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Nitrification Inhibitor, Nitrogen Source, And Herbicide Effects on Soil Nitrogen Transformations and Corn Yield

By

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Nitrification Inhibitor, Nitrogen Source, And Herbicide Effects on Soil Nitrogen Transformations and Corn Yield

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Nitrogen is the most limiting nutrient for crop production; however, its management has been challenging due to increasing nitrogen losses. Increased N losses have led researchers to focus on strategies for improving Nitrogen Use Efficiency (NUE). In this study, we took an integrated approach to compare the effects of N source, nitrification inhibitor and herbicide on nitrification, N loss, crop yield, residual N, and NUE. We first evaluated these factors in a two-year field experiment. A laboratory soil incubation experiment followed. The treatments included a combination of nitrification inhibitor vs. no inhibitor, two nitrogen fertilizer sources (broadcast urea vs. injected aqueous ammonia), and a pre-emergence herbicide vs. no herbicide.

Results indicated that nitrogen source has a more significant effect on NH_4^+-N retention (78-80% higher in anhydrous ammonia vs. urea) than nitrification inhibitor (24-47% higher with inhibitor vs. without inhibitor) and herbicides. Similarly, nitrogen source significantly affected NO_3^--N formation (134-176% lower in anhydrous ammonia vs. urea) than nitrification inhibitor (8-31% lower with inhibitor vs. without inhibitor) and herbicides.

We then evaluated the effect of nitrification inhibitor, nitrogen fertilizer source, and herbicide on (1) soil nitrification through a 25 day-soil incubation and (2) NH_3 volatilization, NO_3^--N leaching, and N_2O emissions through a 31-day soil column study using a loamy sand soil. Results indicated that nitrogen source had a greater effect on reducing nitrification (32.5%

lower with injected aqueous ammonia vs. surface broadcast urea) compared to nitrification inhibitors (4% lower with inhibitor vs. without inhibitor) and herbicide (no effect). Surface broadest urea increased NH_3 volatilization by 673% compared to injected aqueous ammonia. Injected aqueous ammonia had 22% higher NO_3^--N leaching and 33 % higher NH_4^+-N leaching than urea, while nitrification inhibitor had an inconsistent effect on NO_3^--N leaching across both N sources. The results of both experiments indicated that nitrogen source plays a more significant role in regulating soil nitrogen losses than nitrification inhibitors and herbicide.

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1.1 Abstract

Nitrogen fertilizer management continued to be challenging due to potential nitrogen losses under variable weather conditions. The objective of this was to evaluate the performance of nitrification inhibitors, nitrogen sources and herbicides on in-season nitrogen availability and agronomy indicators. A two site-year field experiment was conducted in silty clay loam soil in corn phase of the corn-soybean rotation at Central Nebraska. The treatments included three herbicide (no pre-emergence, Acuron, Resicore) and five nitrogen treatments: 1) control, 2) anhydrous ammonia, 3) anhydrous ammonia without nitrification inhibitor, 4) urea with nitrification inhibitors, and 5) urea without nitrification inhibitors. Results indicated that nitrogen source has a more significant effect on NH_4^+-N retention (78-80% higher in anhydrous ammonia vs. urea) than nitrification inhibitor (24-47% higher with inhibitor vs. without inhibitor) and herbicides. Similarly, nitrogen source significantly affected NO_3^--N formation (134-176% lower in anhydrous ammonia vs. urea) than nitrification inhibitor (8-31% lower with inhibitor vs. without inhibitor) and herbicides. Pre-emergence herbicide increased corn grain yield at one site year, while nitrification inhibitors did not affect agronomic indicators. Within nitrogen source, anhydrous ammonia increased grain yield by 1.06 Mg ha^{-1}, partial factor productivity by 5.7 kg grain kg^{-1} N, agronomic efficiency by 5.5 kg grain kg^{-1} N, aboveground biomass N uptake by 35 kg N ha^{-1}, grain N uptake by 15 kg N ha^{-1}, nitrogen recovery efficiency by 21% and residual total inorganic N by 6-40 kg N ha^{-1} compared to urea. Overall, using the right fertilizer source, followed by nitrification inhibitor and herbicide, might be an effective strategy in conserving nitrogen and improving nitrogen use efficiency in corn.

1.2 Introduction

Balancing the corn nitrogen requirements while maintaining good stewardship of land, air, and water resources is one of the major challenges facing corn producers in Nebraska. Though nitrogen use efficiency of corn production in Nebraska has continuously improved over time (Ferguson, 2015), there are still significant challenges in managing nitrogen. Only one-third to half of the N fertilizer input is recovered in the harvested product (Morris et al., 2018; Mueller et al., 2017), while the unrecovered N is lost to air and water resources. The unrecovered N losses cause a range of environmental problems such as water contamination, biodiversity loss, and greenhouse gas emissions. These N losses are evidenced in groundwater nitrate-N concentrations frequently exceeding the 10 ppm EPA drinking water standard in several wellhead protection areas of Nebraska (NDEQ, 2018). This higher level of nitrate leaching to groundwater are likely due to poor synchrony between N fertilizer applications and crop N demand, excessive nitrogen inputs, and heavy rainfall events during spring fallow periods. Nitrogen losses to groundwater tends to be greatest during wet or warm conditions during the April-June period when soil nitrate is present but without actively growing crops (Bowles et al., 2018). So, N applied prior to corn planting can be lost either to groundwater though nitrate leaching or to air through gaseous emissions. Therefore, protection of nitrogen input is needed to reduce nitrogen losses during early spring.

One strategy to reduce the potential nitrogen losses after fertilizer nitrogen application is the use of nitrification inhibitors. These chemical compounds slow down the soil nitrification, a biological process responsible for the transformation of ammonium (NH_4^+-N) to nitrate (NO_3^--N) (Martens-Habbena et al., 2009). The NO_3^--N form is volatile and can be easily lost through either denitrification or nitrate leaching if not intercepted by crop roots. Therefore, decreasing nitrification is one of the important practices to reduce nitrogen losses during the early season (Laura et al. 2013; Yu et al. 2018). Numerous studies have tested the effect of nitrification inhibitors on nitrogen losses and crop nitrogen uptake (Cahill et al., 2007, 2010; Noellsch et al., 2009; Wang & Alva, 1996). Nitrapyrin (2-chloro-6-(trichloromethyl)-pyridine) is one of the compounds successfully used to reduce nitrification and thus nitrogen losses (Wolt, 2000). However, the performance of nitrapyrin can change depending on the site-specific weather conditions (Maharjan et al., 2017).

Nitrogen fertilizer type and placement can also significantly affect the nitrification process. For example, anhydrous ammonia when injected in band below the soil surface can stabilize nitrogen and improve nitrogen use efficiency. On the other hand, urea is most commonly used fertilizer which when broadcasted over the soil surface, has the potential for substantial N losses through various nitrogen transformation pathways i.e. ammonia volatilization, nitrification and denitrification. Consequently, the performance of the surface broadcasted fertilizer N can substantially vary compared to in band application fertilizer N (Touchton & Hargrove, 1982). However, an effect of conventional fertilizer nitrogen sources vs nitrification inhibitors on soil nitrification has not been tested previously.

Chemical herbicides that are widely used to kill harmful weeds, have also been confirmed to affect soil nitrification in previous studies (Li et al., 2008; Mahia et al., 2008; Zhang et al., 2018). These herbicides vary in their toxicity level to affect microorganisms and rate of soil nitrification. Some of these are more toxic than others to nitrifiers (Debona & Audus, 1978). For example, atrazine and acetochlor have been found to show inhibitory effects on nitrifying bacteria (Li et al., 2008; Mahia et al. 2008). Chen et al. (2015) found that atrazine has both stimulatory and inhibitory effect on soil nitrification. In another study, higher level of atrazine inhibited nitrification, while lower level of atrazine increased nitrification (Laursen & Carlton, 1999). In these studies, the effect of herbicides on nitrification has been mainly studied in laboratories. However, to our knowledge, limited studies have evaluated the herbicide effect on nitrification at field scale.

In this study, we aimed at comparing the effect of nitrification inhibitor, nitrogen source, and herbicide on nitrification, nitrogen use efficiency, and crop yield. We hypothesized that the integrated use of nitrification inhibitor, nitrogen source, and herbicide can improve the synchronization of nitrogen release and crop nitrogen uptake and lead to more crop yield and less potential nitrogen losses. Thus, the objective of this study was to compare the effect of nitrapyrin nitrification inhibitor, two herbicides (Acuron and Resicore), and two nitrogen sources (urea and anhydrous ammonia) on soil nitrification, nitrogen use efficiency, corn grain yield, and post-harvest soil nitrogen.

1.3 Materials and Methods

1.3.1 Experimental Site

The experiment was conducted at the South-Central Agricultural Laboratory (SCAL; 40.540° N; 98.084° W; 538 m elevation) near Clay Center Nebraska over two years (2020 and 2021) with different site each year. Both sites were located within one mile of each other. The soil at both site-years was Hastings silt clay loam (*Udic Argiustoll*) with moderately well-drained to well-drained classification. The sites were under linear irrigation and no-till management. The sites have a sub-humid climate with a 20-year annual average temperature of 10.38 ^{o}C and average precipitation of 514.5 mm yr^{-1}, with significant interannual variability.

Prior to treatment establishment, soil samples were taken at 0-30cm soil depth to determine the basic soil chemical and physical properties. Specific soil properties for each site-year are listed in Table 1. Precipitation data (Figure 1.1.) was collected from the nearest weather station in the High Plains Regional Climate Center network.

1.3.2 Experimental Design and Agronomic Management

The experiment was a split block design with three herbicide and five nitrogen treatments with four replicates. The herbicide was the main plot treatment, whereas nitrogen was the subplot treatment. Each sub plot was 3 m wide by 15 m long. At each site-year, the treatments were applied in corn phase of the corn-soybean rotation. Three herbicide treatments, representing common pre-emergence herbicide programs for Nebraska growers were used for corn crop as following: 1) Control - no herbicide, 2) Acuron - a pre-mix of atrazine/bicyclopyrone/*s*-metolachlor/mesotrione, 3) Resicore - a pre-mix of acetochlor/clopyralid/mesotrione. These pre-emergence herbicides were applied at 6.4 L per hectare. Five nitrogen treatments with two nitrogen sources (Anhydrous Ammonia and urea) with and without nitrification inhibitors and a control were used as following: 1) Control - no nitrogen, 2) AA+I - anhydrous ammonia with nitrification inhibitor of N-Serve, 3) AA+No – anhydrous ammonia without nitrification inhibitor of N-serve, 4) Urea+I - urea with nitrification inhibitor of Instinct or guardian DL, and 5) Urea+No – urea without nitrification inhibitor of Instinct or guardian DL. In each site year, N-serve product from Corteva was used with anhydrous ammonia at a rate of 2.6 L ha^{-1}. N-serve is a nitrification inhibitor product that contains nitrapyrin as an active ingredient to inhibit nitrification and improve NUE (Di & Cameron, 2016; Goring, 1962). Due to unusual circumstances, different nitrification products were used with urea during each year. During 2020, Guardian-DL was used to impregnate urea at the rate of 1.95 L ha^{-1}, while in 2021, Instinct NXTGEN product from Corteva was used at the recommended rate of 1.95 L ha^{-1}. Guardian-DL is a nitrification inhibitor product which contains dicyandiamide (DCD) as active ingredient while Instinct NXTGEN product contains nitrapyrin as active ingredient to inhibit nitrification.

All nitrogen treatments except control received one application rate (168 kg ha^{-1} in year 2020 and 169 kg ha^{-1} in year 2021) based on the University of Nebraska (UNL) nitrogen algorithm. All treatments including herbicide and nitrogen application occurred on the same day at each site year (April 23 2020, April 28 2021). In 2021, the site received half inch of rain within one week following fertilizer application, while in 2021 a 1-acre-inch irrigation was applied within 24 hours following treatment application to incorporate urea and limit ammonia volatilization. Each year, monoammonium Phosphate (MAP) at a rate of 152 kg ha^{-1} was applied during the winter months to meet phosphorus demands of the maize crop that also resulted in addition of 18 kg N ha^{-1} in all plots. Maize with 110-day relative maturity (RM) was no-till planted into soybean residue at a targeted rate of 81,000 seed ha^{-1} on 23 April 2020 and 28 April 2021. A postemergence (POST) herbicide application of a premix of dicamba/tembotrione at a rate of 1.6 L ha^{-1} was made on all plots (June 13 2020, June 1 2021) to reduce weed uptake of N and impact on corn yield. Each year, the corn was irrigated based on the soil moisture percentage, resulting 282 mm of irrigation in 2020 and 155 mm of irrigation in 2021. Management decisions such as hybrid selection and irrigation scheduling were at the discretion of SCAL farm management.

1.3.3 Soil Sampling and Analysis

To evaluate the effect of treatments on soil nitrification following treatment application, weekly soil samples were collected at 0 to 20 cm soil depth during May and June of each year. Different soil sampling strategies were used to collect soil samples in the injected anhydrous ammonia and broadcast urea plots. In anhydrous ammonia plots, 6 soil cores were collected with a 4-cm diameter probe in the in-band and in-row, and kept and analyzed separately for NH_4^+-N and NO_3^--N. The final NH_4^+-N and NO_3^--N values in anhydrous plots were determined using a weighted-average proportional to the lateral dimension of the area with band and without band. In broadcast urea plots, 6 cores were collected from the equivalent positions in each plot and composited. The soil samples were transported in a cooler from the field to laboratory and analyzed immediately to determine soil NH_4^+-N and NO_3^--N. Soil samples were extracted with 2M KCL solution (5:1 solution to soil ratio) after shaking 1 hour at 180 rpm. Extracts were subsequently filtered using pre-leached Whatman # 1 filter paper and analyzed for NO_3^-+NO_2^--N (hereafter NO_3^--N) and soil NH_4^+-N in microplates using the Griess-Ilosvay reaction with vanadium (III) chloride as reducing agent and the Berthelot reaction, respectively (Hood-Nowotny et al., 2010).

To determine residual NH_4^+-N and NO_3^--N, three soil cores between anhydrous bands (same position for urea broadcast treatment) in each plot at a depth of 120 cm were collected using a Gidding hydraulic probe (Giddings, Windsor, CO) after the crop harvest each year. Soil samples were split into depth increments of 0 to 10 cm, 10-20 cm, 20-40 cm, 40-60 cm, 60-90 cm, and 90-120 cm. The soil samples were transported in a cooler from the field to laboratory and analyzed immediately to determine soil NH_4^+-N and NO_3^--N (as above).

1.3.4 Plant Sampling and Analysis

At physiological maturity in year 2021, 6 plant samples were randomly hand harvested in each plot by cutting the stalk at ground level to determine nitrogen concentration in grain and stover (stalk, leaves, cobs). The plants were separated into ears and stover and then weighed. The stover was shredded using a portable woodchipper. Ears and subsamples of chopped corn stover were weighed and dried at 71°C to determine the moisture content. Ears were shelled to separate grain and cobs. Grain and stover were milled and analyzed for total nitrogen using dry combustion method in ward lab at Kearney NE. Hand harvest grain yield at 15.5% moisture, and nitrogen concentration in grain and stover, and plant population were used to calculate total above ground biomass, nitrogen fertilizer recovery efficiency, and nitrogen harvest index. At harvest, a two row combine harvester was used to harvest middle two rows and determine final grain yield at 15.5% grain moisture.

1.3.5 Statistical Analysis

The data was analyzed using SAS version 9.4 (SAS Institute) for each site-year separately because there were significant site-year x treatments interaction. Repeated measure PROC GLIMMIX procedure was used to test the effects of nitrification inhibitor, nitrogen source, herbicide, and time on in-season soil NO_3^--N and NH_4^+-N concentration. The nitrification inhibitor, N source, herbicide, and time were treated as the fixed effects while block and all interactions of block with other terms were used as random effects. PROC GLIMMIX procedure was used to test the effect of nitrification inhibitor, N source, and herbicide on grain yield, partial factor productivity (PFP), agronomic efficiency (AE), and residual NO_3^--N and NH_4^+-N and nitrogen use efficiency indicators including grain N uptake, aboveground biomass N uptake, nitrogen recovery efficiency (NRE), and nitrogen harvest index (NHI) (Equations are provided in Supplementary Table 1). To analyze a complete factorial design (2 N sources, 2 nitrification inhibitors x 3 herbicide), data from the zero N treatment was left out of ANOVA results presented in table 2, 3, and 4. Means from zero N treatment were not included in the main effects to determine significant differences among the treatments. Comparison of the means were conducted by comparing differences in least-square means with significance differences at $P<0.05$.

1.4 Results

1.4.1 Climate

Total seasonal precipitation from March 1 through October 31was 298 mm in 2020 and 386 mm in 2021 (Figure 1.1.). Compared to the Clay County 30-yr average precipitation of 632 mm in the same period, 2020 and 2021 precipitation was low by 53% and 39%, respectively. It is notable that total seasonal water inputs (precipitation plus irrigation) from 1 March through 31 October, being 580 mm in 2020 and 541 mm in 2021, were lower than the 30-yr average precipitation of 632 mm. First month (May) following fertilizer application, monthly precipitation in May was 35% and 17% lower in 2020 and 2021 than Clay County 30-year May average, respectively. Same trend was found in the second month (June) following fertilizer application when June precipitation was 48% and 58% lower in 2020 and 2021 than Clay County 30-year June average, respectively (Figure 1.1.).

1.4.2 In-Season Soil Nitrogen Availability

1.4.2.1 Soil NH_4^+-N concentration

In-season weekly soil NH_4^+-N and NO_3^--N concentration varied significantly over time in both years (Figure 1.2.). Nitrification inhibitor and nitrogen source had a significant but variable main effect on NH_4^+-N concentration in both years. For example, AA+I had significantly higher NH_4^+-N concentration than AA+No on 6 of 8 sampling dates in 2020 while it had significant difference on only 2 of 8 sampling dates in 2021 (Figure 1.2.). When averaged across the entire sampling period, AA+I had 30% and 11% higher NH_4^+-N concentration than AA+No in 2020 and 2021, respectively. Furthermore, nitrification inhibitor and nitrogen source had significant interaction on NH_4^+-N concentration in 2020 only as nitrification inhibitor with AA had a larger effect on NH_4^+-N retention than nitrification inhibitor with urea (Figure 1.2.). Urea+I had significantly higher NH_4^+-N concentration than Urea+No in only 1 of 8 sampling dates in 2020 while no significant difference was found on any sampling date in 2021. When averaged across all sampling dates, Urea+I had 47% and 24% higher NH_4^+-N concentration than Urea+No in 2020 and 2021, respectively. Within nitrogen source, AA had significantly higher NH_4^+-N concentrations than urea across all sampling dates in 2020 and 6 of 8 sampling dates in 2021 (Figure 1.2.). Averaged across all sampling dates, AA had 80% and 78% higher NH_4^+-N concentration than urea in 2020 and 2021, respectively. It is notable that nitrogen source has larger effect on NH_4^+-N retention (78-80% higher in AA vs. urea) compared to nitrification inhibitor with either nitrogen source (24-47% higher with inhibitor vs. without inhibitor).

Though NH_4^+-N concentration was not significantly influenced by herbicide in either year (Figure 1.3., Table 1.2.), it has variable effect within nitrogen source and inhibitor. For example, within AA+I, Acuron had 25% and 24% significantly higher NH_4^+-N concentration than No-PEH and Resicore in 2020, respectively, whereas NH_4^+-N concentration in Acuron and Resicore was 22 and 23% significantly higher than No-PEH in 2021 (Figure 1.3.). However, no significant differences among herbicides were found within other nitrogen treatments (Figure 1.3.).

1.4.2.2 Soil NO_3^--N concentrations

No significant interaction of nitrification inhibitor and nitrogen source was observed on soil NO_3^--N in both years (Figure 1.2., Table 1.2.). However, soil NO_3^--N was significantly influenced by main effects of nitrification inhibitor and nitrogen source. For example, AA+I had significantly lower NO_3^--N than AA+No on 3 of 8 sampling dates in 2020 and 4 of 8 sampling dates in 2021 (Figure 2). When averaged across all sampling dates, AA+I had 31% lower NO_3^--N concentration than AA+No in each year. Similarly, Urea+I had significantly lower NO_3^--N concentration in 3 of 8 sampling dates in 2020 and 4 of 8 sampling dates in 2021. When averaged across all sampling dates, Urea+I had 13% and 8% lower NO_3^--N than Urea+No in 2020 and 2021, respectively. Within nitrogen source, AA had significantly lower NO_3^--N than urea on all sampling dates in both years (Figure 1.2.). Averaged across all sampling dates, AA had 134% and 176% lower NO_3^--N concentration than urea in 2020 and 2021, respectively. It is notable that nitrogen source has a larger effect on NO_3^--N production (134-176% lower in AA vs. urea) compared to nitrification inhibitor with either nitrogen source (8-31% lower with inhibitor vs. without inhibitor).

Herbicide had significant main effect on NO_3^--N in 2020 but no significant effect was found in 2021 (Figure 1.3., Table 1.2.). The significant herbicide effect in 2020 was found within Urea+I and Urea+No treatment only. For example, within Urea+I treatment, Acuron and Resicore had 20% and 25% higher NO_3^--N than No-PEH. Whereas, within Urea+No treatment, Acuron and Resicore had 17% and 11% higher NO_3^--N than No-PEH, respectively.

1.4.3 Agronomic Responses

The effects of nitrification inhibitor, nitrogen source, herbicide, and their interactions on agronomic responses are given in Tables 1.3. In both years, there were no significant three-way interactions of nitrification inhibitor, nitrogen source, and herbicide on grain yield, PFP and AE except AE in 2020. Although, nitrification inhibitor across both sources did not significantly affect grain yield, PFP, or AE, however, these three parameters were slightly lower with nitrification inhibitor than without nitrification inhibitor in 2020, while opposite trend was observed in 2021 where grain yield, PFP, and AE were slightly higher with inhibitor than without inhibitor (Table 1.3.). Furthermore, different nitrogen source response on grain yield, PFP, and AE was observed in each year (Figure 1.4., Table 1.3.). For example, AA had a 1.06

Mg ha^{-1} higher grain yield than urea in 2020, while there was no significant grain yield response in 2021. Similarly, AA had significantly higher PFP (5.7 kg grain kg^{-1} N) and AE (5.5 kg grain kg^{-1} N) compared to urea treatment in 2020. There were no significant differences between AA and urea for grain yield, PFP, or AE in 2021. Within herbicide treatments, there was a variable main effect on grain yield, PFP, and AE in each year (Table 1.3.). For example, in 2020, herbicide had a significant effect on grain yield and PFP. Acuron and Resicore had 1.26 and 1.58 Mg ha^{-1} higher grain yield than no-PEH, respectively. This increase in grain yield was accompanied by an increase of 6.7 and 8.5 kg grain kg^{-1} N PFP with Acuron and Resicore than no-PEH, respectively. Significant but opposite effect of herbicide on AE was found in 2020 where Acuron and Resicore had 12 and 6.8 kg grain kg^{-1} N lower AE than No-PEH. In 2021, no significant effect of herbicide on grain yield, PFP, and AE was found.

Nitrogen indicators including grain N concentration, grain N uptake, aboveground biomass N uptake, NRE, and NHI were measured in 2021 only. The effects of nitrification inhibitor, nitrogen source, herbicide, and their interactions on the nitrogen indicators are given in Table 1.4. Though no significant main or interaction effects of nitrification inhibitor, nitrogen source, and herbicide were observed on grain N concentration or nitrogen uptake, AA had 15 kg N ha^{-1} higher grain N uptake than urea. Resicore had 6 kg N ha^{-1} and 7 kg N ha^{-1} higher grain N uptake than Acuron and No-PEH treatment, respectively. All the nitrogen indicators and their interactions had insignificant effect on aboveground biomass N uptake except nitrogen source where AA increased aboveground biomass N uptake by 35 kg N ha^{-1} compared to urea. Similarly, no significant effect of all N indicators was observed on NRE except nitrogen source where AA increased NRE by 21% compared to urea. All N indicators had insignificant effect on NHI except nitrogen source that had 3% higher NHI in urea compared with AA.

1.4.4 Residual Soil NH_4^+-N and NO_3^--N

Across both years, variable response of residual soil NH_4^+-N and NO_3^--N was observed (Figure 1.5., Table 1.3.). In both years, nitrification inhibitor, nitrogen source, and herbicide did not have a significant main or interaction effect on residual NH_4^+-N except significant main effect of nitrogen source in 2021 where AA had 64% (63 kg N ha^{-1} at 0-1.2m depth) higher residual soil NH_4^+-N than urea (23 kg N ha^{-1} at 0-1.2m depth) (Table 1.3.). During 2020, residual NH_4^+-N did not differ by soil depth, however, soil NH_4^+-N significantly increased with increasing soil depth in 2021. When summed across the both nitrogen sources and whole soil profile at 0-1.2m, AA and urea had 13 and 12 kg NH_4^+-N ha^{-1} in 2020, and 63 and 23 kg NH_4^+-N ha^{-1} in 2021, respectively (Figure 1.5.). There were no significant main or interaction effects of nitrification inhibitor, nitrogen source, and herbicide on residual soil NO_3^--N in both years except nitrification inhibitor effect within AA in 2020 where AA+I had 38% higher residual NO_3^--N across whole soil profile (41 kg NO_3^--N ha^{-1} at 0-1.2m depth) than AA+No (25 kg NO_3^--N ha^{-1} at 0-1.2m depth) (Figure 1.5.). Soil NO_3^--N was significantly higher in upper than lower soil layer in 2020, but no differences across depth were found in 2021. When summed across the both nitrogen sources and whole soil profile, AA and urea had 33 and 27 kg NO_3^--N ha^{-1} in 2020, and 0.4 and 0.49 kg NO_3^--N ha^{-1} in 2021, respectively. Overall, across both years, AA had 6-40 kg N ha^{-1} higher total inorganic nitrogen compared to urea.

1.5 Discussion

1.5.1 The Effect of Nitrification Inhibitors, Nitrogen Source, and Herbicide on In-Season N availability

This study compared the effect of nitrification inhibitor, nitrogen source, and herbicide on early-season soil nitrogen availability. Nitrification inhibitors containing nitrapyrin and DCD are known to reduce the nitrification and delay the conversion of NH_4^+-N to NO_3^--N (Franzen, 2017;

Peng et al., 2015) during early season before the crop can actively take up nitrogen during the early to mid-season. Generally, the potential N losses are more likely during heavy rainfall period in early spring (Loecke et al., 2017; Van Metre et al., 2016). However, in both site-years of this study, early season and cumulative seasonal precipitation was lower than the 30-year average precipitation suggesting a lower probability for nitrogen losses through nitrate leaching. Regardless of weather conditions, nitrification inhibitor can temporarily inhibit nitrification and delay the conversion of NH_4^+-N to NO_3^--N for few weeks after fertilizer application as it has been reported in previous studies (Franzen, 2017). We found similar effect in this study when AA and urea with nitrification inhibitors had 24-47% higher NH_4^+-N and 8-31% lower NO_3^--N than without inhibitors during early season across both study years. During the May and June soil sampling period across both years, NH_4^+-N concentration decreased over time over both nitrogen sources as nitrification gradually increased as expected. At the end of June, assuming no nitrogen loss, we expected similar values of NO_3^--N concentration between AA and urea through nitrification, as these both sources received the same N rate. However, NO_3^--N concentration was quite lower in AA than urea. This was partly due to underestimation of NO_3^--N release from the anhydrous band because we sampled in the anhydrous band and the row (representing outside band area) and missed available NO_3^--N between band and row as nitrate would diffuse away from the band over time (Khengre & Savant, 1977).

Regardless, nitrogen source had a larger effect on inhibiting nitrification than nitrification inhibitors as AA had 78-80% higher NH_4^+-N and 134-176% lower NO_3^--N than urea across both site-years. This lower nitrification with AA than urea could be attributed to knife injection of nitrogen beneath the soil surface as concentrated ammonia band changes soil pH and inhibits the microbial activity for few weeks after anhydrous injection (Biederbeck et al., 1996; Stehouwer &

Johnson, 1990). These results were consistent with previous studies where band application of nitrogen stabilized nitrogen compared to surface broadcast nitrogen application (Biederbeck et al., 1996; Shapiro et al., 2016). However, comparative effect of nitrification inhibitor vs. nitrogen source has not been much focused on field crops previously (Redding et al., 2020). The findings from this study indicates that selecting a right nitrogen source has the higher probability of inhibiting nitrification and protecting potential N loss than nitrification inhibitors during spring period as more wet springs are predicted in the future (Dai et al., 2016; Hatfield et al., 2011). If the potential for nitrogen losses through nitrate leaching and denitrification were higher during this study, more nitrogen would have been lost from urea compared to AA (Stehouwer & Johnson, 1990). Absence of wet year during this study precludes the possibility of evaluating the same treatments for more years. Regardless, the potential of N loss can be minimized by selecting a combination of right nitrogen source and nitrification inhibitors as AA with nitrification inhibitor retained 89% more NH_4^+-N than urea without nitrification inhibitor. This combine nitrification inhibitor and source effect on nitrification was higher than nitrogen source effect of 78-80% as reported above.

Though herbicide did not have a consistent effect on inhibiting nitrification across both sources and site-years, Acuron retained 25% higher NH_4^+-N than No-PEH at the start of the season in 2020 while both herbicides Acuron and Resicore had 22-23% higher NH_4^+-N than No-PEH in 2021, indicating some potential for reducing soil nitrification. These findings are consistent with previous lab studies where herbicide containing atrazine and acetochlor has been reported to inhibit nitrification (Li et al., 2008; Mahia et al., 2008). Herbicide containing atrazine produces non-target effects on microbial community by decreasing soil microbial biomass (Mahia et al., 2008) and altering ammonia oxidizing archaea (AOA) and ammonia oxidizing bacteria (AOB) *amo*A gene abundances which are related to soil nitrification process (Caffrey et al., 2007; Zhang et al., 2018). This is the first study demonstrating the effect of herbicide on nitrification in field conditions. Herbicide provided additive nitrification effect to nitrogen source and nitrification inhibitor in AA+I treatment in both years as both herbicides in AA+I had 92% higher NH_4^+-N than the Urea+No treatment that retained the least NH_4^+-N among all nitrogen treatments in both site-years. This indicates that application of right source with nitrification inhibitor and herbicide can have the cumulative effect to stabilize and protect nitrogen when the conditions can become susceptible to N losses. Compared to herbicide effect on NH_4^+-N, herbicides had an inconsistent effect on NO_3^--N during both years. Higher NO_3^--N concentration with Acuron and Resicore compared to NO-PEH in 2020 (Figure 1.3.) was likely because of N uptake by weeds as this effect was mainly found during Mid-May to end of June when more weeds were found in No-PEH treatment (visually observed) because of delayed post-emergence herbicide application in 2020. Weeds presence can reduce soil NO_3^--N up to 50% by the pollination stage (Lindquist et al., 2010). However, in the same year, it is interesting to note that weeds did not affect nitrate in AA treatment as weeds might not be able to exploit nitrogen in concentrated anhydrous band compared to available nitrogen at the surface broadcast urea, confirming significant nitrogen source and herbicide interaction on NO_3^--N as reported in Table 1.2. In 2021, timely post-emergence herbicide application on June 1 resulted in no weeds (visually observed) and thus no significant effect of either nitrogen source or its interaction with herbicide on NO_3^--N was observed.

1.5.2 The Effect of Nitrification Inhibitors, Nitrogen Source, and Herbicide on Agronomic Responses

Though nitrification inhibitor conserved nitrogen by reducing nitrification during the early part of the growing season, this did not significantly affect agronomic indicators across both site-years. The lack of response of nitrification inhibitor can be due to several reasons. First, this might be due to below than normal precipitation during the growing season which might have lowered the nitrate leaching potential by reducing the effectiveness of nitrification inhibitor. Second, previous studies have suggested that nitrapyrin and DCD are needed in higher concentration than the labelled rate to produce crop yield response (Franzen, 2017). Third, NH_4^+-N concentration in AA are often higher and persist longer at higher rate, thereby diminishing the efficacy of nitrification inhibitors (Hughes & Welch, 1970; Stehouwer & Johnson, 1990). These results are consistent with the previous studies where no effects of nitrification inhibitors on agronomic indicators were found under drier conditions (Franzen, 2017; Sassman, 2014). In this study, it might also be possible that N supply from possibly high N fertilizer rate and soil organic matter mineralization might have compensated the advantage of nitrification inhibitors vs. without inhibitors.

Compared to nitrification inhibitors, nitrogen source interacted with herbicide and had a significant effect on grain yield, PFP, and AE in 2020. Though AA had significantly higher grain yield, PFP and AE than urea, this effect was mainly due to nitrogen source interaction with herbicide where No-PEH in urea with and without nitrification inhibitor had significantly lower grain yield than No-PEH in AA with and without inhibitor. This was possibly because nitrogen from broadcast urea resulted in more weeds (visual estimates) due to a late post-emergence herbicide application in 2020 that likely led to higher nitrogen uptake by weeds and resulted in less nitrogen availability for the corn crop (Lindquist et al., 2010). Meanwhile in AA with and without nitrification inhibitor, weeds might not have exploited the nitrogen from the concentrated anhydrous band to proliferate weed growth in early season that may have resulted in less N uptake by weeds. While in 2021, post-emergence herbicide was applied two weeks earlier following planting than in 2020 (June 13, 2020 than June 1, 2021) and resulted in less weeds with no interaction effects of nitrogen source and herbicide on either grain yield, PFP, or AE. Nevertheless, findings from the current study indicate the advantage of band placement on improving corn yields, thus supporting the previous evidence of higher corn yield with band vs broadcast N application (Howard & Tyler, 1989; Lamond et al., 1991; Stecker et al., 1993).

Plant nitrogen uptake is another indicator that can be used to evaluate the performance of nitrification inhibitors and nitrogen sources. Nitrogen analysis of grain and stover at black layer showed no significant difference in grain N uptake with either nitrification inhibitor or nitrogen source but did show difference in aboveground biomass N uptake and NRE with nitrogen source in 2021. Anhydrous ammonia had 15 kg N ha^{-1} higher grain N uptake, 35 kg N ha^{-1} higher aboveground biomass N uptake and 19% higher NRE compared to urea, showing the advantage of band over broadcast N application. This also provides evidence that inhibiting nitrification with the right source can prolong nitrogen availability in the surface soil, better synchronize soil nitrogen availability with crop nitrogen uptake, and enhance the nitrogen recovery efficiency (Stehouwer & Johnson, 1990). This is similar to findings from Shapiro et al. (2016) who reported 20 kg ha^{-1} increase in aboveground biomass uptake with band vs. broadcast nitrogen treatments. Other studies also reported higher aboveground biomass N uptake with injected than surface broadcast nitrogen (Mengel et al., 1982; Stehouwer & Johnson, 1990). This favorable effect with band vs. broadcast corresponds to lower nitrification rate of AA than urea during early growing season. The higher aboveground biomass N with AA did not lead to higher grain yield in 2021 probably because nitrogen might be available in excess amounts while corn plants tend to partition more nitrogen from grain to stalks when excess nitrogen is available (Shapiro et al., 2016). This also explains why urea treatments had higher NHI, as AA and urea had statistically similar grain yield but higher aboveground biomass N with AA than urea. Regardless, the higher aboveground biomass N and NRE in AA than urea can be beneficial as it leads to more plant N uptake and less seasonal potential N loss through nitrate leaching or denitrification.

1.5.3 The Effect of Nitrification Inhibitors, Nitrogen Source, and Herbicide on Residual Soil Nitrogen

Nitrogen conservation with nitrification inhibitors and nitrogen sources during the growing season is often implied to better synchronize nitrogen availability with crop nitrogen uptake. However, any nitrogen left after the crop harvest is not useful as this can be lost during the winter period (Shapiro et al., 2016). Previous research has shown that enhanced efficiency fertilizers with nitrification inhibitors can leave higher post-harvest soil nitrate nitrogen when used at higher nitrogen rates (Maharjan et al., 2016; Shapiro et al., 2016). In this study, residual nitrate nitrogen across all treatments was lower (0.31-33 kg N ha^{-1}) than generally accepted normal residual NO_3^--N concentration of 50 kg NO_3^--N in fine textured soils of Nebraska (Shapiro et al., 2008). However, we found higher residual NH_4^+-N (11-63 NH_4^+ kg N ha^{-1}) than residual NO_3^--N (0.31-33 kg N ha^{-1}) across all treatments. This could be partly due to drier than normal conditions during both site-years which might have left more NH_4^+-N due to lower N movement in the soil profile. Higher residual NH_4^+-N values from AA than urea in 2021 further indicates nitrogen conservation with band placement than broadcast as reported for in-season N availability earlier. It was interesting to observe higher residual NH_4^+-N in deeper than upper soil layers in 2021 depicting some unknown processes for production of NH_4^+-N in the deep soil layers. This trend is similar to results of a study reported by colleague who found higher NH_4^+-N in the deeper soil layers than the topsoil layer (unpublished results). Nevertheless, these results suggest that in addition to residual NO_3^--N, NH_4^+-N especially under dry conditions should be considered when accounting nitrogen credits for nitrogen requirements for the subsequent cash crop. Furthermore, nitrogen conservation from the nitrification inhibitor and nitrogen sources could be considered when analyzing the value of nitrification inhibitor and nitrogen placement.

1.6 Conclusion

The effects of nitrification inhibitor, nitrogen source, and herbicide on early-season soil nitrogen availability and agronomic indicators were compared in this study. Though we had below than normal precipitation during both study years, but we still observed some significant nitrification inhibitor and source effect on nitrogen indicators in both years. Nitrogen source had a larger effect on in-season nitrogen availability than nitrification inhibitors. Anhydrous ammonia had 78-80% higher NH_4^+-N and 134-176% lower NO_3^--N production than urea in both years, indicating the potential of higher nitrogen conservation with band placement than broadcasted nitrogen. Compared to nitrogen source, nitrification inhibitor had a smaller effect on nitrogen conservation and retained 24-47% higher NH_4^+-N and produced 8-31% lower NO_3^--N than without inhibitor. Herbicide also had smaller effect on in-season nitrogen availability and retained 23-25% higher NH_4^+-N than No-PEH. Furthermore, anhydrous application showed significant advantages as shown by increased grain yield up to 1.06 Mg ha^{-1}, partial factor productivity by 5.7 kg grain kg^{-1} N, agronomic efficiency by 5.5 kg grain kg^{-1} N, aboveground biomass N uptake by 35 kg N ha^{-1}, grain N uptake by 15 kg N ha^{-1}, and nitrogen recovery efficiency by 21%. These improved agronomic indicators might be attributed to higher nitrogen conservation with anhydrous ammonia compared to urea. In addition, residual total soil N was high in anhydrous ammonia by 6-40 kg N ha^{-1} compared to urea, indicating the potential for nitrogen stabilization with application of nitrogen in band than broadcast. These results suggest that nitrogen management in corn can be improved by banding than broadcasting nitrogen in the soil. Though nitrification inhibitors and herbicides had smaller effect on nitrogen conservation compared to nitrogen source, using a right combination of nitrogen source, nitrification inhibitors and herbicide can provide additive effect in conserving soil nitrogen and improving nitrogen use efficiency in corn.

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1.8 Appendix

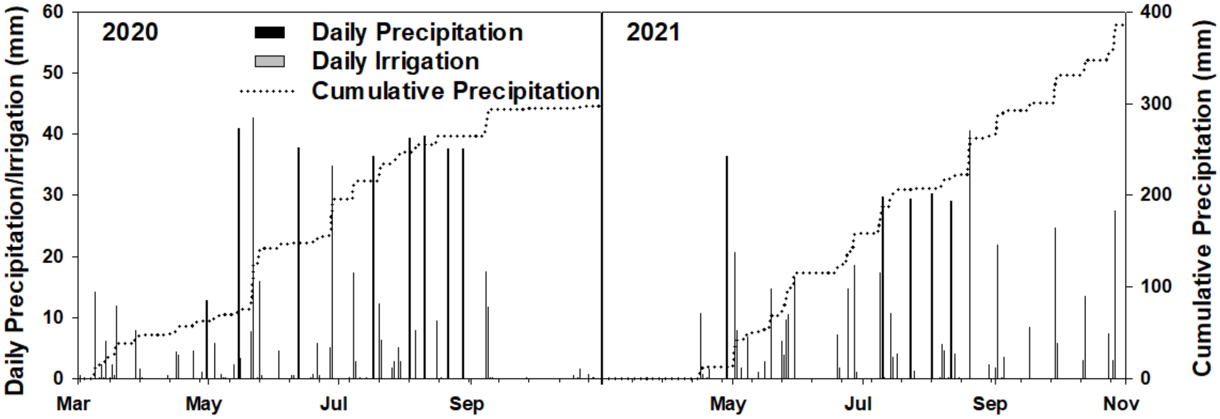

Figure 1.1 Daily precipitation, irrigation, and cumulative precipitation in each site-year of the study.

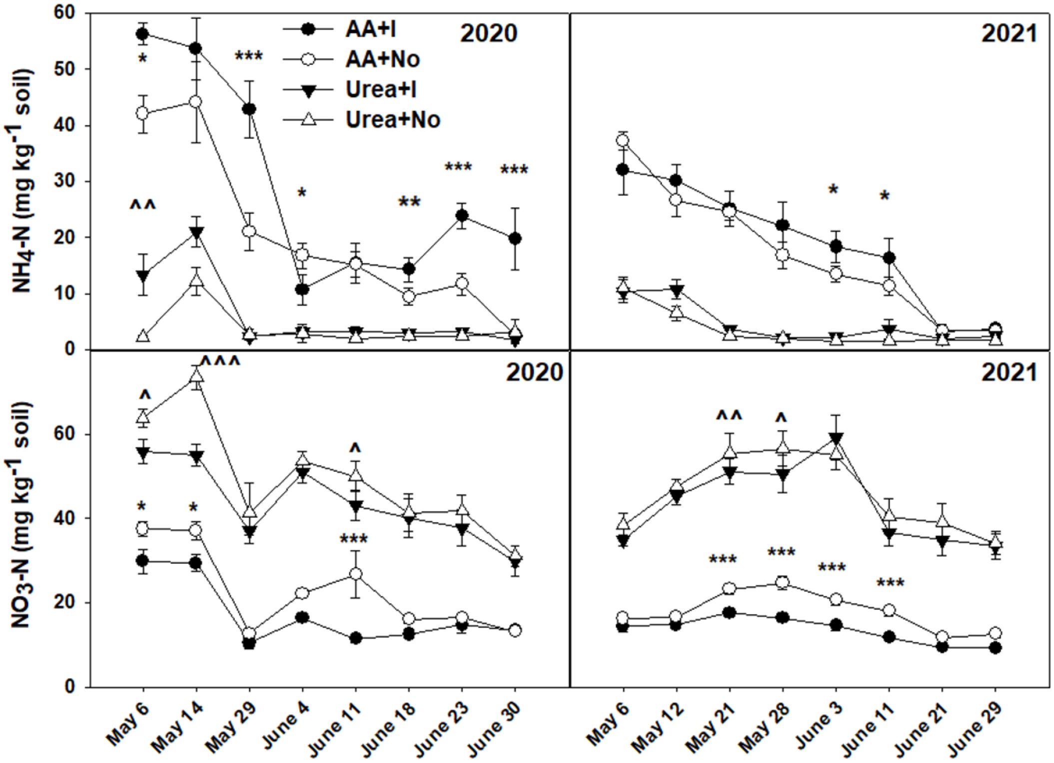

Figure 1.2 Weekly soil NH_4^+-N (upper two panels) and NO_3^--N (lower two panels) concentration in soil sampled at 0-20cm depth for two months (May and June) of 2020 and 2021.

Error bars indicate standard error of the mean. Asterisks indicate significant mean comparison between anhydrous ammonia with inhibitor (AA+I) and anhydrous ammonia without inhibitor (AA+No) at each sampling date within each site-year as determined by least-square means (*≤0.05, **≤0.01, ***≤0.0001). Caret symbols indicate significant mean comparison between urea with inhibitor (U+I) and urea without inhibitor (U+No) at each sampling date within each site-year as determined by least-square means (^≤0.05, ^^≤0.01, ^^^≤0.0001). Significant P values using repeated measure PROC GLIMMIX procedure for the differences of nitrification inhibitor and nitrogen source are shown in table 2.

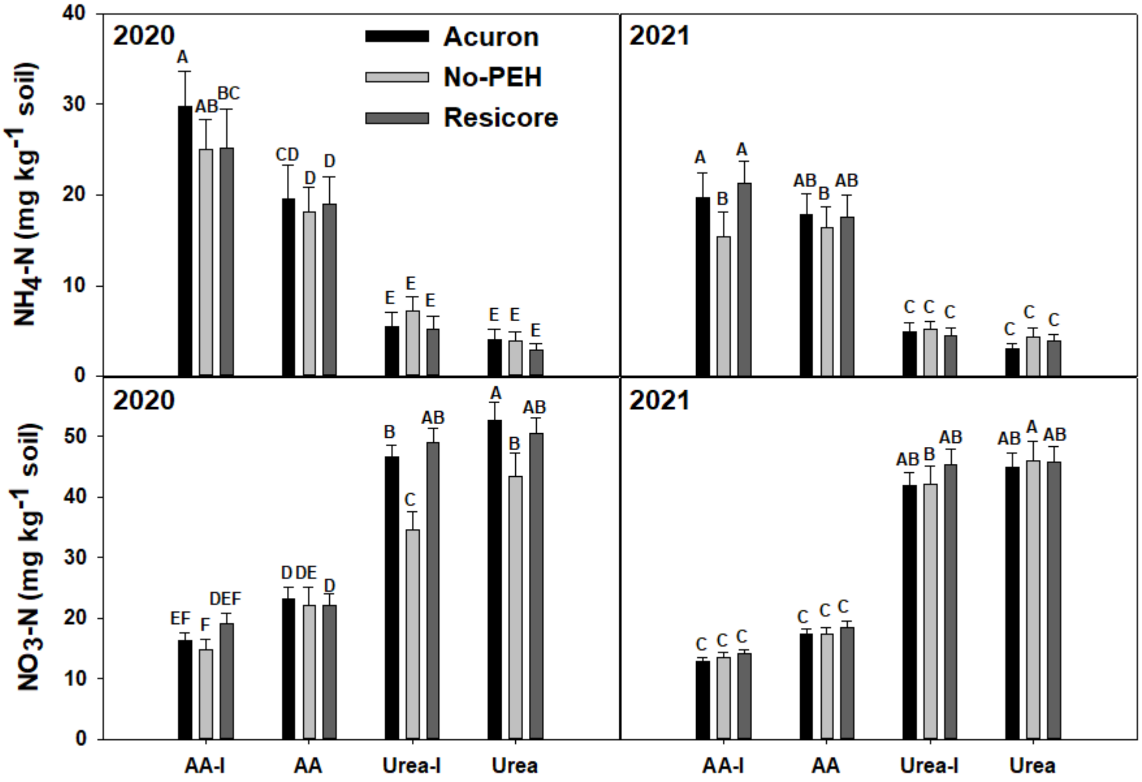

Figure 1.3 Mean soil NH_4^+-N (upper two panels) and NO_3^--N (lower two panels) concentration in soil sampled at 0-20cm depth during two months (May and June) of 2020 and 2021.

Error bars indicate standard error of the mean. Different letters above bars within each source variable indicates significant mean differences between the herbicide treatments as determined by least-square means. No-PEH indicates no preemergence herbicide.

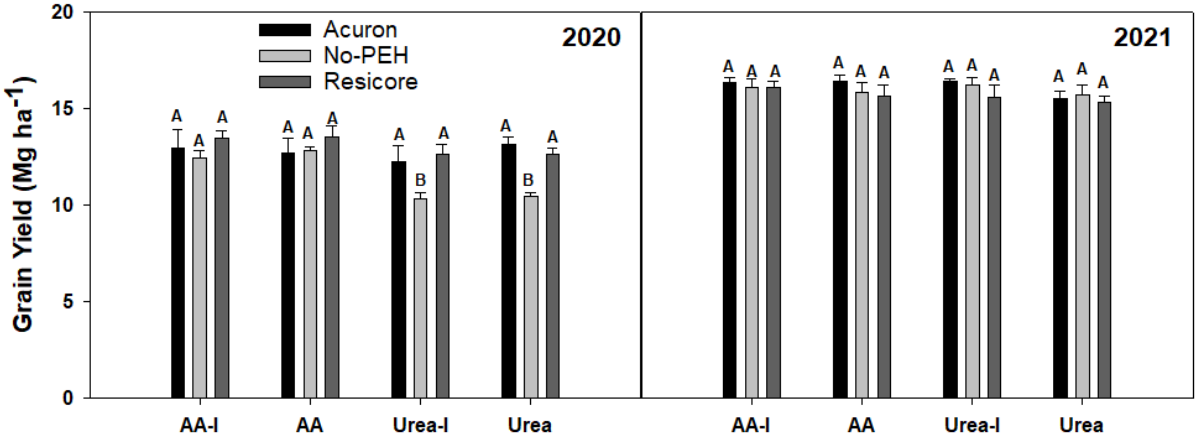

Figure 1.4 The effect of nitrogen inhibitor, nitrogen source and herbicide on corn grain yield in year 2020 and 2021.

The values are mean ± standard error of mean. Different letters above bars indicate significant mean differences between treatments as determined by least-square means. No-PEH indicates no preemergence herbicide.

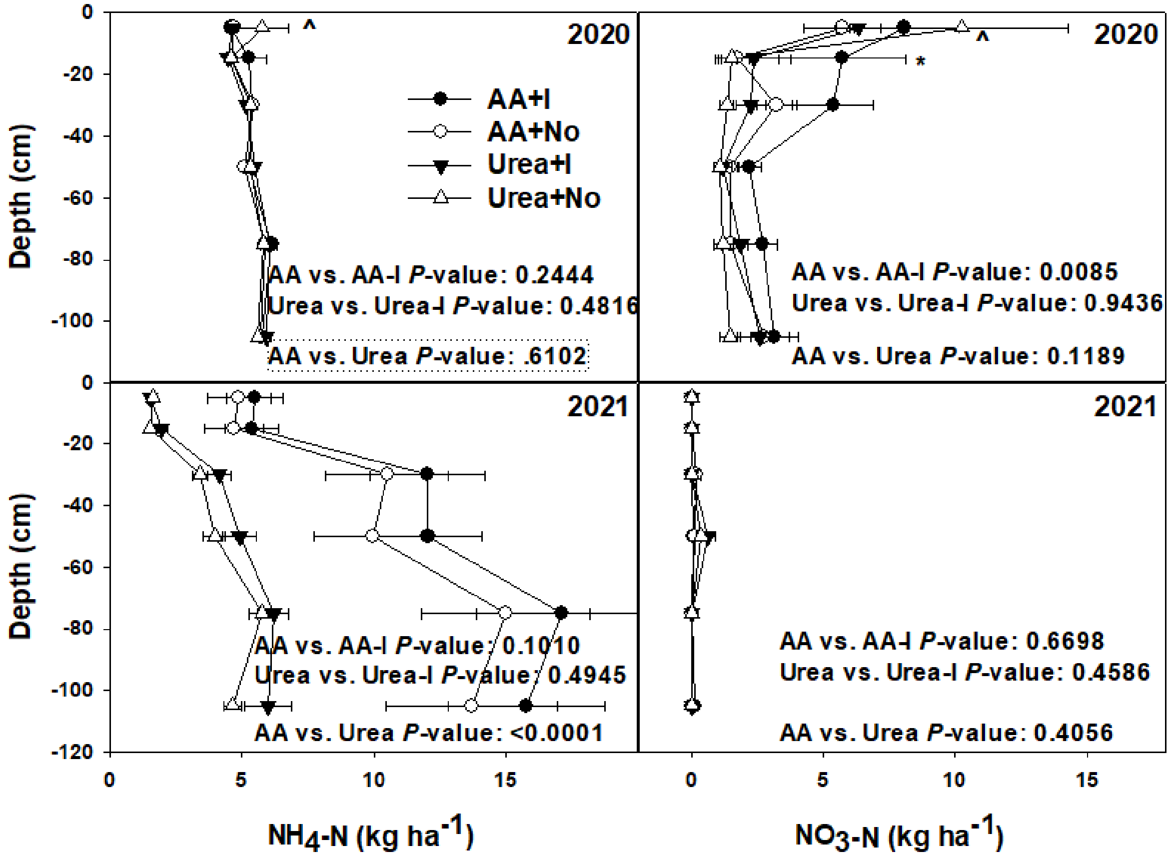

Figure 1.5 Post-harvest soil NH_4^+-N (left two panels) and NO_3^--N (right two panels) concentration in soil sampled at 0-120cm depth in 2020 and 2021.

Error bars indicate standard error of the mean. Asterisks indicate significant mean comparison between anhydrous ammonia with inhibitor (AA+I) and anhydrous ammonia without inhibitor (AA+No) at each sampling depth within each site-year as determined by least-square means (*≤0.05, **≤0.01, ***≤0.0001). Caret symbols indicate significant mean comparison between urea with inhibitor (U+I) and urea without inhibitor (U+No) at each sampling depth within each site-year as determined by least-square means (^≤0.05, ^^≤0.01, ^^^≤0.0001). Significant P values using PROC GLIMMIX procedure for the differences of N Inhibitor and N source are shown in each panel.

Table 1.1 Mean (± standard error) of selected soil chemical and physical properties of the two site-years.

Property	**2020**	**2021**
Soil pH (1:1 soil to water)	6.1 ± 0.1	5.9 ± 0.1
Soil Organic Matter (%)	2.7± 0.03	3.2 ± 0.04
NO_3-N (mg kg^{-1})	12 ± 1	6 ± 1
Mehlich-III P (mg kg^{-1})	50 ± 6.56	46.5 ± 4.09
Mehlich-III K (mg kg^{-1})	336 ± 10	332 ± 23
SO_4 (mg kg^{-1})	8 ± 1	9 ± 1
Ca (mg kg^{-1})	2400 ± 80	2100 ± 60
Mg (mg kg^{-1})	385 ± 25	272 ± 3
Na (mg kg^{-1})	39 ± 5	37 ± 1
Sum of Cations (me $100g^{-1}$)	20.3 ± 0.8	18.3 ± 0.4
Soil Texture	Silty Clay Loam	Silty Clay Loam

Table 1.2 Probability values (repeated measures PROC GLIMMIX) for the main effect of nitrification inhibitor (I), nitrogen source (S), herbicide (H), time, and their interaction on soil NO_3^--N and NH_4^+-N concentrations measured during May-June of 2020 and 2021.

Effect/year	2020		2021	
	NO_3-N	NH_4-N	NO_3-N	NH_4-N
I	<0.0001	<0.0001	0.0015	0.0554
S	<0.0001	<0.0001	<0.0001	<0.0001
H	0.0188	0.3382	0.6944	0.3942
Time	<0.0001	<0.0001	<0.0001	<0.0001
I x S	0.9509	0.0032	0.6704	0.5221
I x H	0.1648	0.3819	0.4576	0.6332
I x time	0.0015	<0.0001	0.2738	0.0274
S x H	0.0221	0.2680	0.7469	0.1317
S x time	<0.0001	<0.0001	<0.0001	<0.0001
H x time	0.0250	0.1842	0.1254	0.6063
I x S x H	0.8382	0.3737	0.6371	0.6467
I x S x time	0.4257	<0.0001	0.3637	0.1877
I x H x time	0.1336	0.3227	0.2555	0.7062
S x H x time	0.0628	0.1603	0.3238	0.4581
I x S x H x time	0.3228	0.3672	0.7846	0.9880

Table 1.3 Means and analysis of variance of grain yield (GY), partial factor productivity (PFP), agronomic efficiency (AE), residual soil nitrate (NO$_3^-$-N), and residual soil ammonium (NH$_4^+$-N) as affected by nitrification inhibitor, nitrogen source, and herbicide treatments.

Sources of effect	2020					2021				
	GY	PFP	AE	NO$_3$-N	NH$_4$-N	GY	PFP	AE	NO$_3$-N	NH$_4$-N
	(Mg ha^{-1})	(kg grain kg^{-1} N)		(kg N ha^{-1})		(Mg ha^{-1})	(kg grain kg^{-1} N)		(kg N ha^{-1})	
N Inhibitor										
With	12.33	66.3	15.8	7.63	9.55	16.13	86.7	22.0	0.10	35.94
Without	12.57	67.6	17.3	6.02	9.40	15.77	84.8	20.0	0.08	30.94
N source										
AA	12.99a	69.9a	19.4a	7.60	9.39	16.09	86.5	21.7	0.07	49.19a
Urea	11.93b	64.1b	13.9b	6.05	9.56	15.81	85.0	20.2	0.11	17.69b
Herbicide										
No-PRE	11.51b	61.9b	22.7a	5.88	10.08	15.99	86.0	20.2	0.05	34.98
Acuron	12.77a	68.6a	10.7b	7.91	8.77	16.19	87.0	21.5	0.14	35.30
Resicore	13.09a	70.4a	15.9b	6.68	9.57	15.67	84.3	21.2	0.08	30.05
Analysis of variance										
I	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
S	***	***	***	ns	ns	ns	ns	ns	ns	***
H	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
I x S	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
I x H	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
S x H	***	***	***	ns	ns	ns	ns	ns	ns	ns
I x S x H	ns	ns	**	ns	ns	ns	ns	ns	ns	ns

Mean within each variable (N inhibitor, N source, Herbicide) and column with different letters are significantly different at $P < 0.05$.

Table 1.4 Means and analysis of variance of grain N concentration, grain N uptake, aboveground biomass N uptake, nitrogen recovery efficiency and nitrogen harvest index as affected by nitrification inhibitor, nitrogen source, and herbicide treatment in 2021.

Sources of effect	Grain N conc.	Grain N uptake	Aboveground biomass N uptake	Nitrogen recovery efficiency	Nitrogen Harvest Index
	(%)	($kg\ N\ ha^{-1}$)		(%)	
Inhibitor					
With	1.26	218	321	67	68
Without	1.28	220	320	67	69
N source					
AA	1.27	226	338a	77a	67b
Urea	1.26	211	303b	58b	70a
Herbicide					
No-PRE	1.26	216	313	71	69
Acuron	1.27	217	310	53	70
Resicore	1.27	223	338	77	66
Analysis of variance					
I	ns	ns	ns	ns	ns
S	ns	ns	*	*	**
H	ns	ns	ns	ns	ns
I x S	ns	ns	ns	ns	ns
I x H	ns	ns	ns	ns	ns
S x H	ns	ns	ns	ns	ns
I x S x H	ns	ns	ns	ns	ns

Mean within each variable (N inhibitor, N source, Herbicide) and column with different letters are significantly different at $P < 0.05$.

Chapter 2: The Effect of Nitrogen Source, Nitrification Inhibitor, and Herbicide on Nitrification and Nitrogen Loss in Loamy Sand Soil

2.1 Abstract

Increased environmental nitrogen losses have prompted the use of enhanced efficiency nitrogen fertilizers including nitrification inhibitors. The comparative effects of these nitrification inhibitors to conventional nitrogen fertilizer sources and herbicides on soil nitrification and nitrogen losses are not well understood. We evaluated the effect of nitrification inhibitor, nitrogen fertilizer source, and herbicide on (1) soil nitrification through a 25 day-soil incubation and (2) NH_3 volatilization, NO_3^--N leaching and N_2O emissions through a 31-day soil column study using a loamy sand soil. In both experiments, the treatments included a combination of nitrification inhibitor (Instinct II) vs. no inhibitor, two nitrogen fertilizer sources (broadcast urea vs injected aqueous ammonia), and a pre-emergence Acuron herbicide vs. no herbicide. Results indicated that nitrogen source had a more significant effect on reducing nitrification (32.5% lower with injected aqueous ammonia vs. surface broadcast urea) than nitrification inhibitors (4% lower with inhibitor vs. without inhibitor) and herbicide (no effect). Surface broadest urea increased NH_3 volatilization by 673% compared to injected aqueous ammonia, while nitrification inhibitor had inconsistent effect on NH_3 volatilization across both N sources. Injected aqueous ammonia had 22% higher NO_3^--N leaching and 33 % higher NH_4^+-N leaching compared to urea, while nitrification inhibitor had inconsistent effect on NO_3^--N leaching across both N sources. Furthermore, no significant treatment effect was observed on N_2O emissions. Herbicide did not affect measured parameters except increasing NO_3^--N leaching by 9% than no-herbicide. These results indicate that nitrogen source plays a significant role in regulating soil nitrogen losses than nitrification inhibitors and herbicide.

2.2 Introduction

Nitrogen is one of the most limiting nutrients required for crop production. Its consumption rate has significantly increased worldwide. International Fertilizer Institute has estimated global nitrogen consumption rate of 1.4% from 2014-2018 (IFASTAT, 2019). This increased fertilizer nitrogen consumption has led to more crop production to meet food and fiber demand for the growing world population (Mueller et al., 2012; Bouwman et al., 2005). At the same time, this nitrogen use has resulted in significant environmental implications in the form of increasing greenhouses gas emissions, and surface and groundwater contamination (Iqbal et al., 2017; Wang et al. 2016). These negative environmental effects are generally associated with excessive nitrogen fertilizer inputs linked with nitrogen losses through soil processes of denitrification, nitrate leaching, and ammonia volatilization (Iqbal et al., 2015; Dawar et al., 2011; Peng et al., 2015). Ammonia volatilization occurs when urea is left on the soil surface for an extend period of time (Keller and Mengel, 1986). Denitrification and nitrate leaching losses are generally associated with excessive or untimely nitrogen application along with excessive rainfall or irrigation (Shapiro et al., 2016). Efficient nutrient management strategies are required to decrease these nitrogen losses and improve nitrogen management to achieve sustainable food production.

Several fertilizer nitrogen additives have been introduced in the market to alter nitrogen transformation processes and reduce N losses. For example, temporarily inhibiting the rate of nitrification is one of the important processes to reduce the nitrogen losses from row crops (Lehtovirta-Morley et al. 2013; Yu et al. 2018). Nitrapyrin is one of nitrification inhibitor that is well known to inhibit nitrification (Wolt, 2000; Decock 2014; Scheer et al. 2014). This compound temporarily deactivate enzyme responsible for the conversion of NH_4^+-N to NO_3^--N

(Misselbrook et al., 2014; Gilsanz et al., 2016) and reduces the potential of nitrous oxide emissions and nitrate leaching under vulnerable N loss conditions (Ruser and Schulz, 2015). Bronson et al. (1992) observed that nitrapyrin decreased nitrous oxide emissions by 66% in an irrigated corn field. In an incubation study, Peng et al. (2015) reported that nitrapyrin decreased nitrate leaching by 26% in a sandy loam soil. However, the use of these nitrification inhibitors to retain N in NH_4^+-N form on the soil surface for an extended period can increase the risk of nitrogen loss through NH_3 volatilization (Ferguson et al., 1984).

Injecting nitrogen fertilizer into the soil is another strategy to improve nitrogen use efficiency and decrease nitrogen losses (Touchton and Hargrove, 1982). In our two-year research study at irrigated corn field in central Nebraska, we found anhydrous ammonia injection improved nitrogen use efficiency and retained 78-80% higher NH_4^+-N than surface broadcast urea during the early part of the growing season (Chapter I). On the other hand, surface broadcast fertilizer can result in significant NH_3 volatilization loss into the atmosphere (Al-Kanani and MacKenzie, 1992), but this loss can be minimized by injecting nitrogen into the soil (Touchton and Hargrove, 1982). Several other studies have reported improved nitrogen use efficiency with fertilizer injection or band placement compared to broadcast urea (Mengel et al., 1982; Stehouwer and Johnson et al., 1990; Shapiro et al., 2016). However, comparative effect of N source placement and nitrification inhibitors on nitrification and nitrogen losses is not well documented.

Some previous studies also reported that chemical herbicides can affect soil nitrification (Li et al. 2008, Mahia et al. 2008; Zhang et al., 2018). These herbicides affect the abundance of nitrifiers population to alter soil nitrification. Hernandez et al. (2011) found that s-triazine herbicide simazine changed ammonium oxidizing bacteria community patterns and inhibited nitrosobacteria. In another study, atrazine showed both inhibition and stimulatory effects on soil nitrification (Chen et al., 2015). As a result, herbicide impact on nitrification can potentially alter nitrogen transformation processes and affect N losses. However, to our knowledge, no previous studies have evaluated the effect of herbicide on nitrogen losses.

In this study, we evaluated the impact of nitrapyrin nitrification inhibitor, nitrogen source, and acuron herbicide on nitrification, NH_3 volatilization, N_2O emissions and NO_3^--N leaching. Based on the result of our previous field study, we hypothesized that effect of nitrogen source on nitrification and nitrogen losses will be much stronger than nitrification inhibitor and herbicide. Thus, the objectives of this study were to evaluate the effect of nitrapyrin nitrification inhibitor, nitrogen source, and acuron herbicide on soil nitrification and nitrogen losses via NH_3 volatilization, N_2O emissions and NO_3^--N leaching.

2.3 Materials and Methods

2.3.1 Soil Characteristics

The soil used for this study was collected from a corn field at Antelope County, Nebraska (42°25'02.3"N, 98°02'52.3"W). The soil at the site is characterized as well-drained Thurman loamy fine sand (sandy, mixed, mesic Udorthentic Haplustolls). The soil was collected at 0-20cm depth, brought to the laboratory, and sieved through a 2-mm screen after air drying. Triplicate sub-samples of air-dried and sieved soil were used to determine soil physical and chemical properties, while the remaining soil was used for two incubation studies: 1) a 31-day soil column study, and 2) a 25-day soil nitrification study. Soil physical and chemical properties are shown in Table 2.1.

2.3.2 Soil Column Study

The air-dried soil was used to determine the soil moisture contents and adjust to 12% gravimetric water content by applying water and mixing thoroughly (Pandey et al., 2020). The adjusted water contents corresponded to 70% of soil field capacity. The soil was packed in clear acrylic columns to a target bulk density of 1.3 Mg m^{-3} and depth of 25cm on top of a ceramic plate placed at the bottom of the column (Peng et al., 2015). Cylindrical acrylic column used in the experiment had a diameter of 5 cm and a height of 35 cm. A porous ceramic plate (10^5 Pa strength) was inserted at the bottom of the column, and Whatman 42 filter paper was placed on top of the ceramic plate to avoid soil clogging into the ceramic plate. The column had lid system placed at either end. The bottom lid had a vacuum port that allowed the suction to be applied during the collection of the leachate. The upper lid had two parts (middle connector and upper lid). The middle connecter was threaded into main column and used to install the NH_3 acid trap, while the upper lid was used to terminate the column with a closed end along with septum port used to collect N_2O gas samples from the headspace above the soil. The soil columns were pre-incubated in an incubator at a daytime temperature of 25C and nighttime temperature of 18C for 10 days to allow for microbial populations to grow. After treatment applications and besides data collection, columns were always kept in the incubator at the same settings.

The treatments were organized in a split block design with four replications. The main block had two herbicide treatments: 1) No herbicide, 2) Acuron. Acuron is a pre-mix of atrazine/bicyclopyrone/*s*-metolachlor/mesotrione. It is a commonly used pre-emergence herbicide in Nebraska. Herbicide was applied at recommended rate of 2.25 µL kg^{-1} soil (equivalent to 5.8 L ha^{-1}). The split block had four nitrogen treatments with two nitrogen sources (aqueous ammonia and urea) with and without nitrification inhibitors as following: 1) AA+I -

aqueous ammonia with nitrification inhibitor of Instinct NXTGEN, 2) AA+No – aqueous ammonia without nitrification inhibitor, 3) Urea+I - urea with nitrification inhibitor of Instinct NXTGEN, and 4) Urea+No – urea without nitrification inhibitor. Instinct NXTGEN is an encapsulated nitrapyrin (2-chloro-6-[trichloromethyl] pyridine) formulation from Corteva that inhibits nitrification. Instinct NXTGEN was applied at a recommended rate of 0.67 µL kg^{-1} soil (Equivalent to 1.7 L ha^{-1}). For aqueous ammonia treatment, ammonium hydroxide was injected with a syringe at 2.5-cm soil depth in soil column to simulate anhydrous ammonia injection in the field. For urea application, urea granules were dissolved in an aqueous solution and evenly spread over the column soil surface through a syringe. In all nitrogen treatments, nitrogen fertilizer was applied at 69.2 mg N kg^{-1} soil which is equivalent to optimal N rate of 225 kg N ha^{-1} for corn crop. Following soil column preparation and treatment application, the water was periodically added during the 31-day incubation period to simulate rainfall (185.17 mm total) of above normal precipitation observed during May 2019 (Figure 2.1) in the nearby High Ag Plains weather station. Water was applied slowly with a syringe to allow infiltration and avoid surface water ponding.

Ammonia volatilization was quantified by using acid trap method described by McGinn & Janzen (1998). Acid trap sponges (5 cm in diameter) were soaked in 5 mL of a glycerol solution (40 mL glycerol, 50 mL concentrated H3PO4 acid, 910 mL DI water) and placed in the column headspace for approximately 24 hours at each sampling time. Sponges were collected and replaced with fresh sponges on Day 1, 2, 3, 4, 5, 6, 7, 10, 13, 18, 22, 26, 30. Upon collection, the sponges were extracted using 50 mL 2M KCl solution. Extracts were frozen until analyzed for NH_4^+-N concentration in microplates using the Berthelot reaction (Hood-Nowotny et al., 2010). Leachate samples were collected following rainfall events on day 7, 8, 9, 12, 15, 19, 21, 22, 24, 27, 28, 29 of the study. Samples collection was facilitated by using a 0.25 hp motor vacuum connected to the bottom lid of each column through the ceramic plate for 1 minute (Peng et al., 2015). Volume of leachate was recorded for each column at each collection date to determine volume-based N losses. The leachate samples were frozen until analyzed for NO_3^--N + NO_2^--N (hereafter NO_3^--N) and NH_4^+-N concentration in microplates using the Griess-Ilosvay reaction with vanadium chloride as a reducing agent and the Berthelot reaction, respectively (Hood-Nowotny et al., 2010).

Nitrous oxide samples were collected following rainfall simulation on days 1, 2, 3, 4, 5, 6, 7, 8, 11, 12, 14, 18, 21, 23, 24, 26, 27, 28 using the septum port on the upper terminal lid. Before gas sampling, NH3 trap was removed, and the soil column left open to equilibrate gas concentration between the headspace and ambient air. A 25-ml syringe was used to collect gas samples at time 1 (0 minute) and time 2 (30 minutes) following upper terminal lid closure. Time 1 gas samples were collected right before closing the lid. The gas samples were transferred into a 12 -ml glass scintillation vial. The gas samples were analyzed for N_2O concentration using a electron capture detector on a gas chromatograph (450-GV, Varion). Daily gas flux (ug N_2O-N /g soil/day) values were calculated by taking the difference of gas concentration between time 1 and time 2.

Upon termination of the study, soil was removed from the columns intact and separated into 5 equal depth increments of 5 cm. The soil in each increment was then homogenized and subsampled for soil gravimetric water content and soil NO_3^--N and NH_4^+-N measurements. For each increment, 12 gram subsample was used for soil gravimetric water contents. The 2nd sub-sample soil was extracted with 2M KCL solution (5:1 solution/soil ratio) by shaking at 180 rpm for 1 hour. The extracts were analyzed for NO_3^--N and NH_4^+-N concentration in microplates as described above.

2.3.3 A 25-Day Nitrification Study

To evaluate the effect of nitrification inhibitor, nitrogen source and herbicide on soil nitrification, a 25-day incubation study was conducted with repeated measures of soil NH_4^+-N and NO_3^--N. The same treatments and experimental design as described above were repeated in multiple 120 mL graduated specimen containers (Medline®). A 20 g of dry-equivalent soil was added to specimen containers at a bulk density of 1.3 g cm^{-1} and brought to 12% gravimetric water content (corresponded to 70% field capacity) by adding DI water followed by treatment application. Urea solution was broadcasted over the soil surface, while aqueous ammonia was injected into the soil at 2-cm depth. Throughout the study, the containers were kept in the incubator with a daytime temperature of 25C and nighttime temperature of 18C. Lids were kept loose on the graduated containers to allow gas exchange and avoid gas build up during the incubation. Soil moisture was periodically checked and maintained by weighing the containers throughout the incubation. There were 9 sets of all treatments combinations with each set being analyzed for soil NO_3^--N and NH_4^+-N on day 0, 2, 5, 9, 13, 17, 25 of the experiment. Each sample was extracted with 100 mL of 2M KCl and analyzed for soil NO_3^--N and NH_4^+-N with procedure described above. Two additional sets at day zero and day 25 were used to determine soil pH (1:1) using a pH meter. Net nitrification rate between the sampling dates was determined by using the following formula (Zhang et al., 2018):

Net nitrification rate = $N2-N1/T2-T1$

Where N1 and N2 were the NO_3^--N contents at time T1 and T2

2.3.4 Statistical Analysis

The data was analyzed using SAS version 9.4 (SAS Institute). Repeated measure PROC GLIMMIX procedure was used to test the effects of nitrification inhibitor, nitrogen source, herbicide, and time on soil nitrification, NO_3^--N and NH_4^+-N leaching, NH_3-N volatilization and N_2O-N emissions. The nitrification inhibitor, N source, herbicide, and time were treated as the fixed effects while block and all interactions of block with other terms were used as random effects. PROC GLIMMIX procedure was used to test the effect of nitrification inhibitor, nitrogen source, herbicide, and depth on soil residual NO_3^--N and NH_4^+-N. Comparison of the means were conducted by comparing differences in least-square means with significance differences at $P<0.05$.

2.4 Results

2.4.1 Nitrate and Ammonium Leaching

The effects of treatment main and interaction effect on nitrate and ammonium leaching are given in table 2. Nitrate and ammonium leaching was significantly influenced over time (Table 2.2.). No leaching occurred until day 7 of the experiment followed by 10 leaching events until the termination of the column study. Highest nitrate leaching was observed on day 8 which decreased over time with most (approximately 93%) of the NO_3^--N leaching occurring in the first 20 days of the study (Figure 2.2.). Nitrogen source and nitrification inhibitor had a significant interaction effect on nitrate leaching (Table 2.2.). Averaged across all sampling dates, AA+I had about 24% (12.1 mg L^{-1}) lower nitrate leaching than AA+No, while no significant difference of nitrification inhibitor occurred in urea treatment. Within nitrogen source, aqueous ammonia had 22.4% (14.6 mg L^{-1}) higher NO_3^--N leaching than urea (Figure 2.2.). Herbicide has significant main as well as interaction effect with the nitrogen source. Averaged across all sampling dates, aqueous ammonia with acuron increased nitrate by 21% (0.34 mg L^{-1}) compared to aqueous ammonia with no-herbicide. There was no significant interaction between herbicide treatments and Urea. Averaged across all treatments, acuron increased nitrate by 8.7% (5.3 mg L^{-1}) compared to no-herbicide.

No interaction effects of treatments were observed on NH_4^+-N leaching; however, nitrogen source and time had a significant main effect on NH_4^+-N leaching. Ammonium leaching increased over time with most of leaching (approximately 87%) occurring on the last four sample collection dates (Figure 2.2.). Aqueous ammonia had 32.6% (1.6 mg L^{-1}) higher NH_4^+-N leaching than urea treatments. Nitrification inhibitor and herbicide had no significant main effect on NH_4^+-N leaching. Overall, cumulative mineral N (NO_3^--N and NH_4^+-N) leaching accounted for 3-27% of applied N in the column. There were significant nitrogen source main effects on cumulative N leaching. Aqueous ammonia had 24% less cumulative nitrogen leaching than urea (Table 2.4.). However, nitrification inhibitor and herbicide did not affect cumulative nitrogen leaching (Table 2.4.).

2.4.2 Nitrogen Loss via Nitrous Oxide Emission and Ammonia volatilization

Nitrous oxide emissions significantly varied over time and ranged from 1.56 to 2 mg (equivalent to 2.84-3.63% of applied N) during the study. N_2O emissions fluctuated over time and peaked on day 11 (Figure 2.3.). No significant main or interaction effects of nitrification inhibitor, nitrogen source, and herbicide were found on N_2O emissions (Table 2.2.) except a single sampling day with significant nitrification inhibitor effect. On day 11, urea+I had 50.7% higher N_2O N emission than Urea+No. Averaged across all treatments, cumulative N loss through N_2O emissions was 1.5-2% of the applied nitrogen (Table 2.8.).

NH_3 volatilization significantly varied over time with peaks observed within one week of incubation (Table 2.2.; Figure 2.4.). About 78% of NH_3 volatilization occurred within the first 5 days of incubation. A significant nitrification inhibitor and nitrogen source interaction effect was observed on NH3 volatilization. Urea+I had 39.7% (3.2 mg kg^{-1} soil) less NH_3 volatilization than Urea+No while there was no significant difference between AH+I and AH+No. Nitrogen source had a significant main effect on NH_3 volatilization. Urea had 673% higher NH_3 volatilization compared to aqueous ammonia (17.9 vs. 2.3 mg). In addition, nitrogen recovery through cumulative NH_3 volatilization was higher in urea (27% of applied N) than aqueous ammonia (3.4 % of applied nitrogen).

2.4.3 Soil Residual NH_4^+-N and NO_3^--N and Fertilizer Nitrogen Recovery

A significant nitrogen source x herbicide x depth interaction was observed for soil residual NH_4^+-N (Table 2.3.). Urea with herbicide had higher soil residual NH_4^+-N at 10-15 cm than urea without herbicide. Furthermore, a significant nitrogen source x depth interaction was observed in soil column. Urea had significantly higher residual soil NH_4^+-N at 5-10 and 10-15cm depth and significantly lower soil residual NH_4^+-N at 20-25 cm than aqueous ammonia (Figure 2.5.). There was a significant main effect of depth on soil residual NH_4^+-N, while the main effects of nitrification inhibitor, nitrogen source, and herbicide were not significant. Soil residual NH_4^+-N increased with depth with the highest concentration at the 15-20 cm and the lowest at 0-5 cm depth.

No significant interaction effects were observed for soil residual NO_3^--N in the column. However, soil residual NO_3^--N was significantly influenced by main effects of nitrification inhibitor and depth. For example, nitrogen fertilizer with nitrification inhibitors had 0.9 mg kg^{-1} soil less NO_3^--N than fertilizer with no nitrification inhibitors (Table 2.3., Figure 2.5.). When separated by depth, soil NO_3^--N was significantly lower at 0-5cm depth compared to the rest of the soil profile (Fig. 2.5.). Soil residual N (NH_4^+-N + NO_3^--N) accounted for a large portion of N recovery (35-38%) in the column (Table 2.4.). However, no significant treatment main or interaction effect on nitrogen recovery of soil residual N in column were observed.

Across all treatments in column, the FNR_{ResN} ranged from 58-74% (Table 2.4.). There were significant main and interaction effects of nitrification inhibitor and nitrogen source on FNR_{RES}. For example, nitrification inhibitor had 9% lower FNR_{RES} compared to no nitrification inhibitor. Aqueous ammonia had 24% lower FNR_{RES} than urea. When averaged across all treatments, no significant effect of herbicide on FNR_{RES} was observed.

2.4.4 Soil Nitrification and pH

In the 2nd soil incubation study, net nitrification was quantified by measuring the changes in soil NO_3^--N pool during the 25 days period. Throughout the incubation study, net nitrification significantly varied over time (Table 2.5.). Net nitrification rates were lower at the start of the study which gradually increased on day 2-13 and then decreased on day 13-17 (Fig. 2.7.). This was followed by a sharp increase in net nitrification rate during the last week of the study. Throughout the incubation period, significant source effect was observed on soil nitrification. Aqueous ammonia had consistently lower nitrification rate throughout the study except on day 13-17 (Fig. 2.7.). When averaged across the entire incubation period, aqueous ammonia had 32.5 % lower net nitrification than urea (Table 2.5.). Nitrification inhibitor did not affect net nitrification rate, though it had a significant effect on day 2-5 only. Similarly, herbicide did not have a significant effect on net nitrification throughout the incubation period.

Figure 2.6. depicts the soil pH of nitrogen source x inhibitor treatments at the beginning and end of the 25-day incubation study. Soil pH of all treatment combinations decreased during

the study as nitrification is an acidifying process. Nitrification inhibitor did not have any effect on soil pH while nitrogen source had significant effect (Figure 2.6.). Aqueous ammonia had significantly higher soil pH than urea at both the beginning and end of the study, although the difference was greater on day 0 than day 25.

2.5 Discussion

2.5.1 Soil Nitrification, Nitrate and Ammonium Leaching

Finding ways to decrease the rate of soil nitrification is an important step for improving nitrogen use efficiency and reducing nitrogen losses under vulnerable nitrogen loss conditions in the Midwest (Lehtovirta-Morley et al 2013, Fisk et al 2015, Yu et al 2018). There have been several calls for a systematic approach in selecting agronomic management practices to improve nitrogen use efficiency (Sahrawat 2008, Sahrawat and Keeney 1985). So, we conducted this study to evaluate the impact of some agronomic practices on soil nitrification and potential nitrogen losses. Nitrification inhibitors and herbicides are known to temporarily inhibit the nitrification process and reduce the potential nitrogen losses as it has been reported in previous studies (Franzen, 2017; Peng et al., 2015; Chen et al., 2015). However, to our knowledge, comparative effect of nitrification inhibitor, nitrogen source, and herbicide on nitrification and nitrogen losses have not been reported.

Generally, the potential N losses through nitrate leaching are more likely after heavy rainfall especially under sandy soils (Loecke et al., 2017; Van Metre et al., 2016; Yahdjian and Sala 2010, Pandey et al., 2020). So, in this study we tested the effect of nitrification inhibitor, nitrogen source, and herbicide on nitrification and potential nitrogen losses under a high precipitation scenario on a loamy sand soil where the performance of nitrification inhibitors is often better than fine textured soils (Barth et al., 2019). During the 25-day nitrification study, nitrification inhibitor slightly decreased nitrification which corresponded with 7.5% lower nitrate leaching with inhibitor than without inhibitor in the soil column study. Reduction in nitrate leaching with nitrification inhibitor agrees with the previous studies (Owens 1987; Walters and Malzer, 1990; Di et al., 2009). However, the slight decrease in nitrification with inhibitor did not change soil pH (Figure 5). Compared to nitrification inhibitor, nitrogen source had a significant effect on inhibiting nitrification as aqueous ammonia had 32.5% lower nitrification rates and significantly higher soil pH than urea. This was similar to findings from our field trial where injected anhydrous ammonia significantly reduced nitrification and had 78-80% higher NH_4^+-N than broadcast urea in May and June of two growing seasons (Chapter I). Other studies also reported lower nitrification with injected band application of nitrogen than surface broadcast nitrogen application (Biederbeck et al., 1996; Shapiro et al., 2016). It is interesting that despite having lower nitrification with aqueous ammonia, it had comparatively higher nitrate leaching than urea in the column study. This might be due to couple of reasons. First, the effective distance between the aqueous ammonia placement at 2.5 cm soil depth and point of collection of leachate in soil column (a simulated vadose zone) was less than the leachate collection distance from surface broadcast of urea. This also led to less N gaseous losses in aqueous ammonia treatment and more nitrate availability for leaching than urea (Table 2.4.). However, it must be noted that this smaller vadose zone or N fertilizer proximity to leachate may not be applicable to real field conditions. Secondly, lower nitrification conserved more nitrogen in the soil profile which eventually led to higher nitrate leaching as nitrogen must move in the soil column upon rainfall simulation given that no plant roots were available to take up the nitrogen. Therefore, nitrate leaching from the two nitrogen sources in soil column study could be different than real field conditions and required field scale research. Nonetheless, the findings from this study indicates that selecting a right nitrogen source has the higher probability of inhibiting nitrification and protecting potential N loss than nitrification inhibitors during spring period as more wet springs are predicted in the future (Dai et al., 2016; Hatfield et al., 2011).

Though herbicides are mostly commonly used to control weeds in the row crops, but previous studies have shown that herbicide containing atrazine and acetochlor can also produce non-target effects on soil microorganism by decreasing soil microbial biomass (Mahia et al., 2008) and altering ammonia oxidizing archaea (AOA) and ammonia oxidizing bacteria (AOB) *amo*A gene abundances which are related to soil nitrification process (Caffrey et al., 2007; Mahia et al., 2008; Zhang et al., 2018). In this study, herbicide did not affect nitrification in the 25-day incubation study, but surprisingly increased nitrate leaching in the soil column study. This contrasting results of herbicide on nitrification and nitrate leaching could be due to variable herbicide response in soil column vs. 25-day incubation study. Previous studies have reported both stimulatory and inhibitory effect of herbicide on nitrification (Chen et al., 2015). In a laboratory study, Laursen & Carlton (1999) reported that higher level of atrazine inhibited nitrification, while lower level of atrazine increased nitrification. So, in this study herbicide might have increase nitrification at low herbicide dose in the soil column, and therefore increased NO_3^--N leaching. Further research is required to ascertain the effect of herbicide on nitrification and NO_3^--N leaching.

2.5.2 Nitrogen Loss via Nitrous Oxide Emission and Ammonia volatilization

N source, nitrification inhibitor and herbicide did not affect N_2O emissions in this study. This insignificant effect of nitrification inhibitor and N source on N_2O emissions might be due to large variability of N_2O emissions in laboratory replicates (Figure 2.3.). N_2O emissions are often highly variable under laboratory and field conditions due to the dynamic nature of its nitrification and denitrification pathway (Panday et al., 2020). Although the variability can be due to specific soil conditions in each study, the higher CV observed in this study failed to detect the differences among the treatments (Patel et al., 2001). It is also important to note that considerable amount of nitrogen moved through soil in the column on day 7-9 or lost through ammonia volatilization within first 5 days before first N_2O peak was observed on Day 11. This implies not enough substrate would have been available at the soil surface to cause a treatment effect.

Surface broadcast N fertilizer can lose nitrogen by 30 to 60% of applied N through ammonia volatilization under favorable soil and environmental conditions (Martha et al., 2004; Franzen et al 2011; Soares et al., 2012; Vaio et al., 2008). Therefore, it is recommended to inject nitrogen into the soil to reduce ammonia volatilization N losses (Dawar et a., 2011, Rochette et al., 2013). In this study, despite having lower soil pH with urea, surface applied urea had significantly higher ammonia volatilization N losses compared to injected aqueous ammonia in the soil column. Ammonia volatilization losses were < 4% of applied N with aqueous ammonia while these were 27% with urea. These results are consistent with previous studies where higher ammonia volatilization losses were reported with surface broadcast urea (Giggs et al., 2007; Faria et al., 2013; Cai et al 2002, Jantalia et al 2012). Furthermore, nitrification inhibitors can also affect ammonia volatilization loss. In this study, nitrification inhibitor either has no effect with aqueous ammonia or reduced ammonia volatilization when used with urea. It was similar to findings from Misselbrook et al. 2014 and Ni et al 2014 who reported that nitrification inhibitors had no consistent effect on NH_3 volatilization. In contrast, Soares et al., 2012 found that nitrification inhibitors increased ammonia volatilization by 5-15% due to the higher soil pH and prolonged presence of NH_4^+-N. However, in this study nitrification inhibitors did not affect soil pH and rather decreased volatilization loss. Though the underlying mechanism for the reduced

NH_3 volatilization with nitrification inhibitor is not well known, soil pH could be a contributing factor in regulating NH_3 volatilization with nitrification inhibitors. In a meta-analysis, Wu et al., 2021 reported that soil pH controlled NH_3 volatilization in nitrification inhibitor studies. In another study, Recio et al., 2018 found no effect of nitrification inhibitors on NH_3 volatilization despite having a large pool of NH_4^+-N in soils with nitrification inhibitor. Nevertheless, the contracting effect of nitrification inhibitors on NH_3 volatilization can be due to specific soil conditions in each study. Herbicide had no significant effect on ammonia volatilization that corresponded with no significant herbicide effect on net nitrification during the incubation study.

2.5.3 Soil Residual NH_4^+-N and NO_3^--N

At the end of the soil column study, significantly higher NO_3^--N and NH_4^+-N at the bottom of the column indicated downward movement of nitrogen in the column (Panday et al., 2020; Pierzynski et al., 2005). Previous studies have shown that 25.4 mm of rainfall or irrigation can transport the nitrate in loamy sand soil (Endelman et al., 1974). In this study, we simulated 185 mm of rain which resulted in both nitrate and ammonium leaching out of the column. However, residual N still had the largest nitrogen recovery of the applied N than other N fractions in the soil column (Table 2.4.). Urea retained higher NH_4^+-N at the 5-15 cm of the column compared to aqueous ammonia which was possibly result of placement of N fertilizer. Surface application of urea might have taken more time to transport nitrogen through the column than aqueous ammonia injected at 2.5cm depth which had less distance to move to the bottom of the column. As a result, less distance between the AA placement at 2.5cm soil depth and point of collection of leachate (a simulated vadose zone), and more availability of residual N corresponded to higher nitrate leaching with aqueous ammonia than urea. Though no effect of nitrogen source, nitrification inhibitor and herbicide were found on residual NH_4^+-N and NO_3^--N, an exception was the significant effect of nitrification inhibitor on residual nitrate. This decreased residual nitrate with nitrification inhibitor might have been a result of slightly reduced nitrification with inhibitor (Table 2.5.; Figure 2.7.) as nitrification inhibitors are well known to reduce nitrification and delay the conversion of NH_4^+-N to NO_3^--N (Franzen, 2017; Peng et al., 2015). This reduced nitrification effect or higher NH_4^+-N retention with nitrification inhibitor can last up to 6 weeks (Singh et al., 2008; Kim et al., 2011), while our 30-day study period was within in that time frame. As a result, significantly lower residual nitrate and slightly higher NH_4^+-N in soil column study was expected (Table 2.3.). This is similar to findings from other studies where higher ammonium retention values with nitrification inhibitor after 4 four weeks of incubation have been reported (Franzen 2017).

2.6 Conclusion

States with high risk of groundwater nitrate contamination or gaseous N losses are promoting the use of nitrification inhibitors to minimize N losses. However, the impact of nitrogen fertilizer sources compared to nitrification inhibitors are not well understood. In this study, we evaluated the effect of nitrification inhibitors, nitrogen sources, and herbicide on N losses and found that nitrogen source had a more significant effect on reducing nitrification than nitrification inhibitors and herbicides. Decreased nitrification with injected ammonia-based fertilizer compared to broadcast urea indicated the potential of higher nitrogen stabilization with injected nitrogen. Furthermore, injected nitrogen significantly decreased NH_3 volatilization losses compared to broadcasted nitrogen, indicating some economic and environmental advantages with injected nitrogen. Compared to broadcasted urea, injected aqueous ammonia increased nitrate leaching after midway of the column study probably because of design of the study with (1) less distance between the aqueous ammonia injection and point of collection of leachate (a simulated vadose zone) than broadcasted urea and the point of collection of leachate, and (2) lack of plant roots to take up the nitrogen as nitrogen has to eventually escape. Herbicide did not affect nitrification or any other measured parameters except an increase in nitrate leaching. Nevertheless, the results of this study suggest that nitrogen management can be improved by minimizing nitrogen losses by injecting ammonia-based fertilizer than broadcasting nitrogen in the soil. Though in this study nitrification inhibitors had smaller effect on nitrogen stabilization compared to nitrogen source, using a right combination of nitrogen source and nitrification inhibitors can provide additive effect in conserving soil nitrogen and improving nitrogen use efficiency in crops. Further evaluation of nitrogen sources vs. nitrification inhibitors through field research on environmental nitrogen is warranted.

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2.8 Appendix

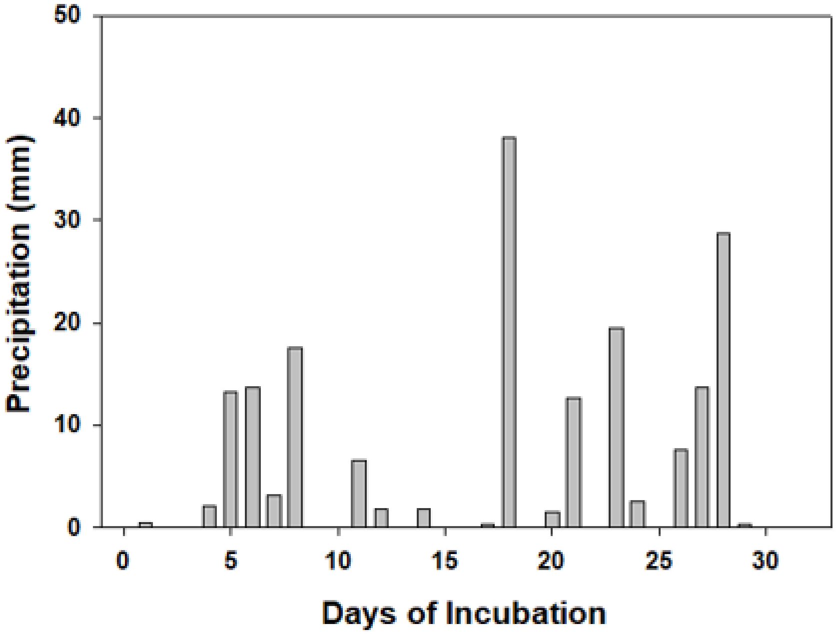

Figure 2.1 Simulated daily precipitation during the column study.

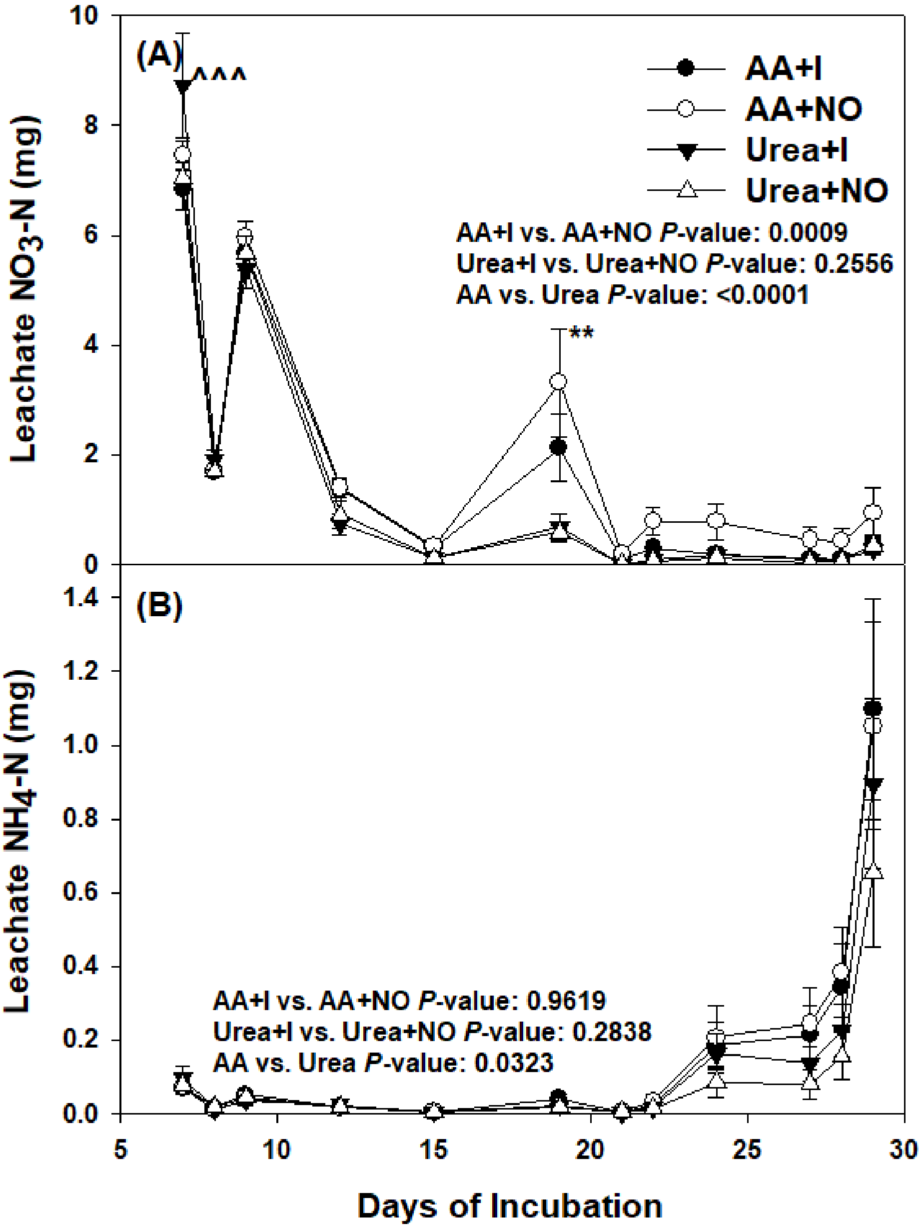

Figure 2.2 Daily NO_3^--N (A) and NH_4^+-N (B) concentrations in leachate collected from soil columns.

Significant P values using repeated measure PROC GLIMMIX procedure for the differences of N Inhibitor and N source are shown. Error bars indicate standard error of the mean. Asterisks indicate significant mean comparison between ammonium hydroxide with inhibitor (AH+I) and ammonium hydroxide without inhibitor (AH) at each sampling date within each site-year as determined by least-square means (*≤0.05, **≤0.01, ***≤0.0001). Caret symbols indicate significant mean comparison between urea with inhibitor (U+I) and urea without inhibitor (U) at each sampling date within each site-year as determined by least-square means (^≤0.05, ^^≤0.01, ^^^≤0.0001).

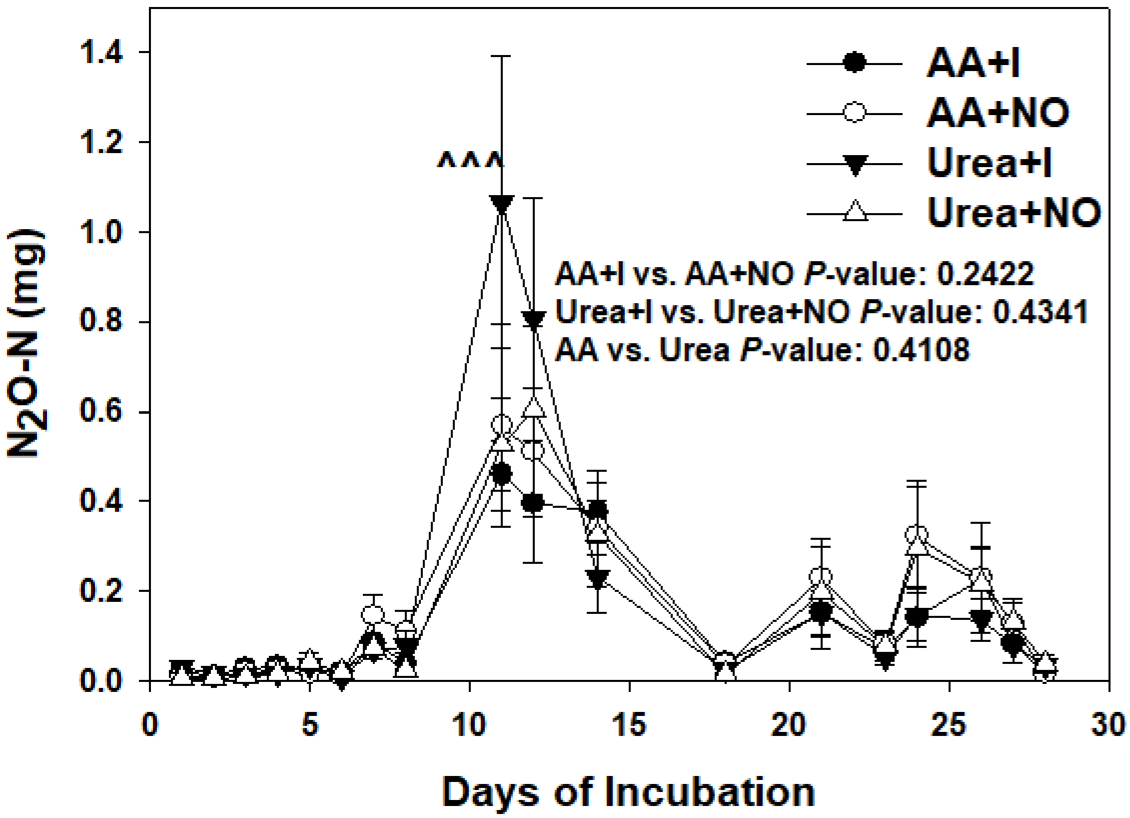

Figure 2.3 Daily N_2O-N emissions under different treatments in soil column study.

Significant P values using repeated measure PROC GLIMMIX procedure for the differences of N Inhibitor and N source are shown. Asterisks indicate significant mean comparison between ammonium hydroxide with inhibitor (AH+I) and ammonium hydroxide without inhibitor (AH) at each sampling date within each site-year as determined by least-square means (*≤0.05, **≤0.01, ***≤0.0001). Caret symbols indicate significant mean comparison between urea with inhibitor (U+I) and urea without inhibitor (U) at each sampling date within each site-year as determined by least-square means (^≤0.05, ^^≤0.01, ^^^≤0.0001).

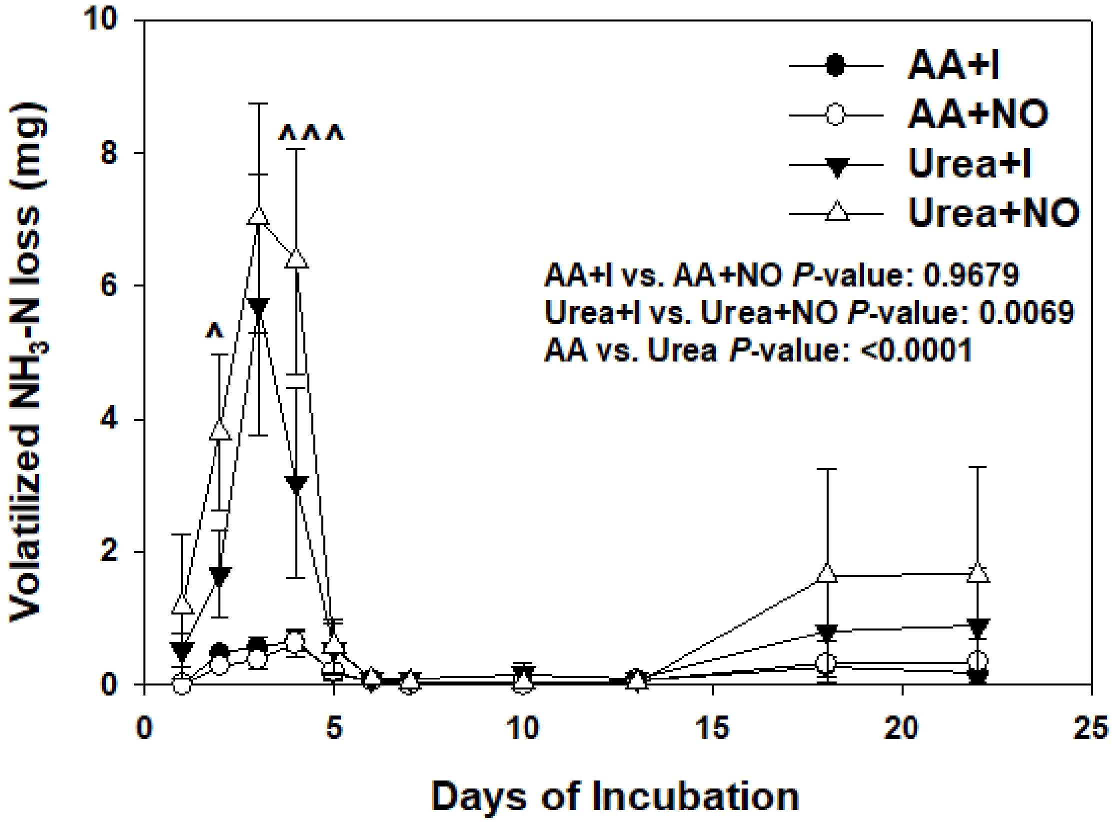

Figure 2.4 Daily NH_3-N volatilization losses from soil column study.

Significant P values using repeated measure PROC GLIMMIX procedure for the differences of N Inhibitor and N source are shown. Asterisks indicate significant mean comparison between ammonium hydroxide with inhibitor (AH+I) and ammonium hydroxide without inhibitor (AH) at each sampling date within each site-year as determined by least-square means (*≤0.05, **≤0.01, ***≤0.0001). Caret symbols indicate significant mean comparison between urea with inhibitor (U+I) and urea without inhibitor (U) at each sampling date within each site-year as determined by least-square means (^≤0.05, ^^≤0.01, ^^^≤0.0001).

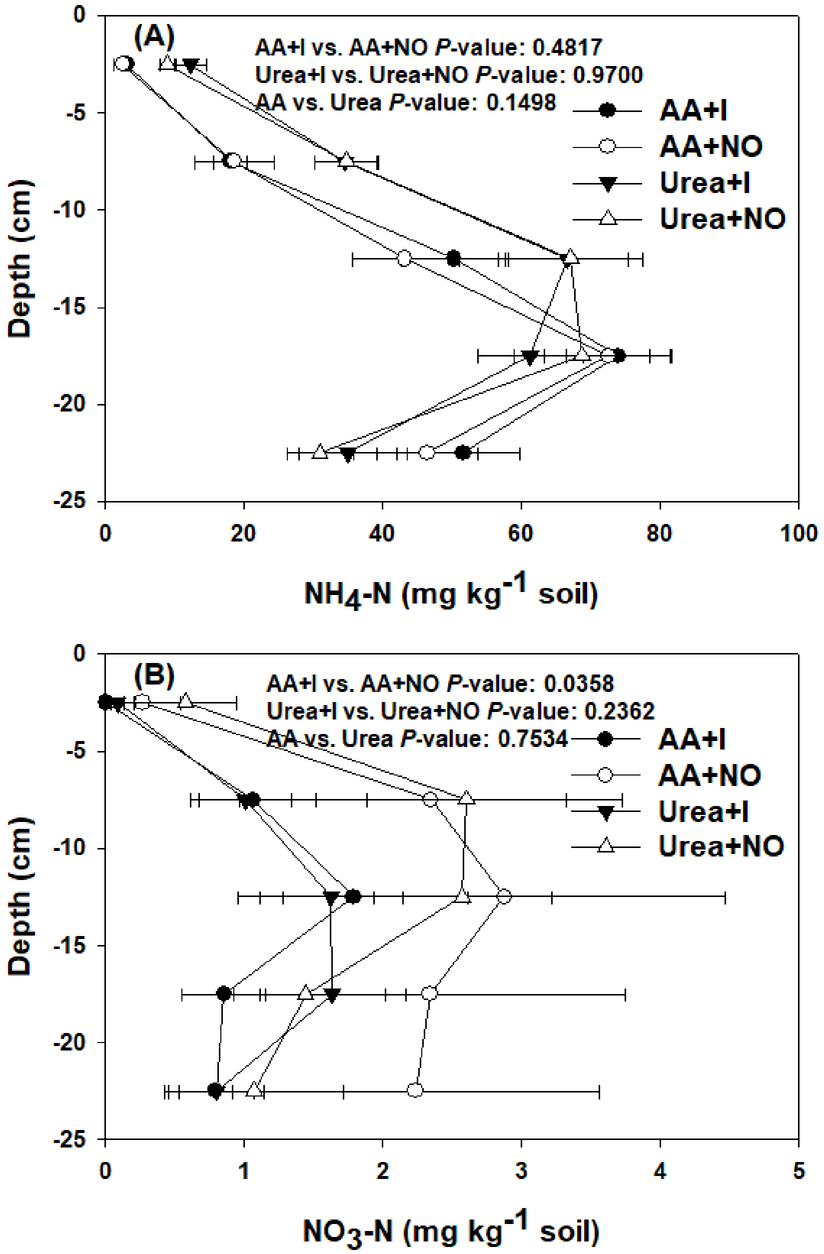

Figure 2.5 Soil Residual NH_4^+-N (A) and NO_3^--N (B) concentration in soil sampled at 5-cm depth intervals upon termination of the soil column study.

Significant P values using repeated measure PROC GLIMMIX procedure for the differences of N Inhibitor and N source are shown. Asterisks indicate significant mean comparison between ammonium hydroxide with inhibitor (AH+I) and ammonium hydroxide without inhibitor (AH) at each sampling date within each site-year as determined by least-square means (*≤0.05, **≤0.01, ***≤0.0001). Caret symbols indicate significant mean comparison between urea with inhibitor (U+I) and urea without inhibitor (U) at each sampling date within each site-year as determined by least-square means (^≤0.05, ^^≤0.01, ^^^≤0.0001).

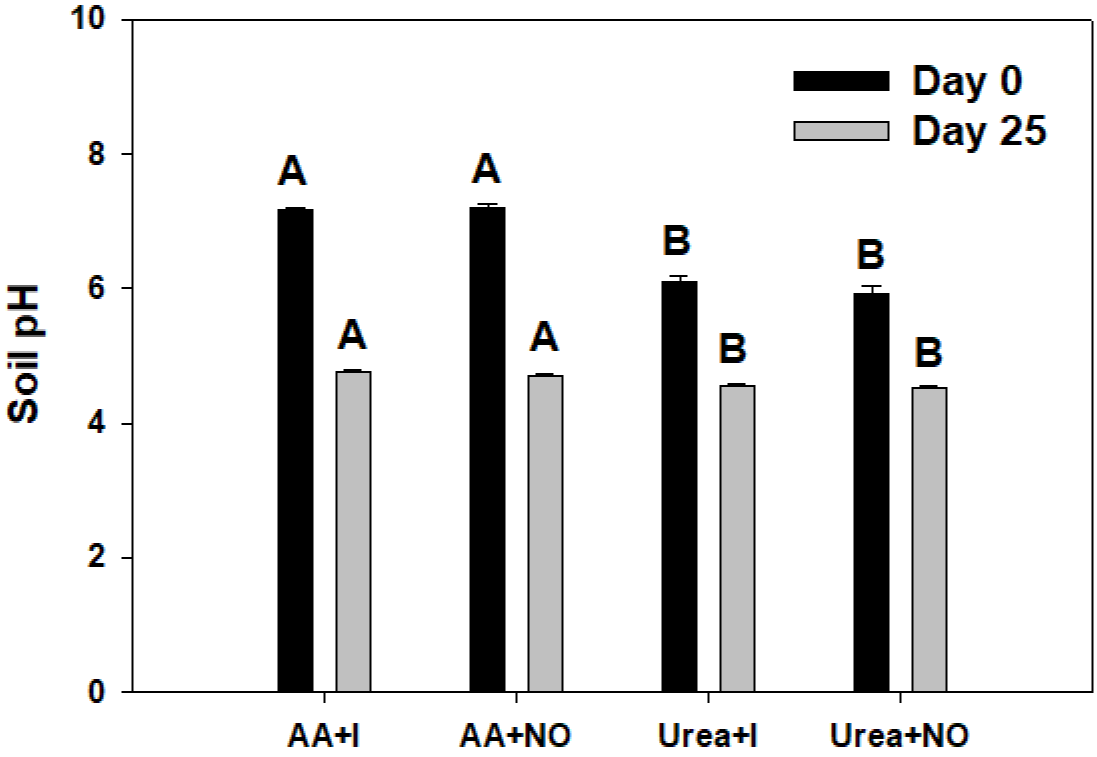

Figure 2.6 Soil 1:1 pH of treatments upon start and termination of specimen cup study.

Error bars indicate standard error of the mean. Different letters above bars within each time period indicates significant mean differences between treatments as determined by least-square means.

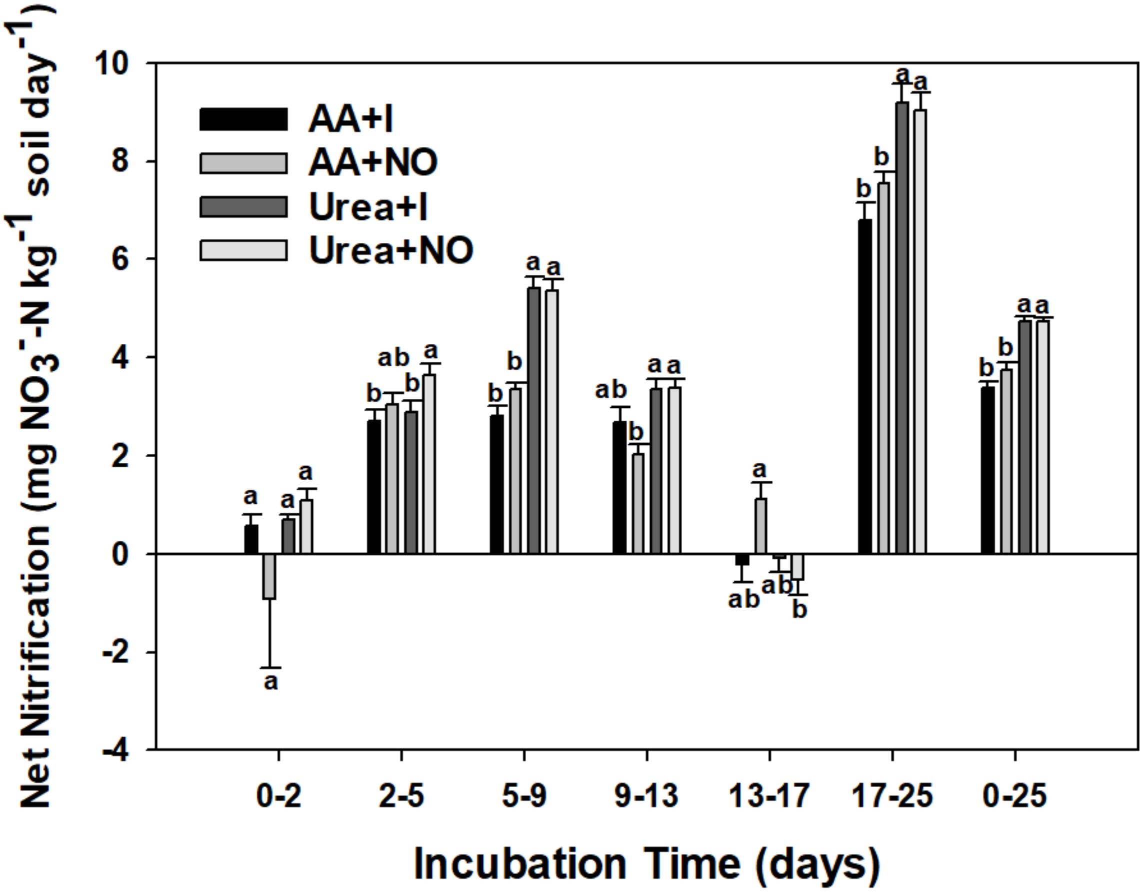

Figure 2.7 Effect of nitrification inhibitors and nitrogen sources on net nitrification rates during 25-day incubation study.

Bars with same letters are not significantly different in LSD test (P≤0.05) reported from PROC GLIMMIX procedure. Data are shown as mean±SE.

Table 2.1 Mean (± standard error) of selected soil chemical and physical properties of the soil used in the study.

Property	**Value**
Soil pH (1:1 soil to water)	6.3 ± 0.1
Soil Organic Matter (%)	1.33 ± 0.1
NO_3-N (mg kg^{-1})	1.13 ± 0.03
Mehlich-III P (mg kg^{-1})	42 ± 8.72
Mehlich-III K (mg kg^{-1})	116 ± 2.16
SO_4 (mg kg^{-1})	10 ± 0.46
Ca (mg kg^{-1})	830 ± 25
Mg (mg kg^{-1})	74 ± 3
Na (mg kg^{-1})	13 ± 1
Sum of Cations (me 100g^{-1})	7.23 ± 0.24
Soil Texture	Loamy Sand

Table 2.2 Probability values (repeated measures PROC GLIMMIX) for the main effect of nitrification inhibitor (I), nitrogen source (S), herbicide (H), time, and their interaction on leachate NO_3^--N, leachate NH_4^+-N, nitrous oxide (N_2O-N) emission, and volatilized NH_3-N in soil column study.

Effect	**Leachate NO_3-N**	**Leachate NH_4-N**	**N_2O – N emission**	**Volatilized NH_3-N**
I	NS	NS	NS	NS
S	***	*	NS	***
H	*	NS	NS	NS
Time	***	***	***	***
I x S	**	NS	NS	*
I x H	NS	NS	NS	NS
I x time	NS	NS	NS	NS
S x H	*	NS	NS	NS
S x time	***	NS	NS	***
H x time	NS	NS	NS	NS
I x S x H	NS	NS	NS	NS
I x S x time	NS	NS	NS	NS
I x H x time	NS	NS	NS	NS
S x H x time	NS	NS	NS	NS
I x S x H x time	NS	NS	NS	NS

NS, not significant.
*Significant at the .05 probability level.
**Significant at the .01 probability level.
***Significant at the .001 probability level.

Table 2.3 Means and analysis of variance of residual soil ammonium (NH_4^+-N) and residual soil nitrate (NO_3^--N) as affected by nitrification inhibitor (I), nitrogen source (s), herbicide (H), soil depth and their interactions in soil column study.

	NH_4^+-N (mg kg^{-1} soil)	**NO_3^--N (mg kg^{-1} soil)**
Inhibitor		
With	40.69	0.97B
Without	39.40	1.83A
Source		
AA	38.05	1.46
Urea	42.04	1.34
Herbicide		
No-PRE	38.36	1.18
Acuron	41.73	1.62
Depth		
0-5cm	6.74E	0.24B
5-10cm	26.50D	1.76A
10-15cm	56.83B	2.22A
15-20cm	69.14A	1.57A
20-25cm	41.01C	1.23AB
	Analysis of Variance	
I	NS	*
S	NS	NS
H	NS	NS
Depth	***	*
I x S	NS	NS
I x H	NS	NS
I x Depth	NS	NS
S x H	NS	NS
S x Depth	***	NS
H x Depth	NS	NS
I x S x H	NS	NS
I x S x Depth	NS	NS
I x H x Depth	NS	NS
S x H x Depth	*	NS
I x S x H x Depth	NS	NS

Mean within each variable (nitrification inhibitor, nitrogen source, herbicide, depth) and column with different letters are significantly different at $P < 0.05$.

NS, not significant.
*Significant at the .05 probability level.
**Significant at the .01 probability level.
***Significant at the .001 probability level.

Table 2.4 Means and analysis of variance of different dependent variables as influenced by nitrification inhibitor (I), nitrogen source (s), herbicide (H), and their interaction in soil column study.

	Soil Residual N#		**Leachate N#**		**Volatilized NH_3-N**		**N_2O-N**		**FNR_{RES}**	
	mg	**%**	**mg**	**%**	**mg**	**%**	**mg**	**%**	**mg**	**%**
Inhibitor										
With	20.4	37.1	5.8	10.5	5.8	10.5	1.9	3.3	33.8B	61.4B
Without	19.9	36.1	6.11	11.1	10.8	19.7	1.8	3.2	38.6A	70.2A
Source										
AA	19.1	34.8	6.9A	12.6A	1.9B	3.4B	1.69	3.1	29.6A	53.9 B
Urea	21.1	38.4	5.1B	9.0B	14.7A	27.0A	1.9	3.4	42.7B	77.6 A
Herbicide										
No-PRE	19.3	35.0	5.7	10.4	7.2	13.1	1.5	2.8	33.7	61.3
Acuron	21.0	38.2	6.2	11.2	9.4	17.1	2.0	3.7	38.6	70.2

Analysis of Variance

I	NS	NS	**	NS	*
S	NS	**	***	NS	***
H	NS	NS	NS	NS	NS
I x S	NS	NS	**	NS	*
I x H	NS	NS	NS	NS	NS
S x H	NS	NS	NS	NS	NS
I x S x H	NS	NS	NS	NS	NS

Mean within each variable (nitrification inhibitor, nitrogen source, herbicide) and column with different letters are significantly different at $P < 0.05$. # These values represent addition of NO_3^--N+NH_4^+-N

NS, not significant.
*Significant at the .05 probability level.
**Significant at the .01 probability level.
***Significant at the .001 probability level.

Table 2.5 Net Nitrification rates (mg kg^{-1} day^{-1}) as influenced by nitrification inhibitor (I), nitrogen source (s), herbicide (H), and their interaction in a 25-day nitrification study.

Incubation days **Treatment**	**0-2**	**2-5**	**5-9**	**9-13**	**13-17**	**17-25**	**0-25**
Inhibitor							
With	0.62	2.79B	4.10	3.02	-0.14	8.00	4.06
Without	0.08	3.34A	4.36	2.70	0.28	8.29	4.23
Source							
AA	-0.18	2.87	3.08B	2.35B	0.45A	7.17B	3.57B
Urea	0.89	3.26	5.38A	3.37A	-0.31B	9.12A	4.73A
Herbicide							
No-PRE	0.21	2.89	4.29	2.76	0.20	8.36	4.20
Acuron	0.49	3.25	4.17	2.95	-0.06	7.93	4.10

Analysis of Variance

I	NS	**	NS	NS	NS	NS	NS
S	NS	NS	***	***	*	***	***
H	NS	NS	NS	NS	NS	NS	NS
I x S	NS	NS	NS	NS	*	NS	NS
I x H	NS	*	NS	NS	NS	NS	NS
S x H	NS	NS	NS	NS	NS	NS	NS
I x S x H	NS	NS	NS	*	NS	NS	NS

Mean within each variable (nitrification inhibitor, nitrogen source, herbicide) and column with different letters are significantly different at P <0.05.

NS, not significant.
*Significant at the .05 probability level.
**Significant at the .01 probability level.
***Significant at the .001 probability level.

Chapter 3: General Conclusions

In this thesis, we used an integrated approach through field and laboratory studies to evaluate the effects of nitrification inhibitor, nitrogen source, and herbicide on early-season soil nitrogen availability, nitrification, agronomic indicators, and pathways for environmental N loss. The two-year field experiment and the laboratory soil incubation experiments indicated that the N source had the most significant effect in stabilizing NH_4^+-N and N availability, followed by nitrification inhibitor use and herbicide. Anhydrous ammonia had 78-80% higher NH_4^+-N and 134-176% lower NO_3^--N production than urea in both years, indicating the potential of higher nitrogen conservation with band placement than broadcasted nitrogen. On the other hand, compared to nitrogen source, nitrification inhibitor had a smaller effect on nitrogen conservation and retained 24-47% higher NH_4^+-N and produced 8-31% lower NO_3^--N than without inhibitor. The herbicide also had a smaller effect on in-season nitrogen availability and retained 23-25% higher NH_4^+-N than No-PEH. Furthermore, the anhydrous application showed significant advantages as shown by increased grain yield up to 1.06 Mg ha^{-1}, partial factor productivity by 5.7 kg grain kg^{-1} N, agronomic efficiency by 5.5 kg grain kg^{-1} N, aboveground biomass N uptake by 35 kg N ha^{-1}, grain N uptake by 15 kg N ha^{-1}, and nitrogen recovery efficiency by 21%. These improved agronomic indicators might be attributed to higher nitrogen conservation with anhydrous ammonia than urea. In addition, residual total soil N was high in anhydrous ammonia by 6-40 kg N ha^{-1} compared to urea, indicating the potential for nitrogen stabilization with application of nitrogen in band than broadcast.

We found that N source also had the largest impact on N losses through a 31-day lab incubation period. Injected nitrogen significantly decreased NH_3 volatilization losses compared to broadcasted nitrogen, indicating some economic and environmental advantages with injected nitrogen. Compared to broadcasted urea, injected aqueous ammonia increased nitrate leaching after midway of the column study probably because of the design of the study with (1) less distance between the aqueous ammonia injection and point of collection of leachate (a simulated vadose zone) than broadcasted urea, and (2) lack of plant roots to take up the nitrogen as nitrogen must eventually escape. Herbicide did not affect nitrification or any other measured parameters except an increase in nitrate leaching. These results suggest that nitrogen management can be improved by minimizing nitrogen losses by injecting ammonia-based fertilizer than broadcasting nitrogen in the soil. Though in this thesis, nitrification inhibitors had a smaller effect on nitrogen stabilization than nitrogen source, using a right combination of nitrogen source and nitrification inhibitors can provide an additive effect in conserving soil nitrogen and improving nitrogen use efficiency in crops. Further evaluation of nitrogen sources vs. nitrification inhibitors through field research on environmental nitrogen is warranted.