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Residual effects of nitrogen fertilization on soil nitrogen pools and corn growth

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RESIDUAL EFFECTS OF NITROGEN FERTILIZATION ON SOIL NITROGEN POOLS AND CORN GROWTH

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James Camberato Chair	
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Approved by: Linda Lee 11/22/2016

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RESIDUAL EFFECTS OF NITROGEN FERTILIZATION ON SOIL NITROGEN POOLS AND CORN GROWTH

A Thesis
Submitted to the Faculty
of
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by
Meghan E. Moser

In Partial Fulfillment of the
Requirements for the Degree
of
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To my parents

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TABLE OF CONTENTS

Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Appendix Figure Page

ABSTRACT

Moser, Meghan E. M.S., Purdue University, December 2016. Residual Effects of Nitrogen Fertilization on Soil Nitrogen Pools and Corn Growth. Major Professor: Jim Camberato.

Given the dynamic nature of soil nitrogen (N), inorganic N fertilization to corn (*Zea mays* L.) has potential to alter N pool balance by creating an accumulation or depletion of soil N. Current corn N recommendations in the common corn-soybean rotation of Indiana strive to find the best N rate that maximizes producer profit. Increasing our understanding of soil N will inform producers if they should adjust fertilizer rates for corn to influence maintenance of organic N and Carbon. Our objective was to determine residual N effects from fertilized corn in a corn-soybean rotation by measuring (1) soil N pools post soybean, (2) soil fertility, (3) growth and yield of corn, and (4) nitrogen removal in rotated corn.

Field-scale corn N trials were established in 2006 at 6 Indiana farms with corn-soybean rotations (near cities of West Lafayette, Farmland, Columbia City, Wanatah, Butlerville, and Lafayette). A randomized complete block design assigned six corn N rates (ranging from starter only to above the minimum needed to maximize yield) to each replication. The design was not re-randomized the next corn year. In 2015, two or three of the replications at each location had only starter fertilizer, thus, allowing for determination of cumulative differences in N fertilization on soil N supply. Soil composites of each plot were collected from 0-20 cm, 20-40 cm, and 40-60 cm post corn planting (<9 days). Initial samples were analyzed for general fertility, inorganic N, and total N. In addition, a 50-day incubation with soils maintained at 25 °C and 33 kPa moisture was used to examine mineralization and nitrification at days 10 and 50 days. Earleaf samples were collected at VT followed by stover and grain samples at maturity; plant samples were analyzed for macro- and micro-nutrient concentrations. Grain yield and total plant N uptake were also determined.

Locations were kept separate for statistical analyses. ANOVAs carried out on general soil fertility data revealed minimal N rate effects. At Lafayette, pH for the 0-20 cm soil decreased linearly beginning at N rate 3 (135 kg ha^{-1}); the acidifying effect of the side-dressed urea-ammonium nitrate (UAN) may be responsible for the pH decrease with increasing N rate. This trend was not observed at other locations. There was no N rate effect on day 0 total inorganic N ($\alpha \leq 0.05$). As N rate increased, total N decreased linearly from 0.9 to 0.8 g kg^{-1} ($R^2 = 0.68$) at Columbia City. When the soil was incubated for 50 days, total inorganic N did not vary by N rate. Generally, soil inorganic and organic N decreased with depth from 0 to 60 cm. When corn was grown and the predominate source of N was derived from the soil, no differences were noted in plant N uptake nor yield for any location.

Spring 2015 had record-breaking rainfall amounts which certainly contributed to residual N loss. Furthermore, the soil's natural N supply, location management practice, and crop N demand are probable cause for the variances noted between locations.

Overall, we conclude that corn N rate has negligible effects on residual N abundance, soil fertility, uptake, and grain yield for Indiana corn-soybean rotations.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

Corn (*Zea mays* L.) is the dominant cash crop in the United States with over 35.6 million ha planted and average grain yield of 10.6 t ha^{-1} in 2015 (USDA-NASS, 2016). Generating immense quantities of corn demands proper management of soil chemical, physical, and biological properties. Oftentimes, corn is grown in rotation with soybean (*Glycine max* L.) to avoid risks such as disease and pest infestation, additional management efforts, and prospective yield decline of continuously cropped monocultures (Bundy et al., 2011; Stalcup, 2012; Sindelar et al., 2013). The soil-plant interface is a dynamic system by way of continuous inputs and outputs. Nitrogen (N), a frequent input, is recognized as the most limiting nutrient to crop growth and is at the forefront of scientific study (Brady and Weil, 2009). In the common corn-soybean rotation of the Midwest, applying N at rate which will achieve maximum profit is crucial (Camberato and Nielsen, 2015). A measure of the N pool distribution in corn-soybean rotations will inform producers of how they may work with the soil and perhaps utilize residual N left by previous crops.

1.2 Corn History

Christopher Columbus reached the New World in 1492 and discovered corn, which was cultivated from Canada to Chile primarily using slash-and-burn agriculture

(Mangelsdorf, 1986). The Indians offered Columbus a seed called Mahiz, or “seed of life,” and taught him how to grow, store, and prepare the crop (Wolf, 2002). Columbus returned to Spain with the seed that would soon be recognized as a valued crop due to its tolerance of the environment and nutritional usefulness to humans and livestock (Staller, 2010). Within two generations of Columbus’ return to the Old World, corn quickly became a global crop (Mangelsdorf, 1986). Prior to the domestication of corn in the New World, the actual ancestry of corn remains a mystery although several theories exist that attempt to resolve whether corn evolved from annual teosinte or wild corn (Beadle, 1980; Mangelsdorf, 1986). Ancestry aside, corn has become a staple in present day agriculture with the U.S. accounting for ~32% of global corn production (USDA-FAS, 2014).

1.3 The Nitrogen Cycle

Nitrogen is essential to life due to its presence in proteins, chlorophyll, and nucleic acids (Brady and Weil, 2009). Nitrogen is found in many forms, or pools, which differ in stability and size depending on environment conditions. The largest and most stable pool is dinitrogen gas (N_2) which comprises 79% of the atmosphere (Weathers et al., 2013). Dinitrogen gas may be fixed from the atmosphere via the man-made Haber-Bosch process; however, natural fixation occurs by means of biological N fixation (BNF) and lightning (Huang et al., 2012). The Haber-Bosch process puts N_2 gas under heat and pressure to create ammonia (NH_3), the building block for most synthetic N fertilizers (Mosier et al., 2004).

Once incorporated into a system, either artificially or naturally, N may be quickly transformed. Nitrogen mineralization is the conversion of organic N to inorganic N while

N immobilization is the conversion from inorganic N to organic N by microorganisms (Huang et al., 2012; Weathers et al., 2013). Within the inorganic pool, the processes of nitrification and denitrification take place. Nitrification is a microbial oxidation process where ammonium (NH_4^+) or ammonia (NH_3) is transformed into nitrite (NO_2^-) followed by nitrate (NO_3^-), while denitrification is a reducing process that converts NO_3^- to nitrous oxide (N_2O), nitric oxide (NO), or N_2 gas (Weathers et al., 2013). The kinetics of the above transformations are driven by factors such as temperature, pH, and moisture (Huang et al., 2012).

In order to maximize crop productivity, plants must find a balance of N that avoids toxicity and deficiency. An excessive supply of NH_4^+ may cause rapid plant growth resulting in decreased plant strength and delayed maturity (Brady and Weil, 2009). In contrast if N is scarce, plants will appear pale yellow with narrow leaves. Various plant response tactics exist to deal with N deficiency; however, in many prolonged cases of inadequate N, plant growth is stunted and overall crop production is compromised (Hawkesford et al., 2011).

1.3.1 Mineralization and Immobilization

Mineralization is the main driver that either directly or indirectly influences N cycle dynamics. Soil temperature, aeration, moisture, pH, and organic matter influence mineralization. In a study by Ros et al. (2011), the size of the soil organic matter (SOM) pool explained 78% of NH_4^+ production and was the primary driver of mineralization. Campbell (1978) noted that soil organic matter was greatest in the topsoil where approximately 1-2% of organic-N was mineralized each year. Soil organic matter is often considered as being comprised of two pools – labile and recalcitrant. The labile pool is available for microbial breakdown while the dominant, recalcitrant pool resists decomposition (Magdoff and Harold, 2000). Rather than looking at total SOM, labile SOM is used as an indicator of soil quality given its rapid turnover and breakdown potential (Haynes, 2005). Labile SOM is greatest in the topsoil (0-10 cm), while recalcitrant SOM dominates the subsoil (20-30 cm) (Fang et al., 2005).

Organic N commonly enters a cropping system via crop residue, sewage sludge, or animal manure applications where it is microbially converted to inorganic NH_4^+. Ideally, an inorganic N fertilization program would consider maintenance and utilization of SOM but research reveals inconsistent results. Cassman et. al (2002) found that 80-240 kg N ha^{-1} yr^{-1} could be supplied from organic matter in a U.S. Corn Belt prairie soil. Carpenter-Boggs et al. (2000) noted net mineralization in a corn-soybean rotation to average 142 kg N ha^{-1} with decreasing mineralization as fertilizer rate increased. They proposed that decreased symbiotic N fixation in the soybean year and difference in residue decomposition may explain the mineralization differences between N rates.

Inorganic N fertilizer additions reduced microbial diversity and abundance because of the acidic environment created by the fertilizer (Zhou et al., 2015). From the 1980s to 2000, Guo et al. (2010) observed an average pH decrease of 0.55 across 154 cash crop fields. In contrast to Zhou et al. (2015), Dick (1992) noted that inorganic fertilizer additions increase microbial activity because of improved nutrient concentrations in post-harvest biomass. The size of the SOM pool is expected to increase as soil mineralization decreases; however, a 13 year continuous corn study found that inorganic N fertilizer rates ranging from 0 to 269 kg N ha^{-1} yr^{-1} and residues ranging from

3.6 to 9.9 Mg dry matter ha^{-1} yr^{-1} had no significant effect on SOM pool size (Brown et al., 2014).

The C:N ratio of residue is one factor that regulates the rate of mineralization and prompts microbes to either mineralize or immobilize nitrogen. A typical topsoil in the Midwest has a C:N ratio ranging from 9:1 to 12:1 (Lakes et al., 1977). Within the soil, microorganisms maintain their bodies at a C:N ratio of 8:1 by utilizing C and N stocks from organic residue (Cleveland and Liptzin, 2007). Organic residue with a high C:N ratio (>30:1) does not have enough N for microbial growth, so organisms will turn to soil inorganic N to meet their demands (immobilization). Residue with a low C:N (<20:1) ratio has enough N to meet microbe demand and permit decomposition (mineralization) (Huang et al., 2012). Although critical points of mineralization-immobilization turnover (MIT) have been noted, it is beneficial to think of MIT on a gradient whereby N transforms from an immobilized to mineralized state in a given time under certain conditions.

Studying patterns and temporal dynamics of MIT is helpful in predicting net mineralization. Douglas and Magdoff (1991) provide an overview of indices used to predict N mineralization. Due to variability of soil and environment conditions, they promote the use of indices as a comparison tool of general mineralization potential. The interaction between temperature and moisture is a key factor controlling microbial activity. Guntiñas et al. (2012) determined that 25 °C and 80-100% of field capacity (33 kPa moisture tension) was optimum temperature and moisture for mineralization in soils obtained from forest, grassland, and cropland systems. Additionally, the study found that temperature had a stronger influence than moisture on N mineralization. Likewise, Kladivko and Keeny (1987) noted maximum mineralization at 25 °C and 33 kPa moisture tension. The transient nature of residue mineralization is described by Honeycutt and Potaro (1990) who studied net mineralization as a function of temperature. They determined that heat units (expressed in heat degree days) could be used as a predictor of residue mineralization.

1.3.1.1 Estimating Mineralization Potential

Organic N is the largest N pool in the soil, but predicting its behavior proves challenging due to small and large-scale variability in organic matter concentrations and microbial populations. The difficult task of creating an accurate soil mineralization test is important for predicting N fertilizer requirements. Biological, chemical, and field-based indices have been developed in attempt to quantify potentially mineralizable N, but the data collected is only a snapshot in time and does not reflect temporal or spatial field variation. Previous literature has reviewed different indices and the potential of each to predict N mineralization (Harmsen and van Schreven, 1955; Bremner, 1965). Generally, the reviews concluded that chemical methods were quick and simple but did not represent soil microorganism activity while on the other hand biological methods closely mimicked in-field microbial activity. Finally, it was determined that field-based studies were expensive yet vital for calibration of laboratory indices.

Biological methods are commonly conducted using incubations where the soil is maintained at a specific temperature and moisture content for a set amount of time. Nitrogen release in the short-term (approximately 2 weeks) is thought to represent the labile, more active pool of organic matter while N released between 2 and 10 weeks is derived from the stabile pool (Bremner, 1965). Stanford et al. (1974) noted that an incubation time of 8 to 10 weeks produced similar results to long-term studies and therefore is sufficient to estimate potentially mineralizable N. If resources allow, field-moist samples are preferred for incubation studies to avoid the large, short-term flush of N release seen with air-dried soil incubations. Beauchamp et al. (1986) found that short-term (7 days) N release in air-dried samples was greater than that from field-moist soil samples. The flush of N in air-dried samples likely came from organisms that died during the air-drying process (Richter et al., 1982). It is reasonable to use air-dried samples for incubation studies when the investigator wishes to make relative comparisons of potentially mineralizable soil N (Bremner, 1965).

1.3.2 Nitrification

Nitrification is a two-step process driven primarily by chemolithoautotrophic bacteria. Heterotrophic bacteria are capable of nitrification in acid soils (De Boer and Kowalchuk, 2001), but at a much slower rate than autotrophs (Prosser, 1989). Betaproteobacteria, planctomycetes, and archaea are ammonia-oxidizing microorganisms that drive step one of soil nitrification - the conversion of NH_3 to NO_2^- via the following reaction (Huang et al., 2012):

$$NH_3 + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O \rightarrow NO_2^- + 5H^+ + 4e^-$$

Nitrospirae, as well as alpha- and beta-proteobacteria are nitrite-oxidizing bacteria which drive step two of nitrification where NO_2^- is transformed into NO_3^- as presented in the following equation (Huang et al., 2012):

$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$

Recently identified bacteria known as “complete ammonia oxidizers”, or comammoxs, are capable of driving both steps of nitrification. Soil bacteria from the genus *Nitrospira* was discovered by Daims et al. (2015) from a deep oil well culture in Russia. The culture contained only *Nitrospira*, however, was capable of complete oxidation of ammonia to nitrate. Further investigation by Daims et al. (2015) noted that the genus is widely distributed in soil, but has been overlooked for decades.

The rate of nitrification is dependent on the size of the microbial community, which is in turn reliant on optimal soil temperature, pH, and moisture. Maximum microbial activity occurs between 20 and 30 °C with decreasing activity at reduced temperatures (Brady and Weil, 2009). Grundman et al. (1995) determined that maximum NO_3^- production in a sandy loam soil occurred at 20 to 25.5 °C while Myers (1975) established that nitrification in a tropical clay loam soil peaked at 35 °C. Ideal nitrifying temperatures may be lower at times due to the adaptation of nitrifying bacteria to present temperatures (Nakos, 1984). Wang et al. (2006) studied nitrification in sandy and silt loam soils from Northern China at -10, 0, 5, 15, 25, and 35 °C. Nitrification rates at each temperature ranked as follows: $35 > 25 > 15 = 5 = 0 = -10$.

Soil nitrifying bacteria are pH sensitive and display peak activity at 7.5 to 8.0 (Prosser, 1989). The typical pH of soil in the Midwestern U.S. ranges from 5.5 to 8.0 (Kyveryga et al., 2004). Kyveryga et al. (2004) noted that a low soil pH can limit the rate of nitrification by decreasing activity of nitrifying bacteria; however, Bramley and White (1990) established that nitrifying bacteria have the ability to adapt to soil pH. Numerous studies have found acid soils that support nitrification, but the mechanism by which nitrifiers adapt has yet to be explained (Robertson, 1982; Pennington and Ellis, 1993; De Boer and Kowalchuk, 2001).

The interplay between soil oxygen and moisture levels may promote or hinder nitrification. Nitrification is limited under water saturated conditions where dissolved oxygen supply is low as well as dry conditions where water is not present to support the reaction (Miller and Johnson, 1964). Optimal soil moisture conditions for nitrifying bacteria occurs at 60% water filled pore space (WFPS) (Bateman and Baggs, 2005).

It is estimated that 65% of global N_2O emissions arise from soil nitrification and denitrification — these processes occur naturally in soils and artificially as a result of fertilization in cropped soils (Smith and Conen, 2004). Crutzen et al. (2008) reported that 3 to 5% of synthetic fertilization is lost as N_2O. In a summary of the literature by Bremner (1997); N_2O emissions from nitrification increased with increasing soil pH, organic matter, water content (from air-dry to field capacity), and temperature (from 5 °C to 40 °C). Furthermore, N_2O may result from nitrification under conditions of low O_2 or carbon supply (Wrage et al., 2001). Rassamee et al. (2011) found that incomplete nitrification occurred primarily from the combination of low dissolved O_2 and high NO_2^- concentrations.

1.3.3 Denitrification

Denitrification is an anaerobic process that occurs when heterotrophic bacteria convert NO_3^- into NO, N_2O, and N_2 gas as shown by the following equation (Brady and Weil, 2009):

$2NO_3^- \rightarrow 2NO_2^- \rightarrow 2NO \rightarrow N_2O \rightarrow N_2$

The amount of each gas produced depends on soil factors such as NO_3^-/ NO_2^- ion concentration, available C, moisture, pH, and temperature. When NO_3^- is reduced into a gaseous form, it moves from the soil to the atmosphere. Although it is an agricultural cost to lose N, loss in the form of N_2 gas is the preferable end product as opposed to NO_3^- moving into the groundwater and compromising environmental health. Additionally, NO_2 and NO are reactive gases that can form nitric acid, contribute to smog formation, and add to the greenhouse effect (Brady and Weil, 2003). Nitrous oxide production may result from both nitrification and denitrification, but a study by Vilain et al. (2014) found that denitrification produces 100 times more N_2O than nitrification using optimum soil conditions for microorganism activity. Soil moisture was shown by Mathieu et al. (2006) to be the key factor in determining whether nitrification or denitrification contributes greater N_2O production. Mathieu et al. (2006) concluded that under water saturated conditions, 85-90% of N_2O was derived from denitrification and in unsaturated conditions, 60% of N_2O was derived from nitrification.

Among the influential soil factors of denitrification, soil C levels are considered the most limiting to the rate of denitrification (Burford and Bremner, 1975; Weier et al., 1993). Weier et al. (1993) measured the effect of soil C and NO_3-N concentrations on rate of denitrification. They concluded that denitrification increased with increasing soil C levels and, in the absence of C, high NO_3-N concentrations had no effect on N_2 production. In contrast, Luo et al. (1999) found that NO_3-N concentrations in a pasture setting limited denitrification. Perhaps the background levels of C in the pasture were above limiting levels. It is apparent that land use (cropped vs. pasture) has an influence on which soil factor limits denitrification. Christensen et al. (1990) found denitrification to occur in “hot-spots”, or denitrifying microsites, across a cropped field that were limited by available C. Similarly, Parkin (1987) used anaerobic incubations to determine that organic C material was the primary factor creating drastic spatial variability of denitrification.

Even though soil pH, temperature, and moisture are not the most limiting factors in the denitrification process, they maintain critical links. A review by Šimek and Cooper (2002) summarized numerous studies regarding the influence of pH on denitrification and concluded that total N gas production was less in acidic conditions. The decreased production may be the result of an indirect effect of pH on organic C concentration. If one avoids extreme high and low soil pH, pH studies on denitrification rates are inconclusive because of confounding factors like C concentration and the adaptability of denitrifying organisms to pH conditions (Parkin et al., 1985; Šimek et al., 2002). Furthermore, Nommik (1956) noted minimal differences in denitrification rates between a soil pH range of 5.6 to 8.0.

Like all N cycle components, moisture and temperature may decrease or enhance the rate of the reaction. Denitrification begins at approximately 60% WFPS and increases as the soil reaches saturation (Nommik, 1956; Brady and Weil, 2003; Amha and Bohne, 2011; Awale and Chatterjee, 2015). Machefert and Dise (2004) conducted an *in situ* study that found the threshold for denitrification to fall between 60-80% WFPS. This range applies to agriculture, pasture, and forest land with an assortment of pH, C content, and temperature characteristics. Denitrification has a positive relationship with temperatures between 15 and 60 °C (Keeney et al., 1979), then slows down above 60 °C (Malhi et al., 1990). Bailey (1976) established that temperature contributes to the form of

N gas produced. In sum, as temperatures cool from 30 °C to 15 °C, 10 °C to 5 °C, and 6 °C to 8 °C, the form of N released changes from N_2 gas to N_2O then NO, respectively.

1.3.4 Leaching

Nitrate and nitrite are negatively charged ions which repel the negatively charged soil (Brady and Weil, 2009). Nitrite is very reactive and does not accumulate readily in soil while nitrate can move with the soil water into subsurface drainage. Leaching occurs primarily via subsurface drains but can be found in surface runoff during large rain events (Angle et al., 1984). Drury et al. (1993) claimed that the volume of water flowing through field tiles is two to four times greater than surface flow.

Nitrate leaching is driven by rainfall exceeding evapotranspiration, thus, peak flow occurs from mid-April until early July in Indiana (NOAA-NWS). Gast et al. (1977) measured tile water NO_3-N concentrations under a range of N fertilizer rates during this time period. They determined NO_3-N leached increased with increased N rate. Andraski et al. (2000) found similar results. Angle et al. (1989) conducted a 3-year study in a continuous corn system with N applied at 136 kg N ha^{-1}. On average, 10 to 20 mg L^{-1} NO_3-N was found in groundwater. Drury et al. (1993) performed a similar study in continuous corn over 3 years and found that average NO_3-N concentration in the tile water averaged between 12 and 17 mg L^{-1} using an economic optimum nitrogen rate (EONR) of 168 kg N ha^{-1}. Kladivko et al. (1991) estimated annual NO_3-N loss through tile in an Indiana silt loam to range between 18 and 70 kg N ha^{-1} depending on tile spacing. In addition to tile spacing, factors such as texture, SOM, temperature, crop, and tillage impact soil hydrology, which directly effects NO_3^- leaching (Dinnes et al., 2002).

More specifically, SOM improves soil structure by increasing aggregate stability — the microbes in the soil decompose the organic matter and excrete polysaccharides which hold aggregates together (Verchot et al., 2011). The large, stable aggregates slow water movement through the soil profile by creating a non-continuous path for water to travel. Additionally, the organic rich soil will expand to fill in cracks/large pores which slows water movement (hydraulic conductivity) through the soil. Hydraulic conductivity is influenced by soil texture; coarse textured sands contain large macropores which easily transport water while fine textured clays contain micropores which may swell and decrease hydraulic conductivity (Klute, 1986).

Excess dissolved NO_3^- in our waterways is detrimental to human and ecosystem health. Agriculture is recognized as the main contributor to NO_3^- loads in the Mississippi River Watershed and resultant hypoxic zone in the Gulf of Mexico (National Research Council, 2009).

1.3.5 Ammonia Volatilization

Although NH_3 emissions to the atmosphere predominately originate from animal waste (Ryden et al., 1987), land application of N fertilizer and decomposing plant residue also contribute to N loss (Scharf, 2015). In fact, 25% of surface-applied urea can be lost as NH_3 gas (Scharf, 2015). This process is a bidirectional reaction expressed as follows (Brady and Weil, 2009):

$NH_4^+ + OH^- \leftrightarrow H_2O + NH_3 \uparrow$

Ammonia abundance and pH primarily control the extent and rate of this reaction; however, buffering capacity, urease activity, moisture, and temperature also influence NH_3 volatilization (Nelson, 1982).

Increased NH_3 loss occurs at high pH because of increased disassociation of NH_4^+ to NH_3 (Fenn and Kissel, 1976). The magnitude of the pH change is dependent upon the buffering capacity of the soil (Brady and Weil, 2009). Ferguson et al. (1984) found that NH_3 volatilization from surface-applied urea decreased as soil buffering capacity increased. Furthermore, the hydrolysis of urea results in a pH range of 7 to 9 in the microzones surrounding urea granules. According to Nelson (1982), the microsite pH zones created by the granules are more important than the overall soil pH in controlling NH_3 volatilization.

Urea and other urea containing fertilizers are hydrolyzed by the enzyme urease. Urease levels in agricultural soils are high enough to support urea hydrolysis due to the soil’s ability to protect the enzyme against degradation and inactivation (Zantua and Bremner, 1976). Crop residue may add to the already high background levels of urease; Mclnnes et al. (1986) determined that urease activity in wheat straw was 20 times greater than the soil beneath the residue. Hargrove (1988) reported that crop residues have high urease activity and maintain soil moisture by providing a barrier between the soil and air, thereby increasing gas production. Although soil moisture is noted to influence volatilization, the extent of moisture impact has varying results due to the fluctuation of field conditions.

Rainfall can reduce or increase NH_3 volatilization. Moisture is necessary to dissolve urea and permit hydrolysis, potentially leading to NH_3 loss; however, rainfall is also necessary to transport N into the soil where volatilization is nominal (Scharf, 2015). According to Craig and Wollum (1985), a light rain event will be enough for urea hydrolysis, but not enough to sufficiently leach urea into the soil. Thinking about soil moisture as a continuum of wetting and drying cycles is more beneficial than studying loss as a snapshot of soil moisture. Field moisture fluctuates daily with dew in the mornings and water loss via evaporation throughout the day, thus, laboratory studies using constant moisture should be viewed with caution. Liu et al. (2007) used an incubation method to determine that NH_3 loss was 2 to 3 times greater at 20% than at 80% field capacity. It is likely that water content was not sufficient to carry urea into the soil. On the contrary, Bouwmeester et al. (1985) conducted a greenhouse study and reported peak NH_3 loss at field capacity. Hargrove (1988) provides a summary of the moisture/volatilization relationship from field studies. Generally, maximum loss occurs when the soil is slowly dried. Slow drying increases soil NH_3 levels, which volatilize in attempt to maintain the equilibrium between NH_3 and NH_4^+. Additionally, NH_3 loss ceased under dry conditions and reached a maximum at field capacity. Contrasting results from lab and field based studies demonstrate the complexity of soil moisture and its relations with many soil and environmental properties.

Numerous lab and field studies have noted the importance of temperature in NH_3 loss to volatilization; specifically, that loss increases as temperature increases (Ernst and Massey, 1960; Gasser, 1964; Prasad, 1976). Temperature primarily affects urease activity which increases with temperature, ultimately increasing urea hydrolysis (Hargrove, 1988). As mentioned previously, increased hydrolysis leads to localized increases in soil pH which promote volatilization. According to Lightner et al. (1990), maximum volatilization occurs midday when the soil has warmed up and morning dew has evaporated.

Urea granules may be physically coated with various substances such as polymers, sulfur, or urease inhibitors in effort to reduce NH_3 volatilization and drive N into the root zone. Polymer and sulfur coatings temporarily reduce the release of urea until either the polymer is broken down, or the sulfur is hydrolyzed by rain. Urease inhibitors slow urease enzyme activity and overall hydrolysis, thus, increasing the chance for rain to drive N into the root zone (Edmeades, 2004). Further management strategies to reduce volatilization are discussed later.

1.3.6 Ammonium Fixation

Nonexchangeable NH_4^+ (NEA), also known as fixed NH_4^+, was first observed by Mcbeth (1917) who demonstrated that added NH_4^+ could not be completely recovered; the portion not recovered was defined as fixed NH_4^+. Pioneered by Page and Baver (1939), the “lattice-hole” theory is a widely accepted mechanism by which NH_4^+ becomes fixed. Page and Baver (1939) explained that fixation occurs in 2:1 clays that consist of hexagonally arranged oxygen ions; the opening in the hexagon provides a snug fit for NH_4^+ and potassium (K^+) ions due to their analogous ionic radii. Clays capable of fixation in order of decreasing ability are vermiculite, illite, and montmorillonite (Nommik and Vahtras, 1982). Release and fixation of NEA is defined by the following equilibrium equation (Nommik and Vahtras, 1982):

Soluble $NH_4^+ \leftrightarrow$ Exchangeable $NH_4^+ \leftrightarrow$ Fixed NH_4^+

The NEA equilibrium is influenced by clay content, K^+ abundance, fertilizer additions, and soil moisture (Nommik and Vahtras, 1982). NEA exists in two forms: native and recently fixed. Native NEA has little impact on crop growth (Allison, 1973) while recently fixed NH_4^+ from fertilizer additions may be used by the plant (Mengel and Scherer, 1981). The recently fixed pool may function as a buffer to replenish N supply to crops when exchangeable sources become depleted.

Competition exists between K^+ and NH_4^+ for available interlayer sites in clays. If K^+ is introduced to the soil before NH_4^+, fixation sites fill with K^+, leaving exchangeable NH_4^+ in solution (Beauchamp, 1982). The blocking effect of K^+ is exemplified at high soil K^+ concentrations; whereas at low K^+ levels, the effect is negligible and NEA is released (Nieder et al., 2011). Walsh and Murdock (1963) found that K^+ and NH_4^+ applied in different soil layers minimized the K^+ blocking effect and increased plant retrieval of N. A study by Feigenbaum et al. (1994) saturated beidellite clay individually with calcium (Ca^{2+}) and K^+. The Ca^{2+} saturated soil allowed for NEA release potentially due to the improper fit of Ca^{2+} in the lattice holes, thereby enabling NEA to exit. The K^+ saturated clay slowed NH_4^+ release because of the perfect fit of K^+ within the lattice.

Increased soil moisture has the potential to expand clays and reduce fixation while reduced moisture contracts the clay layers and fixes NH_4^+ and K^+ cations (Black and Waring, 1972). Gouveia and Eudoxie (2007) used seven Trinidad soils with a range of properties to determine moisture influence on fixation. They found that less fertilizer was fixed in wet soil at 60-80% water holding capacity compared to dry soil at 10-20% water holding capacity.

In light of kinetics, NH_4^+ fixation occurs quickly with 60-90% of total fixation occurring in the first few hours after fertilizer application (Nommik, 1965). NEA is slowly released throughout the growing season as plants consume exchangeable NH_4^+ (Nieder et al., 2011; Scherer and Ahrens, 1996). Research focusing on the contribution of NEA to plant available N shows inconsistent results. Scherer (1993) explains that variable results may be due to the fact that some studies focus on native fixed NH_4^+ while others focus on recently fixed NH_4^+ (fertilizer derived). Drury and Beauchamp (1991) carried out aerobic incubations to determine fixation and release of NH_4^+ additions to soil. They found that fixed NH_4-N doubled when NH_4^+ was added at a rate of 150 mg kg^{-1} compared to 75 mg kg^{-1}. In a continuous wheat study by Black and Waring (1972), 50% of NEA was released to the first wheat crop grown, but had no significant contribution to subsequent crops. Walsh and Murdock (1963) report similar results from a corn greenhouse study concluding that NEA was reduced by cropping. To consider soil and crop variables, Baethgen and Alley (1987) conducted a greenhouse study to determine the contribution of NEA to successive wheat crops grown in a variety of soil types, cropping history, and at various soil depths. They determined that NEA was greater at deeper depths and in soils with intense fertilizer history. Unlike the previous studies mentioned, NEA did not differ between the first and second wheat crop. A downfall to greenhouse studies is the absence of field variables — organic matter mineralization, freeze/thaw cycles, and fertilizer applications have the ability to replenish the NEA supply after NH_4^+ uptake from the first crop (Nieder et al., 2011; Walsh and Murdock, 1963).

From an agronomic standpoint, NEA may be beneficial to plants as a slow release form of N. Environmentally, NEA is unavailable for leaching therefore does not contribute to eutrophication. Further research on recently fixed NH_4^+ and its role in plant

N uptake must be conducted before the NEA pool is considered in N management decisions.

1.3.6.1 Estimating Ammonium Fixation

The generally accepted method to measure NEA was developed by Silva and Bremner (1966) in which soil is treated with potassium hypobromite (KOBr) followed by potassium chloride (KCl) rinse cycles to removed exchangeable and organic compounds. The remaining soil is digested with a hydrofluoric acid (HF) – hydrochloric acid (HCl) solution followed by NEA quantification using steam distillation. A modified Silva and Bremner method was developed by Nieder et al. (1996) and validated by Liang et al. (1999). The modified method eliminates the HF:HCl digestion and replaces it with a combustion step. Nieder reported 100% fixed NH_4^+ recovery with reference to Silva and Bremner's method. Both methods utilize a "gentle" approach to removing organic and exchangeable NH_4^+ so that, hypothetically, native NEA remains in the interlayers. Due to the complexity in quantifying the amount of native NH_4^+ making it into solution, both methods are said to be a measure of total fixed NH_4^+ (native + recently fixed).

Scott et al. (1960) established a method that uses sodium tetraphenolboron ($NaBPh_4$) to extract interlayer K^+. Cox et al. (1996) used the same method with slight modifications to quantify NEA. Cox compared his results with Silva and Bremner and found that the $NaBPh_4$ method released 71% of "total" fixed NH_4^+. Although a smaller percentage was extracted in this manner, it may be a better estimate of recently fixed NH_4^+, which has the potential to become plant available.

1.3.7 Biological Nitrogen Fixation

Biological nitrogen fixation (BNF) is the reduction of N_2 gas to NH_3 (Brady and Weil, 2009). Species involved with BNF include *Rhizobium*, actinomycetes, and cyanobacteria. A corn-soybean cropping system is influenced by the symbiotic relationship between legumes and *Rhizobium*, therefore will be the focus of this section. In short, the N-fixing bacteria infect roots then form nodules where they fix N. The N supplied to the plant from BNF is able to meet 50-60% of plant N requirements for development (Salvagiotti et al., 2008) by fixing on average 160 kg N ha^{-1} (Kinzig and Socolow, 1994). The majority of BNF estimates fall between 25 to 75% (Keyser and Li, 1992), this variability is influenced by soil factors such as soil moisture (Rathore et al., 1981), pH (Cline and Kaul, 1990), temperature (Alexandre and Oliveira, 2013), and fertilizer additions (Brady and Weil, 2009).

Fertilizer N application on soybean is well known to reduce nodule development (Brady and Weil, 2009; Chen et al., 1992; Eaglesham et al., 1982). Nodule activity decreases in late reproductive stages resulting in N shortage during seed fill (Zapata and Danso, 1987). The agronomic benefit to late stage fertilization remains a question. Gutiérrez-Boem et al. (2004) found no impact on soybean grain yield with R3 (50 kg N ha^{-1}) and R5 (100 kg N ha^{-1}) fertilization. They determined that residual N was present in the topsoil and at risk for leaching during the fallow period. Salvagiotti et al. (2008) found that late-season N shortage could be mitigated by a starter fertilizer application of slow-release urea below the root nodulation zone. Maintaining an adequate population of *Rhizobium* via seed inoculation, especially in soils with no history of soybean, is economically the best way to supply nitrogen to the growing legume (Russelle, 2008).

1.4 Influence of Management Practice on Residual Nitrogen

Approximately 95% of N in surface soils is present in organic forms (Legg and Meisinger, 1982). After harvest, residual organic and inorganic N may be left in/on the soil. Factors such as crop rotation, residue, and tillage influence residual N abundance and are discussed below.

1.4.1 Crop Rotation and Residue Cover

Cover crops may be planted between cash crop rotations to improve soil health and tie up residual N. The extent of residual N uptake by cover crops can vary substantially. According to the Root Zone Water Quality Model developed by the USDA-ARS, uptake of residual N by winter cover crops may reduce NO_3^- loss by 20% (Perry, 2015). With farmland contributing up to 46 percent of the Gulf of Mexico NO_3-N, decreasing loss by implementing cover crops is a potential strategy (Perry, 2015).

Field-scale corn-soybean cover crops trials were conducted in Iowa over six growing seasons (Johnson et al., 1998). Treatments consisted of oat and rye combinations that were overseeded in soybean during August. Corn yield following soybean was reduced by an average of 1569 kg ha^{-1} in rye treatments compared to oat which was no different than the no cover crop control. Rye likely reduced yield because it overwinters compared to oat which winter-kills; rye uses a lot of water and competes against the germinating corn for soil moisture and nutrients. Beyond yield, cover crops have the potential to change soil physical properties by reducing bulk density and penetration resistance, as well as increasing aggregate stability and plant available N (Villamil et al., 2006).

Zhu and Fox (2003) led a study on fertilizer rate and crop rotation effects on residual N. In a corn-soybean (CS) rotation, soil residual NO_3-N concentration did not differ as fertilizer rate increased from 0 to 100 kg N ha^{-1}; however, as rate increased from 100 to 200 kg N ha^{-1}, a significant increase in residual N was observed. In zero-N plots, soybean residual N was higher than residual N following corn due to the mineralization potential of the residue. Soybean residue N is easily mineralized and available to the following crop while corn residue N is immobilized for a longer period of time (Power et al., 1986).

The average C:N ratio of soybean and corn stover is 20:1 and 57:1, respectively (Brady and Weil, 2009). The greater mineralization potential of soybean stover is responsible for an average N credit of 45 kg N ha^{-1} given to corn planted after soybean (Gentry et al., 2001). In central Ontario, 30 kg N ha^{-1} less N was recommended after soybean (Ding et al., 1998). Other factors such as nodules and symbiotic N fixation have an insignificant role in N credit determination (Bergerou et al., 2004). Nitrogen credits from soybean are short-lived. Mallarino and Pecinovsky (2006) found a soybean N credit of 59 kg N ha^{-1} and 6 kg N ha^{-1} for first and second year corn, respectively, in a corn-corn-soybean (CCS) system. Additionally, yield in a corn-corn (CC) rotation was similar to second year corn in CCS rotations at N rates ranging from 0 to 270 kg N ha^{-1}. Overall, soybean N contribution to the following corn crop is variable by year and site, thus, accurately predicting the corn grain yield response to soybean N credit is difficult (Schoessow et al., 2010).

1.4.2 Tillage

The quantity of residue remaining after harvest for corn with a yield of 9415 kg ha^{-1} is 9529 kg ha^{-1} while 2242 kg ha^{-1} of soybean residue is left from a soybean yield of 2688 kg ha^{-1} (Plaster, 1997). Tillage is used as a mechanism to incorporate residue into the soil to increase decomposition and mineralization. A review by Blevins and Frye (1993) provides a comprehensive overview of the influence of tillage on soil properties; the gradient from intensive conventional till (CT) to conservation tillage followed by no-till (NT) is discussed in detail. Generally, NT increased residue cover, increased SOM, reduced erosion, increased aggregate stability, improved water quality, and decreased aeration when compared to CT.

Vetsch and Randall (2002) found that CT averaged 25% residue cover after planting while NT and strip till (ST) averaged 40%. Leaving excess residue on the surface via reduced tillage leads to low temperature concerns with spring planting. The residue reflects sunlight and holds moisture, thereby reducing soil temperature and increasing dry down time (Plaster, 1997). The increased moisture has a negative influence on the O_2 dependent reactions of the N cycle. Increased water holding capacity could also reduce NO_3^- leaching. Syswerda et al. (2012) found a 35% reduction in NO_3-N loss with NT compared to CT in a long-term (11 year) corn-soybean-wheat (CSW) rotation in Michigan. They suspect increased surface carbon and corresponding increase in water holding capacity contributed to the reduction in NO_3-N noted with NT.

Doran (1980) studied microbial population changes with reduced tillage in Kentucky, Minnesota, W. Virginia, Nebraska, and three fields in Oregon for a total of seven sites differing in soil characteristics. Microbial populations in the top 0 to 7.5 cm of soil were greater under NT compared to CT. Although greater, microbes were determined to be primarily anaerobic in NT and more aerobic in CT; denitrifier populations expressed as a ratio of NT:CT were 2.7:1 at 0 to 7.5 cm. The cool, moist, and O_2 deficient NT environment in the selected study sites favored denitrification.

Drainage, pest risk, and potential nutrient stratification are important elements for producers considering NT or conservation tillage. Cruse et al. (1983) studied farmer-managed CS plots and noted that poorly drained soils with high rainfall had a negative impact on yield when conservation tillage was used. Additionally, black cutworms damaged 17, 6, and 4% of corn plants in NT, conservation, and CT plots, respectively. Nutrient stratification of K and phosphorus (P) was observed in NT and conservation tillage but not CT. Crozier et al. (1999) noted changes in nutrient stratification in short-term (less than 6 years) and long-term (greater than 6 years) NT systems. They found Ca, manganese (Mn), and sulfur (S) stratification in short-term NT yet similar stratification patterns in CT and NT after 6 years.

1.5 Fertilization Effects on Residual Nitrogen

Application method, rate, source, and timing of N fertilization influence the balance of N pools in the soil. Fertilizer rate was mentioned in the preceding N cycle sections; therefore, our focus will be on the application method, timing, and source of N fertilization.

1.5.1 Nitrogen Application Method

Nitrogen fertilizer may be applied before planting via broadcast or soil injection, at planting via banding or in-row (pop-up), or after planting via topdressing, side-dressing, or fertigation (Plaster, 1997). Literature on residual N abundance as a result of application method is lacking, but many studies focus on N loss differences due to broad or localized fertilizer applications.

Jing et al. (2010) found that a localized fertilizer application of P and NH_4^+ in a calcareous soil increased early stage corn growth and nutrient use compared to broadcast application in North China. The localized fertilizer was applied mid-row and 10 cm deep. Leaf expansion and root length with localized placement was 20 to 50% and 23 to 30%, respectively, greater than broadcast. Ma et al. (2013) confirmed localized fertilizer benefits to corn growth and nutrient uptake by noting a 41-48% increase in nutrient use efficiency in localized compared to broadcast application. The significant increase in nutrient use efficiency was explained by easily accessible nutrients near the root zone, rhizosphere acidification (Jing et al., 2010), and increased lateral root production (Chassot et al., 2001). Localized application and resultant increase in nutrient uptake decreased excess N left after harvest. Low pH soils may not respond as well to localized applications. Rochette et al. (2009) measured ammonia volatilization from banded urea (injected 5 cm below the surface) and broadcast application of coated urea in acidic, dry soil. Banded urea resulted in 27% of N lost to volatilization compared to 9% lost to broadcast. The study concluded that a microsite pH increase from 6.0 to 8.7 was responsible for the increased loss in the banded urea treatment.

1.5.2 Nitrogen Application Timing

Timing the application of fertilizer with crop demand is essential to maximize use and minimize loss of nutrients. Nitrogen management programs strive to provide the plant with available N by considering factors such as soil moisture, soil temperature, and frequency of fertilization. Nitrogen application may occur as preplant in the fall or early spring, at planting, in-season, or in combination (split application). A band of starter fertilizer may be applied at planting to provide the seed with an immediate nutrient source. In northwest and northcentral Indiana, the average agronomic N fertilizer requirement averaged across 26 trials was 203 kg ha^{-1} (Camberato and Nielsen, 2015). Generally, 22-45 kg ha^{-1} is applied as starter (Brouder, 1996) and the remainder as preplant or in-season side-dress. Side-dress N applications match crop demand and occur 6-10 weeks after planting (Vitosh et al., 1995).

Nitrogen applications may be split into two applications in attempt to reduce N loss and provide the plant with easily accessible N. Randall and Schmitt (2007) provide an overview of application time data collected from medium and fine textured soils in Minnesota. Preplant and split applications (preplant + side-dress) were no different at 16 of the 28 sites. Split applications resulted in a positive yield response over preplant at 8 of the sites and a negative yield response at 4 of the sites; previous crops included soybean, oat, corn, and rye. The increased response to split applications was likely due to year and site combinations with above average rainfall. Later N applications reduce loss risk and window of opportunity for leaching and denitrification.

It is recommended that fall application of N fertilizer be made when soil temperatures are consistently below 10 °C and expected to continue falling (Vitosh et al., 1995). Although fall application may be convenient to the producer and fertilizer dealer, there is a risk for N loss during the fallow period. Randall and Vetsch (2005) conducted a six-year study to determine N loss with fall versus spring application on a Minnesota Mollisol. Additionally, they studied the effects of the presence or absence of nitrapyrin (NP), a nitrification inhibitor. Results show 47, 56, 56, and 61% N recovery for fall (no NP), fall (with NP), spring (no NP), and spring (with NP) application, respectively. The study concluded that fall applied N (with NP) and spring applied N (with or without NP) are suitable management practices. According to the Tri-State Fertilizer Recommendations (Vitosh et al., 1995), spring applied N is more effective than fall; this difference is especially noted during favorable fall N loss conditions.

1.5.3 Nitrogen Source

Various forms of N fertilizer are available for producers to use in their N management programs. The forms of N applied may/may not contribute to the final residual N pool distribution. There is currently no published work that looks at soil N pool distribution as a result of N fertilizer source; therefore, this section will focus on loss potential of various N sources. Although all N sources are prone to eventual loss, producers attempt to find the “perfect” N source by considering factors such as application method, cost, nutrient availability, health hazards, and soil properties. Commonly applied fertilizers will be discussed here.

The soil’s capacity to adsorb and exchange cations is known as the cation exchange capacity (CEC) (Hendershot et al., 2007). Soils high in organic matter, and/or clays such as vermiculite and smectite have high CEC values and are able to hang on to cations like NH_4^+ (Huang et al., 2012). Negatively charged ions such as NO_3^- are repelled by the negatively charged CEC sites and remain in solution. The soils capacity to adsorb and exchange anions is known as the anion exchange capacity (AEC) (Hendershot et al., 2007). Soil pH and AEC are inversely proportional; in most Midwest agricultural soils, the pH is too high for substantial AEC effects and NO_3^- is free to move in the solution phase (Lajos, 2008). Furthermore, the AEC's affinity for anions is stronger for phosphate, sulfate, and chloride anions than it is for NO_3^- (Huang et al., 2012); this weak affinity for NO_3^- results in minimal exchange on the AEC. In highly weathered acidic (pH ~3.5) tropical soils where many of the cations have leached away, studies have demonstrated adsorption of NO_3^- on to the AEC (Singh and Kanehiro, 1969; Kinjo and Pratt, 1971).

All NH_4^+ - containing fertilizers have the potential to acidify the soil. In NT environments, soil is susceptible to "acid roof" formation where the surface soil pH is ~1.0 lower than the subsoil pH (Beegle, 1996). As mentioned previously, nitrification is reduced at low pH; consequently, loss of N to leaching and denitrification is suppressed. Anhydrous ammonia (82% N) is the slowest of the N sources to convert to NO_3^- (Vitosh et al., 1995). To reduce loss, it must be injected into the soil before seeding or side-dressed. Shortly after application, NH_3 reacts with soil water to form NH_4^+; the NH_4^+ is then microbially transformed into leachable NO_3^-. Although cost effective, it is hazardous to handle the pressurized liquid. Ammonium sulfate (21% N) is used in starter fertilizers as well as broadcast applications. Topdressing is also acceptable because of its low volatilization potential. Ammonium sulfate can lead to acidification due to the generation of two or four H^+ ions from the conversion of NH_4^+ to NO_3^- (Camberato et al., 2012). Ammonium nitrate (34% N) may be surface applied but is prone to nitrate leaching. Half of the 34% N in ammonium nitrate is NH_4^+ while the other half is NO_3^- , thus, loss of nitrogen to leaching occurs immediately after application (Vitosh et al., 1995). The danger of ammonium nitrate being used as an explosive recently has limited its use.

Urea (46% N) exists in dry and liquid forms and may be used in starter, broadcast, and topdress applications. Urea ammonium nitrate (UAN) solutions (28 to 32% N) may be banded on the soil surface via drop nozzles or directly sprayed followed by incorporation into the soil (Vitosh et al., 1995).

Mulvaney et al. (1997) conducted a 5-day laboratory incubation to determine denitrification of various N sources under waterlogged conditions. The greatest N_2 and N_2O emissions came from anhydrous NH_3 and urea while the lowest came from $NH_4H_2PO_4$ and NH_4NO_3. Increase in pH from anhydrous NH_3 and urea additions likely caused increased emissions.

1.6 Summary

If N fertilizer is applied in excess, N not used by the crop is a cost to the producer and environment. Fertilizer rate recommendations maximize producer profit and minimize leaching, denitrification, and volatilization — pathways that prevent N from reaching the growing crop. Consistent N fertilization to corn in the common corn-soybean rotation of the Midwest has possibly altered soil N pools by creating an accumulation or depletion of soil N relative to N fertilization rate. Quantification and characterization of current corn N pools under a gradient of N fertilization rates will confirm the accuracy of current N balance and fertilizer rate calculations

CHAPTER 2. LONG-TERM EFFECTS OF NITROGEN FERTILIZATION ON SOIL NITROGEN POOLS AND CROP GROWTH

2.1 **Introduction**

Since the 1930s there has been a steady increase in average U.S. corn (*Zea mays* L.) yield that was catalyzed by the introduction of the double-cross hybrid (Nielsen, 2012). Producers are motivated to narrow the gap between the current U.S. corn yield average of 10.6 t ha^{-1} (USDA-NASS, 2016) and maximum hybrid potential of 32.3 t ha^{-1} by finding the seamless combination of management practices, genetics, and soil fertility, while keeping in mind their bottom line (Smith, 2012). The application of nitrogen (N) to corn is essential to crop growth, yet detrimental to the environment and producer profit if applied in excess. Approximately 8.7 million tons of N is applied annually to U.S. crops with corn production accounting for 65% of the total (USDA-ERS, 2011). Substantial N loss from crop production has resulted in decreased ecosystem health including the notable hypoxic zone in the Gulf of Mexico (USGS, 2008).

Predicting crop N demand is difficult given the array of biotic and abiotic factors constantly interacting with N pool balance. Historically (1960s), Indiana N recommendations for corn were based on soil properties and previous crop (Camberato, 2012). Emphasis shifted to yield-based recommendations in the 70’s until the cost of N increased considerably in 2000. In 2006, the economic optimum nitrogen rate (EONR) was proposed as a new N rate recommendation system for Indiana corn growers

(Camberato and Nielsen, 2016); calculations took grain and N price into consideration to find the best N rate with the greatest return in profit. Indiana is currently divided into 9 districts based on USDA-NASS data; each district has an EONR based on numerous field-scale N trials.

Exogenous sources of N may alter N pool balance and possibly create an accumulation or depletion of soil N. Numerous studies have looked at residual N abundance under various climates and cropping systems (White, 1957; Legg and Allison, 1967; Welch et al., 1973; Jokela and Randall, 1989; Jaynes et al., 2001), but none investigated the long-term impact of N fertilization on soil N pools and corn growth. Jaynes et al. (2001) suggested soil N and soil organic matter are depleted in corn-soybean rotations when corn is fertilized at the economic optimum N rate. Increasing our understanding of soil N under the primary cropping system of the Midwest will inform producers if they should adjust fertilizer rates for N carryover. The objective of this study was to determine residual N effects from fertilized corn in a corn-soybean rotation system by measuring (1) soil N pools post soybean, (2) soil fertility, (3) growth and yield of corn, and (4) N removal in rotated corn.

2.2 Materials and Methods

2.2.1 Field-scale Corn Nitrogen Study

Field-scale corn nitrogen trials were established in 2007 at several Purdue farms throughout Indiana, and repeated in 2009, 2011, and 2013. Locations included: West Lafayette, Lafayette, Columbia City, Wanatah, Farmland, and Butlerville. Corn (odd years) was grown in rotation with soybean (even years). A randomized complete block design assigned six corn N rates to each replication. The design was not re-randomized for the repeated trials conducted in subsequent years. All N rates, including the zero N treatment, received starter fertilizer. In 2015, half of the replications of each trial received starter fertilizer only, thus, allowing for the determination of residual N as influenced by repeated fertilizer rates. The number of starter only replicates for each location is presented in Table 1. Nitrogen was ideally side-dressed at V6, but was delayed at some locations due to excessive rainfall. Historic and present starter and side-dress N rates are reported in Table 1. For simplicity, N rates are ranked from 1 (starter only) to 6 — lowest to highest — in subsequent sections. Plot length varied with location, but plot width remained constant (9.15 m). Tillage regime for each location is described in Table 1.

2.2.2 Soil Sampling and Preparation

Soil samples were collected with hand probes from each of the six locations at 0-20 cm, 20-40 cm, and 40-60 cm shortly (1-9 days) after corn planting in each plot of the starter only replicates (Table 1). Only soil samples at 0-20 cm were collected from the fertilized replicates with N rates ranging from zero to above the agronomic requirement. One composite soil sample per depth made up of a representative number of subsamples was obtained from each plot; the number of subsamples collected (Table 1) was dependent upon plot length. Samples were placed in coolers for transport back to Purdue, spread on plastic in a greenhouse to air-dry at ~30.3 °C, then ground to pass a 2 mm sieve. A 30 g subsample of 2 mm ground soil was ground further to <150 µm for several laboratory analyses. Soil series and classification for each location are listed in Table 2.

2.2.3 Texture

Particle-size analysis (PSA) was completed on a composite sample of each replicate (6 plots) for the three separate depths using the Hydrometer Method (Bouyoucos, 1962) (Table 3). Instead of letting the soil sit stagnant in the dispersing solution, samples were agitated on a shaker for 24 hours at 40 rpms to ensure dispersion. Temperature and hydrometer corrections were made according to water temperature and dispersion solution density.

2.2.4 General Soil Fertility

A subsample ground to 2 mm or less soil was sent to A&L Great Lakes Laboratories, Inc. for determination of pH, CEC, SOM, P, K, Ca, S, Mn, iron (Fe), magnesium, sodium, zinc, copper, and boron (S1M3 and S3, Table 4).

2.2.5 Total Nitrogen and Carbon

Soil ground to ≤ 150 µm was analyzed in duplicates for total N and organic C analysis via combustion (Sparks, 1996) using a FlashEA Elemental Analyzer (CE Elantech, Lakewood, NJ; Table 5).

Inorganic carbonate-C was calculated at the Columbia City location for the 40-60 cm soils due to the presence of a carbonate layer at 40+ cm. The Simple Titrimetric Procedure (Bundy and Bremner, 1972) was used to estimate the mass fraction of inorganic carbonate and was calculated as follows:

$$\frac{\text{g C}}{\text{g soil}} = \frac{(\text{mL HCl}_{\text{s}}\text{-mL HCl}_{\text{C}})(\text{mmol}_{\text{c}}\ \text{HCl mL}^{-1})(0.012\ \text{g C mmol}_{\text{c}}^{-1})}{\text{g soil}}$$

where mL HCl_s = mL of standard 0.1 M HCl required to titrate the sample from the phenolphthalein endpoint to the bromcresol green endpoint; mL HCl_C = mL of standard 0.1 M HCl required for this titration in a blank analysis; $mmol_c$ HCl mL^{-1} = concentration of the standardized HCl.

Inorganic carbonate C was calculated as a mass fraction of total carbonates obtained from the combustion analysis, then subtracted to yield organic C.

2.2.6 Soil Incubations to Determine Nitrogen Mineralization and Nitrification

Prior to the aerobic incubation study, soil water retention at 33 kPa was determined using the pressure plate method (Klute, 1986). Water retention measurements were obtained from a composite sample of each replicate (six plots) for each depth (Table 3).

To prepare samples for incubation, five grams of ≤ 2 mm soil was placed into 50 mL centrifuge tubes then brought to 33 kPa tension using nanopure water. Tubes were loosely capped and placed randomly in an incubator at 25 °C. Moisture was maintained at 33 kPa by weighing the tubes and adding the appropriate quantities of nanopure water twice a week. Duplicates were destructively sampled at 10 and 50 days using a 1:10 ratio of soil to 1 M KCl (5:50). Contents of the centrifuge tube were quantitatively transferred to 250 mL Erlenmeyer flasks, agitated on a shaker for one hour at 65 rpm, then filtered with Whatman No. 2 filter paper into 20 mL polyethylene scintillation vials. Initial soil samples to quantify day zero N values were extracted using a 2:20 ratio of soil to 1 M KCl in 250 mL Erlenmeyer flasks. All extracts were preserved with two drops of chloroform and stored at 4 °C until analysis. Extracts were colorimetrically analyzed for NO_3-N + NO_2-N and NH_4-N with a Discrete AQ2 Analyzer (SEAL Analytical, Wisconsin). Total inorganic N was calculated using the summation of NO_3-N + NO_2-N and NH_4-N. Total inorganic N release per day was calculated for the 0 to 10 and 10 to 50 day incubation periods for each soil depth (Table 6). Additionally, N release per day was divided by SOM concentration to express N release per SOM level (Table 6).

2.2.7 Fixed Ammonium

Fixed ammonium is defined as ammonium which is not extractable using a K salt solution (Silva, 1964). The most widely used and accepted method to determine non-exchangeable NH_4^+ (NEA) was developed by Silva and Bremner (1966). The procedure used in the present study was developed by Nieder et al. (1996) who made slight modifications to the Silva and Bremner method. While conducting the Neider method, complications arose with the water bath step; the procedure called to boil the centrifuge tube contents in a water bath, but the solution in the tube appeared to have a higher boiling point than water. To overcome this, tube contents were transferred to 50 mL beakers, covered with a watch glass, and boiled on a hot plate for 10 minutes – as described by the original Silva and Bremner procedure. After boiling and sitting for 24 hours, beaker contents were transferred back to the centrifuge tubes. It should be noted that in all transfer instances, careful attention was allocated to ensuring proper quantitative transfer from centrifuge tube to beaker and vice versa. The remainder of the Neider procedure was carried out as described with a final combustion step used to determine NEA.

The starter only replicates with historic highest and lowest (zero) N rates were selected for NEA extractions. Analysis was conducted in duplicate at all depths. The following calculation was used to convert the analytical result to a whole soil basis:

$$\text{NEA}\left(\text{mg kg}^{-1}\right)=(\text{Z})(10)\left(\frac{\text{Y}}{0.5}\right)$$

where Z = % NEA determined from combustion; Y= weight of dry residue.

2.2.8 Tissue and Grain Sampling

Within a plot, 20 corn ear leaves were randomly sampled at VT, dried at 60 °C for five days, then ground to pass a 2 mm sieve. Samples were sent to A&L Great Lakes Laboratories, Inc. for total N analysis via combustion (AOAC 990.03, Table 7).

At physiological maturity and prior to machine harvest, six consecutive whole plant samples were collected at two or three representative locations within a plot then dried at 60 °C for five days. The number of sample locations per plot at West Lafayette, Lafayette, Columbia City, Wanatah, Farmland, and Butlerville were 3, 3, 2, 2, 3 and 3, respectively. Corn ears were husked, shelled, and separated from stover. Stover was weighed (wet weight) then coarsely ground using a wood chipper. A subsample of coarsely ground stover was weighed (wet weight) then dried at 60 °C to a constant weight (dry weight). The moisture concentration of the subsample was used to adjust the wet weight of the entire sample to dry weight. The coarse subsample was finely ground to pass a 1 mm sieve.

Corn ear samples were dried at 60 °C for five days. Dried ears were shelled, then the kernels were ground with a food processor. Stover samples (1 mm or less) and ground grain samples were sent to A&L Great Lakes Laboratories, Inc. for determination of total N (AOAC 990.03) and nutrient concentrations (Tables 8 and 9).

The middle six rows of each plot were machine harvested and yields obtained from the yield monitor (Table 10). The following calculations were completed using the tissue and grain data:

Grain N (kg) = Grain N concentration * Grain DW

Stover N (kg) = Stover N concentration * Stover DW

Plant N/grain (kg/kg) = (Grain N + Stover N) / Grain DW

A single factor ANOVA was conducted to test for a residual treatment effect on plant N in the grain. No significant effects were noted, therefore an average of plant N in grain — now defined as mean plant N/grain — was obtained for each location to improve the mean estimate. Total plant N uptake was calculated as follows and reported in Table 10.

Total plant N uptake (kg N/ha) = (Mean plant N/ grain DM)* Field grain DM

2.2.9 Weather and Climate

Temperature and precipitation daily averages were obtained from iclimate.org from on- or near-site weather stations. Monthly averages from 2013 to 2015 and 30-year normals are presented in Table 11. Approximately 10% of precipitation data was missing from the Purdue Agriculture Center (PAC) locations, therefore data was obtained from NOAA.gov to fill in the gaps using the same weather stations. Nine precipitation data points remained missing for Butlerville: 3/2/2013 - 3/5/2013, 1/1/2014, 12/3/2014, 1/1/2015, and 4/7/2015. There are two temperature points missing which include Columbia City 8/12/2015 and Butlerville 7/18/2015.

Data for all 30-year normals was gathered from iclimate.org. Wanatah and West Lafayette sites used Purdue Agricultural Center (PAC) data. The remaining four locations used near-by weather stations due to unavailable normal data. For the Butlerville location, a weather station 5 km southwest was used, while the Farmland site used a station 2 km east. The Lafayette site utilized a station 2 km south and the Columbia City location used the closest station which was 10 km northeast of the study field.

2.2.10 Saturated Hydraulic Conductivity

At each location, our field was rotated with another field with the same experimental design from 2006 to 2014, except that corn and soybean were grown in the even and odd years, respectively. Saturated hydraulic conductivity (Ksat) estimates were obtained from Web Soil Survey (http://websoilsurvey.nrcs.usda.gov) using fields from all site-years. Estimates were based on the proportion of each soil series in the experimental area, therefore a weighted Ksat average was calculated for each location.

2.2.11 Irris Scheduler

The Irris Scheduler (http://www.purdue.edu/agsoftware/irrigation) uses soil properties (water holding capacity, texture, organic matter, moisture, and residue cover) from Web Soil Survey, crop information (planting, harvest, and emergence dates), and weather (temperature, precipitation, and evapotranspiration) to estimate “excess” water which is defined as water which may runoff or leach through the soil from planting to harvest. For each field excess water was a weighted average based on the percentage of field area mapped to a particular soil series.

2.2.12 Data Analysis

All statistical analyses were performed with SAS 9.3 (SAS Institute Inc., Cary, NC). Locations were analyzed individually. Simple statistics were conducted on all data to determine mean, standard deviation, minimum, and maximum. A transformation was considered if the range of the means (averaged across N rates and depths per location) divided by grand mean was greater than 0.5. If the majority of data had a range ratio of greater than 0.5, a Box-Cox Regression was carried out to determine log or square root transformation. If a measured variable consisted of numerous zero variances (three or more for locations with three replications, and two or more for locations with two replications), the values were set to missing to run the ANOVA. Error terms in the ANOVA were tested for pooling using PROC GLM — pooling increases the power of the experiment to test the main effects.

Soil N values were experimentally completed in duplicate, but the mean of the duplicates was used for statistical analysis. Day zero inorganic N data and total N values were analyzed as a split-block design using two-way ANOVA in PROC MIXED. The model B and C error terms, depth*block and depth*N rate*block, were pooled; thus, leaving two error terms in the analysis.

General soil fertility data was analyzed as a split-block design using two-way ANOVA in PROC MIXED with N rate as the whole plot and soil depth as the subplot. Least significant (LS) mean separation tests were carried out for significant ANOVA effects/interactions. Square root and log transformations were needed for soil P and Zn, respectively. Least significant (LS) mean separation tests were carried out for significant ANOVA effects/interactions. The error terms N rate*block, depth*block, and depth*N rate*block were not pooled and left separate in the model. Transformed variables were back transformed for presentation. Two depths were dropped from Butlerville P data due to zero variance, thus, simplifying the model to a randomized complete block design, single factor ANOVA. One depth was dropped from West Lafayette Fe data, resulting in a split-plot model.

A single factor analysis of variance (ANOVA) was completed using PROC GLM on earleaf, stover, grain, yield, and N uptake data to determine significance. Least significance difference (LSD) mean separation tests were completed on significant variables.

Soil incubations were conducted in duplicate. The mean of the duplicates was calculated as a better estimate of the mean for statistical analysis. Incubation study variables — NH_4^+-N, NO_3^--N, and total inorganic N — required log transformation. Log transformed variables were analyzed using three-way ANOVA with PROC GLM. LS mean separation tests were requested for significant effects and interactions. Data was back transformed for presentation.

Correlations were completed with plant N uptake against the following independent variables for each location: SOM, pH, total soil N, initial NH_4-N and NO_3-N, and day 50 total inorganic N from the incubation study. Correlations were also completed on soil pH relative to total inorganic N release from the incubation at day 10 and 50. Regressions were carried out on significant correlations. Regression analysis was also completed on historic corn yield data and average Ksat per field at each location — yield data was averaged per field across years. Finally, excess water was regressed with historic yield data for each location.

To compare locations, two-tailed t-tests were completed on SOM, pH, initial day zero NO_3-N concentrations, day 0 and day 50 total inorganic N, plant N uptake, and Ksat. Paired t-tests were used to compare total inorganic N release per day from 0 to 10 and 10 to 50 days. Finally, a single factor ANOVA was completed using PROC GLM on N release per day from 0 to 10 and 10 to 50 days, as well as N release per day from SOM to test for significance within soil depth.

2.3 Results and Discussion

2.3.1 Weather and Climate

From a statewide perspective, Indiana experienced a cooler winter in 2013 than normal; in fact, March broke the record for the coldest since 1895 with an average temperature of 1.6 °C (http://www.iclimate.org). The 2013 growing season from April to October started off wetter than normal with cooler temperatures, transitioned to cooler than normal temperatures in July with below normal rainfall, then ended with average weather patterns at harvest.

The winter of 2014 began with January temperatures of -13.9 °C below the norm of -7.9 °C. The cold temperature pattern continued until March. Precipitation throughout winter 2014 was close to average. The growing season started off warm and wet from April to June, followed by average temperatures and variable precipitation across the state for the remainder of the growing season.

The winter of 2015 maintained lower than normal temperatures and average precipitation. April and May experienced average to slightly above average rainfall; rain events occurred suddenly and often resulted in flooding. These large rain events continued into June where Indiana broke the 1958 rainfall record with an average of 22.8

cm for the month. August showed cool and wet conditions, while September and October were warm and dry.

2.3.2 Texture Classification

Particle-size analysis revealed clay loam textures for all sample depths at Columbia City and Wanatah while Lafayette and Butlerville were silty clay. Farmland 0-20 cm was a silty clay while 20-40 and 40-60 cm depths were clay textures. At West Lafayette, 0-20 and 40-60 cm depths were silty clay loam textures and 20-40 cm was a silty clay (Table 3). Soil texture is a major contributor to N cycle dynamics and will frequently be referred to in subsequent discussion sections.

2.3.3 Soil Fertility

The effect of N rates on soil pH differed by depth (NRxD; $\alpha \leq 0.05$) at Lafayette, West Lafayette, Columbia City, and Wanatah. At Lafayette, pH for the 0-20 cm soil depth decreased linearly beginning at N rate 3 from about 6.0 to 5.4; 20-40 and 40-60 cm depths fluctuated by about ±0.2 (Figure 1). At the lower N rates, pH ranged by about 0.6 between depths, with 0-20 cm being the highest, while the higher N rates ranged by about 0.2. The acidifying effect of the side-dressed UAN may be responsible for the pH decrease with increasing N rate at Lafayette; however, this trend was not observed at other locations. At West Lafayette, Columbia City, and Wanatah, there were significant NRxD interactions but no identifiable patterns — the average range in pH across N rates was 0.4. At Butlerville, 0-20 cm soil pH (5.9) was similar to 20-40 and 40-60 cm soil (5.9 and 5.7, respectively) compared to West Lafayette, Wanatah, Columbia City, and

Farmland where pH increased with depth. The lower pH at 0-20 cm compared to the more basic 20-40 and 40-60 cm soil depths at West Lafayette, Wanatah, Columbia City, and Farmland was still near the ideal Tri-State Fertilizer Recommendation of 6.0 (Vitosh et al., 1995) and thus, not likely limiting to corn growth or N mineralization.

Significant interactions between N rate and depth were present for P and K at Lafayette, but had no identifiable pattern (NRxD; $\alpha \leq 0.05$). For instance, 0-20 cm P at Lafayette began at 35 mg kg^{-1} at N rate 1 then decreased to 20 mg kg^{-1} at N rate 3; a steady increase of P was noted from 21 mg kg^{-1} at N rate 4 to 28 mg kg^{-1} at N rate 6. There were no discernable differences in P levels among N rates at 20-40 and 40-60 cm soil depths. The 0-20 cm soil K began at 149 mg kg^{-1} at N rate 1 then stabilized around 122 ± 4 mg kg^{-1} at the remaining five N rates. The 20-40 and 40-60 cm soils averaged 80 ± 4 mg kg^{-1} and 103 ± 9 mg kg^{-1} respectively across N rates. Additional NRxD effects were present for Mg, Ca, Na, Zn, Fe, and Cu, however did not have discernible patterns.

Phosphorus at Butlerville (13 mg kg^{-1}) fell below the Indiana critical level of 15 mg kg^{-1} for corn. Stratification of S was apparent at Butlerville where S increases with depth from 7 to 19 mg kg^{-1}. Increased oxyhydroxides (not measured), clay abundance, and low pH (5.7) in the subsoil could be responsible for the S stratification patterns at Butlerville (Jez, 2008). Additionally, Butlerville is below the extent of the Wisconsin glacier and consists of older soils which are likely higher in minerals containing oxyhydroxides (Huang et al., 2012). According to the Tri-State Fertilizer Recommendations for corn (Vitosh et al., 1995), all other nutrients were above Indiana critical levels.

2.3.4 Total Nitrogen and Carbon

Nitrogen rate did not affect total soil N concentration at 5 of 6 locations ($\alpha > 0.05$). High N decreased total N at one location, Columbia City, where increased N rate decreased total N linearly from 0.9 to 0.8 g kg^{-1} ($R^2 = 0.68$; Figure 2). Total N decreased by approximately 8% on average from 0 to 60 cm at all locations. Some previous research (Wang et al., 2013; Brown et al., 2014) has found no change in soil N with increased N rate in accord with our findings at 5 locations. However, others have found an increase in soil total N with increasing fertilizer rate (Liu et al., 2005; Mazzoncini et al., 2011; Aula et al., 2016). None have found a decrease with increased N rate like we detected at Columbia City. Total C was unaffected by N rate. As expected, total C decreased by 7.0 g kg^{-1} with depth ($\alpha \leq 0.05$) averaged across locations.

Mazzoncini et al. (2011) conducted a study in Italy to determine the effects of N fertilization and tillage in corn systems. The long-term experiment began in 1993 with corn grown continuously until 1998 when a two-year rotation with wheat began. In 2005, the rotation switched to wheat-corn-wheat-sunflower. In the no-till treatments, they noted a 0.61 and 0.04 Mg ha^{-1} year^{-1} increase in soil organic C and total N while a decrease of 0.06 and 0.04 Mg ha^{-1} year^{-1} was observed in conventional till. Four N fertilizer rates (0 to 300 kg N ha^{-1} for corn) were used to assess N rate effects on soil organic C and total N. They determined that all N rates except for 0 kg N ha^{-1} increased soil C by an average of 0.14, 0.45, and 0.49 Mg C ha^{-1} year^{-1} for 100, 200, and 300 kg N ha^{-1}, respectively . Total N increased by an average of 0.04 Mg N ha^{-1} year^{-1} for two highest N rates. Mazzoncini et al. (2011) concluded that cropping in Italy’s Mediterranean climate with warm, wet winters and hot, dry summers benefits from no-till and N fertilizer inputs — the humid continental climate of Indiana may do best with other practices. Liu et al. (2005) found that corn-soybean-wheat rotations in Northeast China increased soil total C and N compared to continuous corn, soybean, or wheat crops which decreased soil C and N content. Additionally, integrated tillage with moldboard plow for wheat, deep tillage for soybean, and rotary till for corn increased soil C and total N compared to conventional till. The study site was in continental monsoon climate characterized by cold, dry winters and hot, rainy summers.

Contrary to the above findings, Brown et al. (2014) carried out a 13 year continuous corn fertilization study in Iowa to determine the effect of inorganic N fertilization on soil C and total N stocks. No changes in soil C or total N were observed. Brown et al. (2014) concluded the present soil was C saturated, thus, additional residue input would not increase soil C supply. Moving east to Illinois, Stevens et al. (2005) found no differences in 0-30 cm total N and C in continuous corn among N rates that ranged from 0 to 268 kg ha^{-1}. At 336 kg N ha^{-1}, Khan et al. (2007) observed a net decline in soil C in continuous corn in Illinois as the result of 50 years of high N fertilization.

An ^{15}N study by Omay et al. (1998) in Kansas determined the contribution of soybean residue, inorganic N fertilization, and soil N to the subsequent corn crop. They determined that corn recovered 3-14% of total N from soybean residue, <3% from residual fertilizer, and 5-18% from soil and soybean roots. Post corn harvest inorganic fertilizer N was likely lost to the environment or taken up by the previous soybean crop.

2.3.5 Day Zero Ammonium, Nitrate, and Total Inorganic Nitrogen

Ammonium-N concentrations varied by depth at West Lafayette ($\alpha \leq 0.01$) and Farmland ($\alpha \leq 0.05$). The uppermost 0-20 cm soil depth at West Lafayette contained 5.2 mg NH_4-N kg^{-1} and was greater than the statistically similar measurements of 3.7 and 3.3 mg NH_4-N kg^{-1} at 20-40 and 40-60 cm soil depths, respectively. At Farmland, the top two depths were statistically similar to each other (3.3 and 3.1 mg NH_4-N kg^{-1}), yet different from the third depth (2.5 mg NH_4-N kg^{-1}; $\alpha \leq 0.05$). Columbia City, Wanatah, Butlerville, and Lafayette NH_4-N concentrations did not vary by depth. The highest ammonium-N concentrations were found at Wanatah, Lafayette, and Butlerville with averages over all depths of 8.4, 7.0, and 9.0 mg NH_4-N kg^{-1}, respectively. No significant N rate effects were noted; however, a significant NRxD interaction was observed at Wanatah ($\alpha \leq 0.05$; Figure A.1.). The interaction had no identifiable pattern.

Nitrate-N concentration was greatest at 0-20 cm then decreased with depth at West Lafayette ($\alpha \leq 0.05$), Farmland ($\alpha \leq 0.05$), Wanatah ($\alpha \leq 0.05$), Lafayette ($\alpha \leq 0.05$), and Columbia City ($\alpha \leq 0.01$). Nitrate-N concentrations at 0-20 cm were greatest at Lafayette averaging 14 kg N ha^{-1} ($\alpha \leq 0.01$). The higher NO_3-N at this location was likely a product of increased mineralization followed by nitrification after residue was incorporated into the soil (Lafayette was conventionally tilled). Conventional till reduces surface residue and provides aeration, thus, building an ideal warm and oxygen rich environment for nitrifying organisms to rapidly transform ammonium. The concentrations of NO_3-N and NH_4-N were similar at all locations except Lafayette where NO_3-N was double the NH_4-N concentration. The higher soil temperatures following tillage likely resulted in rapid turnover from NH_4^+ to NO_3^-. The Wanatah location was also tilled, but the planting date was much earlier (April 29 vs May 23) when soil temperatures were cooler. Average April and May temperatures were 12 °C and 19 °C for Lafayette, and 7 and 15 °C for Wanatah, respectively (Table 11).

Total inorganic N decreased with depth at all locations except Butlerville ($\alpha \leq 0.01$). Nitrogen rate did not affect total inorganic N, except at West Lafayette and Wanatah where N rate effect differed by depth (NRxD; $\alpha \leq 0.05$; Figures A.2 and A.3). The interactions had no identifiable pattern.

Several studies have investigated the residual impact of fertilization rate on residual N. Legg and Allison (1967) conducted an ^{15}N greenhouse study to trace residual N and subsequent crop uptake in an oat – sudangrass – sudangrass rotation. Oats were fertilized with 50, 100, or 200 mg N kg^{-1}. Following oats, 10-41% of initial N was present in the soil and increased with increasing N rate; after the first sundangrass crop, 4.5% of initial N remained. Jokela and Randall (1989) observed similar findings in a study that evaluated N rate effects on soil residual N abundance in continuous corn in Minnesota. Soil samples (1.5 m) were obtained from all plots following planting and harvest. Residual NO_3-N post-harvest ranged from 150 to 400 kg N ha^{-1}, but was reduced by approximately 60% come spring. The decrease was likely the result of heavy precipitation in fall and spring. In continuous corn plots in Illinois, about 44 and 3 kg N ha^{-1} mineral N was found after harvest from 13 years of high (268 kg N ha^{-1}) and low (67 kg N ha^{-1}) urea application rates, respectively (Stevens et al., 2005).

Scott (2015) conducted a study prior to the present one using the same study sites at Columbia City, Wanatah, and West Lafayette for his 2014 study year. Scott analyzed soils for N carryover from the 2013 corn year. He measured soil nitrate, exchangeable ammonium, and total inorganic N contents for the 2014 soybean growing season at 0-20, 20-40, and 40-60 cm; soils were collected at pre-plant and post-harvest. Pre-plant nitrate-N was significant with previous N rate at Wanatah and Columbia City for 20-40 and 40-60 cm depths, respectively — a concave curvilinear relationship was noted. Scott suggested the wet fallow period at West Lafayette leached away nitrates, as no N rate effect was noted. Post-harvest soil NO_3-N tests revealed no rate effects at West Lafayette and Wanatah. A linear decrease (change in 1.3% or 2.0 kg ha^{-1}) in NO_3-N for Columbia City 20-40 cm soil was noted. No significant N rate effects were noted for ammonium-N or total inorganic N at any location for the 2014 soybean growing season.

Compared to Scott's post-soybean harvest total inorganic N concentrations (2014), our post-corn planting (2015) total inorganic data were greater by 12, 102, and 30% for West Lafayette, Wanatah, and Columbia City, respectively for the 0-20 cm depth. Although different analyzers were used to quantify N, it is clear that soybean residue was mineralized during the fallow period. Overall results from Scott's study indicate minimal significance in nitrate-N, ammonium-N, and total inorganic N from 2013 corn N rates. In sum, the carryover of 2013 corn N application through the soybean year, then to the 2015 corn year did not appear to occur because of a suite of environmental factors encouraging N loss.

Scott (2015) conducted the same experiment in 2013, following the drought of 2012, using different fields at the same locations. Corn grain yield and plant N uptake from the 2012 corn crop were lower than normal at Columbia City and West Lafayette while Wanatah yield and N uptake were similar to previous years. Pre-plant 2013 soil NO_3-N was influenced by N rate (NRxD; $\alpha \leq 0.05$) at Columbia City (all depths) and

West Lafayette (20-40 and 40-60 cm) likely because of lower corn N removal and below average precipitation. Post-harvest NO_3-N was not significant with N rate at any location except Wanatah where the uppermost soil depth increased from 6.7 to 7.7 kg NO_3-N ha^{-1} across N rates. Pre-plant total inorganic N was significant with N rate at Columbia City and West Lafayette at all depths. No differences in total inorganic N related to previous N rate were noted post-soybean harvest.

2.3.6 Nitrogen Release during Aerobic Incubation

A summary of the ANOVAs completed for the 50-day incubations can be found in Table 12. Total inorganic N varied by N rate at Columbia City, Butlerville, and Lafayette (Figure 3; $\alpha \leq 0.05$). At Columbia City, N rate two total inorganic N was about 7 kg ha^{-1} greater than the five other N rate treatments which were statistically similar. Butlerville showed an upward trend in total inorganic N of about 14 kg N ha^{-1} from N rate two to N rate four. Nitrogen rate four at Lafayette had slightly lower total inorganic N (about 3 kg ha^{-1}) than the other N rates. Overall, previous N rate had minimal effects on the amount of total inorganic N released during the 50-day incubation.

Significant depth x day interactions were noted for all locations (Figure 4; $\alpha \leq 0.01$). Total inorganic N increased for all depths at all locations from 0 to 50 days. Compared to 20-40 and 40-60 cm soil depths, the 0-20 cm depth resulted in greater N release. The two lower depths followed a similar trend in N release across time. At Lafayette, all depths showed a decrease in N release at day 10. A similar decrease was found at 20-40 cm and 40-60 cm at Wanatah and 40-60 cm at Butlerville. The decrease may be the result of drying the soil samples, then rewetting for incubation. Studies have demonstrated an initial flush of N from organism death during the soil drying period (Richter et al., 1982; Beauchamp et al., 1986). Similar to the other locations, total inorganic N increased from day 10 to 50.

After 50 days of incubation, total inorganic N at 0-20 cm was greatest at Farmland and Butlerville averaging 183 mg N kg^{-1} while Lafayette had the lowest with 46 mg N kg^{-1} (Figure 5; $\alpha \leq 0.05$). Rate of change in total inorganic N per day between 0 and 10 days was lower than the rate of change between 10 and 50 days at all locations and depths except 0-20 cm at West Lafayette ($\alpha \leq 0.05$; Table 6). In our experiment, temperature and moisture were controlled, but other factors such as SOM and pH may have contributed to the differences in N release between depths and locations. As depth increased from 0 to 60 cm, SOM decreased by an average of 13 g kg^{-1} across locations (Table 4). The observed decrease in cumulative mineralization at the greater soil depths is likely a result of the smaller organic matter pool (Ros et al., 2011), although release from fixed N could occur (Mengel and Scherer, 1981). Further investigation of N release per unit of SOM showed that N release from SOM was greatest at 0-20 cm at all locations except Lafayette (Table 6). Compared across locations, Farmland N release per unit SOM was greater than Butlerville for all depths ($\alpha \leq 0.05$). There was no pH effect on N release, averaged across depth, at 10 nor 50 days at any location except West Lafayette where N release decreased with increasing pH. On day 10, total inorganic N decreased from about 19.3 to 15.6 mg ka^{-1} while pH increased from 6.6 to 6.7 (R^2=0.74; $\alpha \leq 0.05$). On day 50, total inorganic N decreased from about 78.8 to 65.4 mg ka^{-1} while pH increased from 6.6 to 6.7 (R^2=0.73; $\alpha \leq 0.05$). Compared across locations, pH (averaged across depth) was greatest at Farmland (7.0) and lowest at Lafayette (5.6) at $\alpha \leq 0.05$.

Low pH (<6.0) limits microbial activity (Kyveryga et al., 2004) while high pH (7.5 to 8.0) soils display a peak in microbial activity (Prosser, 1989).

The SOM factor is complex; more does not necessarily mean better since the quality (C:N ratio, Section 2.3.4) and availability (labile or recalcitrant) affect overall mineralization potential. Separating SOM into labile and recalcitrant pools is difficult, but studies suggest that the N released in the first 14 days of incubation is derived from labile SOM and the remaining time is from recalcitrant (Bremner, 1965). In our study, the rate of N release between 0 and 10 days may have been less than 0 to 50 days due to size differences of labile and recalcitrant pools. Recalcitrant SOM was previously thought of as unavailable, but recent work suggests that non-native inputs of carbon (i.e. crop residue) may prime the release of recalcitrant SOM by activating microbes that destabilize the C (Derrien et al., 2014). Additionally, the N release may be greater at 0 to 50 days due to the rebound of microbes as soil is rewet or from the mineralization of dead microbes.

Contrary to our results, a study by Carpenter-Boggs et al. (2000) found a decrease in net mineralization from low to high N treatments in a 189-day incubation. They suggest that immobilization or decreased gross mineralization could occur at high N rates; furthermore, such actions could be the result of non-corn year soil processes whereby residual N from high N treatments decreases symbiotic fixation in a legume crop. In general, many studies agree that the size of the organic matter pool is the primary driver of mineralization, but the effect of N rate on the organic pool size has yet to be explained. According to Ros et al. (2011), 78% of mineralization was explained by the size of the SOM pool while 8% was explained by a combination of other soil properties such as pH, CEC, moisture, C:N ratio, N:P ratio, and water holding capacity.

2.3.7 Fixed Ammonium

Nonexchangeable ammonium (NEA), known as fixed ammonium, was measured at all locations in duplicate for the highest and lowest 2013 N rates. The modified Neider method was used however results were inconclusive and are not presented here. Five locations returned zero NEA, but an NEA range of 0-61 mg N kg^{-1} was noted for Farmland. Further statistics were not carried out due to lack of statistical power.

2.3.8 Earleaf Composition

The plant tissue sufficiency levels provided by A&L Great Lakes Laboratories were used to assess present nutrient concentrations. Earleaf N concentrations (Table 7) were well below the lower sufficiency value of 30 g kg^{-1}. Concentrations were unaffected by previous N rate ($\alpha \leq 0.05$) and averaged 15.0, 14.5, 13.4, 18.0, 19.9 and 18.7 g kg^{-1} for West Lafayette, Farmland, Columbia City, Wanatah, and Butlerville, respectively. An N rate effect was noted at Lafayette due to N rate 5 (225 kg N ha^{-1}) showing a lower N concentration (17.9 g kg^{-1}) than the other N rates (Average = 18.9 g kg^{-1}). Phosphorus concentrations were on the lower end of the sufficiency range (2.5 g kg^{-1}) for all locations except Farmland (3.4 g kg^{-1}). Similarly, Mg concentrations at West Lafayette, Farmland, and Columbia City skirted the lower sufficiency level of 1.3 g kg^{-1}. Sulfur fell below the lower sufficiency value of 1.5 g kg^{-1} at all locations with the lowest concentration at Columbia City (1.0 g kg^{-1}). According to the Diagnostic Recommendation Integrated

System (DRIS), the N:S ratio should be ~15:1 for optimum yield. Our N:S ratios averaged 13:1 across locations. Zinc concentrations were below 15 mg kg^{-1} at four locations averaging 14 mg kg^{-1} at Lafayette and 9 mg kg^{-1} at West Lafayette, Farmland, and Columbia City. A significant N rate effect was found for Ca at Columbia City; Ca peaked at N rate one (3.2 g kg^{-1}) then leveled off from N rate two to six (statistically similar).

These earleaf results should be looked at holistically; soil data, weather, and earleaf nutrient concentrations are all interacting factors that should be considered. Heavy rain events after planting likely leached away residual nitrate from the root zone of developing plants. Additionally, soil test NO_3-N levels were below 10 mg kg^{-1} post planting and thus, foreshadowed earleaf N concentrations below sufficient levels. Michigan’s Soil Nitrate Test for Corn (Warncke, 2010) interprets soil test NO_3-N below 10 mg kg^{-1} as normal background concentration, as these levels provide zero N credits to future N application.

Of the small amount of residual NO_3-N present, uptake of N may have been limited by interactions with other macronutrients, specifically P. The N–P interaction is imperative to N uptake (Terman et al., 1977; Pinney et al., 2009). Phosphorus availability is directly influenced by pH and tends to complex with Ca or Mg in alkaline environments (pH 7.5), and Al or Fe in acidic environments (pH 6.0) (Fageria et al., 1995; Huang et al., 2012). At both pH extremes, P solubility is reduced and becomes unavailable to the plant.

Soil test levels of Mg were sufficient, but it is clear that other factors limited plant uptake. The lack of Mg uptake may be attributed to large amounts of K and Ca present in the soil (Fageria, 2009; Hawkesford et al., 2011) or low demand due to N insufficiency.

Sulfur deficiency is common in soils low in organic matter, or soils with external factors that inhibit mineralization. Soil erosion, heavy N and K fertilization, and increased industrial emission standards can reduce soil S stocks (Fageria, 2009). The above factors may contribute to low earleaf S concentrations if N were adequate, but in our case, S uptake was likely sufficient for the amount of N available.

Zinc soil concentrations were adequate for growth, but plant uptake was likely limited at West Lafayette, Farmland, and Columbia City by the wet growing season and overall stunted growth from lack of fertilization (Broadley et al., 2012). Root growth is essential for plants to scavenge immobile soil Zn; the roots form a symbiotic relationship with soil mycorrhizae whereby the roots supply fungi with carbohydrates while the fungi expand hyphae to increase root volume and scavenging ability (Fageria, 2009).

2.3.9 Stover Composition

Previous N rate had no effect on stover N concentration at any location (Table 8, $\alpha \leq 0.05$), suggesting no differences in soil N supply among treatments. When N rates were applied during the growing season the stover tissue N concentration reflected the rate of N applied (data not shown).

Previous N rate had few effects on concentration of other nutrients in stover (Table 8). Stover K and Zn differed among N rates at Wanatah and Zn at Butlerville. In both cases the first N rate above starter-only had the greatest nutrient concentration.

Otherwise there were no differences among N rates. A study by Sindelar et al. (2013) found that in-season N fertilization had a positive effect on stover N concentration, negative or no impact on stover P, and no relationship for K or S.

2.3.10 Grain Composition

Grain N concentration was unaffected by previous N rate at all locations (Table 9, $\alpha \leq 0.05$). Average grain N ranged from 8.2 to 11.8 g kg^{-1} at the six locations, averaging 9.0 g kg^{-1}. According to Boonea et al. (1984), grain N concentrations averaging 16 g kg^{-1} are sufficient. Cerrato and Blackmer (1990) noted that 24 out of 32 papers use grain N concentrations to measure corn yield response to N fertilizer. To determine the reliability of using grain N concentration as an indicator of corn N status, they used continuous corn with 10 N rates applied to Iowa Mollisols. Cerrato and Blackmer (1990) found a poor relationship between grain N and yield and concluded that grain N was an inadequate predictor of corn N status. A recent report by Ciampitti and Vyn (2013) suggests that grain nutrient concentration guidelines do not reflect current management practices and genetic improvements. Ciampitti and Vyn (2013) reported that at an N rate of 224 kg ha^{-1}, corn yield was 13.2 t ha^{-1} and grain N concentration averaged 11.3 g kg^{-1}. A significant N rate effect was noted for K and Zn at Farmland (Table 9) with no identifiable pattern of concentration across N rates and narrow ranges for each nutrient (4.6-5.4 g K kg^{-1} and 22-26 mg Zn kg^{-1}).

2.3.11 Total Plant Nitrogen Uptake

Total plant N uptake was unaffected by previous N rate at all locations (Table 10; $\alpha > 0.05$). Total plant N uptake compared across locations was not correlated with soil total inorganic N release after 50 days of incubation. Plant N uptake was lowest at Farmland with an average of 6 kg N ha^{-1} and highest at Wanatah, Butlerville, and Lafayette with an average of 65 kg N ha^{-1} (Figure 6; $\alpha \leq 0.05$). In contrast, total inorganic N was greatest at Butlerville and Farmland averaging 183 mg N kg^{-1} while Lafayette had the lowest with 46 mg N kg^{-1} (Figure 5; $\alpha \leq 0.05$).

Timmons and Cruse (1991) conducted a study in Ames, IA to measure residual ^{15}N recovery in continuous corn. Recovery was measured for three years after initial application of 200 lb ac^{-1} labeled (^{15}N) urea-ammonium nitrate; in the subsequent three years, corn was fertilized with non-labeled 200 lb N ac^{-1}. Total residual N recovered by the plant during the three years post ^{15}N application was only 2.3, 1.2, and 0.9%, respectively — this is <1% of the cumulative 600 lb N ac^{-1} applied in the three years post ^{15}N application and was not likely significant to crop growth.

When total plant N uptake was regressed against 0-20 cm SOM (Figure 7), a negative relationship was noted at Lafayette ($\alpha \leq 0.05$, $R^2 = 0.68$) while a positive relationship was detected at Farmland ($\alpha \leq 0.05$, $R^2 = 0.66$). At Lafayette, plant N uptake decreased linearly from 71.7 to 59.7 kg N ha^{-1} as SOM increased from 25.7 to 28.3 g kg^{-1}. Increased SOM may have held onto water longer and prevented water loss to evaporation. Prolonged saturation could lead to N loss via denitrification and overall decrease in plant N uptake. In contrast to Lafayette, Farmland plant N uptake increased linearly from 6.0 to 8.0 kg N ha^{-1} as SOM increased from 34.0 to 38.5 g kg^{-1}. Given the poor yield at Farmland and corresponding narrow range in plant N uptake, it is difficult to make any conclusions about the variance in N uptake data at Farmland.

Further regression analyses indicated that plant N uptake at every location was unaffected by 0-20 cm soil pH, total soil N, initial NH_4-N and NO_3-N, and day 50 total inorganic N from the incubation study. Our numeric range of each variable regressed was likely too small to see a trend with plant N uptake.

2.3.12 Yield

Similar to total plant N uptake, previous corn N rates did not influence grain yield at any location (Table 10). The soils natural capacity to supply and maintain a baseline level of N is credible cause for the observed yield differences across locations. Our insignificant relationship between yield and previous corn N rates were no surprise; any carryover of N from the previous corn year was likely utilized by the 2014 soybean, and/or leached as a result of an overly wet spring — no buildup of organic N occurred.

In our study, we contend the interceding soybean crop is a great equalizer of residual N, as studies have demonstrated that soybeans are excellent scavengers of excess N left post corn harvest. Welch et al. (1973) observed that previous corn N rates had no effect on soybean yield. Similarly, Scott (2015) conducted a study prior to the present one using the same study sites at Columbia City, Wanatah, and West Lafayette for his 2014 study year. Scott analyzed soybean yield relative to 2013 corn N rates. Results at Wanatah and West Lafayette showed minimal N rate effects on soybean yield. The Columbia City location had higher soybean yields at lower N rates; this could be attributed to greater residual N abundance in low yielding plots, late 2013 corn planting date, and consequential mineralization as soil temperatures warmed. The soil in the lower N plots may warm faster due to decreased residue on the surface from the previous corn year. Warmer soil temperatures may have promoted early soybean growth and residue decomposition compared to the higher N plots.

Residual soil N effect on yield has been investigated on a variety of crops and climates, but with inconsistent results. Dissertation work by White (1957) explored residual N effects in corn-oat rotations at six Iowa farms. Oat yield was significant with N rate at 4 locations with a linear increase at three and quadratic increase at one location. White concluded that N carryover is possible in areas with “near-normal” rainfall (on average 700 mm for the year) — saturated conditions for prolonged periods promote N loss and reduce residual N effects on successive crops. Rainfall increases from north to south and east to west across the Corn Belt (NOAA). Average winter precipitation (December – February) is 61 mm in northwest Iowa, 205 mm in eastern Ohio, and 256 mm in southern Indiana (U.S. Climate Data). Years of “near-normal” rainfall in Indiana are more than triple those of Iowa which may explain the lack of yield differences in our study. Furthermore, winter precipitation averaged across our study sites was 246 mm for the 2015 winter, much higher than the norm of 179 mm.

Perhaps the lifetime of residual N depends on the properties of the nutrient source. Eghball et al. (2004) conducted an 8-year study in a corn-corn system to determine the residual N impact on grain yield and soil properties from cattle manure. They found that application of cattle manure increased corn grain yield the following corn year, but the effect was short-lived as no yield differences were noted thereafter. Residual N resonated in the soil properties for the remaining three study years; differences observed included:

increased pH, electrical conductivity (EC), and soil NO_3-N concentration. The experimental design contained a control treatment for comparison, however no differences in manure application rate were studied. Other studies agree with the short-term residual N effect on grain yield (Lund and Doss, 1980), and slightly longer effect on soil properties (Wallingford et al., 1975; Ginting et al., 2003).

2.3.12.1 Saturated Hydraulic Conductivity

Saturated hydraulic conductivity (Ksat) values obtained from Web Soil Survey (http://websoilsurvey.nrcs.usda.gov) were highest for Wanatah at 14.1 μm s^{-1}. West Lafayette, Columbia City, and Butlerville Ksat values were similar and ranged from 7.1 to 10.2 μm s^{-1}. Farmland had the lowest Ksat value of 3.3 μm s^{-1}. A positive linear relationship was noted between historic yield averages for starter-only plots and Ksat (R^2= 0.56; Figure 8). Clay concentrations at Farmland and Lafayette were 436 and 280 g kg^{-1}, respectively (Table 3). Clays have a tendency to swell and fill micropores, thus, reducing water movement and overall Ksat.

In contrast to our linear relationship, Keller et al. (2012) found a quadratic increase in crop yield relative to Ksat which ranged from ~4 to 292 μm s^{-1}. A quadratic curve is more plausible because a soil with high Ksat, for example a sand textured soil, will not retain enough water to support the crop; theoretically, yield would decline where rainfall is minimum. In our data, the range of Ksat from 3 to 14 μm s^{-1} is too narrow to make predictions of if/when corn yield plateaus then declines. If a quadratic relationship is assumed, low Ksat values would have the greatest influence on yield. In dry years, a lower Ksat would keep water in the profile longer which could benefit the crop while in wet years, a lower Ksat may keep too much water in the profile and lead to saturated soils. This idea is expanded on in the next section.

2.3.12.2 Irris Excess Water

Excess water appears to be a significant factor on yield at locations with lower Ksat values. A negative relationship was found between excess water and historic yield data from starter-only plots at Farmland (R^2= 0.70; Figure 9) and West Lafayette (R^2= 0.43; Figure 9). Farmland had the lowest Ksat and was likely susceptible to decreased yields in high rainfall years. In contrast, low Ksat was beneficial in low rainfall years when water was maintained in the profile for a longer period of time.

West Lafayette fields were approximately 4 hectares in size and have been compacted by years of cropping. Additionally, the tile in the field is old and does not drain properly. Small-scale bulk density changes are not reflected on Web Soil Survey, thus, it is likely that Ksat values overestimate drainage in these fields. Poor drainage due to compaction and inadequate tile may help describe why excess water reduced yield at West Lafayette. The drought of 2012 was determined to be an outlier at Lafayette. Once 2012 was dropped, a negative relationship was noted between excess water and historic yield from the starter-only plots (R^2= 0.83; Figure 9). Similar to West Lafayette, Lafayette is poorly drained and the Ksat values estimated using Web Soil Survey likely overestimate drainage in the fields.

Saturated hydraulic conductivity can help explain how soil handles excess water. High Ksat soils will primarily leach excess water through the profile and drain quickly while low Ksat soils are slow to drain and may pond or transport water on the surface. Prolonged periods of saturation create an anaerobic environment that is detrimental to plant growth and favorable to N loss. According to Shaver and Ferguson (2014), five days of saturation with soil temperatures between 13 °C and 16 °C can lead to a 10% loss of total N applied via denitrification and ten days of saturation leads to a 25% loss. Furthermore, 60% of total N applied may be lost via denitrification within three days of saturation at 24 °C to 27 °C.

Saturated, anaerobic soils also show decreased mineralization. Stanford and Epstein (1974) demonstrated that optimum soil moisture for mineral N accumulation on a wide array of soil properties occurred between 33 and 10 kPa moisture tension. Beyond 10 kPa, mineral N accumulation was drastically reduced. We conclude that the low 2015 yield and plant N uptake at Farmland was likely due to early onset and prolonged saturation of soil throughout the growing season. The saturated conditions likely decreased mineralization and increased N loss to denitrification, surface runoff, and leaching.

2.4 Conclusion

This is the first long-term (9 year) corn-soybean rotation study to investigate residual N effects on corn growth. The randomized complete block design, which was not re-randomized each corn year, allowed for the determination of any residual N effects. Even though the amount of residual fertilizer N differed substantially due to a range in cumulative N rates over four corn seasons ranging from ~100 to ~1,000 kg N ha^{-1} in a corn-soybean rotation, there were no differences at the beginning of the 5th corn season in soil total N, NO_3-N, or NH_4-N due to cumulative N rate. When soil collected shortly after planting the 5th corn crop was incubated in the laboratory under ideal temperature and moisture, there were no differences in N mineralization across cumulative N rates. When corn was grown in the 5th corn season with starter-only and the predominant form of N available to the crop was derived from the soil, there were no differences in plant N content at physiological maturity relative to cumulative N rate applied in the previous four corn seasons. Nitrogen rates above and below the optimum N rate needed to maximize corn yield in a corn-soybean rotation resulted in no differences in soil N or N supplying capacity in the 5th corn season. Accurately predicting N behavior throughout the growing season is difficult given the range of interactions involved including soil N supply, potential for loss, and weather patterns. Future studies should work on fine tuning N rate recommendations that reflect soil properties, crop demand, and the producer’s bottom line. Increasing the number of N trials to span a wider array of soil types and growing conditions will strengthen our N rate recommendations in the long run.

Table 1. Corn growing location, sampling dates, number of soil subsamples, number of starter only replicates, tillage, and previous N rates.

	West Lafayette	**Farmland**	**Columbia City**	**Wanatah**	**Butlerville**	**Lafayette**
Research Site	Agronomic Center for Research and Education	Davis Purdue Agriculture Center	Northeast Purdue Agriculture Center	Pinney Purdue Agriculture Center	Southeast Purdue Agriculture Center	Throckmorton Purdue Agriculture Center
Soil Sample Date	May 29	May 28	May 20	May 7, May 8	May 13	May 27
Corn Planting Date	May 29	May 22	May 19	April 29	May 4	May 23
Ear Leaf Sample Date	July 28	July 29	July 31	July 14	July 13	July 23
Biomass Sample Date	October 5	October 12	October 13	September 28	September 25	October 6, October 8
Soil Subsamples	15	30	15	15	30	30
Starter Only Replicates	3	2	3	3	3	3
Tillage Regime	No-till in 2015, normally conventional	Strip-till	No-till	Conventional	No-till	Conventional
Previous Corn Nitrogen † Rate Average (kg ha^{-1})	27, 93, 128, 172, 217, 272	33, 93, 138, 183, 227, 272	26, 71, 115, 160, 205, 250	26, 82, 127, 172, 217, 262	32, 83, 127, 172, 217, 262	24, 91, 136, 180, 225, 270
Cumulative Corn Nitrogen Rate (kg ha^{-1})	108, 332, 511, 690, 869, 1048	133, 372, 551, 730, 909, 1089	103, 282, 461, 641, 820, 999	105, 329, 508, 688, 867, 1046	129, 330, 510, 689, 868, 1047	94, 363, 542, 721, 900, 1080

† Nitrogen rates were applied each of 4 previous corn crops.

Table 2. Soil series and U.S. taxonomic classification (in parentheses) for the two dominate soils at each location.

Location	Soil Series (U.S. Taxonomic Classification)
West Lafayette	Chalmers silty clay loam (87 †)
	(Fine-silty, mixed, superactive, mesic Typic Endoaquoll)
	Raub-Brenton complex (11)
	(Fine-silty, mixed, superactive, mesic Aquic Argiudoll)
Farmland	Pewamo silty clay loam (50)
	(Fine, mixed, active, mesic Typic Argiaquoll)
	Blount silt loam (48)
	(Fine, illitic, mesic Aeric Epiaqualf)
Columbia City	Rawson sandy loam (38)
	(Fine-loamy, mixed, active, mesic Oxyaquic Hapludalf)
	Haskins loam (26)
	(Fine-loamy, mixed, active, mesic Aeric Epiaqualf)
Wanatah	Sebewa loam (100)
	(Fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Typic Argiaquoll)
Butlerville	Ryker-Muscatatuck silt loam (35)
	(Fine-silty, mixed, active, mesic Typic Paleudalf)
	Nabb silt loam (32)
	(Fine-silty, mixed, active, mesic Aquic Fragiudalf)
Lafayette	Throckmorton silt loam (32)
	(Fine-silty, mixed, superactive, mesic Mollic Oxyaquic Hapludalf)
	Drummer (23)
	(Fine-silty, mixed, superactive, mesic Typic Endoaquoll)

† Indicates percent soil type in experimental area.

Table 3. Water contents at 33 kPa, saturated hydraulic conductivity (Ksat), and textural classification for all locations.

Depth	**Water Content at 33 kPa**	**Ksat †**	**Sand**	**Silt**	**Clay**	**Textural Class ‡**
cm	--g g^{-1}--	--µm s^{-1}--	-------------- g kg^{-1} --------------			
West Lafayette						
0-20	0.30	8.6	151	480	369	SiCL
20-40	0.31	-	134	463	403	SiC
40-60	0.31	-	147	473	379	SiCL
Farmland						
0-20	0.29	3.3	149	415	436	SiC
20-40	0.32	-	134	340	526	C
40-60	0.31	-	144	330	526	C
Columbia City						
0-20	0.20	8.5	394	333	273	CL
20-40	0.22	-	337	287	376	CL
40-60	0.23	-	347	280	373	CL
Wanatah						
0-20	0.24	14.1	334	343	323	CL
20-40	0.23	-	351	317	333	CL
40-60	0.20	-	424	287	289	CL
Butlerville						
0-20	0.29	8.0	57	647	296	SiCL
20-40	0.29	-	64	597	339	SiCL
40-60	0.29	-	107	557	336	SiCL
Lafayette						
0-20	0.25	10.0	157	563	280	SiCL
20-40	0.28	-	139	533	327	SiCL
40-60	0.30	-	154	467	379	SiCL

† Ksat values at 0-100 cm were obtained from Web Soil Survey and estimated from a weighted average of each soil series per field; averages listed are from 2 or 3 N rate field trials per location.

‡ Hydrometer method (Bouyoucos, 1962); SiCL, silty clay loam; SiC, silty clay; C, clay; CL, clay loam.

Table 4. Soil pH, cation exchange capacity (CEC), organic matter, and Mehlich 3 extractable nutrient concentrations (± standard deviation) averaged across N rates for each location and soil depth.

Depth	pH	CEC	Organic Matter	P	K	Mg	Ca	Na	S	Zn	Mn	Fe	Cu
cm		cmol$_c$ kg^{-1}	g kg^{-1}	----------mg kg^{-1}----------									
West Lafayette													
0-20	6.4 ± 0.2	22.3 ± 1.6	40 ± 6	19 ± 4	136 ± 11	757 ± 44	2486 ± 200	12 ± 1	6 ± 1	1.7 ± 0.1	23 ± 6	32 ± 5	2.1 ± 0.4
20-40	6.7 ± 0.1	22.0 ± 1.1	31 ± 4	3 ± 2	96 ± 7	819 ± 35	2667 ± 162	15 ± 1	4 ± 1	1.2 ± 0.1	27 ± 7	4 ± 4	1.8 ± 0.3
40-60	6.9 ± 0.1	19.3 ± 1.0	23 ± 2	1 ± 0	90 ± 12	753 ± 35	2458 ± 149	16 ± 1	3 ± 1	1.1 ± 0.1	35 ± 3	1 ± 0	1.3 ± 0.2
‡	NRxD*	D**	D**	D**	D**	D**	D**	D**	D**	D**	D*	D** †	D**
LSD, 5%	0.1	1.3	4	0.9	7	44	211	1	2	0.03	7	6	0.4
Farmland													
0-20	6.6 ± 0.3	16.2 ± 1.7	36 ± 4	31 ± 8	136 ± 23	543 ± 45	1938 ± 313	16 ± 6	6 ± 1	2.1 ± 0.3	28 ± 6	63 ± 12	2.5 ± 0.5
20-40	7.0 ± 0.3	19.8 ± 1.4	29 ± 3	3 ± 2	119 ± 17	727 ± 59	2558 ± 320	23 ± 8	4 ± 0	1.6 ± 0.2	31 ± 5	26 ± 7	2.3 ± 0.5
40-60	7.5 ± 0.3	23.2 ± 3.0	25 ± 2	1 ± 0	125 ± 17	817 ± 77	3188 ± 581	27 ± 6	4 ± 1	1.6 ± 0.1	40 ± 4	15 ± 4	2.0 ± 0.4
‡	D**	NS	NS	D** †	NS	D**	NRxD*	D**	NS †	NS	NS	NS	NRxD*
LSD, 5%	2.4			6.4		38	758	25					2.8
Columbia City													
0-20	6.2 ± 0.4	12.1 ± 1.8	29 ± 4	24 ± 7	114 ± 21	274 ± 41	1458 ± 383	12 ± 1	10 ± 1	2.3 ± 0.9	40 ± 7	30 ± 7	1.6 ± 0.4
20-40	6.8 ± 0.3	14.5 ± 2.3	23 ± 3	3 ± 4	92 ± 12	370 ± 68	2058 ± 481	14 ± 2	6 ± 1	1.6 ± 0.6	37 ± 4	19 ± 6	1.6 ± 0.4
40-60	7.2 ± 0.4	20.2 ± 5.3	19 ± 3	2 ± 0	96 ± 15	375 ± 59	3272 ± 1109	16 ± 3	5 ± 1	1.5 ± 0.6	40 ± 4	12 ± 4	1.4 ± 0.3
‡	NRxD*	D**	D**	D** †	NS	D**	D**	D**	D**	NRxD*	NS	NRxD*	D*
LSD, 5%	0.5	2.4	7	1.3		128	361	4	1	0.2		8	0.9

Table 4. Continued.

Depth	**pH**	**CEC**	**Organic Matter**	**P**	**K**	**Mg**	**Ca**	**Na**	**S**	**Zn**	**Mn**	**Fe**	**Cu**
cm		$\text{cmol}_c\ \text{kg}^{-1}$	g kg^{-1}	----------mg kg^{-1}----------									
Wanatah													
0-20	6.4 ± 0.2	19.0 ± 2.0	41 ± 8	17 ± 3	144 ± 19	683 ± 49	2118 ± 209	11 ± 1	5 ± 1	3.4 ± 0.8	7 ± 2	55 ± 12	1.8 ± 0.3
20-40	6.5 ± 0.2	17.4 ± 1.8	24 ± 3	4 ± 1	76 ± 8	666 ± 47	1942 ± 181	12 ± 1	3 ± 1	2.2 ± 0.4	2 ± 1	26 ± 7	2.6 ± 0.5
40-60	6.8 ± 0.3	13.6 ± 1.4	17 ± 2	5 ± 2	77 ± 8	558 ± 58	1594 ± 188	13 ± 1	3 ± 1	2.9 ± 0.5	6 ± 10	20 ± 8	3.4 ± 0.7
‡	D*	D**	D**	D**	D**	D**	D**	D**	D**	NRxD*	NS	D**	D**
LSD, 5%	0.3	2.6	3	0.3	8	37	362	2	1	0.1		21	0.9
Butlerville													
0-20	5.9 ± 0.5	9.0 ± 0.7	28 ± 2	13 ± 7	109 ± 14	193 ± 45	969 ± 216	9 ± 1	7 ± 2	2.8 ± 1.2	73 ± 7	19 ± 4	1.4 ± 0.5
20-40	5.9 ± 0.4	10.1 ± 0.8	21 ± 3	1 ± 0	75 ± 8	226 ± 32	1044 ± 140	10 ± 1	12 ± 4	1.3 ± 0.3	53 ± 10	11 ± 4	0.8 ± 0.1
40-60	5.7 ± 0.4	11.2 ± 1.2	18 ± 2	1 ± 0	78 ± 11	247 ± 24	1078 ± 176	12 ± 1	19 ± 7	1.0 ± 0.2	41 ± 11	7 ± 4	0.6 ± 0.1
‡	D*	D**	D**	NS †	D**	NRxD*	NRxD*	D*	D*	D**	D**	D**	D*
LSD, 5%	1.0	0.5	2		9	45	268	1	7	0.2	8	3	0.3
Lafayette													
0-20	5.8 ± 0.3	12.0 ± 1.2	27 ± 2	25 ± 6	126 ± 20	252 ± 32	1239 ± 198	10 ± 1	7 ± 1	2.3 ± 0.6	39 ± 2	28 ± 5	1.3 ± 0.1
20-40	5.4 ± 0.3	14.5 ± 3.1	25 ± 3	7 ± 5	80 ± 12	263 ± 98	1414 ± 430	12 ± 2	6 ± 2	1.4 ± 0.3	28 ± 4	24 ± 9	1.2 ± 0.2
40-60	5.6 ± 0.4	16.2 ± 3.5	22 ± 3	3 ± 4	103 ± 25	356 ± 147	1625 ± 475	15 ± 3	7 ± 2	1.1 ± 0.1	23 ± 9	19 ± 9	1.1 ± 0.2
‡	NRxD**	NS	D*	NRxD**	NRxD*	NS	NS	D*	NS	D**	D*	NS	NS
LSD, 5%	0.5		3	1.2	27			4		0.1	8		

Significance reported within nutrient column for each location.
‡ Significance of treatment effects. NS = not significant; D = depth; NRxD = N rate x depth interaction
* = $\alpha \leq 0.05$ and ** = $\alpha \leq 0.01$
† Treatments with no variance not included in ANOVA.

Table 5. Soil nitrogen and carbon concentrations (± standard deviation) averaged across previous N rates for each location and sample depth.

Depth	**Ammonium**	**Nitrate**	**Total Inorganic**	**Total Carbon**	**Total Nitrogen**
cm	--------------------------------mg kg^{-1}	-------------------------------		------------------- g kg^{-1}	-------------------
West Lafayette					
0-20	5.2 ± 1.9	6.0 ± 0.8	11.2 ± 2.4	20 ± 2	1.6 ± 0.2
20-40	3.9 ± 1.5	4.9 ± 0.7	8.6 ± 2.0	12 ± 2	1.0 ± 0.2
40-60	3.3 ± 1.8	3.9 ± 0.5	7.2 ± 2.0	6 ± 1	0.6 ± 0.1
‡	D**	D**	NRxD*	D**	D**
LSD, 5%	3.6	1.3	4.2	2.7	0.3
Farmland					
0-20	3.3 ± 1.0	2.9 ± 0.8	6.2 ± 1.5	17 ± 2	1.5 ± 0.2
20-40	3.1 ± 1.0	1.9 ± 0.8	5.0 ± 1.3	11 ± 2	1.0 ± 0.2
40-60	2.5 ± 0.6	1.0 ± 0.3	3.5 ± 0.6	10 ± 2	0.8 ± 0.1
‡	D*	D**	D**	D**	D**
LSD, 5%	3.9	0.4	2.2	1.3	0.2
Columbia City					
0-20	3.8 ± 1.6	5.0 ± 1.6	8.8 ± 2.0	14 ± 2	1.3 ± 0.1
20-40	3.1 ± 1.3	4.4 ± 1.2	7.5 ± 1.6	9 ± 1	0.8 ± 0.1
40-60	3.1 ± 2.0	3.7 ± 1.2	6.8 ± 2.6	8 ± 2	0.6 ± 0.1
‡	NS	D*	D**	D**	NR**, D**
LSD, 5%		1.1	0.8	0.9	0.2
Wanatah					
0-20	9.6 ± 6.0	8.6 ± 2.4	18.2 ± 5.8	21 ± 2	1.6 ± 0.2
20-40	8.6 ± 8.3	6.6 ± 2.3	15.2 ± 8.5	10 ± 2	0.7 ± 0.2
40-60	6.9 ± 5.4	5.9 ± 1.6	12.7 ± 5.4	8 ± 3	0.5 ± 0.1
‡	NRxD**	D**	NRxD*	D**	D**
LSD, 5%	6.7	2.2	6.0	4.4	0.2
Butlerville					
0-20	8.4 ± 3.2	6 ± 1.5	14.4 ± 4.1	10 ± 3	1.1 ± 0.1
20-40	9.5 ± 4.4	6.0 ± 1.5	15.2 ± 5.1	6 ± 2	0.5 ± 0.1
40-60	9.0 ± 3.5	6.1 ± 1.8	15.09 ± 4.7	3 ± 1	0.4 ± 0.0
‡	NS	NS	NS	D**	D**
LSD, 5%				0.6	0.03
Lafayette					
0-20	6.9 ± 1.5	14.3 ± 2.6	21.3 ± 3.5	14 ± 1	1.1 ± 0.1
20-40	6.8 ± 2.4	7.4 ± 1.3	14.3 ± 3.1	10 ± 2	0.9 ± 0.1
40-60	6.8 ± 1.4	5.2 ± 1.2	12.0 ± 1.8	7 ± 2	0.7 ± 0.2
‡	NS	D**	D**	D**	D**
LSD, 5%		0.8	1.3	2.6	0.2

Significance reported within nutrient column for each location.

‡ Significance of treatment effects. NS = not significant; NR = N rate; D = depth; NRxD = N rate x depth interaction

* = α ≤ 0.05 and ** = α ≤ 0.01

Table 6. Total inorganic N release per day (± standard deviation) from the 50 day aerobic incubation study at 25 °C and 33 kPa moisture. Nitrogen release was calculated per day for the 0 to 10 day incubation period, and for the 10 to 50 day incubation period.

Depth (cm)	N release per day 0 to 10 days	N release per day from OM 0 to 10 days	N release per day 10 to 50 days	N release per day from OM 10 to 50 days
cm	------mg N kg^{-1}-----	------mg N g^{-1} OM-----	------mg N kg^{-1}-----	------mg N g^{-1} OM-----
West Lafayette				
0-20	2.0 ± 0.6 a	4.9E-02 ± 7.6E-03 a	2.3 ± 0.4	5.7E-02 ± 2.9E-03 a
20-40	0.5 ± 0.3 b	1.6E-02 ± 6.9E-03 b	1.1 ± 0.2	3.5E-02 ± 3.1E-03 b
40-60	0.03 ± 0.3 c	1.6E-03 ± 9.5E-03 b	0.7 ± 0.2	3.0E-02 ± 7.9E-03 b
‡	D*	D**	NS	D*
Farmland				
0-20	3.1 0.4 a	8.8E-02 ± 9.2E-03 a	3.3 ± 0.4 a	9.3E-02 ± 2.7E-03 a
20-40	1.0 ± 0.2 b	3.5E-02 ± 1.6E-03 b	1.4 ± 0.3 b	4.9E-02 ± 8.6E-03 b
40-60	0.4 ± 0.1 c	1.8E-02 ± 3.9E-03 b	0.6 ± 0.2 c	2.4E-02 ± 8.0E-03 b
‡	D*	D*	D*	D*
Columbia City				
0-20	2.5 ± 0.4 a	8.8E-02 ± 8.9E-03 a	2.9 ± 0.4 a	1.0E-01 ± 8.0E-03 a
20-40	0.9 ± 0.5 b	3.9E-02 ± 1.6E-02 b	1.4 ± 0.5 b	6.2E-02 ± 1.3E-02 b
40-60	0.5 ± 0.5 c	2.2E-02 ± 2.0E-02 b	0.1 ± 0.4 c	5.0E-02 ± 8.8E-03 b
‡	D*	D**	D*	D**
Wanatah				
0-20	1.2 ± 0.8	2.9E-02 ± 1.9E-03 a	2.2 ± 0.4	5.4E-02 ± 2.5E-03 a
20-40	-0.4 ± 0.9	-1.6E-02 ± 1.7E-02 b	0.8 ± 0.1	3.3E-02 ± 2.2E-03 b
40-60	-0.3 ± 0.7	-2.1E-02 ± 3.2E-02 b	0.5 ± 0.1	2.9E-02 ± 8.5E-03 b
‡	NS	D*	NS	D**
Butlerville				
0-20	2.6 ± 1.4 a	9.2E-02 ± 2.8E-02 a	3.9 ± 1.2 a	1.4E-01 ± 1.8E-02 a
20-40	0.03 ± 0.5 b	7.2E-04 ± 1.1E-02 b	1.7 ± 0.3 b	8.0E-02 ± 7.9E-03 b
40-60	-0.2 ± 0.7 b	-1.4E-02 ± 1.6E-02 b	1.5 ± 0.8 b	7.9E-02 ± 2.4E-02 b
‡	D*	D*	D*	D*
Lafayette				
0-20	-1.0 ± 0.3	-3.8E-02 ± 4.6E-03 b	0.9 ± 0.1	3.3E-02 ± 4.6E-03
20-40	-0.5 ± 0.3	-1.8E-02 ± 6.7E-03 a	0.8 ± 0.1	3.2E-02 ± 2.4E-03
40-60	-0.5 ± 0.3	-2.5E-02 ± 1.4E-02 a	0.6 ± 0.2	2.7E-02 ± 6.1E-03
‡	NS	D*	NS	NS

Significance reported within soil depth for each location. Depths with the same letters are not significantly different (α ≤ 0.05).
‡ Significance of treatment effects. NS = not significant; D = depth
* = α ≤ 0.05, and ** = α ≤ 0.01

Table 7. Earleaf at silking nutrient concentration (± standard deviation) averaged across previous N rates (ranked low to high) for all locations. Cumulative N rates are listed in Table 1.

N Rate	N	P	K	Ca	Mg	S	Zn	Mn	Fe	Cu	Al
	g kg^{-1}						mg kg^{-1}				
West Lafayette											
1	14.7 ± 1.3	2.5 ± 0.1	20.4 ± 0.5	2.5 ± 0.2	1.6 ± 0.4	1.2 ± 0.1	8 ± 1	18 ± 1	47 ± 4	4 ± 1	14 ± 8
2	15.4 ± 1.2	2.6 ± 0.2	22.3 ± 1.3	2.3 ± 0.3	1.5 ± 0.3	1.2 ± 0.1	10 ± 3	24 ± 6	47 ± 8	5 ± 1	4 ± 2
3	15.2 ± 0.6	2.5 ± 0.1	20.8 ± 0.6	2.3 ± 0.4	1.5 ± 0.2	1.2 ± 0.1	10 ± 2	23 ± 7	49 ± 11	5 ± 0	9 ± 6
4	14.7 ± 0.9	2.4 ± 0.1	20.6 ± 1.7	2.6 ± 0.2	1.7 ± 0.3	1.2 ± 0.1	8 ± 1	19 ± 6	49 ± 6	5 ± 1	1 ± 1
5	14.5 ± 0.3	2.5 ± 0.3	20.3 ± 0.3	2.4 ± 0.3	1.6 ± 0.4	1.2 ± 0.1	8 ± 1	20 ± 2	48 ± 2	5 ± 1	2 ± 2
6	15.4 ± 0.6	2.6 ± 0.2	21.7 ± 0.7	2.3 ± 0.4	1.6 ± 0.4	1.2 ± 0.1	10 ± 2	26 ± 6	45 ± 6	5 ± 1	2 ± 2
‡	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NR*
Farmland											
1	14.2 ± 0.1	3.6 ± 0.1	23.7 ± 2.5	2.8 ± 0.0	1.7 ± 0.4	1.1 ± 0.1	12 ± 5	32 ± 4	55 ± 7	5 ± 0	11 ± 1
2	15.0 ± 1.6	3.3 ± 0.1	23.1 ± 2.1	2.6 ± 0.5	1.6 ± 0.6	1.2 ± 0.0	9 ± 1	41 ± 13	54 ± 5	5 ± 0	16 ± 11
3	14.0 ± 0.5	3.4 ± 0.0	22.8 ± 2.1	2.5 ± 0.3	1.5 ± 0.5	1.1 ± 0.0	10 ± 1	30 ± 12	53 ± 6	5 ± 1	5 ± 1
4	15.1 ± 0.1	3.4 ± 0.1	22.4 ± 2.1	2.7 ± 0.4	1.9 ± 0.6	1.2 ± 0.1	10 ± 1	44 ± 9	54 ± 6	6 ± 1	6 ± 6
5	14.6 ± 0.1	3.3 ± 0.1	24.0 ± 2.6	2.6 ± 0.5	1.7 ± 0.4	1.1 ± 0.0	9 ± 1	42 ± 2	51 ± 0	5 ± 0	7 ± 8
6	14.0 ± 0.2	3.4 ± 0.1	23.1 ± 2.1	2.7 ± 0.1	1.7 ± 0.5	1.1 ± 0.0	8 ± 0	40 ± 3	51 ± 6	5 ± 1	8 ± 1
‡	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS †	NS
Columbia City											
1	13.7 ± 1.3	2.6 ± 0.2	21.0 ± 0.6	3.2 ± 0.4	1.7 ± 0.2	1.1 ± 0.0	10 ± 1	31 ± 10	47 ± 1	5 ± 1	4 ± 3
2	13.0 ± 1.1	2.6 ± 0.1	22.0 ± 2.5	2.7 ± 0.2	1.3 ± 0.1	1.0 ± 0.1	10 ± 2	31 ± 3	44 ± 4	4 ± 1	10 ± 8
3	13.5 ± 1.6	2.4 ± 0.2	21.1 ± 1.0	2.5 ± 0.3	1.2 ± 0.1	1.0 ± 0.1	9 ± 1	32 ± 1	43 ± 4	4 ± 1	17 ± 2
4	13.4 ± 1.8	2.6 ± 0.2	21.9 ± 0.1	2.5 ± 0.3	1.3 ± 0.2	1.1 ± 0.1	9 ± 0	33 ± 7	53 ± 14	4 ± 1	10 ± 10
5	13.1 ± 1.6	2.5 ± 0.1	21.1 ± 1.7	2.7 ± 0.1	1.4 ± 0.3	1.1 ± 0.1	9 ± 2	36 ± 3	54 ± 15	4 ± 1	6 ± 4
6	13.4 ± 0.9	2.6 ± 0.2	21.2 ± 0.7	2.5 ± 0.3	1.3 ± 0.2	1.0 ± 0.1	9 ± 1	41 ± 3	47 ± 8	4 ± 0	14 ± 16
‡	NS	NS	NS	NR*	NS	NS	NS	NS	NS	NS	NS

Table 7. Continued.

N Rate	N	P	K	Ca	Mg	S	Zn	Mn	Fe	Cu	Al
	g kg^{-1}						mg kg^{-1}				
Wanatah											
1	16.6 ± 1.2	2.7 ± 0.2	24.5 ± 1.4	2.9 ± 0.2	2.3 ± 0.3	1.3 ± 0.2	19 ± 3	16 ± 3	58 ± 7	6 ± 1	8 ± 9
2	18.0 ± 0.9	2.8 ± 0.2	24.0 ± 1.1	3.1 ± 0.3	2.4 ± 0.3	1.3 ± 0.1	20 ± 2	16 ± 5	62 ± 6	6 ± 1	4 ± 2
3	17.6 ± 1.6	2.9 ± 0.1	25.0 ± 0.9	3.0 ± 0.2	2.4 ± 0.3	1.4 ± 0.1	20 ± 3	18 ± 6	61 ± 7	6 ± 1	8 ± 7
4	18.8 ± 1.4	2.9 ± 0.2	24.3 ± 1.4	3.1 ± 0.1	2.5 ± 0.2	1.4 ± 0.2	21 ± 3	19 ± 6	65 ± 8	6 ± 1	9 ± 3
5	18.5 ± 1.0	2.7 ± 0.0	23.7 ± 0.5	3.0 ± 0.3	2.3 ± 0.4	1.3 ± 0.1	19 ± 2	18 ± 3	60 ± 6	6 ± 1	9 ± 7
6	18.5 ± 1.0	2.7 ± 0.2	23.9 ± 1.8	3.4 ± 0.4	2.6 ± 0.2	1.4 ± 0.1	21 ± 3	18 ± 3	63 ± 1	6 ± 1	4 ± 5
‡	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Butlerville											
1	19.8 ± 1.2	2.9 ± 0.1	23.6 ± 0.6	3.6 ± 0.1	1.9 ± 0.0	1.5 ± 0.1	18 ± 2	47 ± 10	70 ± 3	7 ± 1	5 ± 6
2	19.4 ± 3.4	2.9 ± 0.4	23.0 ± 1.7	3.8 ± 0.4	2.2 ± 0.3	1.5 ± 0.3	19 ± 1	44 ± 19	72 ± 15	7 ± 2	3 ± 3
3	18.8 ± 0.7	2.7 ± 0.2	23.8 ± 2.0	3.4 ± 0.5	1.7 ± 0.2	1.4 ± 0.1	19 ± 3	40 ± 2	72 ± 4	7 ± 1	10 ± 3
4	20.7 ± 1.0	2.9 ± 0.1	25.3 ± 0.4	4.0 ± 0.2	2.1 ± 0.4	1.6 ± 0.1	20 ± 3	44 ±7	76 ± 4	7 ± 0	17 ± 2
5	20.6 ± 2.1	2.8 ± 0.1	23.9 ± 1.1	3.7 ± 0.1	1.9 ± 0.2	1.5 ± 0.2	20 ± 1	45 ± 6	77 ± 12	7 ± 1	9 ± 9
6	20.2 ± 1.0	2.9 ± 0.1	24.6 ± 0.1	3.6 ± 0.4	1.8 ± 0.4	1.5 ± 0.1	19 ± 3	52 ± 3	82 ± 18	8 ± 1	14 ± 18
‡	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Lafayette											
1	18.2 ± 3.3	2.8 ± 0.3	24.3 ± 3.8	2.9 ± 0.6	1.8 ± 0.2	1.4 ± 0.3	15 ± 5	40 ± 14	62 ± 13	6 ± 1	10 ± 4
2	19.2 ± 1.6	2.6 ± 0.1	24.2 ± 0.5	2.9 ± 0.5	2.0 ± 0.4	1.4 ± 0.1	14 ± 3	43 ± 5	69 ± 9	6 ± 1	11 ± 7
3	18.4 ± 2.5	2.6 ± 0.2	23.2 ± 2.4	2.9 ± 0.3	1.9 ± 0.2	1.4 ± 0.2	13 ± 3	37 ± 8	63 ± 10	6 ± 1	8 ± 6
4	18.6 ± 2.2	2.5 ± 0.2	23.1 ± 2.5	2.9 ± 0.5	2.0 ± 0.3	1.4 ± 0.2	15 ± 3	43 ± 10	69 ± 23	6 ± 1	16 ± 12
5	17.9 ± 2.2	2.6 ± 0.3	24.0 ± 1.8	3.0 ± 0.4	1.9 ± 0.2	1.4 ± 0.1	13 ± 3	40 ± 5	60 ± 5	6 ± 1	7 ± 10
6	19.9 ± 2.1	2.7 ± 0.2	24.5 ± 1.6	2.8 ± 0.3	1.7 ± 0.1	1.5 ± 0.1	15 ± 3	48 ± 6	64 ± 6	7 ± 1	13 ± 10
‡	NR*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Significance reported within nutrient column for each location.
‡ Significance of treatment effects. NS = not significant; NR = N rate; * = $\alpha \leq 0.05$
† Treatments with no variance not included in ANOVA.

Table 8. Stover at maturity nutrient concentration (± standard deviation) averaged across previous N rates (ranked low to high) for all locations. Cumulative N rates are listed in Table 1.

N Rate	N	P	K	Ca	Mg	S	Zn	Mn	Fe	Cu	Al
	g kg^{-1}						mg kg^{-1}				
West Lafayette											
1	5.0 ± 0.2	2.0 ± 0.7	9.4 ± 0.3	2.8 ± 0.1	2.4 ± 0.1	0.6 ± 0.1	30 ± 11	26 ± 5	59 ± 6	3 ± 1	20 ± 12
2	5.1 ± 0.4	2.1 ± 0.5	9.1 ± 0.5	2.6 ± 0.1	2.3 ± 0.2	0.5 ± 0.1	33 ± 8	25 ± 9	60 ± 8	3 ± 1	25 ± 22
3	5.2 ± 0.4	1.8 ± 0.7	9.0 ± 0.4	2.6 ± 0.3	2.2 ± 0.3	0.5 ± 0.1	28 ± 9	24 ± 6	52 ± 6	3 ± 2	33 ± 14
4	4.9 ± 0.4	1.8 ± 0.3	9.4 ± 0.2	2.8 ± 0.1	2.4 ± 0.1	0.6 ± 0.1	29 ± 5	28 ± 8	69 ± 4	3 ± 0	55 ± 11
5	5.4 ± 0.8	2.1 ± 0.7	9.2 ± 0.3	2.8 ± 0.1	2.4 ± 0.3	0.6 ± 0.1	33 ± 11	25 ± 7	82 ± 27	3 ± 1	48 ± 14
6	5.0 ± 0.3	1.7 ± 0.7	9.1 ± 0.5	2.6 ± 0.2	2.2 ± 0.2	0.5 ± 0.0	25 ± 7	24 ± 6	61 ± 10	3 ± 1	35 ± 19
‡	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS †	NS
Farmland											
1	6.4 ± 0.7	4.5 ± 0.1	12.7 ± 0.2	3.5 ± 0.1	3.4 ± 0.6	0.8 ± 0.1	61 ± 6	50 ± 6	155 ± 63	4 ± 2	115 ± 16
2	6.4 ± 0.5	4.3 ± 0.1	11.8 ± 1.2	3.3 ± 0.0	3.5 ± 0.2	0.8 ± 0.1	64 ± 6	58 ± 5	106 ± 16	3 ± 0	45 ± 11
3	6.3 ± 0.1	4.5 ± 0.1	12.6 ± 1.1	3.3 ± 0.2	3.4 ± 0.5	0.8 ± 0.1	62 ± 5	48 ± 13	89 ± 13	4 ± 0	52 ± 5
4	6.7 ± 0.1	4.2 ± 0.1	12.3 ± 1.6	3.5 ± 0.1	3.4 ± 0.5	0.8 ± 0.0	63 ± 17	53 ± 4	130 ± 28	3 ± 0	56 ± 22
5	6.3 ± 0.1	4.3 ± 0.3	12.3 ± 0.9	3.3 ± 0.2	3.3 ± 0.1	0.8 ± 0.1	63 ± 4	47 ± 7	118 ± 10	3 ± 0	59 ± 13
6	6.2 ± 0.1	4.1 ± 0.7	12.2 ± 0.9	3.3 ± 0.4	3.1 ± 0.4	0.8 ± 0.1	62 ± 11	80 ± 18	133 ± 12	4 ± 1	96 ± 21
‡	NS	NS	NS	NS	NS	NS	NS	NS	NS	NA †	NR*
Columbia City											
1	4.7 ± 0.5	2.5 ± 0.7	15.5 ± 3.9	2.9 ± 0.4	2.0 ± 0.6	0.7 ± 0.2	29 ± 13	46 ± 21	168 ± 120	3 ± 0	61 ± 60
2	4.7 ± 0.5	2.1 ± 0.3	13.5 ± 1.2	2.8 ± 0.2	1.7 ± 0.2	0.6 ± 0.0	27 ± 7	44 ± 14	89 ± 3	3 ± 1	38 ± 5
3	4.9 ± 0.6	2.2 ± 0.4	14.6 ± 1.6	2.5 ± 0.3	1.8 ± 0.4	0.6 ± 0.1	31 ± 8	48 ± 7	93 ± 38	2 ± 1	48 ± 25
4	5.3 ± 0.6	2.9 ± 1.1	13.7 ± 2.4	2.6 ± 0.1	1.9 ± 0.3	0.7 ± 0.1	38 ± 17	45 ± 6	117 ± 20	3 ± 1	62 ± 34
5	5.1 ± 0.5	2.9 ± 0.6	14.0 ± 1.9	2.6 ± 0.3	2.0 ± 0.3	0.7 ± 0.1	39 ± 12	48 ± 7	106 ± 51	3 ± 0	64 ± 33
6	5.0 ± 0.4	2.6 ± 1.0	13.7 ± 1.6	2.7 ± 0.2	2.2 ± 0.3	0.8 ± 0.2	39 ± 19	48 ± 3	98 ± 21	3 ± 1	57 ± 24
‡	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS †	NS

Table 8. Continued.

N Rate	N	P	K	Ca	Mg	S	Zn	Mn	Fe	Cu	Al
	---------- g kg^{-1} ----------						---------- mg kg^{-1} ----------				
Wanatah											
1	4.0 ± 0.3	1.6 ± 0.3	14.0 ± 0.9	2.1 ± 0.1	1.9 ± 0.2	0.4 ± 0.0	19 ± 4	15 ± 7	225 ± 95	3 ± 1	90 ± 23
2	4.2 ± 0.2	2.3 ± 0.5	15.6 ± 0.9	2.2 ± 0.1	2.1 ± 0.1	0.4 ± 0.1	30 ± 5	19 ± 7	296 ± 204	3 ± 1	92 ± 35
3	4.1 ± 0.2	1.7 ± 0.7	13.6 ± 0.9	2.1 ± 0.2	2.0 ± 0.3	0.4 ± 0.1	24 ± 5	16 ± 6	224 ± 130	2 ± 1	72 ± 33
4	3.9 ± 0.3	1.4 ± 0.5	13.8 ± 0.4	2.0 ± 0.2	1.9 ± 0.2	0.4 ± 0.0	22 ± 6	16 ± 7	194 ± 59	3 ± 0	66 ± 14
5	4.1 ± 0.1	1.7 ± 0.7	14.1 ± 1.1	2.1 ± 0.1	2.0 ± 0.1	0.4 ± 0.0	26 ± 3	16 ± 3	212 ± 74	3 ± 1	65 ± 12
6	4.1 ± 0.3	1.3 ± 0.2	12.7 ± 0.9	2.1 ± 0.1	2.1 ± 0.1	0.4 ± 0.1	21 ± 3	16 ± 7	235 ± 102	3 ± 1	81 ± 27
‡	NS	NS	NR*	NS	NS	NS †	NR*	NS	NS	NS	NS
Butlerville											
1	4.3 ± 0.1	1.5 ± 0.5	15.5 ± 0.1	2.3 ± 0.3	1.5 ± 0.3	0.6 ± 0.1	23 ± 3	55 ± 10	68 ± 17	2 ± 0	36 ± 15
2	4.3 ± 0.3	1.7 ± 0.6	14.7 ± 2.6	2.2 ± 0.2	1.6 ± 0.5	0.6 ± 0.1	30 ± 9	57 ± 1	70 ± 27	3 ± 0	17 ± 13
3	4.4 ± 0.2	1.7 ± 0.2	17.4 ± 1.9	2.2 ± 0.2	1.4 ± 0.2	0.6 ± 0.0	21 ± 3	47 ± 8	51 ± 3	4 ± 0	13 ± 16
4	4.2 ± 0.1	1.6 ± 0.3	16.7 ± 2.4	2.2 ± 0.3	1.5 ± 0.3	0.6 ± 0.1	24 ± 2	50 ± 9	76 ± 20	4 ± 0	21 ± 17
5	4.4 ± 0.2	1.1 ± 0.3	13.9 ± 1.7	2.2 ± 0.3	1.4 ± 0.4	0.6 ± 0.1	21 ± 2	61 ± 7	62 ± 17	3 ± 1	16 ± 12
6	4.4 ± 0.3	1.6 ± 0.5	16.1 ± 0.6	2.4 ± 0.1	1.5 ± 0.2	0.6 ± 0.1	24 ± 3	56 ± 7	50 ± 10	3 ± 1	15 ± 24
‡	NS	NS	NS	NS	NS	NS	NR*	NS	NS	NS	NS
Lafayette											
1	4.9 ± 0.6	1.5 ± 0.6	15.2 ± 3.5	2.6 ± 0.4	1.5 ± 0.3	0.5 ± 0.1	18 ± 3	60 ± 12	61 ± 8	4 ± 2	10 ± 16
2	4.6 ± 0.1	0.8 ± 0.2	13.0 ± 1.6	2.2 ± 0.3	1.5 ± 0.2	0.4 ± 0.1	12 ± 4	57 ± 4	58 ± 12	3 ± 0	9 ± 7
3	4.8 ± 0.2	1.2 ± 1.0	14.6 ± 1.8	2.6 ± 0.3	1.7 ± 0.3	0.5 ± 0.0	16 ± 9	57 ± 4	59 ± 7	3 ± 1	12 ± 17
4	4.9 ± 0.1	1.2 ± 1.1	12.8 ± 2.3	2.6 ± 0.1	1.7 ± 0.4	0.5 ± 0.1	20 ± 9	55 ± 10	70 ± 3	3 ± 1	10 ± 9
5	4.5 ± 0.3	0.9 ± 0.5	13.9 ± 1.0	2.5 ± 0.2	1.6 ± 0.2	0.4 ± 0.1	12 ± 4	52 ± 6	66 ± 9	2 ± 1	12 ± 12
6	5.0 ± 0.4	1.2 ± 1.2	13.7 ± 1.1	2.7 ± 0.3	1.7 ± 0.4	0.5 ± 0.1	17 ± 10	63 ± 12	59 ± 7	3 ± 1	16 ± 13
‡	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Significance reported within nutrient column for each location.

‡ Significance of treatment effects. NS = not significant; NR = N rate; NA = Not applicable because 4 treatments had zero variance; * = $\alpha \leq 0.05$

† Treatments with no variance not included in ANOVA.

Table 9. Grain at maturity nutrient concentration (± standard deviation) averaged across previous N rates (ranked low to high) for all locations. Cumulative N rates are listed in Table 1.

N Rate	N	P	K	Mg	S	Zn	Mn	Fe	Al
	g kg^{-1}					mg kg^{-1}			
West Lafayette									
1	9.4 ± 0.5	2.9 ± 0.4	4.9 ± 0.7	1.2 ± 0.2	0.9 ± 0.1	19 ± 3	6 ± 1	12 ± 3	21 ± 9
2	8.8 ± 0.5	3.0 ± 0.4	5.1 ± 0.5	1.2 ± 0.2	0.9 ± 0.2	20 ± 3	5 ± 1	12 ± 1	20 ± 15
3	8.9 ± 0.5	2.9 ± 0.4	4.9 ± 0.4	1.2 ± 0.2	0.8 ± 0.1	20 ± 1	6 ± 2	13 ± 3	12 ± 3
4	9.2 ± 0.4	3.1 ± 0.3	5.3 ± 0.5	1.3 ± 0.1	0.8 ± 0.1	21 ± 2	6 ± 2	12 ± 3	26 ± 12
5	9.5 ± 0.9	3.4 ± 0.1	5.4 ± 0.2	1.4 ± 0.1	0.9 ± 0.1	22 ± 1	6 ± 1	14 ± 2	4 ± 5
6	9.0 ± 0.1	2.9 ± 0.2	5.1 ± 0.3	1.2 ± 0.1	0.9 ± 0.1	20 ± 2	7 ± 2	13 ± 2	21 ± 11
‡	NS	NS	NS	NS	NS	NS	NS	NS	NS
Farmland									
1	10.2 ± 0.4	3.2 ± 0.1	4.8 ± 0.2	1.3 ± 0.0	1.0 ± 0.0	22 ± 1	4 ± 2	13 ± 2	10 ± 12
2	11.8 ± 1.2	3.3 ± 0.4	4.6 ± 0.3	1.4 ± 0.3	1.0 ± 0.1	23 ± 3	6 ± 1	16 ± 4	6 ± 7
3	10.5 ± 0.6	3.5 ± 0.1	5.0 ± 0.3	1.5 ± 0.1	1.0 ± 0.1	23 ± 2	6 ± 1	17 ± 1	2 ± 1
4	12.0 ± 1.1	3.8 ± 0.1	5.4 ± 0.1	1.6 ± 0.1	1.0 ± 0.0	25 ± 1	7 ± 1	16 ± 0	13 ± 6
5	11.6 ± 0.5	3.7 ± 0.6	5.3 ± 0.1	1.6 ± 0.3	1.0 ± 0.1	26 ± 2	8 ± 1	17 ± 4	2 ± 1
6	10.4 ± 0.7	3.4 ± 0.3	4.8 ± 0.3	1.5 ± 0.1	1.0 ± 0.0	23 ± 1	6 ± 0	17 ± 2	4 ± 4
‡	NS	NS	NR*	NS	NA †	NR*	NS	NS	NS
Columbia City									
1	9.2 ± 1.2	2.9 ± 0.4	4.9 ± 0.2	1.2 ± 0.2	0.9 ± 0.1	18 ± 3	4 ± 2	12 ± 2	9 ± 4
2	8.8 ± 0.5	3.3 ± 0.2	5.4 ± 0.6	1.3 ± 0.1	0.9 ± 0.1	19 ± 2	4 ± 1	13 ± 1	3 ± 3
3	9.0 ± 0.4	3.0 ± 0.6	4.9 ± 0.5	1.2 ± 0.2	0.9 ± 0.1	21 ± 3	4 ± 1	13 ± 2	3 ± 2
4	9.1 ± 1.3	3.5 ± 0.6	5.2 ± 0.7	1.4 ± 0.2	0.9 ± 0.1	22± 2	5 ± 2	14 ± 1	9 ± 9
5	9.3 ± 0.2	3.4 ± 0.2	5.3 ± 0.2	1.4 ± 0.1	0.9 ± 0.1	21 ± 0	5 ± 2	14 ± 1	6 ± 9
6	9.4 ± 0.8	3.4 ± 0.4	5.4 ± 0.1	1.5 ± 0.2	0.9 ± 0.1	23 ± 3	6 ± 2	14 ± 2	5 ± 4
‡	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 9. Continued.

N Rate	N	P	K	Mg	S	Zn	Mn	Fe	Al
	g kg^{-1}					mg kg^{-1}			
Wanatah									
1	8.8 ± 0.3	3.1 ± 0.1	5.4 ± 0.3	1.1 ± 0.1	0.8 ± 0.0	20 ± 2	7 ± 2	14 ± 1	11 ± 17
2	8.2 ± 0.5	2.5 ± 0.6	4.9 ± 0.2	0.9 ± 0.2	0.7 ± 0.1	17 ± 3	6 ± 2	11 ± 1	7 ± 6
3	8.5 ± 0.4	3.0 ± 0.1	5.1 ± 0.3	1.1 ± 0.0	0.8 ± 0.1	19 ± 0	7 ± 2	15 ± 1	32 ± 10
4	8.7 ± 0.4	3.0 ± 0.3	5.1 ± 0.5	1.1 ± 0.1	0.8 ± 0.0	19 ± 1	4 ± 1	14 ± 2	28 ± 26
5	8.5 ± 0.4	2.8 ± 0.1	4.8 ± 0.1	1.0 ± 0.1	0.7 ± 0.1	18 ± 1	5 ± 2	13 ± 1	18 ± 9
6	8.6 ± 0.7	3.1 ± 0.2	5.2 ± 0.3	1.2 ± 0.1	0.8 ± 0.0	20 ± 3	7 ± 3	16 ± 2	16 ± 13
‡	NS	NS	NS	NS	NS †	NS	NS	NR*	NS
Butlerville									
1	8.8 ± 0.7	3.2 ± 0.3	5.8 ± 0.4	1.2 ± 0.1	0.8 ± 0.1	19 ± 2	7 ± 2	13 ± 1	18 ± 15
2	9.0 ± 1.0	3.5 ± 0.4	6.1 ± 0.2	1.2 ± 0.2	0.8 ± 0.1	21 ± 2	6 ± 1	14 ± 3	16 ± 7
3	9.2 ± 0.9	3.1 ± 0.1	5.5 ± 0.1	1.2 ± 0.1	0.8 ± 0.0	20 ± 4	7 ± 4	13 ± 2	15 ± 10
4	8.4 ± 0.5	3.2 ± 0.5	5.8 ± 0.4	1.2 ± 0.2	0.8 ± 0.0	19 ± 2	7 ± 2	13 ± 1	17 ± 16
5	8.6 ± 0.3	2.8 ± 0.4	5.5 ± 0.5	1.0 ± 0.2	0.8 ± 0.0	18 ± 3	7 ± 1	15 ± 2	15 ± 20
6	9.0 ± 0.3	3.0 ± 0.1	5.5 ± 0.1	1.1 ± 0.0	0.8 ± 0.1	18 ± 1	6 ± 3	14 ± 1	10 ± 16
‡	NS	NS	NS	NS	NS †	NS	NS	NS	NS
Lafayette									
1	8.8 ± 0.6	2.8 ± 0.6	5.0 ± 0.8	1.1 ± 0.3	0.8 ± 0.1	17 ± 3	5 ± 2	12 ± 3	1 ± 0
2	8.6 ± 0.2	2.8 ± 0.1	5.3 ± 0.2	1.1 ± 0.0	0.8 ± 0.1	18 ± 1	7 ± 2	14 ± 1	2 ± 2
3	9.7 ± 0.9	3.2 ± 0.2	5.5 ± 0.2	1.2 ± 0.1	0.8 ± 0.1	19 ± 0	7 ± 1	16 ± 1	1 ± 0
4	9.2 ± 0.9	2.9 ± 0.7	5.1 ± 0.7	1.1 ± 0.3	0.8 ± 0.1	20 ± 6	7 ± 1	14 ± 2	2 ± 2
5	8.8 ± 0.3	2.7 ± 0.3	5.0 ± 0.4	1.0 ± 0.1	0.8 ± 0.0	18 ± 1	5 ± 2	15 ± 1	5 ± 6
6	9.2 ± 0.3	2.9 ± 0.5	5.3 ± 0.4	1.2 ± 0.2	0.8 ± 0.1	19 ± 4	7 ± 2	15 ± 3	1 ± 0
‡	NS	NS	NS	NS	NS	NS	NS	NS	NS †

Significance reported within nutrient column for each location.

‡ Significance of treatment effects. NS = not significant; NR = N rate; NA = Not applicable because 3 treatments had zero variance; * = $\alpha \leq 0.05$

† Treatments with no variance not included in ANOVA.

Table 10. Plant N content and grain yield at 50% moisture (± standard deviation) across previous N rates (ranked low to high) for all locations. Cumulative N rates are listed in Table 1.

N Rate	**Total Plant N** (kg N ha^{-1})	**Grain Yield** (kg ha^{-1})	**Total Plant N** (kg N ha^{-1})	**Grain Yield** (kg ha^{-1})
	West Lafayette		**Wanatah**	
1	33 ± 11.0	2464 ± 816	59 ± 7.3	5779 ± 716
2	29 ± 9.2	2182 ± 732	62 ± 12.8	5522 ± 400
3	32 ± 5.1	2383 ± 420	62 ± 1.0	5553 ± 851
4	39 ± 8.9	2899 ± 691	65 ± 5.7	5920 ± 1358
5	32 ± 17.6	2379 ± 1355	63 ± 3.3	5662 ± 912
6	40 ± 9.2	3004 ± 735	62 ± 4.1	6043 ± 399
‡	NS	NS	NS	NS
	Farmland		**Butlerville**	
1	8 ± 0.2	503 ± 11	67 ± 10.7	5801 ± 528
2	6 ± 2.6	400 ± 170	62 ± 11.5	5401 ± 406
3	7 ± 1.6	457 ± 105	62 ± 4.9	5495 ± 305
4	7 ± 2.4	447 ± 156	66 ± 10.6	5832 ± 423
5	7 ± 2.0	443 ± 132	68 ± 12.9	5814 ± 642
6	8 ± 1.6	529 ± 104	66 ± 5.5	5890 ± 289
‡	NS	NS	NS	NS
	Columbia City		**Lafayette**	
1	38 ± 21.5	3015 ± 1564	60 ± 31.0	5934 ± 3099
2	31 ± 8.6	2555 ± 716	72 ± 21.4	7143 ± 2107
3	34 ± 10.9	2818 ± 903	65 ± 36.3	6396 ± 3624
4	28 ± 10.5	2222 ± 969	68 ± 31.5	6823 ± 3199
5	29 ± 8.2	2250 ± 757	70 ± 27.0	6981 ± 2650
6	27 ± 6.1	2242 ± 496	68 ± 31.8	6784 ± 3192
‡	NS	NS	NS	NS

Significance reported within nutrient column for each location.

‡ Significance of treatment effects. NS = not significant

Table 11. Air temperature and precipitation monthly averages, and 30-yr normal from previous corn year (2013) through last corn year (2015) for all locations.

Location	2013	2014	2015	30-yr normals	2013	2014	2015	30-yr normals
Month	------------Precipitation (mm)--------------				---------------Temperature (°C)--------------			
West Lafayette								
Jan	112	48	53	49	-2	-8	-5	-4
Feb	61	121	30	47	-2	-7	-7	-2
Mar	24	78	60	66	1	0	2	4
Apr	161	56	63	91	10	11	11	11
May	77	82	88	121	18	17	18	16
Jun	107	102	201	104	21	22	21	22
Jul	68	78	163	107	22	20	22	23
Aug	44	170	28	92	21	22	21	22
Sep	89	129	78	72	19	17	20	18
Oct	37	100	24	77	12	12	13	12
Nov	52	35	53	82	4	1	8	5
Dec	137	60	209	62	-2	1	5	-2
Farmland								
Jan	132	22	43	53	-2	-8	-5	-4
Feb	40	25	20	49	-2	-6	-8	-2
Mar	38	7	197	72	0	0	2	3
Apr	151	155	110	93	10	10	11	10
May	54	117	7	112	18	16	18	16
Jun	52	166	226	108	21	22	21	21
Jul	29	63	110	121	22	20	22	23
Aug	19	183	62	90	21	21	21	22
Sep	96	63	98	75	18	17	20	18
Oct	77	55	51	75	12	11	12	11
Nov	100	79	73	86	4	1	8	5
Dec	136	38	327	66	-1	1	5	-1
Columbia City								
Jan	60	41	44	58	-2	-9	-5	-5
Feb	37	63	22	54	-3	-7	-8	-3
Mar	23	84	87	73	0	-2	1	3
Apr	194	98	177	93	8	10	11	9
May	46	79	59	109	18	16	18	15
Jun	196	108	218	113	21	22	21	20
Jul	65	54	163	104	22	21	22	22
Aug	120	84	87	98	21	22	21†	21
Sep	44	118	71	81	19	17	20	17
Oct	60	81	32	76	12	12	13	11
Nov	73	75	43	85	4	2	8	5
Dec	63	62	92	69	-2	1	5	-2

Table 11. Continued.

Location	2013	2014	2015	30-yr normals	2013	2014	2015	30-yr normals
Month	------------Precipitation (mm)-------------				---------------Temperature (°C)--------------			
Wanatah								
Jan	75	49	28	49	-3	-9	-6	-5
Feb	40	56	43	44	-3	-9	-9	-3
Mar	27	30	0	61	0	-2	0	3
Apr	150	71	56	87	8	9	7	9
May	86	95	95	98	16	16	15	15
Jun	242	248	120	108	20	21	19	20
Jul	63	83	114	109	22	20	21	22
Aug	112	234	86	104	21	21	20	21
Sep	78	68	91	85	18	19	17	17
Oct	136	80	28	87	11	11	9	11
Nov	77	76	62	89	3	1	7	4
Dec	39	30	128	59	-4	1	4	-3
Butlerville								
Jan	103	83†	96†	77	1	-5†	-1†	-1
Feb	51	82	43	75	0	-2	-5	2
Mar	84†	47	162	95	3†	3	5	7
Apr	102	173	185†	114	12	13	13	13
May	97	118	65	128	18	18	19	18
Jun	154	110	160	109	22	23	22	22
Jul	40	108	283	115	23	21	23†	24
Aug	19	43	74	109	22	23	22	23
Sep	103	70	38	80	20	18	20	20
Oct	106	92	120	93	13	13	14	14
Nov	66	57	123	102	5	3	9	8
Dec	138	98†	123	90	1	3†	7	1
Lafayette								
Jan	86	0	0	53	-2	-7	-4	-4
Feb	67	0	0	50	-2	-7	-7	-2
Mar	31	0	0	69	1	1	2	4
Apr	168	50	17	87	10	11	12	11
May	37	81	70	118	18	17	19	17
Jun	57	69	152	116	22	23	22	22
Jul	50	65	144	104	22	21	23	23
Aug	41	67	36	100	22	22	22	22
Sep	22	77	74	71	20	18	21	19
Oct	28	128	43	69	12	12	13	12
Nov	54	50	56	85	4	2	8	6
Dec	1	0	176	68	-2	2	5	-2

† Monthly average contains up to three missing daily precipitation or temperature points.

Table 12. ANOVA summary table for soil total inorganic nitrogen from a 50 day aerobic incubation at 25 °C and 33 kPa moisture for all locations. Seven N rates ranging from 24 to 272 kg N ha^{-1}, three soil depths at 0-20, 20-40, and 40-60 cm, and three incubation sample times at 0, 10, and 50 days were used in the ANOVA.

Variable	**West Lafayette**	**Farmland**	**Columbia City**	**Wanatah**	**Butlerville**	**Lafayette**
	-- Level of significance --					
N rate			*		**	**
Depth	**	**	**	**	**	**
N rate*Depth						
Day	**	**	**	**	**	**
N rate*Day						
Depth*Day	**	**	**	**	**	**
N rate*Depth*Day						

Significance reported within column for each location.
* = $\alpha \leq 0.05$ and ** = $\alpha \leq 0.01$
Log_{10} transformation was used for total inorganic nitrogen at all locations.

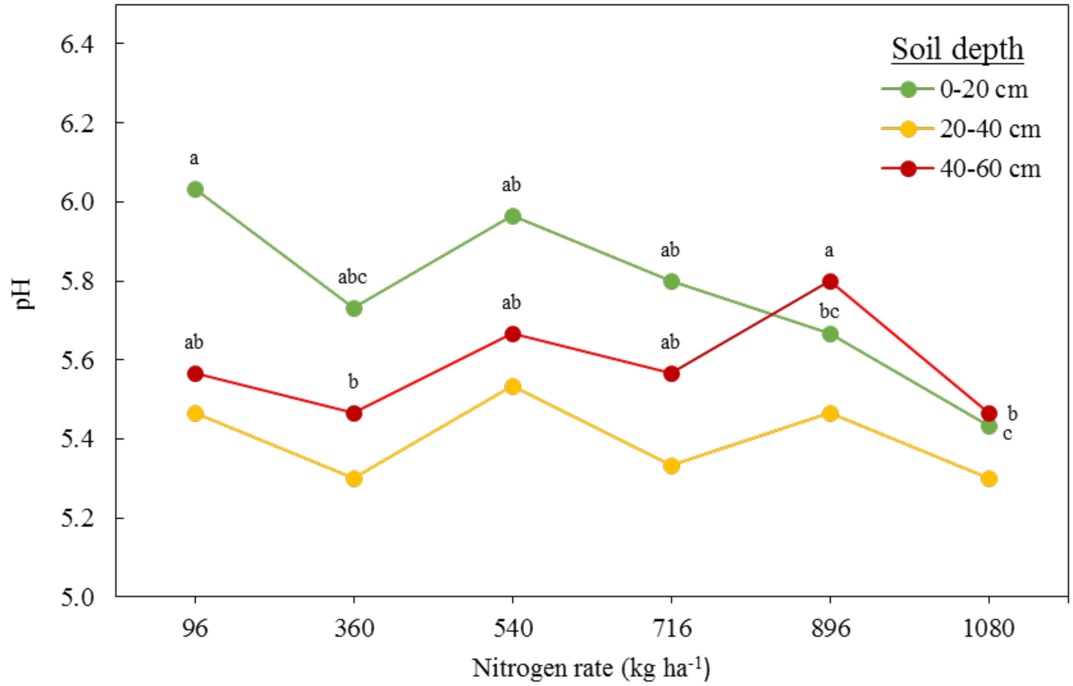

Figure 1. Cumulative nitrogen rate by depth interaction for pH at Lafayette (α ≤ 0.05). Soil pH was unaffected by N rate at the 20-40 cm soil depth. Similarity letters represent significant differences between N rate at α ≤ 0.05. Each point represents the mean of three replicates.

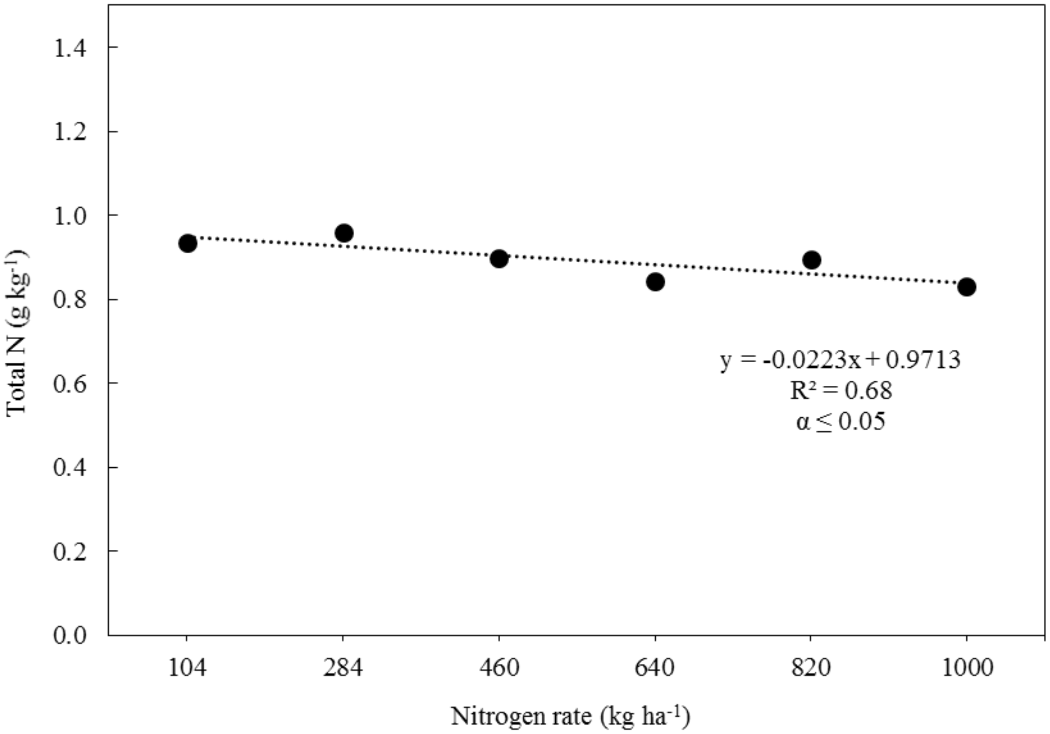

Figure 2. Significant N rate effect on total N averaged over depths of 0-20, 20-40, and 40-60 cm relative to cumulative N applied to four previous corn years grown in rotation with soybean at Columbia City ($\alpha \leq 0.01$).

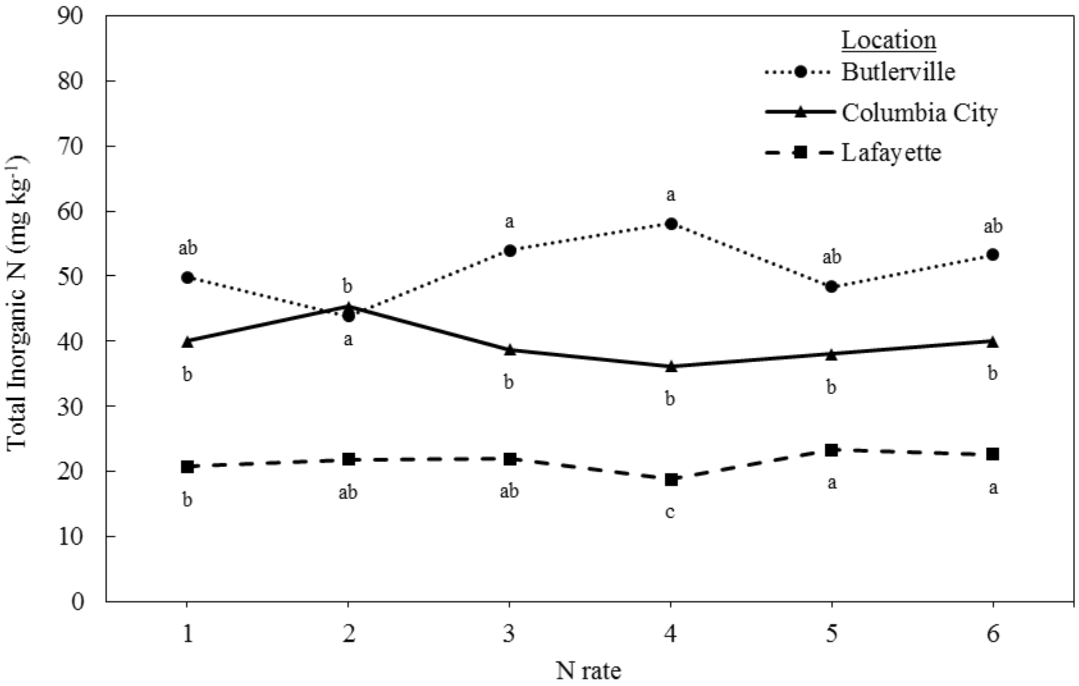

Figure 3. Total inorganic N from the 50-day aerobic incubation relative to cumulative N applied (ranked low to high) to four previous corn years grown in rotation with soybean for Butlerville, Columbia City, and Lafayette. Rates of cumulative N applied are listed in Table 1. Similarity letters represent significant differences between N rate at $\alpha \leq 0.05$. Total inorganic N refers to the sum of NO_3^--N and NH_4^+-N.

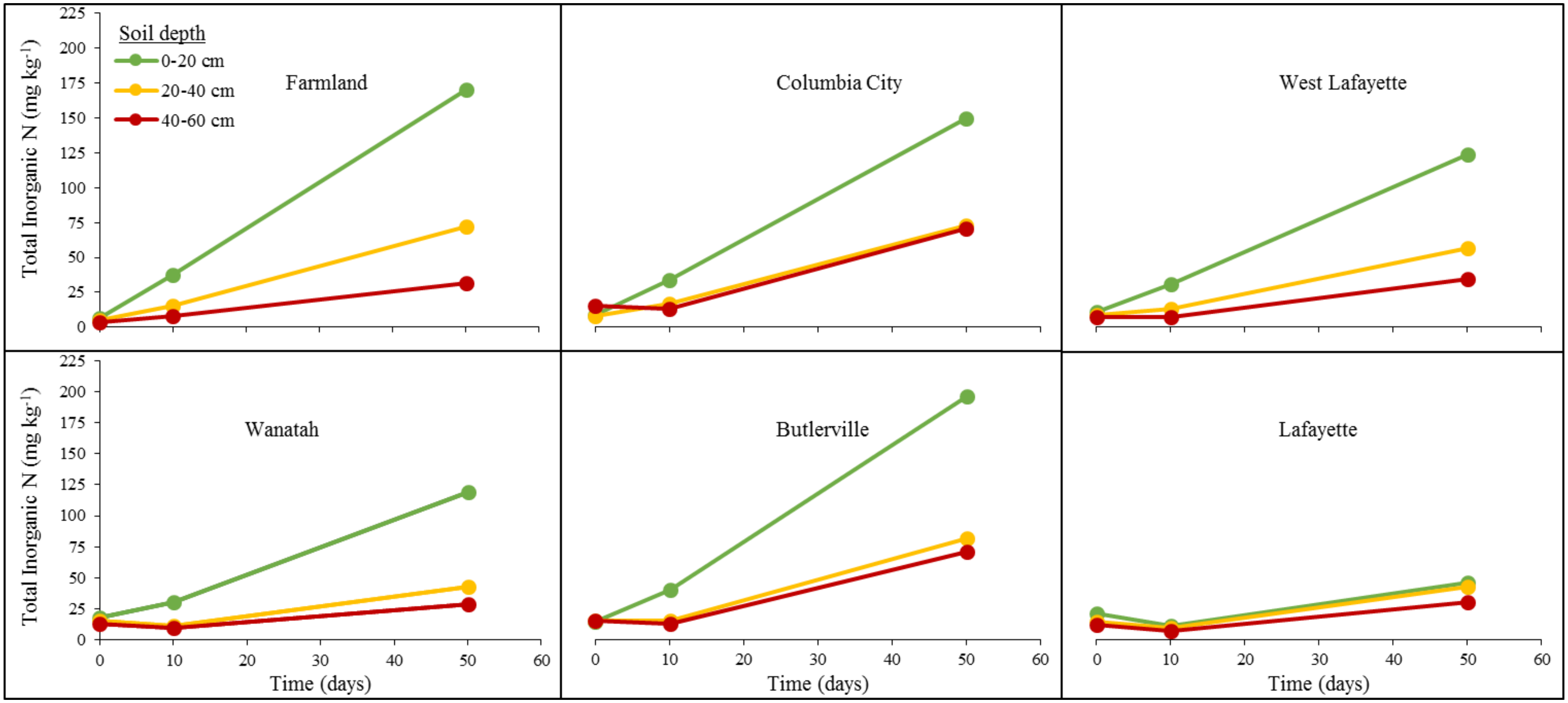

Figure 4. Total inorganic N by depth relative to incubation time for all locations (depth x day; $\alpha \leq 0.01$). Total inorganic N refers to the sum of NO_3^--N and NH_4^+-N. Soil samples were aerobically incubated at 25 °C and 33 kPa moisture tension then destructively sampled at 0,10, and 50 days.

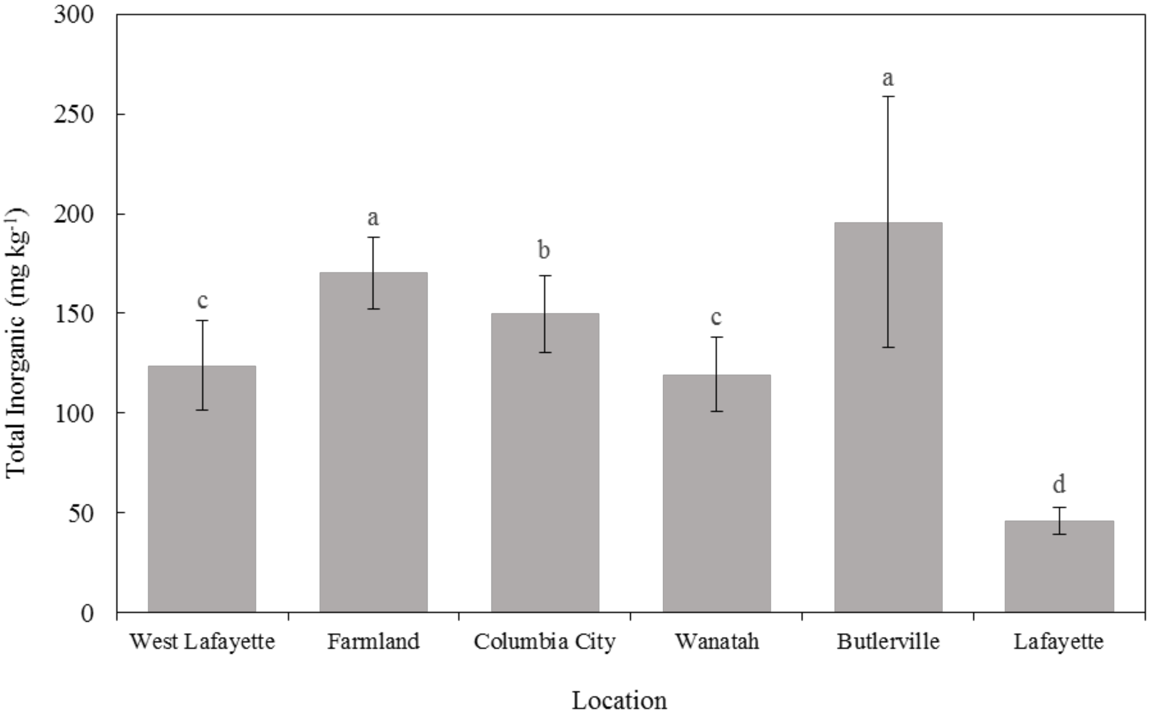

Figure 5. Total inorganic N at 0-20 cm after 50 days of incubation compared across locations. Similarity letters represent significant differences between locations at $\alpha \leq 0.05$. Total inorganic N refers to the sum of NO_3^--N and NH_4^+-N.

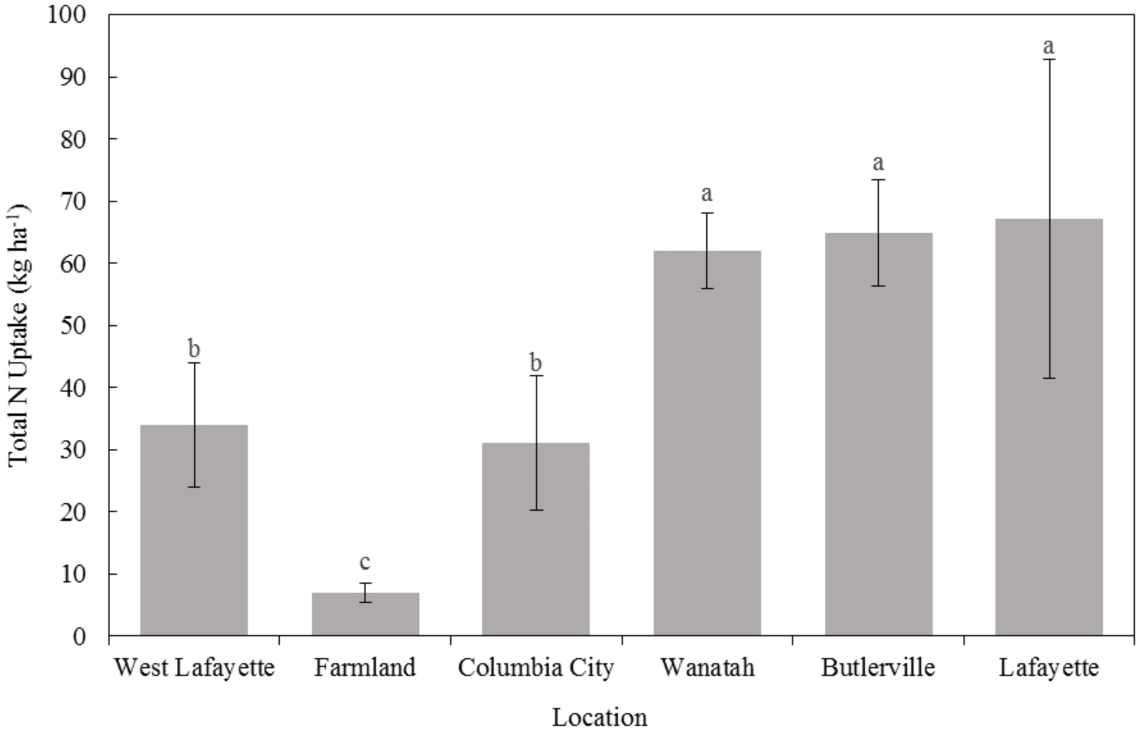

Figure 6. Total plant N uptake in 2015 compared across locations. Similarity letters represent significant differences between locations at $\alpha \leq 0.05$.

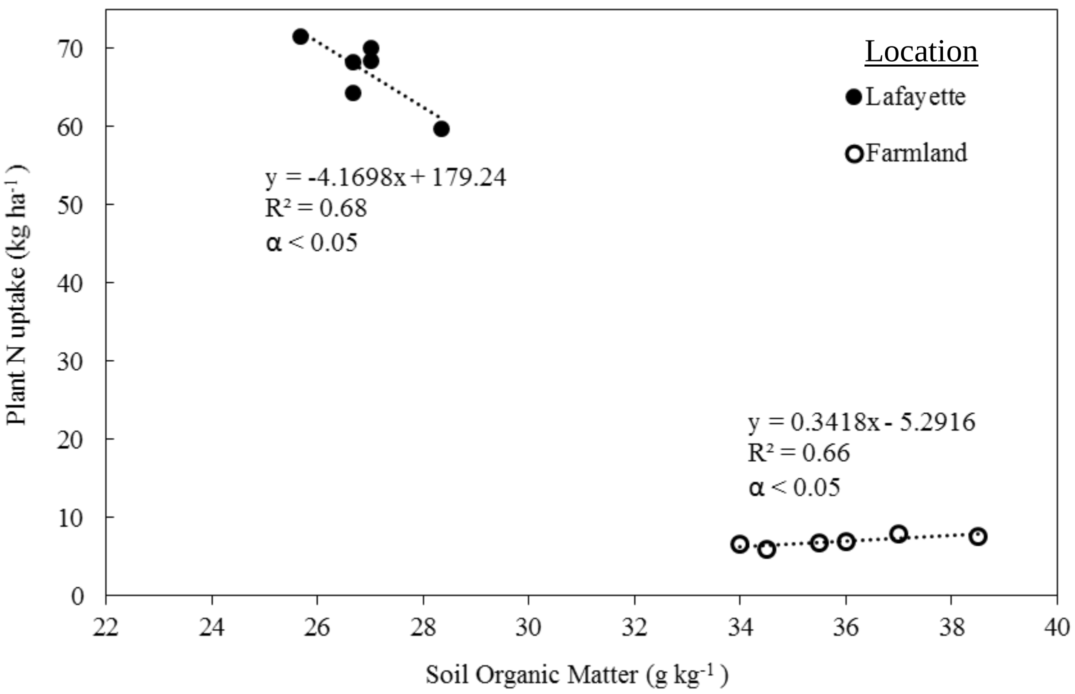

Figure 7. Total plant N uptake in 2015 relative to soil organic matter concentration at the 0-20 cm depth at Lafayette and Farmland. Plant N uptake showed a linear decrease with increasing soil organic matter at Lafayette while a linear increase was noted for Farmland ($\alpha \leq 0.05$). Each point represents the mean of each N rate across three replicates at Lafayette and two replicates at Farmland.

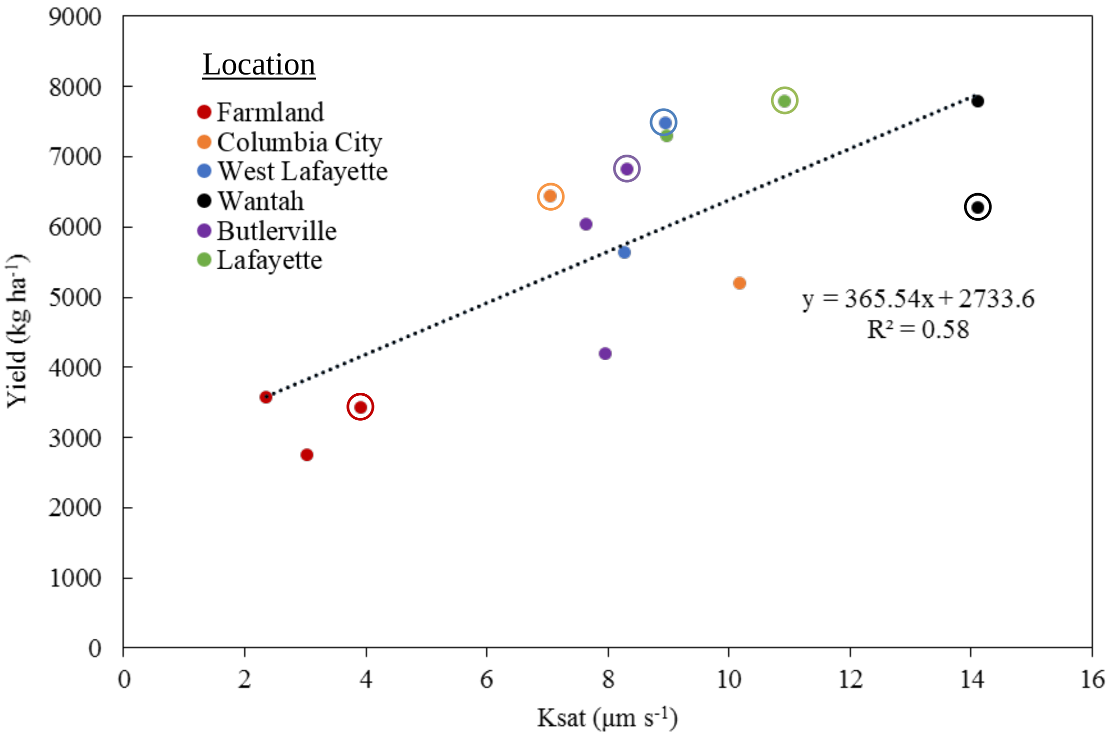

Figure 8. Historic (2006 to 2015) yield averages (averaged across years) from starter-only plots per field relative to saturated hydraulic conductivity (Ksat) for all locations ($\alpha \leq 0.05$). Starter-only N rates ranged from 24 to 33 kg N ha^{-1}. Saturated hydraulic conductivity was estimated using Web Soil Survey based on the proportion of each soil series in the experimental area, therefore a weighted Ksat average was calculated for each field. Circled points refer to the fields used in our study from 2007 to 2015 where corn was grown in the odd years and soybean in the even. Our study fields were rotated with another with the same experimental design except corn was grown in the even years and soybean in the odd.

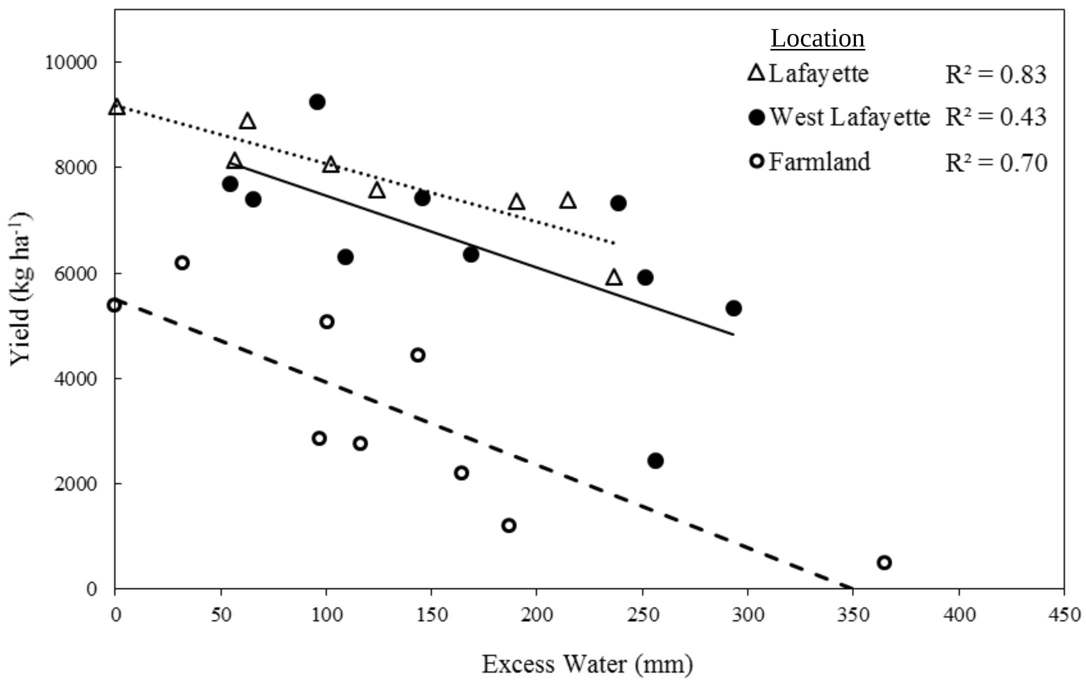

Figure 9. Lafayette, West Lafayette, and Farmland historic (2006 to 2015) yields for starter-only plots relative to sum of excess water from planting to harvest ($\alpha \leq 0.05$). Each point represents one year at a specified location. Starter-only N rate ranged from 24 to 33 kg N ha^{-1}. The Irris Scheduler was used to estimate excess water which is defined as water which may runoff or leach through the soil from planting to harvest. Irris uses soil properties from Web Soil Survey, crop information, and weather to calculate excess water. For each field excess water was a weighted average based on the percentage of field area mapped to a particular soil series.

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APPENDIX

APPENDIX

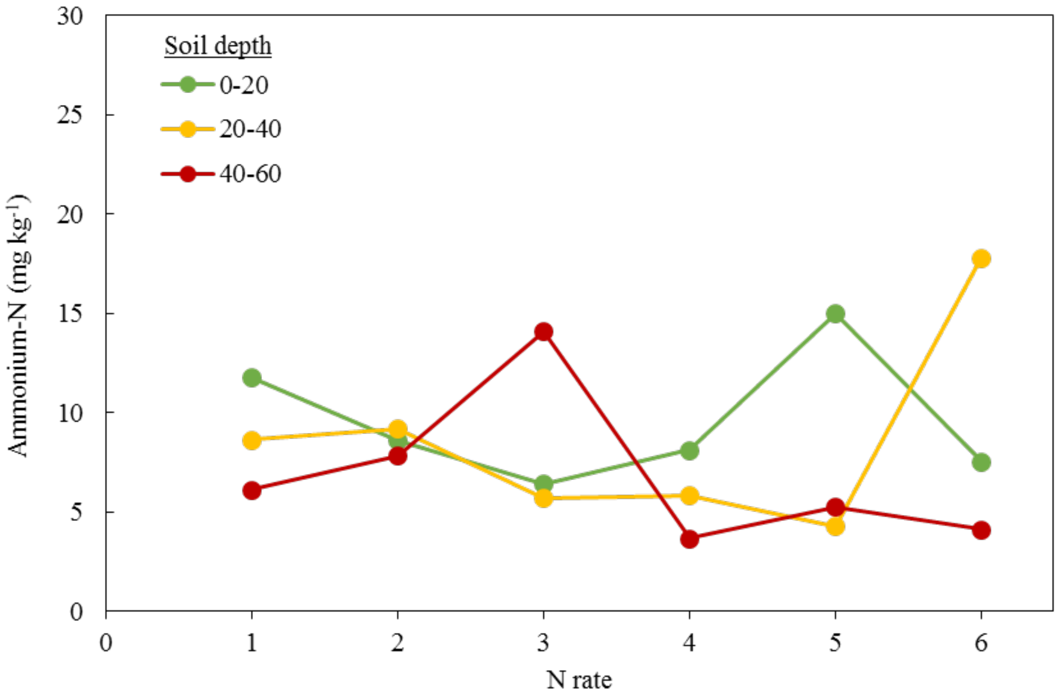

Figure A.1. Ammonium at day zero relative to cumulative N applied (ranked low to high) to four previous corn years grown in rotation with soybean at Wanatah. Graph illustrates the significant N rate x depth interaction at $\alpha \leq 0.05$. Actual rates of cumulative N applied are listed in Table 1.

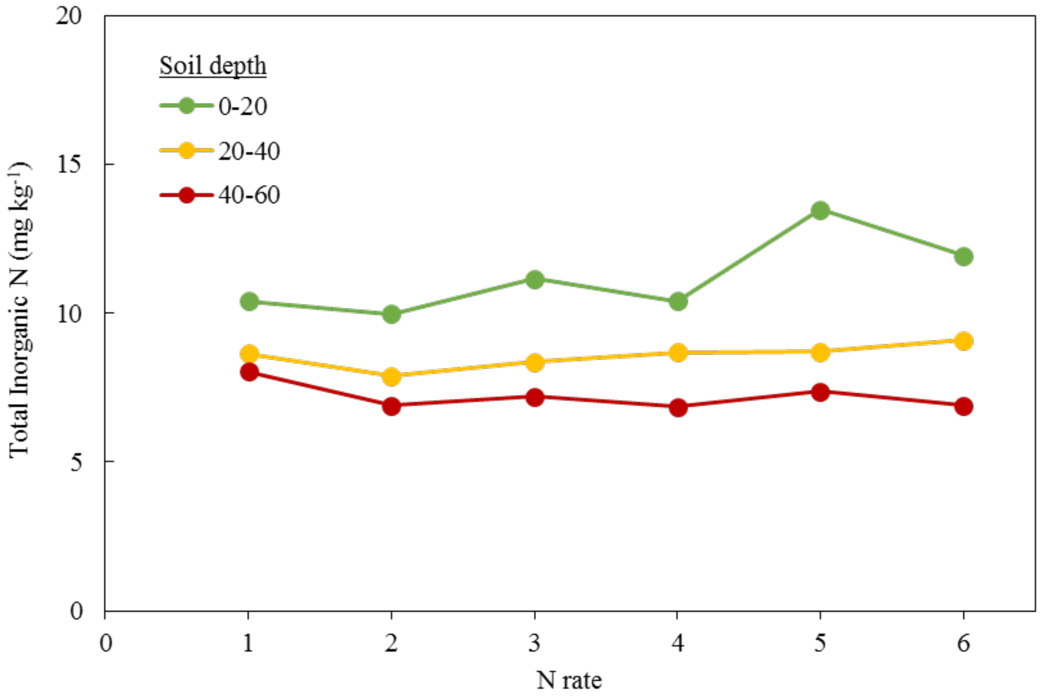

Figure A.2. Total inorganic N at day zero relative cumulative N applied (ranked low to high) to four previous corn years grown in rotation with soybean at West Lafayette. Graph illustrates the significant N rate x depth interaction at $\alpha \leq 0.05$. Total inorganic N refers to the sum of NO_3^--N and NH_4^+-N. Actual rates of cumulative N applied are listed in Table 1.

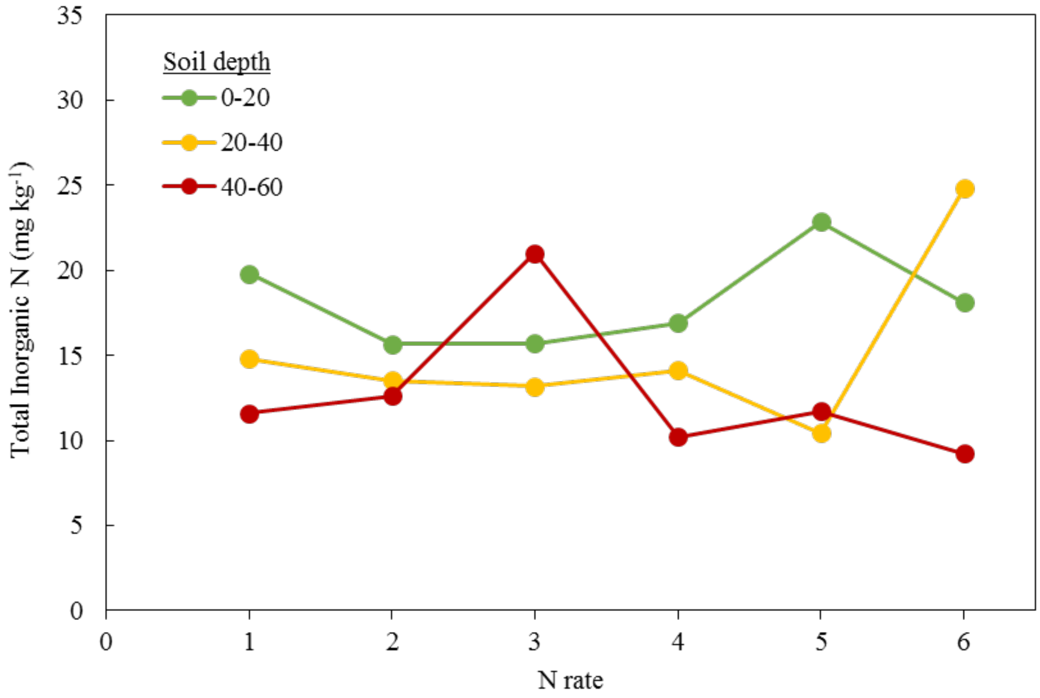

Figure A.3. Total inorganic N at day zero relative cumulative N applied (ranked low to high) to four previous corn years grown in rotation with soybean at Wanatah. Graph illustrates the significant N rate x depth interaction at $\alpha \leq 0.05$. Total inorganic N refers to the sum of NO_3^--N and NH_4^+-N. Actual rates of cumulative N applied are listed in Table 1.