and 127 to 219 mm at sites established in 2015. These losses represented 37 to 100% of the cumulative water loss from the time of fertilization through PHY1. In the upper Midwest USA, the majority of N fertilizer applied to corn occurs in the spring months of April through June (Bierman et al., 2012) when approximately 33% of annual precipitation (Spackman et al., 2019) and 62% (Randall and Vetsch, 2005) to 72% (Lawlor et al., 2008) of annual subsurface drainage occurs. Likewise, on an Arvilla sandy loam soil, (Struffert et al., 2016) observed that averaged over three growing seasons, 73% of the total season-long NO_3–N leaching occurred by the end of June. This represents a critical time for N losses on upper Midwest corn cropping systems because developing corn plants have low demand for soil N and water (Dinnes et al., 2002).

Precipitation at Waseca15 and precipitation plus irrigation at Becker sites successfully maintained a low soil water deficit from July through PHY1 soil sampling that resulted in cumulative water loss of 474, 423, and 450 mm at Becker14, Becker15, and Waseca15, respectively (Figure 3.1). These volumes of water loss were in line with subsurface drainage volumes reported by Lawlor et al. (2008) in Iowa in years of similar total annual precipitation. The volume of water lost below the rooting zone at these sites suggests that soil NO_3–N was likely leached down the soil profile soon after nitrification and potentially became inaccessible to the corn roots. This is in line with observations of NO_3–N leaching loads closely following cumulative water drainage amounts (Struffert et al., 2016).

When available, supplemental irrigation is typically applied before the soil water deficit reaches 50% to avoid the risk of water limiting conditions (Steele et al., 2010). Drier-than-normal conditions from mid-July through August 2014 increased the soil water deficit to >50% for 22 d at Clara City14 and 40 d at Waseca14. Infrequent precipitation events during the same period in 2015 resulted in 19 d of soil water deficits >50% at Lamberton15. On non-irrigated sites, the greatest soil water deficits typically coincided with corn tasseling. Inadequate soil moisture during corn tasseling and grain fill can limit grain yield potential and N uptake by the roots (Abendroth et al., 2011) as well as N mineralization by soil microbes (Griffin, 2008). Additional water loss was minimal from July through PHY1 soil sampling at all non-irrigated sites except for Waseca15 where 191 mm of water was lost from August through September due to excess precipitation. During this period and following harvest, unsaturated soil conditions allow inorganic N to accumulate in the soil profile that may be available for the following year's crop (Jokela and Randall, 1997). However, excessive precipitation during the fallow months may flush soil NO_3–N below the rooting zone or out through tile-drainage for low soil inorganic N content the following spring (Cameron et al., 1978; Bauder and Montgomery, 1979; Sanchez and Blackmer, 1988; Randall and Vetsch, 2005; Lawlor et al., 2008).

Initial Soil Nitrogen Content

Before initiating the study, soil TIN in the top 60 cm of the soil profile was 29 to 98 kg N ha^{-1} across field sites (Table 3.3). Of the TIN in the soil profile, 59 to 79% was NH_4–N at Becker and Waseca field sites. The predominance of NH_4–N in the soil may be indicative of organic matter mineralization from the following fall and early spring. However, it could also indicate leaching or denitrification of soil NO_3–N from the soil profile effectively enriching the NH_4–N pool relative to the NO_3–N pool. In contrast, soil NH_4–N was only 44 and 24% of TIN at Clara City and Lamberton, respectively, and may indicate retention of soil NO_3–N from the following fall as these sites had the greatest TIN content in the study. Due to an east-to-west decreasing precipitation gradient, soil NO_3–N may accumulate in western Minnesota soils (Schmitt and Randall, 1994; University of Minnesota Extension, 2018). For this reason, the University of Minnesota guidelines suggest quantifying and crediting residual N in the spring for the following crop in the western region. Following the University of Minnesota guidelines, Lamberton14 was the only site that had a residual N credit (35 kg N ha^{-1}).

Nitrogen Rate

Total and Inorganic Fertilizer-Derived Nitrogen

There was a significant N treatment by soil sampling event interaction for FD_{TN} and FD_{TIN} in the soil profile at each field site (Table 3.5). The total mass of FD_{TN} and FD_{TIN} measured in the soil was greatest at PA and increased with increasing N fertilizer application rate (45, 135, and 225 kg N ha^{-1} treatments). The mass of FD_{TN} increased with increasing N rate at all soil sampling events except at PHY1 at Becker14, Becker15, and Waseca15 and PHY2 at all field sites (Table 3.6) similar to Stevens et al. (2005b). In contrast, the mass of FD_{TIN} increased with increasing N fertilizer application rate at the PA and V8 soil sampling events at Becker and Waseca sites and PA, V8, and R1 soil sampling events at Clara City14 and Lamberton15, but there were no differences between N rates at later sampling events. Although the mass of FD_{TN} increased with increasing fertilizer rate, generally there was no difference in FR measurements for FD_{TN} between N rate treatments at each sampling event (Table 3.5; 3.6). This may indicate that FDN loss and immobilization in the soil is proportional to the applied N rate in these sites.

This finding contrasts others who observed decreasing FR values with increasing N rates and attributed the response to increased FDN losses and a limited capacity of the soil to immobilize FDN (Sanchez and Blackmer, 1988).

The mass of FD_{TN} in the soil profile and their respective FR values were greatest at PA, closest to the time of ^{15}N enriched fertilizer application (Table 3.6). Averaged across the three N rates, FR for FD_{TN} at PA was 59, 100, 38, 89, 84, and 96% for Becker14, Becker15, Clara City14, Lamberton15, Waseca14, and Waseca15, respectively. Of the total FD_{TN} recovered at PA, only 10 to 28% across N rates and sites was FD_{TIN} (Table 3.6) with 48 to 96% of it being NO_3–N (Table 3.7; 3.8). The low recovery of FD_{TN} at Becker14 was likely influenced by N leaching. Like NO_3^-, urea is highly soluble and moves with soil water. Becker14 received 36 mm of irrigation plus precipitation immediately after fertilization that likely favored FDN leaching through soil macropores (Balasubramanian et al., 1973), especially as 3 to 18 kg FD_{TN} ha^{-1} was observed in the 30- to 60-cm depth at PA (Table 3.7). In contrast, Becker15, which received only 8 mm of water during the same period, had 100% FDN recovery, though 15 to 19% of the applied FDN rate was in the 30- to 60-cm depth. This illustrates that urea and NO_3^- can readily move through the soil with a small amount of water and, likely in 2014, there was sufficient water to move N below the sampling depth.

Clara City14's low FD_{TN} recovery at PA was likely due to excessive volatilization. Of all the sites, Clara City14 had the most alkaline surface soil with a pH of 7.7 (Table 3.2). Following ^{15}N enriched urea application, the fertilizer was incorporated using a hand rake and 19 mm of precipitation 3 d after fertilization whereas urea was incorporated with ≥6 mm of irrigation within 3 h of application at the other five sites. Because the enriched ^{15}N urea was applied as a solution to the entire microplot soil surface, urea was in intimate contact with the soil and the urease enzyme. Researchers have observed that hydrolysis is rapid with ≤ 4% of the applied N rate still in the urea form after 48 h (Francis and Haynes, 1991) or 8 d (Recous et al., 1988a) of application, although hydrolysis can be delayed on acid soils (Sahrawat, 1992). Following urea hydrolysis, NH_3 volatilization is favored on warm and moist soils that are drying, especially with high pH (7.0 to 9.0) (Francis et al., 2015). Clara City14 had all of these conditions following fertilization. Under field conditions, reported volatilization loss has been as great as 50% of the applied N rate but is reduced when urea is incorporated by tillage, precipitation, or irrigation (Hargrove, 1988; Engel et al., 2011). Hargrove (1988) reported that surface-applied urea should be incorporated 5 to 10 cm in the soil profile to minimize volatilization loss. It is evident that at Clara City14, the hand rake was inadequate to sufficiently incorporate the urea to prevent volatilization. Although a 19 mm precipitation event occurred 3 d after fertilization, the majority of the urea had likely already hydrolyzed and been subject to volatilization and nitrification processes so that by PA, 94% of FD_{TIN} was NO_3–N averaged across N rate treatment and sampling depth (Table 3.7).

When FD_{TN} FR was analyzed with only the three N rate treatments, there was not a significant treatment by sampling event interaction and only the main effect of sampling event was significant at all field sites (Table 3.9). Across all field sites and averaged across treatments, FR decreased from PA to V8 by 11 to 76 percentage points with the greatest reduction occurring at Becker15 and Waseca15 and the smallest reduction occurring at Clara City14, due to significant losses before PA. A small amount (1 to 28%) of the unaccounted FD_{TN} was in corn uptake at Becker and Waseca sites (Chapter 2), but leaching and denitrification were likely the major N loss mechanisms due to the low soil water deficit from PA to V8 (Figure 3.1) and the rapid appearance of FD_{TN} in the 30- to 60-cm depth (Table 3.7). Others have reported similar results (Cameron et al., 1978; Kowalenko, 1978; Sanchez and Blackmer, 1988; Timmons and Cruse, 1990). One key exception to this finding occurred at Lamberton15 where corn FDN uptake was equivalent to or greater than unaccounted for FD_{TN} in the soil (Chapter 2; Table 3.6). This result was largely due to rapid corn growth where, averaged across treatments, 60% of the final dry-weight biomass was accumulated by V8 and contrasts the other five sites that had accumulated only 12 to 20% (data not shown). While there are likely many underlying and uncontrollable factors that favored vigorous biomass production at Lamberton15, this result indicates that corn management practices that promote early corn growth may be more likely to reduce early season fertilizer N losses and improve fertilizer use efficiency.

Because Becker14, Becker15, and Clara City14 sites were subject to significant FD_{TN} losses before V8, their FR values were similarly low from V8 through PHY2 (Table 3.9). Averaged across the three sites and rate treatments, FR was 26% at V8 and R1 and decreased to 12% at PHY1. In contrast, a greater proportion of the applied N was retained in the soil profile longer into the growing season at Lamberton15, Waseca14, and Waseca15. Averaged across treatments and the three sites, FD_{TN} FR values were 52% at V8, 56% at R1, and 21% at PHY1 (Table 3.9). Improved retention of FD_{TN} at Lamberton15, Waseca14, and Waseca15 relative to Becker sites was likely primarily due to differences in soil texture and soil moisture. Unlike the soil at the Becker sites with small aggregates and large pores, the finer-textured soil at Lamberton15 and Waseca sites may have reduced the downward movement of FD_{TN} in the profile. As excess soil water moved quickly through macropores, dissolved organic N compounds and NO_3–N were likely protected from leaching in the micropores within soil aggregates (Balasubramanian et al., 1973). Following a flushing event, soil NO_3–N and dissolved organic N compounds diffuse from the inter-aggregate spaces into the water-filled macropore spaces, but only until reaching equilibrium. Thus, although the cumulative water loss pattern was similar for Becker and Waseca15 sites (Figure 3.1), the reduction of FD_{TN} from Waseca15's soil system was more gradual throughout the growing season. Dry conditions at Lamberton15 and Waseca14 from V8 through R1 further helped to retain FD_{TN} in the soil profile as soil water was not lost below the root zone, leaving FD_{TIN} available for corn N uptake (Chapter 2).

Organic Fertilizer-Derived Nitrogen

The response of FD_{ON} was nearly identical to FD_{TN} at each sampling event and location because across rates, 72 to 90% of FD_{TN} was FD_{ON} at PA, 81 to 98% at V8, 86 to 100% at R1, 83 to 98% at PHY1, 92 to 99% at PPY2, and 95 to 100% at PHY2 (Table 3.6). These values were in line with (González-Prieto et al., 1997) who found FD_{ON} accounted for 70% of FD_{TN} 7 d after fertilization and 98% after 107 d. Others have also found the majority of FD_{TN} in the soil at harvest was FD_{ON} (Legg et al., 1971; Allen et al., 1973; Olson, 1980; Timmons and Cruse, 1990; Reddy and Reddy, 1993). In this study, FD_{ON} is the difference between FD_{TN} and FD_{TIN}. Based on the previous discussion, it is likely that <4% FD_{ON} was in the urea form at PA or later. Likewise, corn roots likely contained ≤1.3% of FDN (Broadbent and Nakashima, 1968; Chichester and Smith, 1978; de Oliveira et al., 2018).

Clay-fixation of fertilizer-derived NH_4–N ($FD_{NH4–N}$) may account for a significant amount of the FD_{ON} pool. Researchers have shown that clay-fixation is rapid and, depending on soil texture, can account for 11 to 68% of the applied NH_4–N within the first few minutes to days after fertilization (Kowalenko and Cameron, 1976; Kowalenko, 1978; Sowden et al., 1978; Drury et al., 1989; Beauchamp and Drury, 1991; Chantigny et al., 2004). Clay mineralogy affected NH_4–N fixation and retention where NH_4–N retention decreased in the order of vermiculite, illite, and smectite (Allison et al., 1952; Doram and Evans, 1983). Clara City, Lamberton, and Waseca soils were formed from glacial till where the predominant clay minerals have an interatomic spacing of 14 Å, indicative of vermiculite and montmorillonite (Smith et al., 2019) that can potentially fix large amounts of FDN. Further, clay content affects the soils' ability to fix NH_4–N as contrasted on clay loam and sandy loam soils that fixed 34 and 11%, respectively, of ^{15}N labeled swine (*Sus scrofa*) slurry 1 d after application (Chantigny et al., 2004). While the rate of clay-fixation of $FD_{NH4–N}$ is rapid, the rate of release is much slower in response to the concentration of NH_4^+ and K^+ in soil solution, rates of nitrification and denitrification, plant roots, and microflora (Nieder et al., 2011). Recent clay-fixed $FD_{NH4–N}$ is likely bound near the outer edges of the clay interlayer and is preferentially released relative to soil-derived clay-fixed NH_4–N that is likely bound deeper in the clay interlayers (Nommik and Vahtras, 1982). In a 120 d incubation study, clay-fixed $FD_{NH4–N}$ fluctuated with exchangeable $FD_{NH4–N}$ suggesting a dynamic equilibrium between the two N pools (Kelley and Stevenson, 1987). In a field study, (Kowalenko, 1978) observed that 66% of recent clay-fixed $FD_{NH4–N}$ was released over an 86 d period and the remainder was held tightly until the experiment concluded 426 d later. Although this study did not specifically measure clay-fixed $FD_{NH4–N}$, a similar pattern was observed where 52 to 88% of the FD_{ON} was released over approximately 160 d from PA to PHY1 and then changed little through PHY2 (Table 3.6).

While clay-fixation may initially account for a significant percentage of the FD_{ON} pool, abiotic and biotic immobilization may also be significant sinks. As urea is hydrolyzed, fertilizer-derived NH_3 may react directly with organic matter to form heterocyclic aromatic compounds that are resistant to microbial attack and are not water-soluble, exchangeable in a salt solution, or removed by leaching with acid (Foster et al., 1985; Thorn and Mikita, 1992; Myrold and Bottomley, 2015). This could be one pathway by which FDN is immediately incorporated into the stable soil organic N fraction. Soil microbial competition can also rapidly immobilize significant amounts of FDN. In a lab study, (Kelley and Stevenson, 1987) observed approximately 14 to 26% of the applied rate was clay-fixed $FD_{NH4–N}$ within 1 h of applying ^{15}N labeled ammonium sulfate but decreased to 3 to 5% within 10 d of incubation. Simultaneously, FDN immobilization was maximized within 6 to 20 d of fertilization with the majority of FD_{ON} as amino acids and unidentified acid-soluble N, suggestive of microbial biomass. Over time, amino acid and unidentified acid soluble ^{15}N decreased and acid-insoluble ^{15}N increased suggesting that immobilization-mineralization interactions condensed FD_{ON} to more stable organic forms and that humification of immobilized FDN begins within the first days following immobilization (Kelley and Stevenson, 1987). Similar FD_{ON} stabilization patterns were also obtained in field studies (Allen et al., 1973; González-Prieto et al., 1997); indicating that FDN stabilized in organic matter is not readily mineralized or subject to leaching.

In this study, it is hypothesized that the combined effects of microbial immobilization and clay-fixation are responsible for the high percentage of FD_{TN} as FD_{ON} at each sampling event (Table 3.6). Because the majority of soil microbial activity occurs in the top 20 cm of the soil profile (Wolf and Wagner, 2005) and NH_3 fixation is rapid, the majority of FD_{ON} was found in the top 0- to 15- or 0- to 30-cm at each sampling event and each location (Table 3.7), similar to (Reddy and Reddy, 1993). Because clay content was ≤23% and because immobilization is greatest in soils where inorganic N content is low (Azam et al., 1994), immobilization likely accounted for the majority of FDN_{ON} at Becker sites. Clay-fixed $FD_{NH4–N}$ and immobilized FDN can increase with application rate, but studies have shown that the percentage fixed or immobilized decreased with increasing application rate (Doram and Evans, 1983), suggesting a critical concentration at which additional NH_4–N will not be incorporated into the organic fraction (Sanchez and Blackmer, 1988). In this study, FD_{ON} similarly increased with increasing N rate across sites, but there was generally no difference in the proportion of FDN incorporated into the organic N fraction between N rates (Table 3.5; 3.6) suggesting the soils had the additional capacity to assimilate additional NH_4–N. Fertilizer-derived NH_4–N levels were generally low across sites with the majority of FD_{TIN} as NO_3–N, indicating a rapid nitrification rate that may have favored the release of clay-fixed $FD_{NH4–N}$ to soil solution (Table 3.7). From V8 to PHY2, it is hypothesized that the majority of FD_{ON} was in the microbial biomass fraction or various forms of semi-stable soil organic matter, similar to previous observations by (Kelley and Stevenson, 1987). This organic fraction likely stabilized over time as FD_{ON} was reduced by only 30% averaged across N rates and field sites (excluding Becker14) from PHY1 to PHY2 (Table 3.6). Additional analysis of these soils is needed to verify the accuracy of these hypotheses.

As was previously described, FD_{TN} FR decreased significantly from R1 to PHY1 across all sites (Table 3.9) and was primarily due to a reduction of FD_{ON} from the entire soil profile (Table 3.7). Dry conditions from mid-July through the end of August at Lamberton15 and Waseca14 produced many large cracks in the soil that may have desiccated soil microbes and aerated much of the top 30 cm of the soil profile. Following September precipitation events, rapid, saturated flow through these macropores and cracks likely became the dominant drainage pathway (Chichester and Smith, 1978; Grossman and Udluft, 1991) potentially leaching recently mineralized FDN and lysed microbial cell organic N compounds below the 90 cm depth. By PHY1, 10 to 25% of the applied FD_{TN} averaged over treatments was accounted for in the top 90-cm of the soil profile (Table 3.9). These results are similar to those of Sanchez and Blackmer (1988) and Reddy and Reddy (1993) and less than those reported by Allen et al. (1973), Olson

(1980), and Jokela and Randall (1997). Lower recovery values in those studies were often associated with higher N rates that had significant leaching or denitrification losses while greater recoveries were associated with lower N rates or drier-than-normal conditions. Of the total FD_{ON} in the soil at PHY1, 54 to 71% at Becker sites and 78 to 88% at the fine-textured sites was in the top 30 cm of the soil profile (Table 3.7). Fertilizer-derived organic N in the deeper soil depths could be due to cracks as previously described, immobilization of leached inorganic FDN, or incorporation of organic FDN residues by soil fauna. Additionally, in agricultural settings, the soluble organic N–N pool is of the same magnitude, and often of equal size, as TIN and subject to similar processes of mineralization, immobilization, leaching and plant uptake (Murphy et al., 2000). The greater dispersion of the FD_{ON} through the soil profile at PHY1 at Becker sites relative to the fine-textured sites may be due to the larger pore spaces between soil aggregates that allow downward movement of soluble and insoluble organic N.

Total FDN FR increased from PHY1 to PPY2 at all sites except Becker15 and Waseca15 (Table 3.9). Because the recognizable surface corn residue was brushed aside before soil sampling, this increase is due to the partial breakdown and incorporation of corn biomass residue (Table 3.9). Others have indicated that significant FDN leaching loss occurs from PHY1 to PPY2 (Cameron et al., 1978; Bauder and Montgomery, 1979; Sanchez and Blackmer, 1988; Randall and Vetsch, 2005; Lawlor et al., 2008). In this study, the PPY2 soil sample was deeper than the other sampling events but across rates and sites, 0.2 to 5.9 kg FD_{ON} was recovered in the 60- to 120-cm sampling depth, representing 12 to 19% of FD_{TN} recovered in the entire soil profile at Becker14 and 2 to 5% of the FD_{TN} recovered in the soil profile at all other sites (Table 3.7). The low percentage of FDN in the 60- to 120-cm depth likely indicates that FDN leaching loss from PHY1 to PPY2 was minimal.

By PHY2, only 12% of the applied N rate on average across sites and rates was still accounted for in the soil with the majority as FD_{ON}. The lack of difference between FD_{ON} from PHY1 to PHY2 likely indicates that FDN had been stabilized in more recalcitrant forms of soil organic matter (Allen et al., 1973; Kelley and Stevenson, 1987; González-Prieto et al., 1997; Stevens et al., 2005b). Timmons and Baker (1992) observed that after the first year, approximately 2% of the original FDN was re-mineralized and assimilated into the grain over two additional growing seasons leaving 18 to 24% of the original FDN in the soil at the end of the third year. Long-term stabilization was also demonstrated by Allen et al. (1973) that accounted for 15 to 18% of ^{15}N enriched urea after five growing seasons in the top 25 cm of the soil profile. As time progressed, the relative fraction of FDN distribution in the various organic N pools (amino acids, amino sugars, acid-soluble N, acid-insoluble N, etc.) became similar to the native soil N pools indicating that the transformation and turnover rates were in equilibrium.

Nitrogen Timing

Split-applying N fertilizer at two times during the growing season produced a different pattern of FD_{TIN}, FD_{ON}, and FD_{TN} in the soil relative to a single application at planting. Generally, across sites, the 45/90 kg N ha^{-1} treatment FD_{TIN}, FD_{ON}, and FD_{TN} values were similar to the 45 kg N ha^{-1} treatment at PA and the 135 kg N ha^{-1} treatment from V8 to PHY2 (Table 3.6). This generalized response reflects the 45 kg N ha^{-1} band application at planting followed by the 90 kg N ha^{-1} broadcast application at V4. By split-applying, 90 kg N ha^{-1} were protected from N loss mechanisms until the crop was actively growing and significantly improved fertilizer N uptake and translocation to the grain relative to a single pre-plant application (Chapter 2). However, the 45/90 kg N ha^{-1} treatment FD_{TIN} content was only greater than the 135 kg N ha^{-1} treatment at V8 and no different at any later sampling events with the majority (67 to 88% at PA;

76 to 88% at V8) of FD_{TN} rapidly incorporated into the organic fraction as previously described (Table 3.6). Following incorporation, FD_{ON} was re-mineralized at a slower rate than it was immobilized because the organic soil N pool was two to three orders of magnitude greater than the applied N rate (data not shown). However, because recently immobilized FD_{ON} re-mineralizes two- to ten-fold faster than native soil organic matter (Legg et al., 1971; Allen et al., 1973; Clay et al., 1990), the inorganic N pool was enriched in ^{15}N and likely accounts for the low (≤4.0 kg FDN ha^{-1}) but constant amount of FD_{TIN} in the soil from R1 through PHY2 (Table 3.6).

Some sites' responses varied from the generalized pattern described above. As was previously mentioned, ^{15}N enriched urea was evenly applied to the entire microplot area for the single-application N rate treatments but the pre-plant 45 kg N ha^{-1} portion of the 45/90 kg N ha^{-1} treatment was banded over the seed row. On an application area basis, this banded area (approximately 2.5 cm wide per row) would have been equivalent to applying 1019 kg N ha^{-1} at Becker and Lamberton sites, 827 kg N ha^{-1} at Clara City14, and 1122 kg N ha^{-1} at Waseca sites. As previously described, the single-application N rate treatments at Clara City likely experienced significant volatilization losses due to inadequate incorporation of broadcast urea. However, because the 45 kg N ha^{-1} band of the 45/90 kg N ha^{-1} treatment was in contact with a smaller volume of the soil than the 45 kg N ha^{-1} broadcast treatment, volatilization loss was likely less (Francis et al., 2015) with FD_{TIN} values and distribution through the soil being similar to the 135 kg N ha^{-1} treatment at PA (Table 3.6; Table 3.7).

At PA at Becker15, the mass of FD_{TN} recovered in the 45/90 kg N ha^{-1} treatment was 11% relative to the 45 kg N ha^{-1} pre-plant treatment that was 111% (Table 3.6). Likely, the lower recovery associated with the 45/90 kg N ha^{-1} treatment is due to soil sampling error rather than application error or N losses. The soil sampling strategy was to collect one soil sample core from within the corn row for every three cores collected between the corn rows. However, despite best efforts during soil sampling, if one or more soil cores in the composite sample missed or only partially intercepted the fertilizer band in the 45/90 kg N ha^{-1} treatment, the between corn row cores (that did not receive any ^{15}N enriched fertilizer) would have diluted the sample ^{15}N signal indicating poor recovery of FDN. This may have also occurred at Lamberton15 at PA and R1 when FD_{TN} values for the 45 and 45/90 kg N ha^{-1} treatments were not statistically different (Table 3.6). Although the 45/90 kg N ha^{-1} treatment FD_{TN} value at R1 was less than the 135 kg N ha^{-1} treatment, the mass of FDN corn uptake was nearly identical between the two treatments (Chapter 2) further supporting the hypothesis of the soil sampling technique diluting the soil sample rather than N loss. This error may be avoided by modifying the soil sampling technique to a subplot excavation approach where the microplot is subdivided into blocks and excavated at predetermined times (Hauck et al., 1994). Following excavation, the soil is mixed, subsampled, and excess soil is returned to the plot at the proper depth and repacked to the original bulk density (Moraghan et al., 1984a). Because the banded fertilizer application creates irregularities of FDN distribution laterally across the soil surface, the excavation areas should span both the banded and non-banded areas. Using the microplot design for Becker and Lamberton sites as an example, one approach may be performed by excavating a 15 cm wide by 75 cm long area perpendicular to and centered on the corn row. The subplot excavation method better accounts for soil heterogeneity relative to the soil core technique reducing the sampling error, but is significantly more time and labor-intensive as a larger volume of the soil must be processed at each sampling event (Hauck et al., 1994). Additionally, the microplot area must be sufficiently large to avoid confounding FDN cycling dynamics within the other sampling blocks.

Additional deviance from the pattern described above was observed for sites where excessive precipitation in May favored N loss. At Becker14, Becker15, Clara City14, and Waseca14 at V8, the FD_{TIN} values for the 45/90 kg N ha^{-1} treatment were greater than or equal to the 225 kg N ha^{-1} pre-plant treatment, indicating improved FDN availability to the developing corn crop. This observation supports the N management concept that split-applications avoid early-season loss, which improves FDN corn uptake and FR relative to a single application at planting (Chapter 2; Bigeriego et al., 1979). However, at V8, 37 to 56% of the FD_{TIN} and 8 to 28% of the FD_{ON} in the soil profile at these sites was in the 30- to 60-cm depth (Table 3.7) indicating that FDN losses may still be significant when split-applications are used.

By PHY2, FR for FD_{TN} for the 45/90 kg N ha^{-1} treatment was 10 to 15% at all sites except Becker14 which was 22% (Table 3.6). On the fine-textured soils, there was no difference in FD_{TN} or FR values between the 45/90 kg N ha^{-1} treatment and the 135 kg N ha^{-1} treatment (Table 3.5). Because nearly 100% of the FD_{TN} was FD_{ON}, long-term FDN immobilization and stabilization on fine-textured soils may be in proportion to the applied N rate rather than the application timing, similar to Jokela and Randall (1997). However, on the coarse-textured soils at Becker14 and Becker15, the 45/90 kg N ha^{-1} FR FD_{TN} values decreased with increasing N rate but the 45/90 kg N ha^{-1} treatment had the greatest FR value, similar to the 45 and 135 kg N ha^{-1} treatments (Table 3.5; 3.6). The greater FR values associated with the 45/90 kg N ha^{-1} treatment at PHY2 are likely due to greater FDN uptake in corn biomass (Chapter 2) that subsequently increased the amount of FDN returned to the soil as corn residue following harvest the first year. An increase in soil organic matter is often considered to improve the soils' ability to supply mineral N to future crops (Robertson and Vitousek, 2009). Thus, field management that maximizes biomass production and fertilizer recovery in the crop not only contributes to reducing N loss, but it may be an important strategy for building long-term soil carbon and increasing soil N fertility (Liang and Mackenzie, 1992).

CONCLUSIONS

The goal of N fertilization is to supplement the soil N supply to ensure optimal corn production and grain yield. This study demonstrated that irrespective of soil texture, fertilizer rate, or application timing, FD_{TIN} availability was greatest immediately after fertilization and decreased rapidly as the result of N loss, corn uptake, or incorporation into the soil organic pool. Immediately after application, FD_{TIN} content in the soil increased with the application rate, but by V8 or R1, FD_{TIN} availability was low regardless of the N fertilizer treatment. This indicates that FDN_{TIN} availability to the corn crop is greatest immediately after fertilization and decreases with time as immobilization and N loss processes decrease FDN_{TIN} content in the soil. If a producer's goal is to maximize fertilizer N recovery in the final marketable product (i.e. grain), delaying fertilizer applications until the corn crop is actively growing will likely achieve that goal, but not necessarily increase total grain yield over a single pre-plant application. However, in most cases, corn producers are primarily concerned with final grain yield rather than grain protein so this is likely of little concern.

Immediately following fertilization, the majority of FD_{TN} is incorporated into the soil organic fraction within the top 15 cm of the soil profile, likely primarily due to rapid clay-fixation of FD_{NH4-N} and microbial immobilization. While this is positive in the sense that pre-plant fertilizer applications are temporarily protected from loss to the environment during the spring when N loss potential is generally highest, the rate of re-mineralization or de-fixation is variable and may not be well synchronized to meet corn demand potentially resulting in substantial N loss. While immobilization is rapid, fertilizer N was observed in all sampling depths at all locations by PA indicating that leaching of FD_{NO3-N} and soluble organic N compounds can be a significant N loss pathway irrespective of soil texture. Although unintentional, this study also demonstrated at Clara City14 that urea broadcast sprayed on and left near the soil surface can result in large amounts of unaccounted FDN, likely due to volatilization. Further, Becker sites validated the University of Minnesota guidelines that split-applications should be performed in place of pre-plant urea on coarse-textured soils as only 27% of FD_{TN} (averaged across pre-plant N treatments) was present in the top 60 cm by V8 (Table 3.6) and total FDN accounted for in the corn and soil at PHY1 was 87% greater on average across Becker14 and Becker15 for the 45/90 kg N ha^{-1} treatment relative to the single 135 kg N ha^{-1} pre-plant application.

Over time, the FD_{ON} content in the soil decreased with the remaining FD_{ON} fraction and became increasingly stable with little net change in FD_{ON} content from PHY1 to PHY2. Split-applications may increase the amount of FDN immobilized in the soil, but this is likely due to a greater amount of FDN taken up by the corn crop during the season that is then re-incorporated into the soil profile at harvest rather than the soil's greater ability to immobilize FDN. Based on these results, it is possible to retain ≤22% of the applied fertilizer rate in the soil profile after two growing seasons. While immobilized FD_{ON} means less FDN is available for recovery by the crop in the short term, it may represent improved soil fertility and mineralization capacity for future crops.

Table 3.1 Site locations and soil classification for studies conducted over two consecutive growing seasons in Minnesota.

Site	Start Year	Coordinates	County	Soil series classification
Becker14	2014	45˚23’32"N, 93˚52’57"W	Sherburne	Hubbard loamy sand (Sandy, mixed, frigid Entic Hapludolls)
Becker15	2015	45˚23’31"N, 93˚52’57"W	Sherburne	Hubbard loamy sand (Sandy, mixed, frigid Entic Hapludolls)
Clara City14	2014	44˚58’14"N, 95˚22’25"W	Chippewa	Bearden-Quam silty clay loam (Fine-silty, mixed, superactive, frigid Aeric Calciaquolls)
Lamberton15	2015	44˚14’41"N, 95˚18’1"W	Redwood	Normania loam (Fine-loamy, mixed, superactive, mesic Aquic Hapludolls)
Waseca14	2014	44˚04’15"N, 93˚31’16"W	Waseca	Nicollet clay loam (Fine-loamy, mixed, superactive, mesic Aquic Hapludolls)- Webster clay loam (Fine-loamy, mixed, superactive, mesic Typic Endoaquolls)
Waseca15	2015	44˚03’35"N, 93˚31’20"W	Waseca	Nicollet clay loam (Fine-loamy, mixed, superactive, mesic Aquic Hapludolls)- Webster clay loam (Fine-loamy, mixed, superactive, mesic Typic Endoaquolls)

Table 3.2 Initial soil physical and chemical properties in the top 15 cm of the soil profile for six Minnesotan field sites.

Site	Sand, Silt, Clay	pH	SOM	CEC	P†	K†	Ca†	Mg†
	%	Water	g kg^{-1}	$cmol_c$ kg^{-1}	—— mg kg^{-1} ——			
Becker14	72, 6, 22	6.1	15.9	4.3	26	95	615	113
Becker15	74, 3, 23	6.2	15.0	4.4	22	94	649	100
Clara City14	10, 48, 42	7.7	71.5	46.7	48	531	7313	963
Lamberton15	35, 25, 40	5.1	47.0	16.3	30	112	2375	385
Waseca14	19, 35, 46	5.6	66.8	29.6	23	161	4571	746
Waseca15	23, 30, 47	6.0	56.0	26.0	26	212	4042	612

† Bray-1 P (pH ≤ 7.2) or Olsen P (pH > 7.2). Potassium, calcium, and magnesium were extracted with ammonia acetate.

Table 3.3 Initial soil NO_3–N and NH_4–N measured at the 0- to 30- and 30- to 60-cm depths and total inorganic N–N (TIN) across depths for six Minnesotan field sites.

Site	NO_3–N		NH_4–N		TIN
	0-30	30-60	0-30	30-60	0-60
	———kg ha^{-1}———				
Becker14	4	2	14	9	29
Becker15	9	6	17	14	45
Clara City14	15	20	16	12	62
Lamberton15	45	27	10	14	98
Waseca14	5	11	22	7	46
Waseca15	15	7	19	10	51

Table 3.4 Soil bulk density at multiple depth increments for six Minnesotan field sites.

Site	0–15 cm	15–30 cm	30–60 cm	60–90 cm	60–120 cm
	Mg m^{-3}				
Becker14	1.53	1.63	1.64	1.58	1.62
Becker15	1.53	1.63	1.64	1.58	1.62
Clara City14	0.97†	1.31	1.30	1.38	1.36
Lamberton15	1.25	1.38	1.55	1.58	1.59
Waseca14	1.13	1.25	1.34	1.41	1.47
Waseca15	1.22	1.29	1.34	1.41	1.43

† Recent tillage resulted in an unrealistically low value. The 15- to 30-cm depth value was used for calculations involving bulk density as it is more representative of the actual soil surface bulk density for this soil (Soil Survey Staff, 2020).

Table 3.5 Tests of fixed effects for the mass or fertilizer recovery efficiency (FRE) of fertilizer-derived N (FDN) in the soil profile as total inorganic (FD_{TIN}) (2 *M* KCl extractable $FD_{NH4–N}$ and $FD_{NO3–N}$), organic (FD_{ON}) (immobilized and clay-fixed NH_4–N), and total FDN (FD_{TN}) for the 45, 135, 225, and 45/90 kg N ha^{-1} treatments. Soil sampling depths were 0- to 60-cm except at post-harvest (0–90 cm) and pre-plant (0–120 cm).

Field Site	Variable		Treatment (N)	Time (T)†	N × T
			——— *P* > *F* ———		
Becker14	Mass	FD_{TIN}	<0.001	<0.001	<0.001
		FD_{ON}	<0.001	<0.001	<0.001
		FD_{TN}	<0.001	<0.001	<0.001
	FR	FD_{TIN}	0.149	<0.001	<0.001
		FD_{ON}	0.037	<0.001	0.032
		FD_{TN}	0.162	<0.001	0.008
Becker15	Mass	FD_{TIN}	<0.001	<0.001	<0.001
		FD_{ON}	<0.001	<0.001	<0.001
		FD_{TN}	<0.001	<0.001	<0.001
	FR	FD_{TIN}	<0.001	<0.001	<0.001
		FD_{ON}	<0.001	<0.001	<0.001
		FD_{TN}	<0.001	<0.001	<0.001
Clara City14	Mass	FD_{TIN}	<0.001	<0.001	<0.001
		FD_{ON}	<0.001	<0.001	<0.001
		FD_{TN}	<0.001	<0.001	<0.001
	FR	FD_{TIN}	0.281	<0.001	0.288
		FD_{ON}	0.208	<0.001	0.061
		FD_{TN}	0.094	<0.001	0.008
Lamberton15	Mass	FD_{TIN}	<0.001	<0.001	<0.001
		FD_{ON}	<0.001	<0.001	<0.001
		FD_{TN}	<0.001	<0.001	<0.001
	FR	FD_{TIN}	0.003	<0.001	<0.001
		FD_{ON}	<0.001	<0.001	0.001
		FD_{TN}	0.001	<0.001	0.008
Waseca14	Mass	FD_{TIN}	<0.001	<0.001	<0.001
		FD_{ON}	<0.001	<0.001	<0.001
		FD_{TN}	<0.001	<0.001	<0.001
	FR	FD_{TIN}	0.204	<0.001	0.233
		FD_{ON}	0.080	<0.001	0.871
		FD_{TN}	0.108	<0.001	0.869
Waseca15	Mass	FD_{TIN}	<0.001	<0.001	<0.001
		FD_{ON}	<0.001	<0.001	<0.001
		FD_{TN}	<0.001	<0.001	<0.001
	FR	FD_{TIN}	0.105	<0.001	0.273
		FD_{ON}	0.060	<0.001	0.061
		FD_{TN}	0.143	<0.001	0.266

† Time of soil sampling as six events over two consecutive growing seasons, except at Waseca14 that had only five sampling events.

Table 3.6 Mass of fertilizer-derived N (FDN) in the soil profile as total inorganic (FD_{TIN}) (2 *M* KCl extractable $FD_{NH4–N}$ and $FD_{NO3–N}$), organic (FD_{ON}) (immobilized and clay-fixed NH_4–N), and total FDN (FD_{TN}) at six sampling events over two consecutive growing seasons. Soil sampling depths were 0- to 60-cm except at post-harvest (0–90 cm) and pre-plant (0–120 cm).

Site	Treatment	PA†	V8	R1	PHY1	PPY2	PHY2
Becker14				kg N ha^{-1}			
				FD_{TIN}			
	45	5.1cA‡	0.5cB	0.1aB	0.2aB	0.3aB	0.1aB
	135	21.8bA	3.1bB	0.2aC	0.2aC	0.3aC	0.3aC
	225	33.3aA	7.0aB	0.4aC	0.3aC	0.2aC	0.6aC
	45/90	3.7cB	9.4aA	0.5aC	0.4aC	0.3aC	0.5aC
				FD_{ON}			
	45	19.2cA	16.1cA	14.6cA	6.0aA	11.6bA	5.9bA
	135	56.3bA	29.4bAB	30.1abB	11.2aC	29.5aAB	17.7abBC
	225	112.7aA	53.0aB	42.3aBC	14.0aE	31.3aCD	24.3aDE
	45/90	16.1cA	30.0bA	28.7bA	18.4aA	33.3aA	29.4aA
				FD_{TN}			
	45	24.3cA	16.5cA	14.7bA	6.2aA	11.9bA	6.0bA
	135	78.1bA	32.5bB	30.4aBC	11.4aCD	29.8aBC	18.0abC
	225	146.0aA	60.0aB	42.7aC	14.3aE	31.5aCD	24.8aDE
	45/90	19.8cB	39.4bA	29.2aAB	18.8aB	33.6aA	29.9aAB
Becker15							
				FD_{TIN}			
	45	10.4cA	0.3cB	0.0aB	0.2aB	0.1aB	0.0aB
	135	24.8bA	1.1bcB	0.1aB	0.4aB	0.2aB	0.0aB
	225	45.0aA	3.3bB	0.1aC	0.5aC	0.3aC	0.0aC
	45/90	1.5dB	5.3aA	0.1aB	0.5aB	0.2aB	0.0aB
				FD_{ON}			
	45	39.7cA	13.5cB	14.3bB	8.5aB	11.9bB	4.0aB
	135	88.3bA	26.9bB	22.7bB	15.1aBC	21.4bB	9.6aC
	225	187.0aA	44.2aB	42.1aB	25.2aC	34.1aBC	11.9aD
	45/90	3.6dB	20.3bcA	21.4bA	15.1aA	17.8bA	13.4aA
				FD_{TN}			
	45	50.1cA	13.8bB	14.3bB	8.6aB	12.0bB	4.0aB
	135	113.1bA	28.0bB	22.8bBC	15.5aC	21.7abBC	9.6aC
	225	232.0aA	47.4aB	42.2aB	25.7aCD	34.3aBC	11.9aD
	45/90	5.1dA	25.6bA	21.5bA	15.6aA	18.0bA	13.4aA

† PA, soil sampling within eight days post-^{15}N fertilizer application; V8, vegetative development stage eight; R1, reproductive stage one; PHY1, post-harvest year 1; PPY2, pre-plant year 2; PHY2, post-harvest year 2.

‡ Within site, column, and FDN form, treatment means followed by the same lowercase letter are not significantly different ($P \leq 0.05$). Within site, treatment, and row, treatment means followed by the same upper case letter are not significantly different ($P \leq 0.05$).

Table 3.6 Continued.

Site	Treatment	PA	V8	R1	PHY1	PPY2	PHY2
Clara City14				kg N ha^{-1}			
				FD_{TIN}			
	45	2.5cA	1.3cA	0.3bA	0.3aA	0.5aA	0.0aA
	135	10.4bA	5.5bB	1.5bC	0.5aC	0.6aC	0.2aC
	225	25.4aA	11.5aB	5.6aC	2.0aD	2.7aCD	1.1aD
	45/90	8.8bA	11.6aA	3.5abB	0.4aC	0.9aBC	0.2aC
				FD_{ON}			
	45	13.4cA	9.7cA	13.5dA	6.1bA	8.2cA	4.4aA
	135	32.9bA	34.4bA	28.5cAB	15.7abB	25.7bAB	13.5aB
	225	77.7aA	48.1aB	55.0aB	25.5aC	45.8aB	19.0aC
	45/90	17.8cCD	45.4abA	41.7bAB	10.7bD	29.8bBC	15.9aD
				FD_{TN}			
	45	15.9cA	11.0cA	13.8dA	6.4bA	8.7cA	4.5aA
	135	43.3bA	39.9bAB	30.0cAB	16.2abCD	26.3bBC	13.7aD
	225	103.1aA	59.6aB	60.6aB	27.4aC	48.5aB	20.0aC
	45/90	26.5cC	57.0aA	45.3bAB	11.1bD	30.7bBC	16.1aCD
Lamberton15							
				FD_{TIN}			
	45	7.4bA	1.8dB	1.0cB	0.4aB	0.3bB	0.2aB
	135	21.7aA	15.8bB	10.9bC	3.4aD	2.3abD	0.2aD
	225	25.9aA	23.1aA	23.1aA	5.1aB	6.5aB	0.5aC
	45/90	4.1bB	8.5cA	4.0cB	0.8aB	1.6bB	0.4aB
				FD_{ON}			
	45	33.4bA	22.1cA	33.3cA	7.4aA	19.4bA	5.9aA
	135	117.0aA	77.8bB	108.1bA	16.2aC	37.6bC	12.0aC
	225	136.0aA	130.1aA	138.9aA	32.6aC	72.4aB	25.7aC
	45/90	15.7bB	61.3bA	54.7cA	4.9aB	29.9bB	13.2aB
				FD_{TN}			
	45	40.8bA	24.0cA	34.3cA	7.9aA	19.6bA	6.0aA
	135	138.7aA	93.6bB	118.9bB	19.6aC	39.9bC	12.3aC
	225	161.9aA	153.2aA	162.0aA	37.7aC	79.0aB	26.2aC
	45/90	19.8bB	69.9bA	58.7cA	5.7aB	31.5bB	13.5aB

Table 3.6 Continued.

Site	Treatment	PA	V8	R1	PHY1	PPY2	PHY2
Waseca14				kg N ha^{-1}			
				FD_{TIN}			
	45	4.5cA	0.7cB	0.4aB	0.7aB	-	0.2aB
	135	8.3bA	3.4bB	1.1aC	0.9aC	-	0.5aC
	225	15.1aA	6.9aB	2.0aC	3.2aC	-	0.8aC
	45/90	5.2cB	7.9aA	2.6aB	1.7aC	-	0.4aC
				FD_{ON}			
	45	41.9cA	22.6bA	31.5cA	12.9bA	-	9.7aA
	135	76.0bA	51.5aB	65.2bAB	24.9bC	-	23.3aC
	225	142.9aA	57.8aC	94.6aB	50.8aC	-	36.6aC
	45/90	39.2cBC	56.6aB	63.7bA	16.2bC	-	20.2aC
				FD_{TN}			
	45	46.4cA	23.3bAB	32.0cAB	13.5bB	-	9.9aB
	135	84.3bA	55.0aB	66.3bAB	25.9bC	-	23.9aC
	225	158.0aA	64.7aC	96.7aB	54.0aCD	-	37.5aD
	45/90	44.4cBC	64.5aAB	66.3abA	17.9bC	-	20.6aC
Waseca15							
				FD_{TIN}			
	45	4.5cA	1.3cA	0.2aA	0.2aA	0.2aA	0.2aA
	135	20.2bA	6.6bB	0.7aC	0.5aC	0.4aC	0.2aC
	225	37.3aA	15.6aB	2.6aC	1.2aC	0.4aC	0.3aC
	45/90	6.6cA	8.6bA	0.6aB	0.6aB	0.2aB	0.2aB
				FD_{ON}			
	45	34.4cA	29.2bAB	19.4cAB	12.5aB	19.3cAB	5.9aB
	135	109.5bA	57.9aB	46.2abB	22.4aC	41.4abB	17.7aC
	225	185.4aA	71.7aB	63.3aB	37.2aC	55.8aB	24.3aC
	45/90	38.5cA	33.4bA	33.3bcA	24.1aA	24.8bcA	29.4aA
				FD_{TN}			
	45	38.9cA	30.5cAB	19.6bcABC	12.7aBC	19.5cBC	6.1aC
	135	129.7bA	64.6bB	46.9bBC	23.0aD	41.7abCD	17.9aD
	225	222.6aA	87.3aB	65.9aC	38.4aD	56.2aC	24.6aD
	45/90	45.1cA	42.0cA	33.9bA	24.7aA	25.0bcA	29.6aA

Table 3.7 Mass of fertilizer-derived N (FDN) in the soil as NO_3–N ($FD_{NO3–N}$), total inorganic N (FD_{TIN}), and organic (FD_{ON}) (immobilized and clay-fixed NH4–N) at six sampling events over two consecutive growing seasons.

Site	Stage†	Depth	Fertilizer treatment											
			45	135	225	45/90	45	135	225	45/90	45	135	225	45/90
		cm	kg N ha^{-1}											
Becker14			$FD_{NO3–N}$				FD_{TIN}				FD_{ON}			
	PA	0–6	2.8	12.2	15.0	1.2	3.3	16.0	23.8	1.9	14.1	43.3	85.2	10.9
		6–12	0.7	1.4	2.3	0.3	0.9	1.9	3.4	0.5	2.6	6.3	12.5	2.6
		12–24	0.6	2.0	2.9	0.5	0.9	3.4	5.6	1.1	1.9	5.6	12.5	2.1
	V8	0–6	0.1	0.2	0.5	1.3	0.1	0.3	0.7	1.5	10.5	18.8	38.3	17.9
		6–12	0.0	0.2	0.6	2.6	0.1	0.3	0.8	2.7	2.5	4.4	8.0	7.6
		12–24	0.2	1.9	4.2	4.7	0.3	2.0	4.4	4.8	2.2	4.0	5.5	4.0
	R1	0–6	-	-	-	-	0.1	0.1	0.2	0.2	10.4	22.0	29.7	17.7
		6–12	-	-	-	-	0.0	0.0	0.0	0.1	2.0	2.2	4.4	5.6
		12–24	-	-	-	-	0.0	0.1	0.1	0.1	1.8	5.0	5.8	4.2
	PHY1	0–12	-	-	-	-	0.1	0.1	0.1	0.2	3.7	8.9	9.1	13.2
		12–24	-	-	-	-	0.0	0.0	0.0	0.0	0.4	0.7	1.2	1.0
		24–36	-	-	-	-	0.1	0.0	0.1	0.1	1.7	1.3	3.0	2.7
	PPY2	0–6	-	-	-	-	0.1	0.1	0.1	0.1	7.1	16.3	22.0	25.4
		6–12	-	-	-	-	0.0	0.0	0.0	0.0	1.1	2.9	2.0	2.8
		12–24	-	-	-	-	0.1	0.0	0.0	0.0	0.6	2.0	0.9	1.2
		24–48	-	-	-	-	0.1	0.1	0.0	0.1	1.2	5.0	3.5	5.9
	PHY2	0–12	-	-	-	-	0.1	0.2	0.3	0.3	3.4	14.4	19.7	23.2
		12–24	-	-	-	-	0.0	0.0	0.1	0.1	0.2	1.0	2.2	2.4
		24–36	-	-	-	-	0.0	0.0	0.0	0.1	0.9	1.4	2.1	2.6

† PA, eight days post-^{15}N fertilizer application; V8, vegetative development stage eight; R1, reproductive stage one; PHY1, post-harvest year 1; PPY2, pre-plant year 2; PHY2, post-harvest year 2.

Table 3.7 Continued.

Site	Stage	Depth	Fertilizer treatment											
			45	135	225	45/90	45	135	225	45/90	45	135	225	45/90
		cm	kg N ha^{-1}											
Becker15			$FD_{NO3–N}$				FD_{TIN}				FD_{ON}			
	PA	0–6	4.2	8.0	14.9	0.4	5.5	14.4	29.5	0.6	26.5	53.8	130.9	2.4
		6–12	0.9	1.3	2.6	0.2	1.6	2.8	5.1	0.3	7.0	15.0	27.3	0.7
		12–24	1.8	3.1	3.6	0.5	3.1	7.5	9.4	0.6	5.5	18.4	25.2	0.4
	V8	0–6	0.1	0.2	0.2	0.8	0.1	0.3	0.4	0.9	11.0	21.0	34.7	13.9
		6–12	0.0	0.1	0.2	1.3	0.1	0.1	0.3	1.3	1.5	3.9	6.0	4.0
		12–24	0.1	0.6	2.1	2.7	0.1	0.7	2.2	2.8	0.9	1.6	3.2	1.5
	R1	0–6	-	-	-	-	0.0	0.1	0.1	0.1	10.9	16.5	30.7	14.7
		6–12	-	-	-	-	0.0	0.0	0.0	0.0	1.8	3.4	6.3	4.1
		12–24	-	-	-	-	0.0	0.0	0.0	0.0	1.4	2.7	4.6	2.0
	PHY1	0–12	-	-	-	-	0.1	0.2	0.3	0.3	5.4	9.8	15.9	10.9
		12–24	-	-	-	-	0.0	0.1	0.1	0.1	0.5	1.4	1.6	1.1
		24–36	-	-	-	-	0.0	0.1	0.1	0.1	1.8	3.0	6.9	2.3
	PPY2	0–6	-	-	-	-	0.0	0.1	0.1	0.1	10.6	18.5	30.0	12.4
		6–12	-	-	-	-	0.0	0.0	0.1	0.1	0.6	1.4	1.5	1.5
		12–24	-	-	-	-	0.0	0.1	0.1	0.0	0.4	0.5	0.8	0.6
		24–48	-	-	-	-	0.0	0.0	0.0	0.1	0.2	0.4	1.1	0.9
	PHY2	0–12	-	-	-	-	0.0	0.0	0.0	0.0	3.9	9.0	14.8	12.0
		12–24	-	-	-	-	0.0	0.0	0.0	0.0	0.0	0.3	0.4	0.7
		24–36	-	-	-	-	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1

Table 3.7 Continued.

Site	Stage	Depth	Fertilizer treatment											
			45	135	225	45/90	45	135	225	45/90	45	135	225	45/90
		cm	kg N ha^{-1}											
Clara City14			FD_{NO3-N}				FD_{TIN}				FD_{ON}			
	PA	0–6	1.2	5.0	13.4	4.8	1.3	5.3	13.9	4.8	9.7	22.9	51.7	11.6
		6–12	0.4	1.3	3.7	1.0	0.4	1.4	3.8	1.1	1.6	4.1	11.7	3.9
		12–24	0.7	3.4	5.9	2.1	0.7	3.5	6.1	2.1	1.1	4.6	8.0	1.8
	V8	0–6	0.1	0.5	0.9	3.0	0.1	0.6	1.0	3.1	4.9	17.2	23.8	20.4
		6–12	0.3	1.5	2.8	2.4	0.3	1.6	2.8	2.5	2.1	8.3	13.0	11.0
		12–24	0.8	2.8	7.1	4.5	0.8	2.8	7.2	4.9	2.3	6.6	9.8	12.3
	R1	0–6	-	-	-	-	0.1	0.1	0.3	1.9	8.3	15.8	35.4	30.8
		6–12	-	-	-	-	0.0	0.2	0.7	0.3	2.5	6.2	11.5	5.0
		12–24	-	-	-	-	0.2	1.0	4.2	1.0	2.0	5.2	7.0	4.7
	PHY1	0–12	-	-	-	-	0.1	0.1	0.2	0.1	4.7	10.6	20.2	8.0
		12–24	-	-	-	-	0.1	0.2	0.7	0.1	0.7	1.6	3.1	1.3
		24–36	-	-	-	-	0.1	0.2	0.9	0.2	0.4	1.4	1.8	0.8
	PPY2	0–6	-	-	-	-	0.1	0.1	0.3	0.2	5.2	17.6	32.6	20.6
		6–12	-	-	-	-	0.0	0.1	0.3	0.1	1.4	3.5	12.0	3.1
		12–24	-	-	-	-	0.1	0.2	1.5	0.2	0.7	1.2	3.8	1.3
		24–48	-	-	-	-	0.2	0.1	0.7	0.3	0.3	0.5	0.5	0.8
	PHY2	0–12	-	-	-	-	0.0	0.1	0.2	0.1	3.8	9.0	17.6	12.7
		12–24	-	-	-	-	0.0	0.0	0.5	0.0	0.3	1.2	2.9	0.6
		24–36	-	-	-	-	0.0	0.0	0.4	0.0	0.0	0.4	1.2	0.2

Table 3.7 Continued.

Site	Stage	Depth	Fertilizer treatment											
			45	135	225	45/90	45	135	225	45/90	45	135	225	45/90
		cm	kg N ha^{-1}											
Lamberton15			$FD_{NO3–N}$				FD_{TIN}				FD_{ON}			
	PA	0–6	2.2	5.9	6.4	0.9	4.1	13.1	15.3	1.7	26.2	94.5	102.8	11.5
		6–12	0.6	1.7	1.5	0.3	0.8	2.3	2.3	0.5	3.4	6.8	8.9	1.3
		12–24	1.7	4.5	4.1	1.3	2.3	6.0	7.0	2.1	3.1	11.3	11.2	2.1
	V8	0–6	0.2	2.0	5.7	1.8	0.7	3.5	9.5	4.0	15.2	34.7	90.6	43.3
		6–12	0.1	2.0	2.6	0.4	0.3	2.9	3.5	0.6	2.3	11.9	17.3	6.2
		12–24	0.5	5.8	6.5	2.6	0.8	8.2	9.8	3.3	3.3	18.3	20.1	10.8
	R1	0–6	-	-	-	-	0.2	1.5	5.1	1.2	14.8	53.1	68.6	27.2
		6–12	-	-	-	-	0.1	0.8	3.3	0.5	6.1	14.4	28.3	9.3
		12–24	-	-	-	-	0.6	4.3	13.8	2.2	10.9	35.5	40.5	15.5
	PHY1	0–12	-	-	-	-	0.3	1.8	2.3	0.3	6.0	14.1	27.3	3.8
		12–24	-	-	-	-	0.1	0.6	1.5	0.2	0.7	1.3	2.8	0.7
		24–36	-	-	-	-	0.0	0.6	1.1	0.3	0.1	0.6	1.0	0.3
	PPY2	0–6	-	-	-	-	0.1	0.1	0.3	0.1	17.4	28.7	59.5	23.3
		6–12	-	-	-	-	0.0	0.2	0.3	0.1	0.9	3.2	4.9	1.8
		12–24	-	-	-	-	0.1	0.6	1.7	0.5	0.5	2.5	3.6	1.3
		24–48	-	-	-	-	0.1	1.2	3.9	0.9	0.3	1.7	1.7	2.3
	PHY2	0–12	-	-	-	-	0.1	0.2	0.4	0.2	5.5	10.1	20.8	11.5
		12–24	-	-	-	-	0.0	0.0	0.1	0.1	0.2	1.1	1.9	1.0
		24–36	-	-	-	-	0.0	0.0	0.1	0.1	0.0	0.5	1.3	0.2

Table 3.7 Continued.

Site	Stage	Depth	Fertilizer treatment											
			45	135	225	45/90	45	135	225	45/90	45	135	225	45/90
		cm	kg N ha^{-1}											
Waseca14			$FD_{NO3–N}$				FD_{TIN}				FD_{ON}			
	PA	0–6	1.1	1.8	3.6	0.7	2.2	3.5	8.2	1.0	22.7	36.2	92.1	15.8
		6–12	0.4	0.6	1.1	0.6	0.7	0.9	1.6	0.9	5.9	9.3	16.3	4.1
		12–24	0.8	2.0	2.7	1.8	1.2	3.5	4.9	2.5	6.4	23.7	30.5	9.3
	V8	0–6	0.2	1.0	1.5	3.5	0.4	1.8	3.0	2.8	18.4	39.5	42.9	26.7
		6–12	0.0	0.2	0.7	1.3	0.1	0.3	0.9	0.8	2.0	4.2	5.8	8.5
		12–24	0.2	1.0	2.6	2.6	0.2	1.2	3.0	2.1	1.7	5.1	6.8	11.0
	R1	0–6	-	-	-	-	0.2	0.5	1.3	1.5	23.7	46.5	70.8	49.7
		6–12	-	-	-	-	0.0	0.1	0.0	0.2	3.1	6.6	7.7	12.4
		12–24	-	-	-	-	0.2	0.5	0.5	1.0	3.2	9.0	11.4	7.0
	PHY1	0–12	-	-	-	-	0.4	0.6	1.5	1.2	11.0	20.9	37.7	13.0
		12–24	-	-	-	-	0.1	0.2	0.8	0.4	0.7	1.6	4.8	1.8
		24–36	-	-	-	-	0.2	0.2	0.7	0.1	0.9	1.2	3.6	1.0
	PPY2	0–6	-	-	-	-	-	-	-	-	-	-	-	-
		6–12	-	-	-	-	-	-	-	-	-	-	-	-
		12–24	-	-	-	-	-	-	-	-	-	-	-	-
		24–48	-	-	-	-	-	-	-	-	-	-	-	-
	PHY2	0–12	-	-	-	-	0.1	0.5	0.7	0.4	7.0	19.2	30.1	15.2
		12–24	-	-	-	-	0.0	0.0	0.1	0.0	0.6	1.7	3.3	1.5
		24–36	-	-	-	-	0.0	0.0	0.1	0.0	0.3	0.8	2.5	1.0

Table 3.7 Continued.

Site	Stage	Depth	Fertilizer treatment											
			45	135	225	45/90	45	135	225	45/90	45	135	225	45/90
		cm	kg N ha^{-1}											
Waseca15			$FD_{NO3–N}$				FD_{TIN}				FD_{ON}			
	PA	0–6	2.3	10.1	18.0	2.7	2.8	14.7	30.0	3.9	28.4	89.2	159.8	30.1
		6–12	0.4	1.0	1.5	0.9	0.4	1.2	1.9	1.1	2.6	7.3	8.7	1.8
		12–24	0.9	2.2	3.1	0.8	1.0	2.7	4.5	1.0	2.7	7.6	10.5	2.4
	V8	0–6	0.1	0.7	2.6	2.4	0.1	0.8	2.9	2.7	14.6	26.0	41.6	14.2
		6–12	0.1	0.9	3.0	1.6	0.2	1.0	3.0	1.7	4.9	13.7	14.1	9.9
		12–24	1.0	4.6	9.5	3.0	1.0	4.6	9.6	3.0	6.8	9.7	13.2	2.9
	R1	0–6	-	-	-	-	0.1	0.2	0.4	0.2	11.6	30.5	40.3	18.5
		6–12	-	-	-	-	0.0	0.1	0.1	0.1	3.7	6.6	8.5	4.7
		12–24	-	-	-	-	0.1	0.4	1.8	0.3	3.0	7.3	11.4	5.4
	PHY1	0–12	-	-	-	-	0.1	0.4	0.4	0.4	10.6	16.6	28.3	14.5
		12–24	-	-	-	-	0.1	0.1	0.1	0.1	0.9	2.8	5.0	3.0
		24–36	-	-	-	-	0.0	0.1	0.6	0.1	0.7	1.5	2.7	1.8
	PPY2	0–6	-	-	-	-	0.0	0.1	0.1	0.0	6.4	29.0	44.5	16.8
		6–12	-	-	-	-	0.0	0.1	0.0	0.0	1.4	5.1	4.9	2.8
		12–24	-	-	-	-	0.0	0.1	0.1	0.1	1.2	3.1	3.9	2.5
		24–48	-	-	-	-	0.0	0.1	0.2	0.1	0.5	2.1	1.7	0.8
	PHY2	0–12	-	-	-	-	0.1	0.1	0.2	0.1	5.4	11.1	23.5	10.3
		12–24	-	-	-	-	0.1	0.0	0.0	0.0	0.5	1.4	2.5	1.8
		24–36	-	-	-	-	0.1	0.0	0.0	0.1	0.2	0.9	1.4	0.8

Table 3.8 Tests of fixed effects for the mass of fertilizer-derived N (FDN) in the soil profile as NO_3–N ($FD_{NO3–N}$), total inorganic (FD_{TIN}) (2 *M* KCl extractable $FD_{NH4–N}$ and $FD_{NO3–N}$), and organic FDN (FD_{ON}) (immobilized and clay-fixed NH_4–N) for the 45, 135, 225, and 45/90 kg n ha^{-1} treatments. Soil sampling depths were 0- to 15-, 15- to 30-, and 30- to 60-cm except at post-harvest (0–30, 30–60, 60–90 cm) and pre-plant (0–15, 15–30, 30–60, 60–120 cm).

Field Site	Source of Variation	PA†	V8	R1	PHY1	PPY2	PHY2
		P > F					
Becker14		$FD_{NO3–N}$					
	Treatment (N)	<0.001	<0.001				
	Depth (D)	<0.001	<0.001				
	N × D	<0.001	0.007				
		FD_{TIN}					
	Treatment (N)	<0.001	<0.001	0.001	0.098	0.135	<0.001
	Depth (D)	<0.001	<0.001	<0.001	<0.001	0.007	<0.001
	N × D	0.001	0.003	0.869	0.600	0.037	0.004
		FD_{ON}					
	Treatment (N)	<0.001	<0.001	<0.001	0.005	0.02	0.002
	Depth (D)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	N × D	0.016	0.338	0.042	0.202	0.700	0.100
Becker15		$FD_{NO3–N}$					
	Treatment (N)	<0.001	<0.001				
	Depth (D)	<0.001	<0.001				
	N × D	<0.001	0.013				
		FD_{TIN}					
	Treatment (N)	<0.001	<0.001	<0.001	0.0085	0.004	0.148
	Depth (D)	<0.001	<0.001	<0.001	<0.001	<0.001	0.002
	N × D	<0.001	0.0196	<0.001	0.024	<0.001	0.018
		FD_{ON}					
	Treatment (N)	<0.001	<0.001	<0.001	<0.001	0.038	<0.001
	Depth (D)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	N × D	0.003	0.152	0.096	0.317	0.007	<0.001
Clara City14		$FD_{NO3–N}$					
	Treatment (N)	<0.001	0.002				
	Depth (D)	<0.001	<0.001				
	N × D	0.482	0.0046				
		FD_{TIN}					
	Treatment (N)	<0.001	0.001	0.002	0.001	<0.001	0.001
	Depth (D)	<0.001	<0.001	<0.001	0.001	<0.001	0.796
	N × D	0.5207	0.0395	<0.001	0.002	0.001	0.059
		FD_{ON}					
	Treatment (N)	<0.001	<0.001	<0.001	0.001	<0.001	0.0019
	Depth (D)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	N × D	0.615	0.808	0.212	0.790	0.002	0.010

† PA, eight days post-^{15}N fertilizer application; V8, vegetative development stage eight; R1, reproductive stage one; PHY1, post-harvest year 1; PPY2, pre-plant year 2; PHY2, post-harvest year 2.

Table 3.8 Continued.

Field Site	Source of Variation	PA	V8	R1	PHY1	PPY2	PHY2
		P > *F*					
Lamberton15		$FD_{NO3–N}$					
	Treatment (N)	0.001	<0.001				
	Depth (D)	<0.001	<0.001				
	N × D	0.903	0.034				
		FD_{TIN}					
	Treatment (N)	<0.001	<0.001	<0.001	<0.001	<0.001	0.001
	Depth (D)	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
	N × D	0.034	0.031	0.053	0.246	<0.001	0.004
		FD_{ON}					
	Treatment (N)	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
	Depth (D)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	N × D	0.128	0.604	0.592	0.001	0.269	0.634
Waseca14		$FD_{NO3–N}$					
	Treatment (N)	0.029	<0.001				
	Depth (D)	<0.001	0.001				
	N × D	0.095	0.300				
		FD_{TIN}					
	Treatment (N)	0.0137	<0.001	0.002	0.001	-	<0.001
	Depth (D)	<0.001	0.002	<0.001	<0.001	-	<0.001
	N × D	<0.001	0.875	0.041	0.413	-	<0.001
		FD_{ON}					
	Treatment (N)	0.012	<0.001	<0.001	<0.001		0.002
	Depth (D)	<0.001	<0.001	<0.001	<0.001		<0.001
	N × D	0.430	0.271	0.465	0.535		0.759
Waseca15		$FD_{NO3–N}$					
	Treatment (N)	0.001	<0.001				
	Depth (D)	<0.001	<0.001				
	N × D	0.232	0.013				
		FD_{TIN}					
	Treatment (N)	<0.001	<0.001	<0.001	0.004	0.001	0.476
	Depth (D)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	N × D	0.002	0.020	<0.001	0.014	0.016	<0.001
		FD_{ON}					
	Treatment (N)	0.001	0.001	0.001	0.003	0.003	0.002
	Depth (D)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	N × D	0.682	0.199	0.756	0.872	0.439	0.511

Table 3.9 Fertilizer recovery and tests of fixed effects of total fertilizer-derived N in the soil profile at six sampling events over two consecutive growing seasons for the 45, 135, and 225 kg N ha^{-1} treatments. Soil sampling depths were 0- to 60-cm except at post-harvest (0–90 cm) and pre-plant (0–120 cm).

Site	Treatment	PA†	V8	R1	PHY1	PPY2	PHY2
Becker14	kg N ha^{-1}	——————————————% ——————————————					
	45	54	37	33	14	27	13
	135	58	24	23	8	22	13
	225	65	27	18	6	14	11
Becker15	45	112	31	32	19	27	9
	135	84	21	17	11	16	7
	225	104	21	19	11	15	5
Clara City14	45	35	24	31	14	19	10
	135	32	30	22	12	20	9
	225	46	27	27	12	24	10
Lamberton15	45	91	54	77	18	44	13
	135	103	70	81	15	30	9
	225	72	68	72	17	35	12
Waseca14	45	104	52	71	30	-	22
	135	63	41	49	19	-	18
	225	70	29	43	24	-	17
Waseca15	45	87	68	44	28	33	15
	135	96	48	35	17	31	11
	225	99	39	29	17	25	13

Tests of Fixed Effects

Source of Variation	Becker14	Becker15	Clara City14	Lamberton15	Waseca14	Waseca15
Treatment (N)	0.084	0.002	0.535	0.479	0.022	0.074
Time (T)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
N×T	0.149	0.071	0.267	0.128	0.711	0.136

Figure 3.1 Daily precipitation, irrigation, soil water deficit, and cumulative water losses (runoff and deep percolation) for the 225 kg N ha^{-1} treatment at each site during the first year. The timing of treatment plot (F) and ^{15}N enriched microplot (f) fertilization at planting, soil sampling eight days post-^{15}N fertilizer application (PA), vegetative and reproductive development stages (V4, V8, R1, and R6), harvest (H), and post-harvest soil sampling (PH) are also shown.

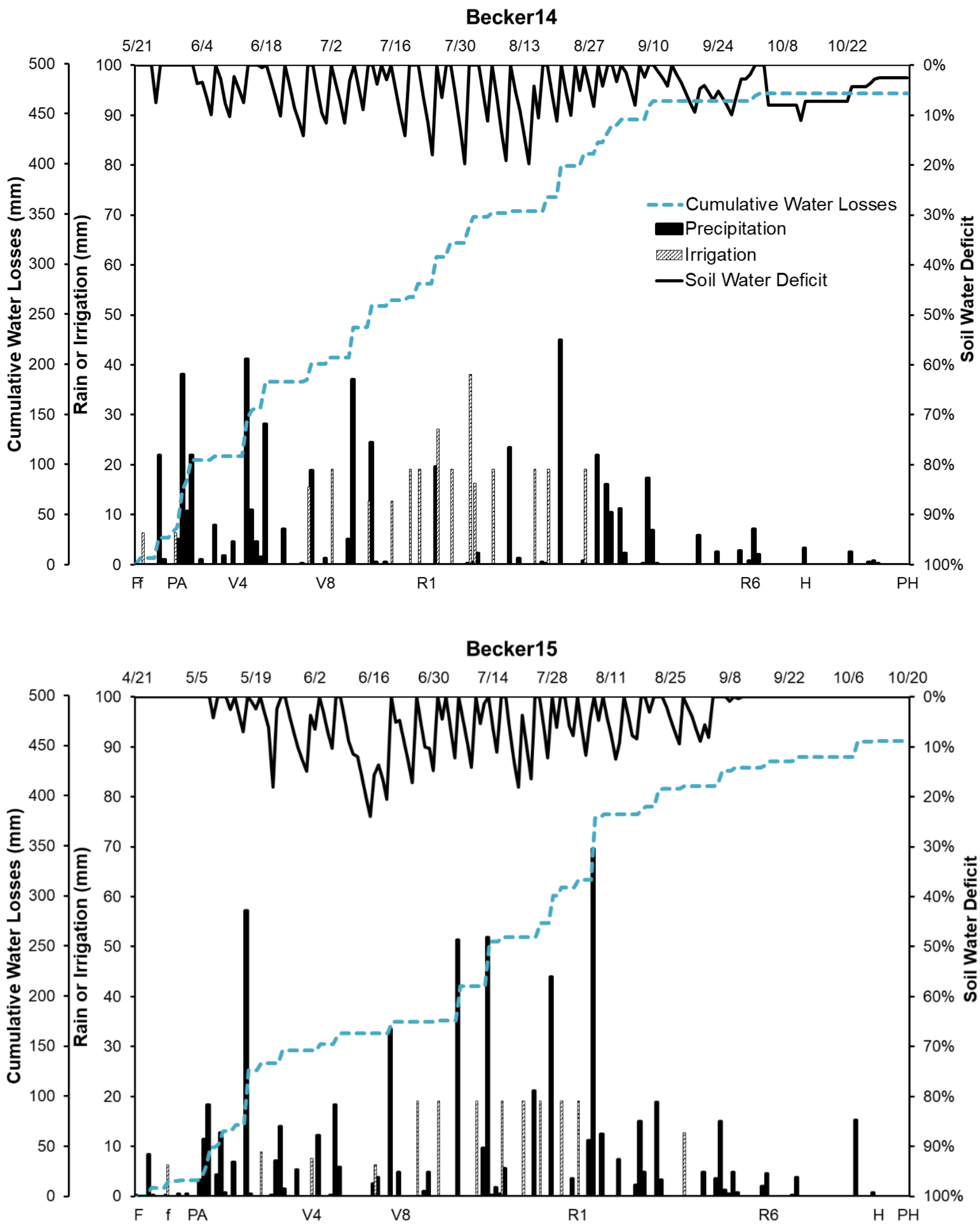
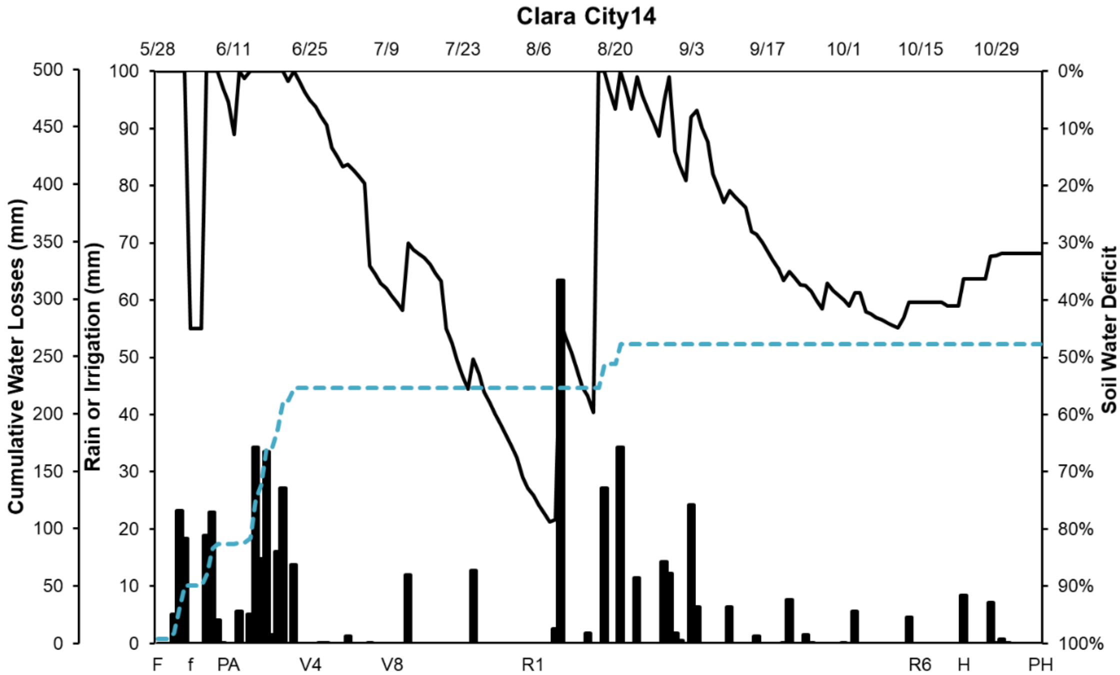


Figure 3.1 Continued.



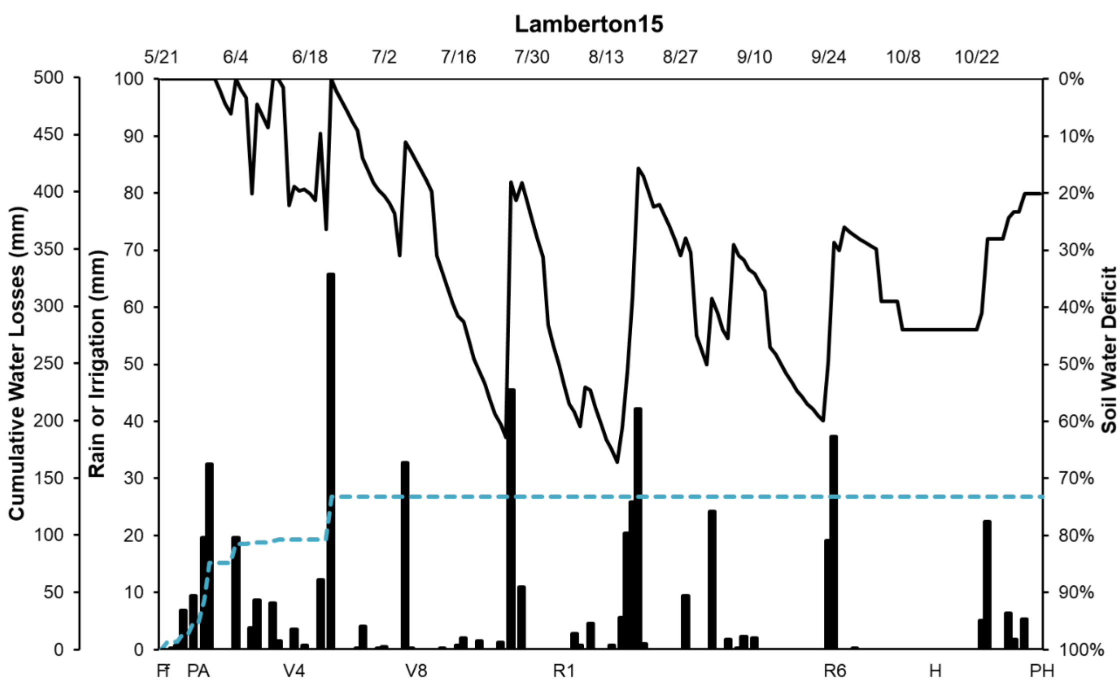
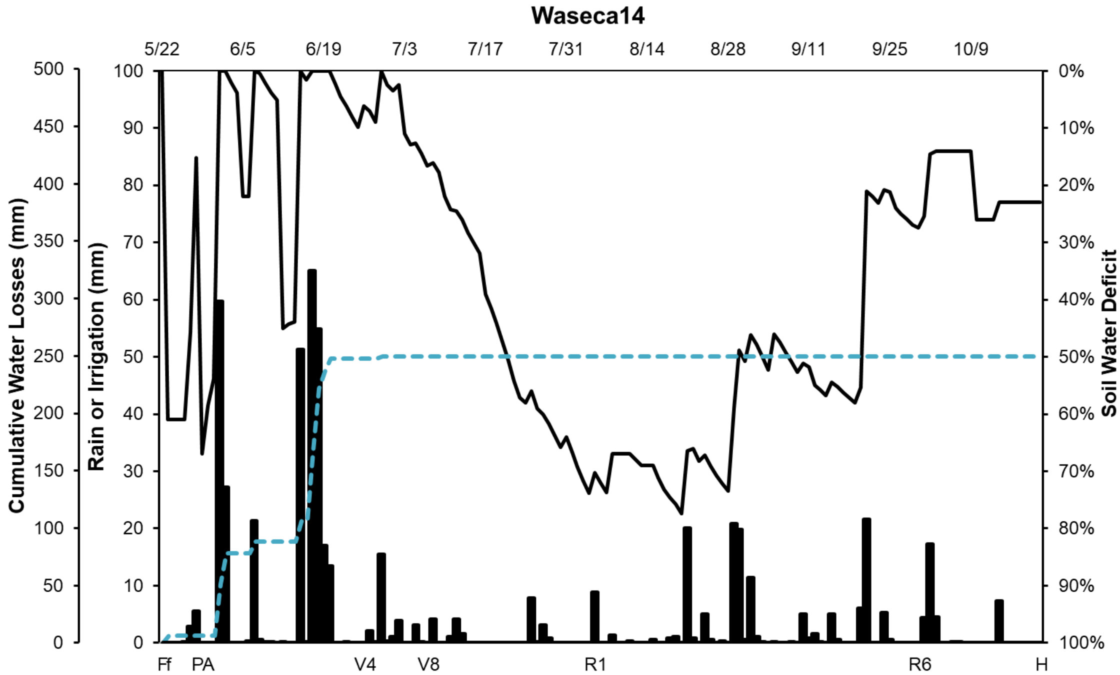
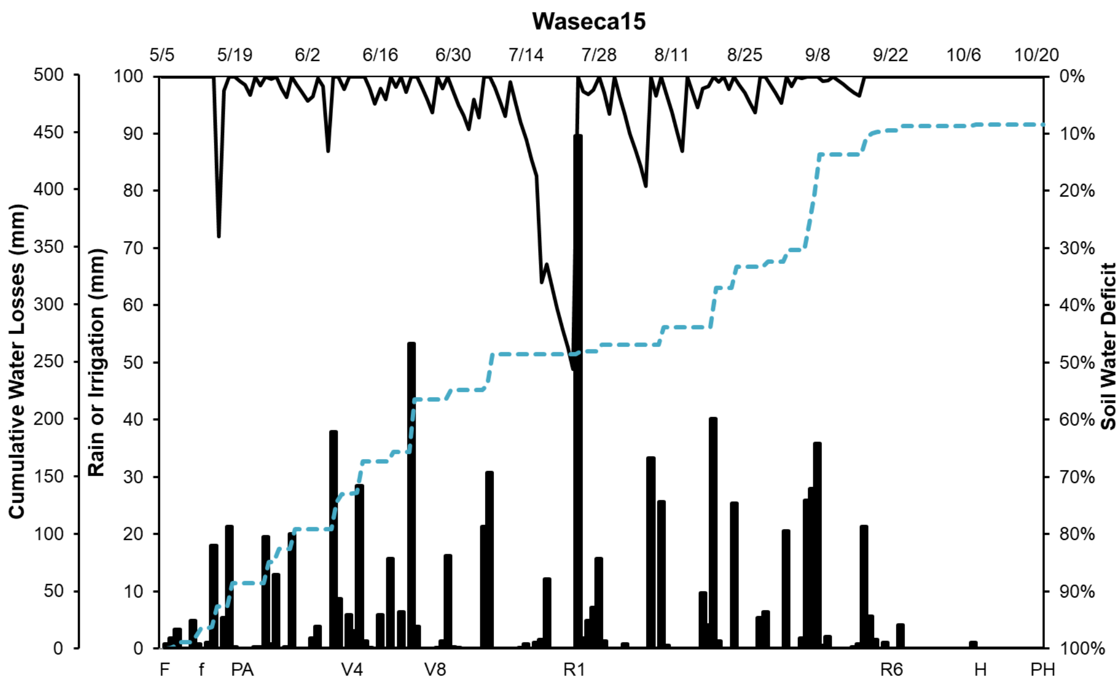


Figure 3.1 Continued.





CHAPTER 4: SUMMARY AND FUTURE WORK

Fertilizer nitrogen (N) is an important component of high-yielding corn production but it can be difficult to manage because it exists in multiple forms that may be transported from the soil-crop system into the environment. These transformations are affected by soil texture, weather patterns, and the timing and rate of fertilizer application (Van Cleemput et al., 2008). Fertilizer N enriched with stable ^{15}N isotopes allow researchers to differentiate between fertilizer-derived N (FDN) and soil-derived N (SDN) and to assess total fertilizer N recovery and loss to the environment under field conditions. This study allowed the author to investigate how urea rate and timing impacted FDN distribution and form in the soil profile over time as well as N uptake patterns and partitioning to the crop over two growing seasons.

Urea has multiple advantages as a fertilizer N source because it is easily stored and transported, it has a high concentration of N (46% N by mass), and it is water-soluble for rapid availability to the crop. However, this study demonstrated that urea fertilizer applied at planting is prone to loss, especially when spring conditions are wet. Urea hydrolysis and nitrification were rapid and likely favored leaching processes on all soils, and more especially on coarse textures (such as loamy sands) that have high water permeability and drainage. This result reinforces the current University of Minnesota guidelines that urea applications should not be made at planting on coarse-textured soils. Ponded soils and saturated micropores on fine-textured soils likely favored denitrification losses. When urea fertilizer was applied to the soil surface and insufficiently incorporated, approximately 60% of the fertilizer derived N (FDN) was unaccounted and was likely volatilized. By the end of the first year, <63% of the applied FDN was accounted for in the soil-crop system that represents a substantial economic loss and reactive N load to the environment.

Split-applications had the greatest improvement for coarse-textured soils because two-thirds of the FDN was not subject to N loss or immobilization processes for 21 to 34 days relative to fertilizer applied at planting. This delay allowed the corn crop to become established and more competitive for inorganic N assimilation. On most fine-textured soils, the single- and split-applications had similar FDN uptake and yield, likely because large soil aggregates and small micropores protected inorganic and soluble organic N from leaching losses that predominantly occur in macropores following large precipitation events (Balasubramanian et al., 1973). Despite split-applications improving FDN uptake in the crop, unaccounted FDN for the split-application was ≥44% of the applied rate across all sites at the end of the first year. Further delaying the split-application until V6 or V8 may avoid additional leaching events and further improve FDN recovery in the soil-crop system.

This study demonstrated that fertilizer N supplements the soil N supply to meet corn N uptake demand. However, ultimately, SDN was primarily responsible for most of the N taken up by the crop irrespective of the soil type or its mineralization potential. This result re-emphasizes the importance of native soil N for sustainable cropping systems. If soil N reserves are depleted, additional crop inputs will likely be needed to achieve similar yields. Nitrogen fertilization may build soil organic matter and soil N reserves, but only if there is a net increase of soil N and carbon from one growing season to the next. At the Broadbalk long-term N fertility plots, annual applications of 225 kg N ha^{-1} as manure accrued 58 kg N ha^{-1} yr^{-1} over the first 50 years of the experiment, representing about 25% of the applied N rate (Meisinger et al., 2008). Given that ≤24.7 kg FDN ha^{-1} was returned to the soil as corn residue in the 225 kg N ha^{-1} treatment in this study (Table 2.6), FDN_{ON} accrual will likely only be noticeable after many years and should be evaluated over five or more years to determine if a management practice is building soil organic matter and soil N content (Meisinger et al., 2008), which is outside the scope of the present study. Long-term N rate studies that establish a new ^{15}N enriched microplot each year and maintain previous year's microplots would also be useful for determining how annual growing season variability impacts ^{15}N recovery by the crop and stabilization into soil organic matter. This information would also allow researchers to document long term recovery of FDN in the soil system and could quantify FDN buildup in the soil.

While this study successfully quantified the FDN recovered in the soil and the aboveground crop, it is missing two pieces of information that would improve our understanding of the effect fertilization has on soil N availability and the fate of FDN. The first shortcoming of this study was that it could not quantify the mechanism of FDN loss. The N loss mechanisms described above are no better than educated guesses based on the observed position of FDN and the known soil conditions that favor denitrification, leaching, or volatilization. If this study was repeated, it would be informative to collect estimates of denitrification using static gas collection chambers within the microplots (Fernández et al., 2016). Additionally, leaching was assumed to be a significant N loss mechanism due to the volume of water lost from the soil that was estimated using the water balance approach. Lysimeters or tile-drainage water could improve estimates of the volume of water moving through the soil and its total N and FDN load. Estimates of NH_3 volatilization would likely be most valuable during the first two weeks following ^{15}N application at planting and again for the two weeks following the V4 application. Volatilization from aboveground crop tissues should also be quantified from R1 through R6 as Francis et al. (1993) postulated that 7 to 34 kg FDN ha^{-1} was lost from aboveground corn biomass following anthesis due to inefficient conversion of NH_4^+ to organic compounds within the plant tissues. At Lamberton15, volatilization from vegetative biomass may partially explain why the reduction of fertilizer derived total N (FD_{TN}) from R1 to R6 (69% to 13% averaged across treatments suggesting mineralization) was not accompanied by an increase of FDN uptake in the crop. Because the soil water deficit was greater than 15% from R1 through post-harvest the first year (PHY1), leaching was probably not the N loss mechanism.

The second shortcoming of this study is that it could not quantify how much total N and SDN were lost from the soil-corn system. As was discussed in Chapter 1 and Chapter 2, added N interactions (ANI) may occur following fertilization where SDN uptake in fertilized plants may be the same, greater-than, or less-than SDN uptake in the unfertilized plants. These ANIs occurred primarily due to pool substitution where labeled fertilizer N substituted for unlabeled fertilizer N during denitrification, immobilization, or crop uptake processes. Fertilization may stimulate mineralization of soil organic matter, increasing the total inorganic N content in the soil. Each soil sampling event was a snapshot of the N fertility level in the soil, but sampling events were not frequent enough to accurately estimate total SDN loss from the system. Further, because the focus of Chapter 3 was on FDN recovery in the soil, this dissertation touched only briefly on the amount of inorganic SDN in the soil but did not discuss the effect of fertilization on inorganic SDN content in the soil. Future work will address this topic. If this study was repeated where total N lost from the system was quantified (i.e., continuous measurements of soil water in tile drainage, etc.), researchers could identify if fertilization increases the total inorganic N supply and N lost from the soil. This information would also allow researchers to determine if fertilization results in a net increase, decrease, or no change in total soil N following fertilization.

One line of work that this dissertation theorized on but did not have data to verify was regarding FDN in the organic N fraction (FD_{ON}). This study observed that the majority of FD_{TN}

was FD_{ON} within eight days of application (PA) and at each subsequent sampling event until post-harvest the second year (PHY2). Based on others' work, it was assumed that FD_{ON} resided in two major pools: clay-fixed fertilizer derived NH_4–N ($FD_{NH4–N}$) and immobilized into the soil microbial fraction. Other researchers have demonstrated that $FD_{NH4–N}$ replaced clay-fixed soil-derived NH_4–N resulting in pool substitution (Mengel and Scherer 1981) and that clay-fixed $FD_{NH4–N}$ was released within a few weeks (Kelley and Stevenson, 1987) to years (Kowalenko, 1978). The ability of the soil to fix and retain $NH4^+$ ions may be an N preservation strategy for improved N use efficiency. Because the soils in this study are of contrasting textures, analyzing each site's soil samples for clay-fixed $FD_{NH4–N}$ would inform how tightly it is retained over time. Additionally, the soils from this study could be analyzed using acid hydrolysis to fractionate soil N into the amino acids, amino sugars, soluble organic N, and insoluble organic N fractions. Changes of FD_{ON} pool ratios over time would be informative of how active the microbial biomass pool is and how quickly FDN is stabilized into more recalcitrant forms of $FD_{ON.}$

The acid hydrolysis analysis would also improve our understanding of how soil texture affects FDN retention in the soil. Over time, FD_{ON} was depleted from the soil at different rates where coarse-textured soils' FDN_{TN} was <37% by V8 whereas similar levels were not observed on some fine-textured soils until PHY1. Additionally, across all sites, FD_{ON} values did not differ from V8 to R1. At Lamberton15 and Waseca15, soil FD_{ON} was rapidly depleted from R1 to R6 which did not correspond to an equivalent increase in FDN uptake by the crop. Based on these observations, the acid hydrolysis analysis could answer the following questions. Was FD_{ON} loss more rapid on coarse-textured sites due to rapid microbial biomass turnover? Was clay-fixation responsible for high FD_{ON} values on fine-textured soils? Was clay-fixed $FD_{NH4–N}$ released as the soil NH_4^+ concentration decreased? Was FD_{ON} bound in more recalcitrant forms by V8 at

Lamberton15 that then mineralized from R1 to R6? Why was there consistently no difference in FD_{ON} from V8 to R1 and were the relative ratios of the FD_{ON} pools the same between these two sampling events?

This study found that because FDN recovery in the crop increased with increasing N rate in the first year, more FDN was returned to the plot in corn residue. It was assumed that FDN recovered by the crop the second year was primarily due to first-year corn residue decomposition since there was little change in FD_{ON} from PHY1 to PHY2. In a future study, this assumption could be tested by replacing the ^{15}N enriched microplot corn stover with an equivalent mass of unlabeled corn stover from the same treatment at PHY1. The ^{15}N enriched microplot biomass could then be applied to a new microplot. At PHY2, the mass of FDN recovered in the second year crop from the original microplot would be re-mineralized FD_{ON} and corn roots while the mass of FDN recovered in the crop from the new microplot would be the mass of FDN from mineralized aboveground corn residue. Comparisons of the FD_{ON} in both the original and new microplot would also be useful for determining if FDN from corn residue builds soil organic N in the soil.

To further improve this study, additional sampling events during periods of high N loss or FDN cycling, such as at V4 and R3, would be informative. The V4 sampling event would be used to help quantify leaching and denitrification losses during wet springs and would be especially useful on coarse-textured soils. The R3 event would improve our understanding of later season mineralization of FD_{ON}. Combined with the acid hydrolysis analysis previously prescribed, this sample would help us to know if the FD_{ON} is decreasing due to mineralization of soil microbial biomass or some other pool. Regarding plant N uptake, this sample could be used to learn about N translocation from the leaves and stalk to the cob and grain. An additional suggestion is to take soil samples from the 0- to 15-, 15- to 30-, 30- to 60-, 60- to 90-, and 90- to 120-cm depths at PHY1, pre-plant in the second year, and PHY2. This will improve estimates of FDN leaching through the soil profile.

Finally, the original project plan included an additional treatment of 135 kg N ha^{-1} of polymer-coated urea applied at planting. Due to timing constraints, the ^{15}N enriched fertilizer source was not secured before initiating the study. Other research has shown that polymer-coated urea performed similar to anhydrous ammonia or split-applications and significantly improved corn grain yield relative to urea when applied at planting on coarse-textured soils but there was little yield improvement on fine-textured soils (Spackman et al., 2018). The polymer-coated urea treatment would have some unique challenges relative to the other fertilizer treatments such as ensuring an even distribution of fertilizer over the microplot and ensuring the soil sampling technique is representative of the bulk soil. In this study, ^{15}N enriched urea fertilizer was dissolved in water and spray-applied to the microplot. This is not possible for polymer-coated urea because of the polymer coating. One potential solution would be to overlay a grid pattern over the microplot and, at each gridline intersection, press a polymer-coated urea prill into the soil at the desired depth. This process would likely be extremely time-consuming. Additionally, this fertilizer strategy could create soil sampling issues as the fertilizer concentration will likely be greatest immediately around the prill and more dilute further away from the prill. During soil sampling, the researcher will not know where each prill is located and may not obtain a representative estimate of FDN in the soil. The excavation technique described in Chapter 3 may be a better soil sampling strategy for this fertilizer treatment.

While much work has already been performed investigating the fate of N in the soil-crop system, this research demonstrated that there are still many questions to be answered. Future work should seek to better understand the processes of FDN immobilization immediately following application and factors that affect when and how much FDN is re-mineralized or de-fixed from clay. Additional work should focus on quantifying and determining if fertilization increases, decreases, or does not affect total N lost from the soil-crop system. This information would further improve crop models and nutrient management strategies for corn production and environmental protection.

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