

USE OF ^{15}N -LABELED ANHYDROUS NH_3 TO DETERMINE THE EFFICIENCY OF FALL
N FERTILIZATION FOR CORN PRODUCTION

BY

KELSEY LUELLA GRIESHEIM

THESIS

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Adviser:

Professor Richard L. Mulvaney

ABSTRACT

Fall application of anhydrous ammonia (NH_3) is a common practice for corn (*Zea mays* L.) production in the Midwestern USA, but evaluations to date have relied entirely on yield comparisons that provide no means of distinguishing fertilizer from soil N uptake. A more rigorous evaluation of this practice requires the use of $^{15}\text{NH}_3$, which has long been impeded by the difficulties and safety hazards inherent to a liquefied gas that must be handled and applied under pressure. A manifold system is described for transferring known quantities of NH_3 from labeled and unlabeled sources to obtain a desired ^{15}N enrichment, and for collecting the mixture cryogenically in a tank specifically configured for knifed applications using a tractor-mounted tool bar. Enrichments of 1.2–1.5 atom % ^{15}N were obtained for 3 kg of NH_3 prepared within a normal working day by a fifteen-fold dilution with 10 atom % ^{15}N as the starting label. A collection capacity of such magnitude represents a 3000% upscaling over systems previously described for this purpose, providing an essential prerequisite for field plot research to realistically assess the fate and fertilizer value of anhydrous NH_3 . In using this system, six field trials were conducted between 2016 and 2018 by applying 224 kg N ha^{-1} with and without the use of nitrapyrin (NP) or an experimental alternative (EP) for inhibiting nitrification. Significant grain yield response to fall N fertilization averaged 46% at five of the six sites studied in both years of the experiment. Isotopic estimates of fertilizer N uptake efficiency (FNUE) ranged from 12 to 34% for grain and from 16 to 42% for total aboveground biomass, while in both cases N derived from fertilizer (NDFE) never exceeded 50%. A significant increase in FNUE occurred with NP but not EP at only two sites studied, whereas site differences in soil organic C and potentially mineralizable N had a much larger effect on crop uptake of ^{15}N . The results show that, even with the addition of NP, the majority of the N applied in the fall as anhydrous NH_3 is

not taken up by the following corn crop. If this practice is to be used, uptake efficiency can be improved by accounting for soil N mineralization.

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CHAPTER 1: General Introduction

The importance of synthetic fertilizer nitrogen (N) for agricultural systems is apparent from the rapid increase in cereal yields following the implementation of the Haber-Bosch process. Although the majority of ammonia (NH_3) generated by this process is used for urea and other synthetic fertilizers, direct application accounts for a large portion of fertilizer N used in the Midwestern USA (Illinois Department of Agriculture, 2019; Iowa Department of Agriculture, 2019).

While most anhydrous NH_3 is applied in the spring, fall application has long been a topic of interest as evidenced by coverage in the Illinois Agronomy Handbook since 1969 and is incentivized by lower prices (Schnitkey, 2018) and soil conditions that are often more conducive to field work than would exist in the spring when application equipment is in high demand. These applications, however, raise serious environmental concerns as the fertilizer N is subject to soil N cycle processes for a prolonged period before crop N uptake, with the risk of nitrate (NO_3^-) loss via leaching or denitrification.

A common strategy to reduce this type of loss following both spring and fall applications is with the use of a nitrification inhibitor such as nitrapyrin (NP; 2-chloro-6-(trichloromethyl)pyridine), which retards autotrophic NH_3 oxidation, thereby delaying the accumulation of fertilizer-derived NO_3^- (Meisinger et al., 1980). NP was first proved effective for decreasing nitrification by Goring (1962a,b) and its efficacy for this purpose has been verified in many subsequent studies (e.g., Campbell and Aleem, 1965; Bundy and Bremner, 1973; Touchton et al., 1979; Fisk et al., 2015). Despite this finding, numerous field studies have been conducted in which no significant yield benefit was found with the addition of NP (Boswell, 1976; Hendrickson et al., 1978; Malzer and Randall, 1985; Hanson et al., 1987;

Randall and Vetsch, 2005a; Pittelkow et al., 2017) and in some cases NP has caused corn yield decreases (Touchton, et al., 1979; Hoelt, 1984; Cerrato and Blackmer, 1990; Wolt, 2004).

This disparity may be due to factors affecting the persistence of NP activity including soil organic matter content, which has been shown to decrease the effectiveness of NP (Goring, 1962a,b; Redemann et al., 1964; Bundy and Bremner, 1973) via sorption (Hendrickson and Keeney, 1979). Increases in temperature promote the degradation of nitrapyrin and also reduce its effectiveness by enhancing the activity of nitrifying microorganisms (Goring, 1962b; Bundy and Bremner, 1973; Touchton et al., 1979). Similarly, increases in soil pH reduce the efficacy of NP (Kyveryga et al., 2004), owing to the stimulating effect of alkalinity on nitrification (Goring, 1962a; Alexander, 1965; Focht and Verstraete, 1977) Regardless of these factors, NP has been found to increase immobilization of fertilizer N (Lewis and Stefanson, 1975; Söchtig and Salfeld, 1977), which could explain yield decreases, especially on soils with high carbon from sources such as continuous corn.

Several yield-based studies have been conducted to assess the efficiency of fall versus spring NH_3 applications, with or without the addition of NP. In studies evaluating timing, spring applications yielded significantly higher than fall applications (Boswell, 1997; Hendrickson et al., 1978; Touchton et al., 1979; Warren et al., 1980; Maddux et al., 1984; Malzer and Randall, 1985; Stehouwer and Johnson, 1990; Vetsch and Randall, 2004; Randall and Vetsch, 2005; Pittelkow et al., 2017), except in the two cases of Chalk et al. (1975) and Hanson et al. (1987) where application timing was not a significant factor. Similarly, yield effects have been inconsistent for the use of nitrapyrin with fall-applied NH_3 (Warren et al., 1975, 1980; Boswell, 1977; Hendrickson et al., 1978; Touchton et al., 1979; Malzer and Randall, 1985; Hanson et al., 1987; Stehouwer and Johnson, 1990; Randall and Vetsch, 2004; Pittelkow et al., 2017).

Fertilizer N uptake efficiency (FNUE; i.e., the percentage of fertilizer N taken up by the crop), is often estimated from the difference in crop N uptake with and without N fertilization, but a far better approach is to use ^{15}N as a tracer so that fertilizer N can be distinguished from soil-derived N. Due to difficulties in handling a liquefied gas, $^{15}\text{NH}_3$ has mainly been used in laboratory studies involving quantitative micro-injections to soil samples, and the findings cannot be extrapolated in any meaningful way for field-scale comparisons (Norman et al., 1987; He et al., 1990, 1991). A major obstacle to the use of $^{15}\text{NH}_3$ is the need to lower commercial sources of highly enriched ^{15}N (10 to 99 atom % ^{15}N) to a suitable working enrichment that is not only much more economical but also improves data quality by minimizing cross contamination during sample preparation and analysis. This complication led Norman and Kurtz (1986) to generate $^{15}\text{NH}_3$ by alkalizing a mixture of labeled and unlabeled $(\text{NH}_4)_2\text{SO}_4$, but a safer and more convenient option was later described by Vanden Heuvel (1988), in which a vacuum manifold was used in diluting commercially obtained $^{15}\text{NH}_3$ with an unlabeled source, such that both gases were transferred cryogenically to a single sampling cylinder. Unfortunately, neither system provided the capacity needed to allow applications to multiple microplots in field studies with ^{15}N .

Even with a suitable enrichment and an adequate quantity of $^{15}\text{NH}_3$, the application process presents major challenges due to the plot scale that is necessarily limited in isotopic research. Sanchez and Blackmer (1987) were the first to face these challenges, and their response was to design a system whereby $^{15}\text{NH}_3$ was dispensed from a capillary tube pulled with a hand-drawn winch through a slit previously made with a conventional applicator knife. This system was subsequently employed in the only field study involving the use of $^{15}\text{NH}_3$, which was applied in the spring with and without the addition of nitrapyrin (Blackmer and Sanchez, 1988).

The only alternative, described by Vanden Heuvel and Harrold (1990), allows more realistic knife injections with the use of a vehicle-mounted winch but has not been adopted in any field studies using $^{15}\text{NH}_3$.

The objective of this two-year study was to evaluate fertilizer N uptake efficiency for fall-applied $^{15}\text{NH}_3$, with and without the addition of NP or EP. Toward this end, a manifold system was designed that would be capable of diluting high enrichments of $^{15}\text{NH}_3$ obtained commercially to working enrichments of 1 to 1.5 atom % ^{15}N in quantities sufficient for field plot research. The resulting mixtures were collected in specially designed tanks that allowed the addition of a nitrification inhibitor and were compatible with a tractor mounted tool-bar. After verifying accuracy and precision, this system was used for conducting field trials on six sites in which 224 kg N ha^{-1} was applied with or without NP or EP. In both years of the study, two of the sites were cropped to a corn-soybean rotation on soils of contrasting productivity (Mollisol and Alfisol), whereas the third site was under continuous corn. Total aboveground biomass (grain, leaves, stalks, and husks) was harvested and carefully processed to collect data for estimating dry matter production, N derived from fertilizer (NDFF) and soil (NDFS), and FNUE determined by the difference method as well as from ^{15}N recovery.

CHAPTER 2: System for Preparing and Applying $^{15}\text{NH}_3$ in Field Plot Research

2.1 Introduction

The importance of anhydrous NH_3 to modern agriculture is apparent from the dramatic increases achieved in cereal yields with global dependence on the Haber-Bosch process since the 1960s. The massive output of NH_3 is largely directed toward the manufacture of urea and other N fertilizers, although direct application is a major source of fertilizer N used for corn production in the Midwestern USA (Illinois Department of Agriculture, 2019; Iowa Department of Agriculture, 2019).

Owing to its occurrence as a liquefied gas that boils at -78°C and can be fatal at high concentrations, anhydrous NH_3 presents substantial challenges in ^{15}N -tracer research to quantify fertilizer N uptake efficiency (FNUE) as well as the fate and behavior of this fertilizer in the soil-plant system. In some cases, aqueous $^{15}\text{NH}_3$ has been utilized to simplify the application process (Chalk and Keeney, 1975a,b) despite the risk of artifacts in soil-fertilizer reactions due to physical properties that are very different for a nitrogenous solution than for a pressurized gas. In others, micro-scale injections of $^{15}\text{NH}_3$ have been made for laboratory incubation experiments (Norman et al., 1987; He et al., 1990, 1991), using a microliter syringe equipped with a propipette and cooled with dry ice in a cold jacket (Norman and Kurtz, 1986). Although the use of a syringe allows quantitative injections, there are obvious limitations that preclude upscaling to field studies.

A fundamental complication in research utilizing $^{15}\text{NH}_3$ arises because a lower enrichment is often desired than can be obtained commercially, and this will necessarily entail dilution of labeled with unlabeled NH_3 . One option is to carry out the dilution after generating $^{15}\text{NH}_3$ from a labeled NH_4^+ -salt, as was done by Norman and Kurtz (1986) in alkalizing

$(\text{NH}_4)_2\text{SO}_4$ with NaOH. In subsequent work by Vanden Heuvel (1988), $^{15}\text{NH}_3$ was obtained commercially instead of being generated, and dilution was carried out using a vacuum manifold for cryogenic transfer. The latter technique is simpler and less hazardous than the former but is subject to the same constraint, in that the amount of $^{15}\text{NH}_3$ produced is far too limited for application to field plots typically measuring 5-10 m².

Even with a sufficient supply of $^{15}\text{NH}_3$, the application process is inherently more problematic than with conventional field equipment because of the difficulties involved in achieving uniform flow over the very limited distances encountered in microplot-scale research with ^{15}N . These difficulties led Sanchez and Blackmer (1987) to devise a method to simulate subsurface band applications of anhydrous NH_3 , in which liquid $^{15}\text{NH}_3$ is dispensed from an ice-cooled cylinder connected to a capillary tube that is pulled with a hand-drawn winch through a channel previously created by a conventional applicator. Following preliminary studies to evaluate plot size in relation to ^{15}N movement (Sanchez et al., 1987), this method was successfully used to estimate ^{15}N uptake with and without nitrapyrin (Blackmer and Sanchez, 1988; Sanchez and Blackmer, 1988), and subsequently allowed an assessment of NH_4^+ fixation before and after soil drying (Thompson and Blackmer, 1993). Unfortunately, the technical complexities inherent to the method of Sanchez and Blackmer (1987) were not conducive to further field studies using $^{15}\text{NH}_3$, nor was there any tendency in this direction after the development by Vanden Heuvel and Harrold (1990) of a more realistic design utilizing a winch-drawn applicator knife.

If progress is to be made in field plot research with $^{15}\text{NH}_3$, the labeled material must be available in sufficient quantity, along with the means to mimic a conventional knifed application that may also supply a nitrification inhibitor to reduce nitrate (NO_3^-) loss by leaching or

denitrification. The present paper describes and evaluates a manifold system and collection tank with kilogram capacity, as well as a field applicator specifically configured for plot-scale research.

2.2 Methods

Components

Dilution manifold

The manifold used to dilute ^{15}N -labeled with unlabeled NH_3 is depicted schematically by Fig. 2.1 and consists of the following components connected by either galvanized or stainless steel tubing (see Note 1):

1. A cylinder of $^{15}\text{NH}_3$, which in our work supplied an enrichment of 10 atom % as obtained from Isotec Inc., Miamisburg, OH in a cylinder equipped with a source valve (SV) having a 12.7 mm (0.5 in.) tap.
2. A cylinder of unlabeled NH_3 (Size 150A; Airgas, Radnor, PA).
3. A stainless steel regulator with maximum delivery pressure of 690 kPa (e.g., Model 3810; Matheson Gas Products, Basking Ridge, NJ).
4. Two stainless steel pressure gauges that read to 690 kPa (PG_1 and PG_2).
5. A 20-L stainless steel spray tank used for expansion of $^{15}\text{NH}_3$. This tank was modified by Vanden Heuvel (1988) with sheet metal reinforcements epoxied to the outer wall and two stainless steel fittings welded to the top of the tank, one a male pipe weld connector (Cat. No. SS-400-1-4W; Swagelok[®], Solon, OH) for access to the manifold and the other an Ultra-Torr[®] (Swagelok[®] SS-2-UT-A-4) adapter to allow insertion of a thermometer probe.

6. A digital thermometer (Model 8517-00; Cole-Parmer, Vernon Hills, IL) secured below a support plate clamped to a ring stand (DT).
7. A 303-L (80-gal.) vertical air receiver (Cat. No. 302417; Manchester Tank & Equipment Co., Franklin, TN) used as an expansion vessel for unlabeled NH₃.
8. A two-stage rotary pump (DuoSeal[®] Model 1402; Welch Vacuum Technology, Niles, IL) with a thermocouple vacuum gauge.
9. A Styrofoam cooler (31 × 31 × 30.5 cm) used to allow immersion of the collection tank in liquid N₂. To support the weight of the tank, a polyethylene sheet (3.2 mm thick) was fitted into the bottom of the cooler.
10. Two O-ring face seal fittings (OF₁ and OF₂)(Swagelok[®] SS-4-VCO-6-400) and a compression fitting (CF)(Swagelok[®] SS-40-6), each in stainless steel and sized at 6.4 mm (¼ in.).
11. Eight stainless steel ball valves, of which seven were 6.4 mm (¼ in.) with compression fittings and one 12.7 mm (½ in.) with female NPT fittings (V₁-V₈).

Collection tank

A key element of the system described is the collection tank (Fig. 2.2), which was specifically configured for connection not only to the dilution manifold, but also to the application toolbar. The design utilized the following components, connected via appropriate adapters and pipe sealant (see Note 2):

1. An 11.4-L (3-gal.) horizontal air receiver (Cat. No. A21008016400; Penway Inc., Edinburgh, IN) rated at 2758 kPa (400 psi) and equipped for connection of a sidearm and access valve.

2. A sidearm assembly comprised of a galvanized 19 mm ($\frac{3}{4}$ in.) street run elbow, two galvanized nipples (10 and 20 cm in length) joined via a 6.4 mm ($\frac{1}{4}$ in.) stainless steel needle valve (TV₂)(Swagelok[®] SS-1RM4), a stainless steel ball valve (TV₃)(Model VSS075; BANJO[®], Crawfordsville, IN), and a stainless steel street run elbow terminating in a steel male ACME adaptor (not shown in Fig. 2.2)(Cat. No. ST-M526-6; Squibb-Taylor, Dallas, TX).
3. A stainless steel ball valve (BANJO[®] VSS075) for accessing the collection tank (TV₁), which is fitted with a street run elbow connected to a steel male ACME adapter (not shown in Fig. 2.2)(Squibb-Taylor ST-M526-6), for use during application.
4. A VCO[®] tube adapter gland (Swagelok[®] SS-4-VCO-3-4TA) with female nut (Swagelok[®] SS-4-VCO-4), for use during the loading step (OF₁ or OF₂).

Field applicator

The applicator used in our work consists of a toolbar with five knife assemblies, which was mounted to a utility tractor (Massey Ferguson 4610M; AGCO, Duluth, GA) via a Category II three-point hitch. Before use, the toolbar was equipped with a bracket to support the collection tank described previously, modifications were made to minimize dead volume between the tank and injection knives, and the two outside knives were removed to confine applications within a microplot scale. A schematic diagram is presented in Fig. 2.3 that depicts most of the components listed below:

1. A customized 15 × 15 cm toolbar adjustable to a maximum width of 4.1 m, which in our work was fully contracted to a width of 2.6 m that accommodated three C-shanks spaced 76 cm apart.
2. Three mole knives and double disc sealers mounted to the C-shanks behind rolling coulters fastened to the toolbar. As is common in conventional field applications, knife placement resulted in NH₃ bands 15 cm below the soil surface.
3. A flow regulator consisting of a hydraulically actuated regulator (Cat. No. C-2500-6572, Continental NH₃ Products, Dallas, TX) and a multiple-outlet distributor fitted with restricting nipples (2.4 mm ID, 9.5 mm OD) for connection to ethyl vinyl acetate tubing of uniform length (9.5 mm ID, 15.9 mm OD) that delivers NH₃ to each of the knives.
4. A cylinder (size Q) mounted to the toolbar and fitted with a two-stage regulator (ProStar PRS 302243; Praxair, Danbury, CT) that supplies N₂ gas to the collection tank through a reinforced hose (4.8 mm ID, 7.9 mm OD).
5. Female ACME adaptors (Squibb-Taylor A1130FS) to connect the regulator at TV₁ and the N₂ supply at TV₃ of the collection tank.

2.3 Protocol

Preparation of ¹⁵NH₃

With the fixed volume design of the manifold system depicted by Fig. 2.1, different quantities of NH₃ can be collected by varying a measured pressure. In our experience, beginning the collection process with labeled rather than unlabeled NH₃ is somewhat more convenient owing to the much smaller quantity of material involved, although if desired, this sequence may

be easily reversed. To initiate the transfer of $^{15}\text{NH}_3$, the source cylinder must be connected to the regulator and the collection tank to the manifold via the fitting identified as CF in Fig. 2.1 (see Note 3), after which V_3 is opened for evacuation (to < 5 Pa) with SV closed to isolate the source cylinder and the collection tank configured with TV_{1a} open and TV_{2a} closed. Of the remaining valves pertinent to the transfer of $^{15}\text{NH}_3$, V_1 , V_4 , and V_6 are open while V_2 , V_5 , and V_8 are closed.

Prior to admitting $^{15}\text{NH}_3$ into the manifold and expansion tank 1, V_6 is closed to isolate the collection tank, and V_3 is closed to discontinue evacuation. The valve on the $^{15}\text{NH}_3$ cylinder (SV) is then opened and the regulator adjusted to supply a pressure of 550-600 kPa as measured with PG_1 , while heating the source cylinder with a heat gun. Expansion causes NH_3 to warm as expansion tank 1 is pressurized, which is monitored with the digital thermometer (DT) connected to the top of the tank. When the temperature reading has dropped to approximately 25°C while maintaining the desired pressure (see Note 4), the source valve (SV) is closed and V_6 is opened. As the $^{15}\text{NH}_3$ freezes into the collection tank a rapid decrease occurs in the manifold pressure, during which the liquid N_2 boils vigorously and must be replaced to maintain the original level. The transfer of $^{15}\text{NH}_3$ is complete when PG_1 has dropped below 0 kPa (base pressure), and at this point the collection tank is isolated from the manifold by closing V_6 and TV_{1a} .

If additional $^{15}\text{NH}_3$ is to be collected, any residual pressure is relieved into the fume hood by briefly opening V_2 , the manifold is then evacuated through V_3 while the collection tank remains isolated, and the protocol described in the previous paragraph is subsequently repeated. Otherwise, the O-ring fitting (OF_1) is disconnected and the tank carefully removed from the Styrofoam cooler for weighing (see Note 5) or for dilution of labeled with unlabeled NH_3 .

To proceed with dilution, the collection tank is removed from the Styrofoam cooler (see Note 6) and both are relocated to allow connection of the tank via OF₂, while maintaining liquid N₂ in the cooler as previously described. After closing SV, the ¹⁵NH₃ source cylinder is disconnected at CF, and the regulator is removed for connection between a much larger cylinder of unlabeled NH₃ and the greater length of tubing needed for coupling to CF. The manifold is then configured for evacuation to < 20 Pa with V₂, V₄, V₆, TV_{1b}, and TV_{2b} closed and V₁, V₃, V₅, V₇, and V₈ open. To begin the transfer process, V₃ and V₈ are closed before opening SV and adjusting the regulator so as to obtain a pressure of 550-600 kPa on PG₁ and PG₂, while applying a heat gun to the source cylinder (see Note 4). After closing SV, TV_{1b} is opened to begin the transfer of NH₃ to the collection tank, requiring the addition of liquid N₂ to replace what was lost from the cooler. When there is no further decrease in pressure as measured with PG₂, TV_{1b} is closed to isolate the collection tank.

Weight data may be collected (see Note 7) at this point by closing V₇ and disconnecting the collection tank at OF₂ (see Note 5) unless more unlabeled NH₃ is to be loaded, in which case the protocol described in the previous paragraph would be repeated starting with the evacuation step.

When loading is complete for both labeled and unlabeled NH₃, the collection tank is allowed to warm to room temperature and then inverted to thoroughly mix the contents. To check the ¹⁵N enrichment, the sidearm on the collection tank is modified at the ball valve (TV₃) by securely connecting a second needle valve (e.g., Swagelok[®] SS-1RM4) equipped to dispense NH₃ via a section of vinyl tubing. With the added needle valve closed and TV₃ open, TV₂ is briefly opened to allow NH₃ to fill the volume between the needle valves. After confirming that TV₂ is closed, the open end of the outlet tube is immersed in 20 mL of 1 mol L⁻¹ H₂SO₄ pre-

weighed in a 50-mL Erlenmeyer flask, and the entire NH_3 aliquot is bubbled into the acid by cautiously opening the added needle valve. This process is repeated until a weight gain of 0.02 to 0.03 g is achieved, which allows isotope-ratio analysis to be conveniently performed after alkaline diffusion of a 1-mL aliquot containing approximately 1 mg of N (Mulvaney et al., 1990; Khan et al., 1997).

Calibration and Usage of Field Applicator

The NH_3 flow rate is calibrated by measuring the weight gained by buckets of water during timed collections dispensed from each knife with the outlet positioned below the water surface. In our work, these measurements were made using a collection tank fitted with a gauge to monitor headspace pressure, which was loaded with fertilizer-grade NH_3 and connected to the field applicator via both ACME fittings. In the normal configuration with the N_2 regulator adjusted to 1100 kPa, TV_2 was opened to pressurize the collection tank (see Note 8) before allowing NH_3 expansion to the flow regulator via TV_1 . With pre-weighed buckets of water carefully positioned, the toolbar was lowered so as to immerse the knives to a depth of approximately 20 cm and a reading was taken from the pressure gauge on the tank. Using hydraulic controls inside the tractor cab, the flow of NH_3 was initiated, and subsequently terminated after 20 s, while precisely tracking the time duration to within 0.01 s. After raising the toolbar, the buckets were removed from under the knives and weighed (see Note 7) to determine the flow achieved at a given regulator setting and the consistency among the three knives.

For applications of $^{15}\text{NH}_3$ with a nitrification inhibitor, the elbow and ACME fitting connected to TV_3 (Fig. 2.2) are removed after loading the collection tank, and the inhibitor is introduced (via syringe) to the sidearm through TV_3 , which is then closed before reconnecting

the elbow and ACME fitting for connection to the toolbar. The inhibitor is incorporated into the NH_3 when the collection tank is pressurized with N_2 , and mixing is completed by agitation during field transport.

Besides calibrating the flow rate, care is also called for in maintaining the low ground speeds ($0.4\text{-}0.6 \text{ m s}^{-1}$) required to deliver fertilizer N rates typically applied in production agriculture. In our work, this was accomplished by 1) using a tractor equipped with a real-time kinematic global positioning system (RTK GPS) to ensure that velocity was unaffected by soil conditions, 2) lowering the knives to the depth of injection as the tractor moved toward the beginning of band application, 3) providing visual confirmation to aid the operator in obtaining precise start and stop positioning, and 4) weighing the collection tank to check that the quantity of NH_3 applied was consistent with the plot area fertilized.

To validate the method of application, a three-band injection of $^{15}\text{NH}_3$ was made to bare soil in a single pass 3 m in length, using the toolbar described previously. At 0.75-m intervals relative to the midpoint of the pass, slices (25 cm deep, 20 cm wide, 2.5 cm thick) centered perpendicular to each band were collected with a tractor-mounted Giddings probe (Giddings Machine Co., Windsor, CO) equipped with a stainless steel rectangular tube sampler specifically designed for transecting NH_3 bands. The samples were transported to the laboratory in air-tight polyethylene bags that were stored for 1 day in a refrigerator at 5°C to ensure complete absorption of NH_3 , followed by transfer to paper bags for drying in a forced-air oven at 50°C . While taking care to prevent isotopic cross-contamination, the dried samples were crushed with a mechanical grinder (Custom Laboratory Equipment, Orange City, FL) and then thoroughly homogenized, and a subsample (approximately 25 g) was subsequently crushed with a mortar and pestle to $< 2 \text{ mm}$. Extractions to recover inorganic N were performed by shaking 12 g of soil

with 120 mL of 2 mol L⁻¹ KCl on a reciprocal shaker for 1 h, followed by vacuum filtration through Whatman no. 42 filter paper (GE Healthcare, Buckinghamshire, UK) that had been washed (Mulvaney, 1996) to prevent contamination by any mineral N initially present. A 20-mL aliquot of extract was diffused with MgO and Devarda's alloy to determine (NH₄⁺ + NO₃⁻ + NO₂⁻)-N (Khan et al., 1997), which was subsequently processed for automated N-isotope analyses (Mulvaney et al., 1990).

2.4 Results and Discussion

The system described addresses the two obstacles that have long limited progress toward utilizing ¹⁵NH₃ in plot-scale research to improve the management of a major N fertilizer, namely, the kilogram quantities of labeled material required and the need for a realistic method of application that is also simple and convenient. The former was overcome by using a manifold system with adequate capacity for dilution of labeled with unlabeled NH₃, and the latter by carrying out applications through injection knives on a tractor-mounted toolbar. Crucial to both processes is a specially designed tank that collects and dispenses the labeled NH₃.

Table 2.1 verifies that the manifold system described has ample capacity for plot-scale research, demonstrating that more than 3 kg of ¹⁵NH₃ can be collected, a 3000% increase relative to the quantities prepared by Vanden Heuvel (1988). Table 2.1 also summarizes the enrichments obtained in preparing four batches of ¹⁵NH₃, relative to what was expected from the weight gains measured upon collecting labeled and unlabeled NH₃. In all but one case, the actual enrichments were higher than expected, which can be explained by frost buildup on the collection tank during multiple transfers of labeled NH₃ prior to a prolonged loading period for unlabeled NH₃. These discrepancies could be reduced by minimizing the number of transfers required, by carrying out

collections in a dehumidified environment, and/or by N₂ enrichment of the airspace surrounding the collection tank (see Note 5).

Owing to the quantities of NH₃ involved, considerable care should be taken to ensure that the manifold system is completely leak-tight before use, which can be verified after applying soap solution to every joint and checking for the formation of bubbles with He pressurization. Special attention should be given to those joints on the collection tank that will be in direct contact with liquid N₂, as leakage will occur from differing contraction rates if stainless steel components are used to connect the sidearm to the carbon steel tank. The use of liquid N₂ presents a further hazard if the collection tank is not kept frozen throughout the entire loading process, due to the expansion of NH₃ upon refreezing (Vanden Heuvel, 1991).

So that a single transfer can be completed within 30 minutes, the manifold is evacuated to < 20 Pa before the introduction of labeled or unlabeled NH₃, the source cylinder is heated to reduce the loading period, freezing is carried out with liquid N₂ rather than dry ice, and unloading of the 303-L expansion tank occurs through high-conductance tubing. To expedite leak-tight coupling of the collection tank to the manifold, both connecting ports are equipped with a zero-clearance O-ring fitting.

An important feature of the collection tank is the sidearm, originally added for the sole purpose of preventing the valves from coming into contact with liquid N₂ during the loading process. The presence of a sidearm allows the introduction of a nitrification inhibitor after the loading of NH₃, in lieu of the much more complicated technique described by Sanchez and Blackmer (1987). The sidearm is also utilized in sampling the NH₃ for ¹⁵N analysis and in pressurizing the collection tank with N₂ during the application process.

To validate the precision of field application, calibration tests were performed at three different regulator settings, with and without N₂ pressurization, by measuring the NH₃ dispensed from the three-knife toolbar used in our work. The results (Table 2.2) show higher flow rates and better precision with than without N₂, except for the final trial where the data obtained were much lower than with the two previous replicates and were removed after evaluation via the *Q*-test. This anomaly may have been caused by water loss from the collection buckets, but a more likely factor was a low NH₃ level in the source tank that could have caused erratic delivery to the knives. Regardless of the regulator setting used, and with or without headspace N₂, the coefficient of variation (CV) between knives averaged 10%, which compares favorably with corresponding CV values of 14 to 31% reported by Hanna et al. (2002).

Table 2.3 shows the results from a further evaluation of the toolbar applicator, in which ¹⁵NH₃ was injected into bare soil for the purpose of quantifying spatial uniformity among and along the bands. The data show considerably more variability than was observed by bucket testing (Table 2.2), which is not surprising in view of the physical heterogeneity inherent in making knifed applications to soil. This variability would have been exacerbated by the low speed required to deliver the N rate desired (224 kg N ha⁻¹) and by the limited sampling intensity achieved in collecting only 2% of the band applied. Regardless, the variability documented by Table 2.3 raises concerns about the validity of bucket testing for calibrating anhydrous NH₃ applications, and emphasizes the need for care in harvesting ¹⁵NH₃ microplots.

2.5 Notes

1. Brass is attacked by NH_3 and cannot be used.
2. Galvanized and carbon steel components were connected using formula 55 pipe thread compound (WM Harvey Manufacturing Co., Omaha, NE), while Teflon[®] tape was used to seal stainless steel pipe joints.
3. Before making the connection to this fitting, the collection tank was carefully placed in the Styrofoam cooler, and sufficient liquid N_2 was subsequently added to maintain the liquid level in the cooler no more than 7 cm above the elbow connecting the sidearm (Fig. 2.2).
4. Maximum loading capacity is achieved at 600 kPa, but if necessary the manifold pressure may be reduced to effect a proportional reduction in the quantity of NH_3 collected. The source cylinder will cool as NH_3 expands into the manifold, which prolongs the transfer process unless heat is applied with a heat gun.
5. Weighing of the collection tank is recommended before and after the transfer of NH_3 , in order to utilize manifold pressure measurements in estimating the mass of NH_3 collected. Frost accumulation on the tank leads to positive weighing errors that intensify over time, so weighing should be done quickly after removing as much frost as possible. The frost buildup problem can be reduced by covering the collection tank with a plastic bag fitted to the Styrofoam cooler, such that water vapor is excluded as gaseous N_2 accumulates in the bag.
6. The tank must be kept frozen throughout the loading process, so as to avoid any safety hazard caused by expansion that occurs as liquid NH_3 freezes in a confined volume (Vanden Heuvel, 1991).

7. The scale used must have a capacity of at least 20 kg with 0.01 kg readability. A Torrey (Houston, TX) Model EQB 50/100 bench scale proved satisfactory.
8. For trials without N₂ pressurization, TV₂ was left closed.

2.6 Figures

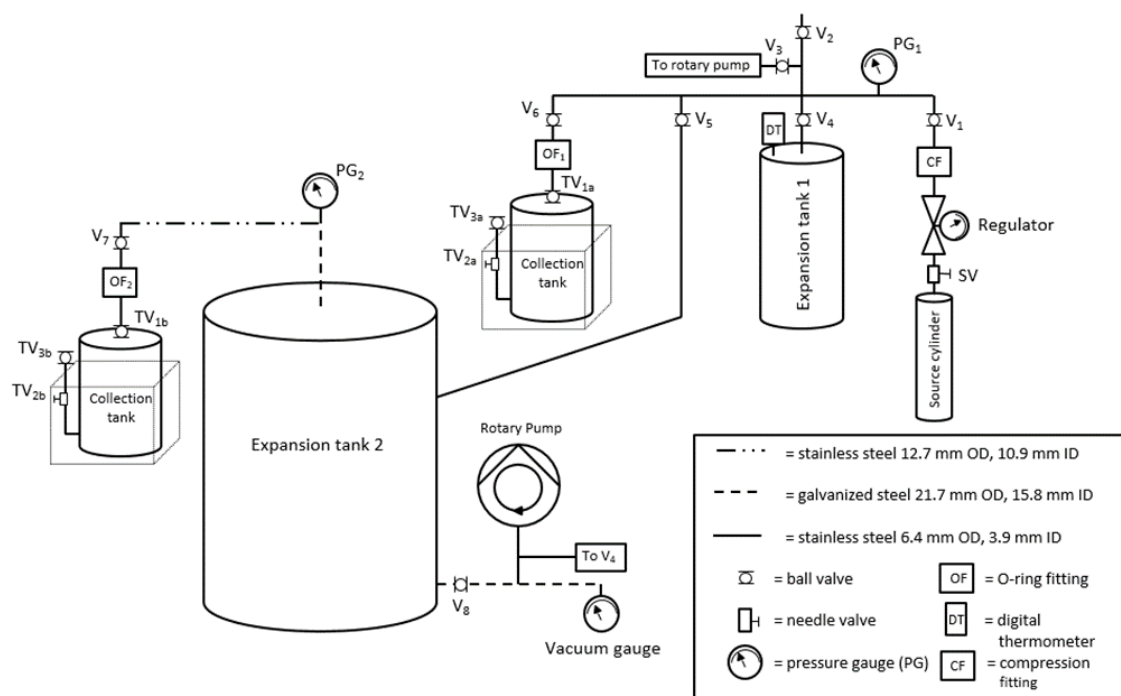


Fig. 2.1. Schematic diagram of dilution manifold, consisting of components located inside (CF, OF₁, V₁-V₆, PG₁, expansion tank 1, DT, and collection tank with tank valves TV_{1a}-TV_{3a} and Styrofoam cooler), or adjacent to a laboratory fume hood. To clarify the protocol for manifold dilution of NH₃ supplied from a cylinder equipped with a source valve (SV), two collection tanks are depicted schematically whereas during use a single collection tank is transferred between O-ring fittings OF₁ and OF₂.

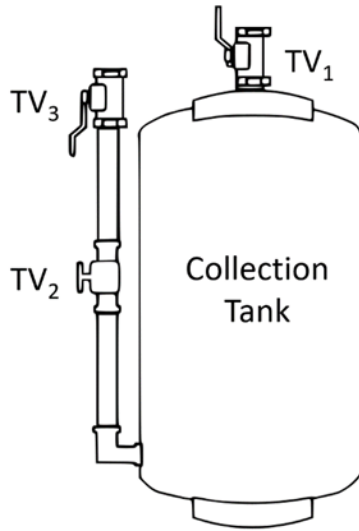


Fig. 2.2. Collection tank shown without fittings on the ball valves. For loading, a VCO[®] fitting is connected to TV₁. For application, elbows terminating in ACME fittings are connected to TV₁ and TV₃.

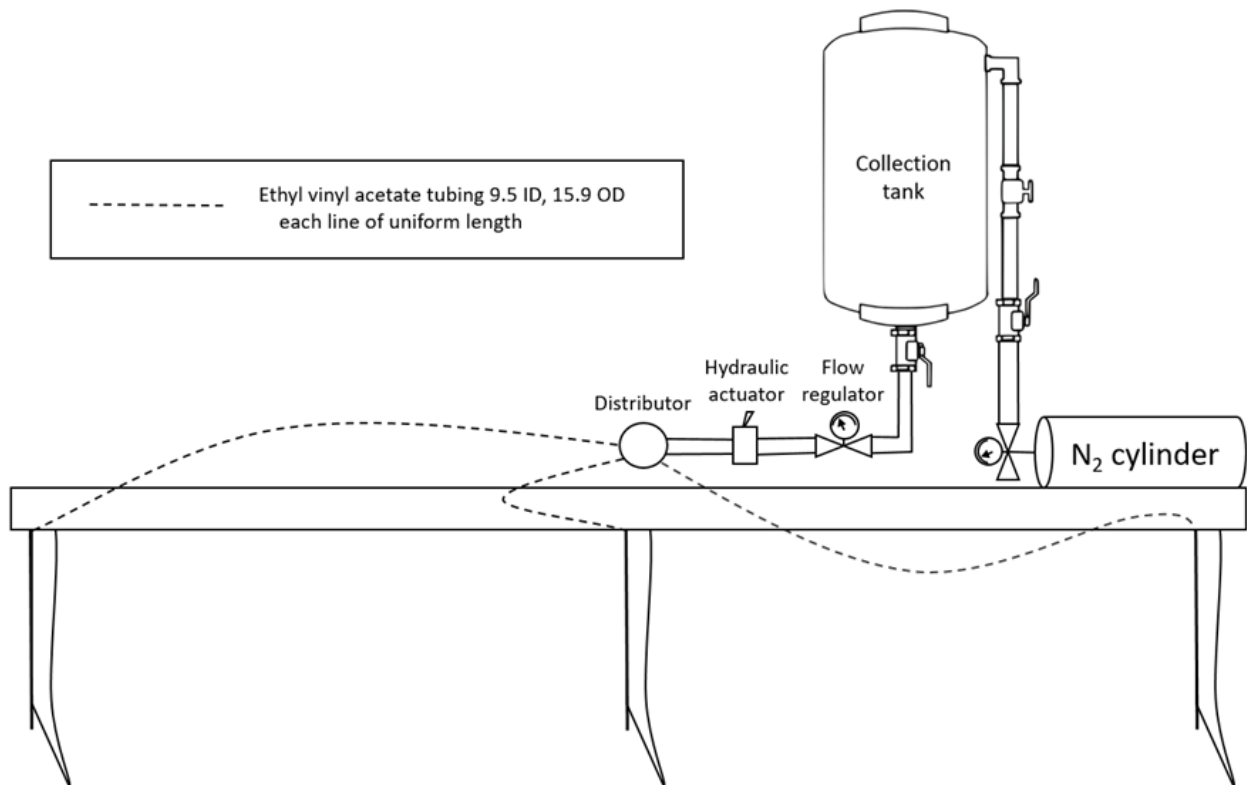


Fig. 2.3. Schematic diagram of field applicator system.

2.7 Tables

Table 2.1. Data illustrating use of dilution manifold for preparing kilogram quantities of $^{15}\text{NH}_3$.

| Trial | Labeled NH_3 collected† | Unlabeled NH_3 collected | ^{15}N enrichment | | |
|-------|-------------------------------------|--------------------------------------|----------------------------|--------|--------------|
| | | | Expected‡ | Actual | Discrepancy§ |
| | | g | atom % ^{15}N | | |
| 1 | 210 | 2940 | 1.009 | 1.539 | 0.530 |
| 2 | 185 | 2665 | 0.992 | 1.204 | 0.212 |
| 3 | 260 | 2765 | 1.194 | 1.196 | 0.002 |
| 4 | 824 | 980 | 4.767 | 5.774 | 1.007 |

†Enriched to 10 atom % ^{15}N as reported by Isotec Inc (Miamisburg, OH).

‡As calculated by an isotope dilution equation: $E_M = \frac{Q_L A_L + Q_U A_U}{Q_U + A_U}$,

where E_M is the atom % ^{15}N expected by mixing the specified quantities (Q in grams), assuming atom % ^{15}N (A) values of 0.3663 for unlabeled (U) and 10 for labeled (L) NH_3 .

§Values obtained as the difference between actual and expected atom % ^{15}N .

Table 2.2 Precision of anhydrous NH₃ delivery with field applicator.

| Regulator setting [†] | Headspace N ₂ [‡] | Replicate test | NH ₃ collected | | | | | | | |
|--------------------------------|---------------------------------------|----------------|---------------------------|---------|---------|--------------|-----------------|-----------------------------|-----------------------|-----------------|
| | | | Knife 1 | Knife 2 | Knife 3 | Among knives | | Total per test [¶] | Among replicate tests | |
| | | | | | | Mean | SD [§] | | Mean | SD [§] |
| kg min ⁻¹ | | | | | | | | | | |
| 250 | – | 1 | 1.02 | 0.90 | 0.81 | 0.91 | 0.11 | 2.72 | 3.24 | 0.46 |
| | – | 2 | 1.20 | 1.16 | 1.07 | 1.14 | 0.07 | 3.43 | | |
| | – | 3 | 1.40 | 1.14 | 1.02 | 1.19 | 0.19 | 3.57 | | |
| | + | 1 | 1.38 | 1.09 | 1.24 | 1.24 | 0.15 | 3.71 | 3.85 | 0.13 |
| | + | 2 | 1.49 | 1.22 | 1.26 | 1.32 | 0.15 | 3.97 | | |
| | + | 3 | 1.51 | 1.22 | 1.13 | 1.29 | 0.20 | 3.86 | | |
| 375 | – | 1 | 1.13 | 1.16 | 1.16 | 1.15 | 0.02 | 3.45 | 3.80 | 0.41 |
| | – | 2 | 1.57 | 1.39 | 1.29 | 1.42 | 0.14 | 4.25 | | |
| | – | 3 | 1.60 | 1.31 | 1.34 | 1.42 | 0.16 | 3.71 | | |
| | + | 1 | 1.78 | 1.62 | 1.71 | 1.70 | 0.08 | 5.11 | 5.00 | 0.13 |
| | + | 2 | 1.76 | 1.64 | 1.63 | 1.68 | 0.07 | 5.03 | | |
| | + | 3 | 1.42 | 1.93 | 1.51 | 1.62 | 0.27 | 4.86 | | |
| 500 | – | 1 | 2.12 | 1.54 | 1.72 | 1.79 | 0.30 | 5.37 | 5.27 | 0.13 |
| | – | 2 | 1.96 | 1.43 | 1.73 | 1.71 | 0.27 | 5.12 | | |
| | – | 3 | 2.10 | 1.53 | 1.71 | 1.78 | 0.29 | 5.33 | | |
| | + | 1 | 2.94 | 2.97 | 2.67 | 2.86 | 0.17 | 8.57 | 8.54 | 0.04 |
| | + | 2 | 2.85 | 2.78 | 2.88 | 2.84 | 0.05 | 8.51 | | |
| | + | 3 [#] | 2.38 | 2.41 | 1.95 | 2.24 | 0.26 | 6.74 | | |

[†]Values reported as dial settings calibrated in pounds of N dispensed per hour, but not literally quantitative due to distributor modifications.

[‡]Zero (–) or 1100 (+) kPa.

[§]SD, standard deviation.

[¶]Reported as a total dispensed from the three knives used in each test.

[#]The abnormally low data obtained in this replicate test were excluded by the *Q*-test (Dean and Dixon, 1951) when calculating the mean and SD values reported for the highest regulator setting using headspace N₂.

Table 2.3. Spatial uniformity of $^{15}\text{NH}_3$ injection with the field applicator. †

| Distance from band origin | ^{15}N recovery‡ | | | | |
|------------------------------|---------------------------|---------|---------|-------|------|
| | Knife 1 | Knife 2 | Knife 3 | Mean | SD |
| m | mg kg ⁻¹ | | | | |
| 0.75 | 173.8 | 170.5 | 104.7 | 149.7 | 39.0 |
| 1.50 | 218.3 | 147.7 | 105.0 | 157.0 | 57.2 |
| 2.25 | 198.8 | 181.4 | 148.7 | 176.3 | 25.4 |
| Mean | 197.0 | 166.5 | 119.5 | | |
| SD | 22.3 | 17.2 | 25.3 | | |

†Transect samples were collected from each of three bands following the injection of $^{15}\text{NH}_3$ made with a regulator setting of 250 for a 3-m distance on bare soil. Data collected by total inorganic ^{15}N analyses performed in duplicate. SD, standard deviation.

‡As calculated by an isotope dilution equation: $N_F = N_L \left(\frac{A_L - A_U}{A_F - A_U} \right)$,

where N_F is the quantity (mg kg⁻¹) of fertilizer N recovered, N_L is the total inorganic N determined for the labeled soil, and A is the atom % ^{15}N measured for the labeled soil (L), unfertilized soil (U), and fertilizer

CHAPTER 3: Nitrogen-15 Evaluation of Fall-Applied Anhydrous NH₃ for Nitrogen Uptake by Corn

3.1 Introduction

Besides serving an essential role in the production of urea and other synthetic nitrogen (N) fertilizers, anhydrous ammonia (NH₃) continues to dominate N consumption through direct application for corn (*Zea mays* L.) production in the Midwestern USA (Illinois Department of Agriculture, 2019; Iowa Department of Agriculture, 2019). This extensive usage reflects economics inherent to the primary product of the Haber-Bosch process, further enhanced by an unmatched N content (820 g kg⁻¹) that reduces transportation, storage, and distribution costs.

Although anhydrous NH₃ is often applied in the spring prior to planting or by sidedressing between V4 and V7, fall applications are also common when post-harvest weather conditions are conducive to field work. The latter practice is favored by NH₃ prices that tend to be lower in the fall than in the spring (Schnitkey, 2018), and by greater availability of the necessary application equipment. As additional incentives, there are fewer tasks to delay planting in the spring, the risk of soil compaction is reduced, and in-season N application can be avoided.

Unfortunately, fall NH₃ applications are subject to the inherent limitation that crop N uptake occurs long after the fertilizer N becomes accessible to N cycling by soil microorganisms, increasing the risk that NO₃⁻ losses by leaching or denitrification will lower fertilizer N uptake efficiency (FNUE). This limitation has motivated numerous yield response studies comparing fall- and spring-applied NH₃, sometimes showing no significant effect due to time of application (Chalk et al., 1975; Hanson et al., 1987) but more often inconsistencies in documenting a significant yield advantage for spring application (Stevenson and Baldwin, 1969; Boswell, 1977; Hendrickson et al., 1978; Touchton et al., 1979; Warren et al., 1980; Maddux et al., 1984; Malzer

and Randall, 1985; Stehouwer and Johnson, 1990; Vetsch and Randall, 2004; Randall and Vetsch, 2005a; Pittelkow et al., 2017). Similar inconsistencies are documented by yield studies comparing fall NH_3 applications with and without the addition of nitrapyrin (NP) as a nitrification inhibitor to control NO_3^- losses by leaching or denitrification (Warren et al., 1975, 1980; Boswell, 1977; Hendrickson et al., 1978; Touchton et al., 1979; Malzer and Randall, 1985; Hanson et al., 1987; Stehouwer and Johnson, 1990; Vetsch and Randall, 2004; Pittelkow et al., 2017). In both cases, the disparities reflect not only diverse weather conditions, but also the spatial variability inherent to soil N supplying power (Mamo et al., 2003; Ruffo et al., 2005).

A more rigorous evaluation of application timing for anhydrous NH_3 fertilization requires the use of ^{15}N tracer techniques, but due to technical difficulties very little progress has been made in this direction. These difficulties prompted Sanchez and Blackmer (1987) to dispense $^{15}\text{NH}_3$ into a knife slit from a capillary tube pulled by a hand-drawn winch, which was subsequently used in estimating FNUE for spring applications of NH_3 (Blackmer and Sanchez, 1988). To our knowledge, no comparable studies have been reported that involve fall fertilization, reflecting the obvious complications that arise when using a labeled fertilizer in the form of a liquefied gas that must be prepared, handled, and applied on a scale suitable for microplot research.

These complications have been reduced considerably with the recent development by Griesheim et al. (2019) of a system that allows the use of a tractor-mounted toolbar for plot-scale applications of $^{15}\text{NH}_3$, with or without the addition of a nitrification inhibitor. In the work reported here, this system was used to quantify FNUE from fall NH_3 applications for corn production, through comparative studies that involved contrasting soils, two different crop rotations, as well as the presence and absence of two nitrification inhibitors.

3.2 Materials and Methods

Experimental sites

The six sites studied between 2016 and 2018 were located on production fields under mulch tillage in De Witt and Piatt Counties in Illinois, and had been cropped to either continuous corn or a corn-soybean (*Glycine max* L. Merr.) rotation. Site locations varied between the two crop years, but represented the same contrasting set of two Mollisols (Sable and Elburn) and an Alfisol (Birkbeck). Table 3.1 shows the soil type at each site as well as physicochemical properties determined by profile sampling from unamended locations. After crushing soil samples to pass through a 2 mm screen, pH was determined with a glass electrode (soil:water ratio, 1:1), organic C by dichromate oxidation (Mebius, 1960), total N and ^{15}N by Kjeldahl digestion (Bremner, 1996) followed by diffusion and N-isotope analysis (Stevens et al., 2000), potentially mineralizable N as estimated by the Illinois Soil N Test (ISNT) originated by Khan et al. (2001) and detailed by Technical Note (2019a), and Bray-1 P using the Fiske-Subbarow colorimetric technique (Frank et al., 2011).

Climatic conditions were monitored throughout the study period, by collecting precipitation and air temperature data using a Vantage Pro2 Weather Station (Davis Instruments Corp., Hayward, CA) located within 17 km of all six experimental sites. Daily data were summed to obtain total monthly precipitation between November 2016 and September 2018, and averaged to compile a record of monthly air temperature. Historical norms for comparison were accessed through public records posted by the Illinois State Climatologist (2019a), which also served as the source of climatic data for the final month of the study (Illinois State Climatologist, 2019b).

At each site in areas unaffected by tile drainage, nine plots measuring 3.05×2.29 m were established to accommodate four corn rows spaced 76 cm apart in the east-west direction, such that the outer rows would coincide with the two longer edges of the plot. Adjacent plots were separated by a 1.5-m border in the north-south direction, and were so positioned that two corn rows would provide a 2.29-m border in the longitudinal direction. The nine plots served as experimental units in a randomized block design involving three replications of three treatments, and additional plots of the same size were established to provide unfertilized controls for estimating FNUE by the difference method. For the first growing season, at least three unfertilized plots were located within 32 m of the experimental area, whereas for the second, the three extra plots were incorporated into the randomization scheme as an additional treatment.

¹⁵NH₃ Application and cultural practices

Using the manifold system described by Griesheim et al. (2019), NH₃ enriched to 10 atom % ¹⁵N as supplied by Isotec Inc. (Miamisburg, OH) was diluted with unenriched NH₃, producing a final enrichment between 1.2 and 1.6 atom % ¹⁵N that was precisely measured for each mixture. To avoid any shortage in the supply of ¹⁵NH₃ to the field applicator, the quantity collected exceeded the amount needed by at least 80%, which was confirmed by weighing each of the three collection tanks before and after the loading process. For treatments involving a nitrification inhibitor, NP or an experimental product for inhibiting nitrification (EP) was introduced to the sidearm on the collection tank as described by Griesheim et al. (2019), at a rate supplying 2.34 (NP) or 0.37 (EP) L ha⁻¹.

All applications of ¹⁵NH₃ were performed using the same three-knife toolbar described by Griesheim et al. (2019), after bucket testing with unlabeled NH₃ to determine the flow rate and

thus the appropriate speed for the N rate desired. In order to maximize application accuracy, this speed was maintained by lowering the knives to create a constant draft load before entering the plot and by using a real-time kinematic global positioning system (RTK GPS) for precise monitoring of ground speed. As a means of ensuring that applications were made to the entire plot but not beyond, the driver was provided with flag signaling for starting and stopping the flow of $^{15}\text{NH}_3$. The collection tank was weighed before and after completing all applications of a given treatment, for verifying consistency between weight loss and the intended N rate applied.

In keeping with common guidelines for fall N fertilization (Fernández et al., 2009), $^{15}\text{NH}_3$ applications were delayed until after the soil temperature at 10 cm had decreased to $< 10^\circ\text{C}$, and occurred on 17 November 2016 and 28 November 2017. In each year, the N rate was 224 kg ha^{-1} , applied with or without NP ($0.56 \text{ kg a.i. ha}^{-1}$) or EP ($0.15 \text{ kg a.i. ha}^{-1}$) as a nitrification inhibitor. For the 2016 applications, enrichments for the $^{15}\text{NH}_3$ were 1.556 (no inhibitor), 1.303 (NP), and 1.288 atom % ^{15}N (EP), while the corresponding enrichments in 2017 were 1.539, 1.196, and 1.204 atom % ^{15}N . Following the application of 22 kg P ha^{-1} as triple superphosphate and 28 kg K ha^{-1} as potassium chloride (KCl), planting was done on 18 May in both growing seasons studied, using Agrigold 6462 STXRIB ($87700 \text{ plants ha}^{-1}$) in 2017 and Agrigold A6499 STXRIB ($86500 \text{ plants ha}^{-1}$) in 2018 (Agrigold, St. Francisville, IL). For weed control, all sites received multiple post-emergence spot treatments of glyphosate [*N*-(phosphonomethyl)glycine]. Additionally, a pre-plant application of atrazine [2-chloro-4(ethylamino)-6-(isopropylamino)-1,3,5-triazine] was made to all sites in 2018, which was tank-mixed with either (i) thiencarbazone-methyl (methyl-4-[[[4,5-dihydro-3-methoxy-4-methyl-5-oxo-1H-1,2,4-triazol-1-yl]carbonyl]amino]sulfonyl]-5-methyl-3-thiophenecarboxylate) and isoxaflutole [5-cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)isoxazole] or (ii) glyphosate, mesotrione {[2-[4-

(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione}], and *S*-metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide].

Sample collection and processing

After reaching physiological maturity (R6), corn was hand-harvested from all plots on 12 October 2017 and 4 October 2018. In 2017, the entire aboveground biomass was collected from two plants near the middle of each of the two center rows, followed by separation of the ears, leaves, and stalks. The harvest strategy was modified in 2018, such that all plants were harvested from the center 2.4 m of the two middle rows. This change was made based on spatial variation documented for knife injections of $^{15}\text{NH}_3$ (Griesheim et al., 2019), with the aim of better representing crop ^{15}N uptake for the entire plot area. After separating all plant parts collected in the second harvest, all the leaves and ears were retained along with subsamples consisting of four husks and ten stalks. In both years, fresh weights of all plant parts were determined within 8 h after collection, for subsequent use in calculating FNUE, N derived from fertilizer (NDFE), and N derived from soil (NDFS). Stalks selected were mechanically shredded on the day of harvest, taking care to minimize cross-contamination by thoroughly cleaning the shredder (SKU#69293, Harbor Freight, Calabasas, CA) between samples. After shelling the ears by hand (2017) or with a hand-powered corn sheller (2018), the grain was mixed, a subsample (~100-g) collected, and grain moisture measured using a Dickey-John Model M-3G or MiniGAT moisture tester (Dickey-John, Auburn, IL). All plant samples were transferred to paper bags for drying in a dehumidified room heated to between 38 and 54°C while tracking moisture changes by measuring weight loss. In order to reduce sample volume, dried leaf material from the 2018

harvest was cut into 5-cm pieces with careful cleaning after each sample, and the resulting fragments were mixed thoroughly for subsampling.

When dry, the grain was ground to < 0.7 mm using a KitchenAid® stand mixer equipped with a grain mill (KitchenAid, Benton Harbor, MI), while all other plant parts were ground to < 0.5 mm with a Model 4 Wiley mill (Thomas Scientific, Swedesboro, NJ). In each case, the mill was scrupulously cleaned to prevent cross-contamination, and the ground sample was dried further after transfer to a paper bag that was placed in a forced-air oven (50°C).

Plant analyses

Total N analyses of dried plant material were performed by Kjeldahl digestion and diffusion (Technical Note, 2019b), using a block digester (Model BD50; Seal Analytical, Mequon, WI) for semimicro digestion involving a KMnO_4 -reduced Fe pretreatment to recover $(\text{NO}_3^- + \text{NO}_2^-)$ -N. The digestion technique was modified by including a 3-h pre-digestion period at 90°C after addition of catalyst and 18 M H_2SO_4 , so as to control frothing and foaming during the clearing step at 250°C. Following quantitative determinations by acidimetric titration, the diffused N was processed for ^{15}N analyses using an isotope-ratio mass spectrometer equipped with an automated Rittenberg system (Mulvaney et al., 1990).

Calculations

For each dried plant sample analyzed, total N (T_N in μg) was obtained as $(S - B)M \times 28013$, where S is the milliliters of H_2SO_4 used in titrating NH_4^+ -N liberated from the sample digest, B is the volume titrated for a blank digest, and M is the molarity of the titrant. Values of T_N were divided by the corresponding sample weight to determine total N

concentration ($\mu\text{g g}^{-1} = \text{mg kg}^{-1}$), which was upscaled to kg N ha^{-1} using weight data collected at harvest.

Fresh weights measured at harvest were adjusted to determine dry matter production for grain and stover (leaves, stalks, and husks) with and without fall NH_3 fertilization. Values obtained for grain (DMG) were used in estimating FNUE by the difference method:

$$\text{FNUE}_{\text{DIF}} = 100 \times \frac{DMG_F T_{N(F)} - DMG_U T_{N(U)}}{224}, \quad [1]$$

where F denotes plot-specific data collected for fertilized treatments and U represents the mean values for unfertilized plots at the corresponding location.

Percentage values of NDFFF were obtained using the equation,

$$\text{NDFFF} = 100 \left(\frac{A_P - A_B}{A_F - A_B} \right), \quad [2]$$

where A is atom % ^{15}N measured for the plant sample under analysis (P), the $^{15}\text{NH}_3$ applied (F), and the unfertilized background soil (B), the latter quantity being obtained as an average of the depth-specific values reported in Table 3.1. The percentage of NDFS was calculated from the difference, $100 - \text{NDFFF}$. The following equation was used in determining percentage values of $\text{FNUE}_{^{15}\text{N}}$:

$$\text{FNUE}_{^{15}\text{N}} = \left(\frac{T_N \times \text{NDFFF}}{224} \right), \quad [3]$$

where the value in the denominator is the application rate (in kg N ha^{-1}) of $^{15}\text{NH}_3$.

Statistical analyses

Because not all locations were used in each year of data collection, years were analyzed and reported separately. Statistical analyses were conducted using the MIXED procedure of SAS 9.4 (SAS, 2013) to detect treatment differences. The type 3 test of fixed effects was used to

determine significance at $P < 0.05$. Orthogonal polynomial contrast statements were utilized for mean separation of treatment effects. Results of interest were correlated with the use of the SAS CORRELATION procedure.

3.3 Results and Discussion

The six sites studied (Table 3.1) were selected so that each growing season would include an Alfisol and a Mollisol cropped to a corn-soybean rotation, plus an additional Mollisol under continuous corn. Each of the four corn-soybean rotations had been in place for at least 16 years, while the two monoculture sites represented the second (2017) or fourth (2018) year of continuous corn after previous cropping in rotation with soybean. All six sites were level to gently sloping with no manuring for at least five years, and had received regular inputs of fertilizer and limestone prior to the study period.

As documented by annual data in Table 3.2, both of the two years studied were average in air temperature, whereas total precipitation was lower than the historical norm. There were, however, substantial monthly differences that would have had ramifications for fertilizer N losses and crop N uptake. The first of these differences occurred during the winter and early spring following application, when nitrification and other N cycle processes would have been promoted by temperatures that were considerably higher in year 1 than in year 2. Another factor was greater rainfall during the second growing season, which would have helped the crop avoid moisture stress while augmenting the supply of mineral N by stimulating soil N mineralization.

Dry matter production determined at harvest (Table 3.3) was consistent with county grain yields for the two growing seasons studied (USDA/NASS, 2019). Except for one of the Sable sites (no. 1), anhydrous NH_3 was effective for increasing grain yield when applied in the fall

without a nitrification inhibitor (–NI). Relative to the unamended controls (UTC), N response ranged from 20 to 75% (46% on average), which is comparable to what has been reported in previous yield-based evaluations involving fall NH₃ applications to corn (Stevenson and Baldwin, 1969; Warren et al., 1975, 1980; Hendrickson et al., 1978; Vetsch and Randall, 2004; Randall and Vetsch, 2005a). Greater yield responses have been reported for fall-applied NH₃, but only when check-plot yields were depressed in static-plot studies (Boswell, 1977; Maddux et al., 1984; Hanson et al., 1987; Stehouwer and Johnson, 1990; Pittelkow et al., 2017).

Because of better growing conditions, grain yields were higher in 2018 than in 2017 (Table 3.3), although this cannot be substantiated for the unfertilized check plots at the Sable (no. 1 and 4) and Birkbeck (no. 2 and 5) sites. A likely explanation in the latter case can be found in Table 3.1, as profile levels of potentially mineralizable N were much lower for site 5 than for site 2. Table 3.1 suggests a trend in the same direction for the Sable sites, according to somewhat lower subsoil ISNT levels that were determined for site 4 than 1. Regardless of the site studied in 2018, stover biomass at harvest was lower than in 2017 due to more complete drying in the field.

As can be seen from Table 3.3, the two nitrification inhibitors studied were of little benefit for increasing the fertilizer value of fall-applied NH₃. The only significant effect was a 12% increase in stover production that occurred with NP in 2017, concurrent with a nonsignificant 7% increase in grain yield obtained at site 2 with EP. Such ineffectiveness is by no means surprising considering the erratic performance in previous yield-based studies to evaluate NP for use with fall-applied NH₃ (Warren et al., 1975, 1980; Boswell, 1977; Hendrickson et al., 1978; Touchton et al., 1979; Malzer and Randall, 1985; Hanson et al., 1987; Stehouwer and Johnson, 1990; Randall and Vetsch, 2005a; Pittelkow et al., 2017).

Table 3.4 shows area-based estimates of total N recoveries in aboveground biomass as obtained from dry matter production and N concentration for each plant part. Significant treatment and site effects were observed for both years when these estimates were analyzed statistically, the most noticeable trend arising from the negative impact of the unfertilized control (UTC) on total N concentrations if not also dry matter production.

Closer examination of Table 3.4 provides evidence that total N recoveries can be affected by the use of a nitrification inhibitor, although the effect varied between the two cropping systems studied. With continuous corn, there were negative ramifications from the addition of NP that were more pronounced in 2018 (site 6) than in 2017 (site 3), presumably reflecting an increased tie-up of NH_4^+ -N through immobilization in the presence of ample residue C. The opposite trend was observed in comparing total N recoveries with and without NP when corn followed soybean at sites 2, 4, and 5, whereas NP showed no tendency to increase total N when applied at site 1, which is consistent with the high level of potentially mineralizable N (Table 3.1) and the lack of fertilizer N response (Table 3.3). In comparing treatment effects with and without the use of a nitrification inhibitor in the first growing season, the only significant difference occurred from the higher total N recovery obtained with NP than EP. No such difference was found in 2018 because the two inhibitors had both positive and negative effects on total N recovery when compared to the -NI treatment, resulting in a significant treatment \times site interaction.

Considering that unfertilized yields always exceeded the magnitude of yield increase observed after fall NH_3 applications, fertilizer N would have presumably been a lesser source for crop uptake, as compared to soil-derived N. This is indeed confirmed by Tables 3.5 and 3.6, which compare NDFP and NDFS for the six sites studied. In all cases and for either separate

plant parts or total aboveground biomass, NDFS exceeded NDFE when evaluated at harvest. The same trend has previously been documented by numerous field studies involving the use of ^{15}N to evaluate fertilizer N management for corn production (e.g., Chichester and Smith, 1978; Bageriego et al., 1979; Olson, 1980; Kitur et al., 1984; Jokela and Randall, 1987; Blackmer and Sanchez, 1988; Timmons and Cruse, 1990; Omay et al., 1998).

Because rainfall was less limiting (Table 3.2), soil N mineralization should have been more extensive for the second than the first growing season studied, which would be expected to increase NDFS and decrease NDFE. Some evidence of this pattern can be found by comparing data reported in Tables 3.5 and 3.6 for the two Sable sites (no. 1 and 4), whereas the opposite trend is apparent for the Birkbeck sites (no. 2 and 5) and no consistent differences were observed in comparing the Elburn sites (no. 3 and 6). The former disparity reflects the marked differences in potentially mineralizable N documented for sites 2 and 5 (Table 3.1), while the latter may be related to low levels of soil test P (Table 3.1) that could have limited crop N uptake (Magalhães et al., 1998). Interestingly, these low soil test levels produced no symptoms of P deficiency, nor did those at site 2 or 4 that were also well below the soil P test goal of 50 kg ha^{-1} (0-18 cm) according to the *Illinois Agronomy Handbook* (Fernández and Hoelt, 2009).

With the greater rainfall in year 2, significant site and treatment effects were far more common than in year 1. Examination of Table 3.6 reveals that site was a more important factor than treatment, such that NDFE increased in the order site 4 < site 6 < site 5 as NDFS decreased in the same order. Significant treatment effects were observed only for grain and total aboveground biomass, whereby the addition of NP resulted in an increase in NDFE and a decrease in NDFS.

Tables 3.7 and 3.8 document a similar pattern of statistical significance that was observed in estimating FNUE from ^{15}N recoveries. For the Sable and Elburn sites (no. 1, 3, 4, and 6), FNUE values were lower in a more favorable growing season (year 2), a pattern documented previously (Meisinger et al., 1985) that can be attributed to greater soil N mineralization promoted by additional rainfall. In contrast, there was a substantial increase in FNUE between years 1 and 2 for the Birkbeck sites, which is consistent with the much lower ISNT values obtained for site 5 than for site 2 (Table 3.1).

In view of the warmer winter that prevailed during the first year of the study, the use of a nitrification inhibitor was expected to be more beneficial in 2017 than in 2018; however, the only significant effect was an increase in FNUE that was observed for stalks with the use of NP. This pattern was in fact more prevalent in 2018, when the addition of NP led to a significant increase in FNUE for grain, stalks, and total aboveground biomass. As would be expected considering that the N rate applied was more optimal for a low-fertility Alfisol than for highly fertile Mollisols, an increase in FNUE was much more evident for site 5 than for site 4 or 6, and coincided with a similar trend documented for total N recovery (Table 3.4) but not dry matter production (Table 3.3). Interestingly, each inhibitor caused a numerical decrease in FNUE when applied to site 3 under continuous corn, perhaps reflecting immobilization promoted by prolonging the supply of NH_4^+ in the presence of abundant residue C (Lewis and Stefanson, 1975; Söchtig and Salfeld, 1977; Crawford and Chalk, 1993).

Relative to the isotopic estimates reported for grain in Tables 3.7 and 3.8, estimates of FNUE by the difference method tended to be lower in 2017, and higher in 2018. Such inconsistencies can readily be found in the literature relating to cropping experiments with ^{15}N , either from individual studies that document both trends (e.g., Westerman and Kurtz, 1974;

Olson, 1980; Torbert et al., 1992; Jokela and Randall, 1997) or from the diametric differences that can arise in separate studies estimating FNUE by the two methods (e.g., Moraghan et al., 1984; Schindler and Knighton, 1999). The tendency toward lower FNUE values in 2018 by ^{15}N - than yield-based estimation would logically be ascribed to greater rainfall that promoted pool substitution of labeled for unlabeled N through mineralization-immobilization turnover; however, this view must be questioned for two reasons. First, this tendency was least apparent for the Elburn site (no. 6) under continuous corn that would have accentuated the input of residue C, thereby stimulating heterotrophic N immobilization. Secondly, NP should have exacerbated the isotopic underestimation of FNUE owing to the heterotrophic preference for NH_4^+ over NO_3^- (Jansson, 1958; Aulakh and Rennie, 1984; Rice and Tiedje, 1989; Recous et al., 1990; Jensen, 1997), yet no such effect was observed for either growing season studied.

As documented by Tables 3.7 and 3.8, estimates of FNUE by the isotopic method ranged from 12 to 34% (20% on average) for grain and from 16 to 42% (27% on average) for total aboveground biomass. These estimates can only be compared to results obtained by Blackmer and Sanchez (1988) in a study involving spring application of $^{15}\text{NH}_3$ at the same rate used in the present project (224 kg N ha^{-1}). When calculated from NDFE and total N data reported in their paper, the range of FNUE for grain was from 13 to 20% (average of 17%) with 26 to 43% (average of 31%) FNUE for total aboveground biomass.

Considering that NO_3^- leaching losses tend to be higher (Randall et al., 2003; Randall and Vetsch, 2005b) and corn yields lower (e.g., Hendrickson et al., 1978; Stehouwer and Johnson, 1990; Pittelkow et al., 2017) for fall than spring NH_3 applications, lower FNUE values would be expected from our work, as compared to those obtained from Blackmer and Sanchez (1988). This expectation is at odds with the consistency documented in the previous paragraph;

however, the Iowa study was conducted on soils ranging in organic matter (0-25 cm) from 50 to 70 g kg⁻¹ according to site-specific soil survey data (USDA/NRCS, 2019), as opposed to 20 to 45 g kg⁻¹ reported for the current study sites by the same online data source. The implication is that the similarity between FNUE values for spring (Blackmer and Sanchez, 1988) and fall (present study) NH₃ fertilization is more apparent than real because soil N mineralization would have been more extensive in the Iowa study, enhancing the dilution of fertilizer ¹⁵N by soil-derived N. The importance of such dilution is readily apparent from Tables 3.7 and 3.8, which show that FNUE values were highest for an Alfisol (site no. 5) that was much lower in organic C and N, and also in potentially mineralizable N (Table 3.1), as compared to the soils at the other experimental sites. This finding is further supported by the negative correlation ($r = -0.56$) that was obtained in relating FNUE with site-specific ISNT levels for surface samples, which implicates soil N mineralization as a key factor in reducing FNUE by dilution of the fertilizer N applied.

3.4 Tables

Table 3.1. Characterization of study sites.†

| Site no. | Soil Series‡ | Soil Subgroup | Sampling depth | Textural class§ | pH | Organic C | Total N | | PMN¶ | Available P |
|-------------|----------------|---------------------|----------------|-----------------|-----|--------------------|------------------------|---------------------------|------|-------------|
| | | | | | | | Concentration | ¹⁵ N abundance | | |
| | | | m | | | g kg ⁻¹ | atom % ¹⁵ N | mg kg ⁻¹ | | |
| <u>2017</u> | | | | | | | | | | |
| 1 | Sable (C-S) | fine-silty, mixed, | 0–0.3 | sil | 6.3 | 20.9 | 1.76 | 0.371 | 276 | 21.0 |
| | | superactive, mesic | 0.3–0.6 | sil | 6.6 | 9.1 | 0.85 | 0.372 | 134 | 4.3 |
| | | Typic Endoaquolls | 0.6–0.9 | sicl | 7.0 | 6.3 | 0.61 | 0.371 | 91 | 3.2 |
| 2 | Birkbeck (C-S) | fine-silty, mixed, | 0–0.3 | sicl | 5.9 | 12.3 | 1.21 | 0.371 | 191 | 9.7 |
| | | superactive, mesic | 0.3–0.6 | sicl | 5.5 | 6.1 | 0.68 | 0.372 | 104 | 2.2 |
| | | Oxyaquic Hapludalfs | 0.6–0.9 | sicl | 6.2 | 3.9 | 0.47 | 0.372 | 72 | 3.0 |
| 3 | Elburn (C-C) | fine-silty, mixed, | 0–0.3 | sil | 6.4 | 18.3 | 1.68 | 0.372 | 259 | 73.7 |
| | | superactive, mesic | 0.3–0.6 | sicl | 6.2 | 9.7 | 0.93 | 0.372 | 146 | 11.1 |
| | | Aquic Argiudolls | 0.6–0.9 | sicl | 7.3 | 5.6 | 0.59 | 0.373 | 87 | 3.9 |
| <u>2018</u> | | | | | | | | | | |
| 4 | Sable (C-S) | fine-silty, mixed, | 0–0.3 | sil | 6.6 | 20.9 | 1.71 | 0.371 | 271 | 7.1 |
| | | superactive, mesic | 0.3–0.6 | sicl | 7.1 | 9.1 | 0.83 | 0.372 | 119 | 2.4 |
| | | Typic Endoaquolls | 0.6–0.9 | sicl | 7.5 | 4.9 | 0.50 | 0.372 | 67 | 1.4 |
| 5 | Birkbeck (C-S) | fine-silty, mixed, | 0–0.3 | sicl | 6.3 | 6.3 | 0.71 | 0.371 | 108 | 14.9 |
| | | superactive, mesic | 0.3–0.6 | sicl | 5.4 | 4.0 | 0.48 | 0.371 | 73 | 6.4 |
| | | Oxyaquic Hapludalfs | 0.6–0.9 | sicl | 5.7 | 3.5 | 0.38 | 0.371 | 52 | 8.6 |
| 6 | Elburn (C-C) | fine-silty, mixed, | 0–0.3 | sil | 6.6 | 20.3 | 1.69 | 0.371 | 281 | 4.9 |
| | | superactive, mesic | 0.3–0.6 | sicl | 6.5 | 10.2 | 0.84 | 0.372 | 148 | 8.9 |
| | | Aquic Argiudolls | 0.6–0.9 | sicl | 7.5 | 5.5 | 0.56 | 0.372 | 82 | 1.5 |

†All analytical data are reported as the mean of three replicate determinations.

‡Crop rotation indicated in parentheses: C = corn (*Zea mays* L.), S = soybean (*Glycine max* L. Merr.).

§As determined by the hydrometer method (Gee and Or, 2002): sil = silt loam, sicl = silty clay loam.

¶Potentially mineralizable N, estimated by alkaline diffusion using the Illinois Soil N Test (ISNT)(Khan et al., 2001).

Table 3.2. Precipitation and temperatures during the study period, in relation to historical norms.

| Month | Total precipitation | | | Average air temperature | | |
|--------------|---------------------|--------|-------------|-------------------------|--------|-------------|
| | Year 1 | Year 2 | Historical† | Year 1 | Year 2 | Historical† |
| | mm | | | °C | | |
| November | 90 | 56 | 72 | 8 | 5 | 5 |
| December | 27 | 2 | 62 | -2 | -2 | -2 |
| January | 44 | 25 | 41 | 0 | -5 | -5 |
| February | 11 | 109 | 42 | 5 | 0 | -2 |
| March | 66 | 78 | 75 | 5 | 3 | 4 |
| April | 100 | 64 | 87 | 14 | 7 | 11 |
| May | 85 | 70 | 100 | 16 | 22 | 17 |
| June | 40 | 103 | 101 | 23 | 23 | 22 |
| July | 49 | 75 | 97 | 24 | 23 | 24 |
| August | 85 | 63 | 101 | 20 | 23 | 23 |
| September | 38 | 89 | 72 | 19 | 21 | 19 |
| October | 100 | 58‡ | 65 | 14 | 12‡ | 12 |
| Annual data§ | 735 | 792 | 915 | 12 | 11 | 11 |

†As reported for Farmer City by Illinois State Climatologist (2019a).

‡As reported for Champaign-Urbana by Illinois State Climatologist (2019b).

§Reported as a sum for precipitation and as a mean for temperature.

Table 3.3. Dry matter production after fall NH₃ fertilization that supplied 224 kg N ha⁻¹ with no nitrification inhibitor (-NI), nitrapyrin (NP), or an experimental product (EP) with inhibitory properties.†

| 2017 | | | | 2018 | | | |
|-------------------------------|------------|-----------|--------|-------------------------------|------------|-----------|---------|
| Site no. | Treatment‡ | Grain§ | Stover | Site no. | Treatment‡ | Grain§ | Stover |
| ———— Mg ha ⁻¹ ———— | | | | ———— Mg ha ⁻¹ ———— | | | |
| 1 | -NI | 10.6 (4) | 8.6 | 4 | -NI | 11.5 (55) | 6.2 |
| | NP | 10.8 (6) | 9.0 | | NP | 11.9 (61) | 6.3 |
| | EP | 9.9 (-3) | 8.6 | | EP | 11.1 (50) | 6.2 |
| | UTC | 10.2 | 8.5 | | UTC | 7.4 | 4.6 |
| 2 | -NI | 10.6 (20) | 9.4 | 5 | -NI | 13.3 (75) | 7.3 |
| | NP | 10.9 (24) | 10.3 | | NP | 13.9 (83) | 8.2 |
| | EP | 11.4 (30) | 9.6 | | EP | 13.8 (82) | 7.2 |
| | UTC | 8.8 | 10.2 | | UTC | 7.6 | 5.8 |
| 3 | -NI | 9.4 (38) | 10.0 | 6 | -NI | 11.8 (44) | 5.2 |
| | NP | 9.8 (44) | 11.9 | | NP | 10.2 (24) | 4.8 |
| | EP | 8.5 (25) | 8.2 | | EP | 11.2 (37) | 5.5 |
| | UTC | 6.8 | 8.6 | | UTC | 8.2 | 4.4 |
| <u>Statistics</u> | | | | | | | |
| Treatment | | 0.03 | 0.02 | Treatment | | <0.0001 | 0.0003 |
| Site | | 0.003 | 0.03 | Site | | <0.0001 | <0.0001 |
| Treatment × site | | NS | NS | Treatment × site | | 0.003 | NS |
| <u>Treatment effect</u> | | | | <u>Treatment effect</u> | | | |
| | -NI | 10.2a | 9.3b | | -NI | 12.2a | 6.2a |
| | NP | 10.5a | 10.4a | | NP | 12.0a | 6.4a |
| | EP | 9.9a | 8.8b | | EP | 12.0a | 6.3a |
| | UTC | 8.6b | 9.1b | | UTC | 7.7b | 4.9b |
| <u>Site effect</u> | | | | <u>Site effect</u> | | | |
| | 1 | 10.4a | 8.7b | | 4 | 10.5b | 5.8b |
| | 2 | 10.4a | 9.9a | | 5 | 12.2a | 7.1a |
| | 3 | 8.6b | 9.7a | | 6 | 10.4b | 5.0c |

†Primary data (upper nine rows) reported as a mean from triplicate plots.

‡UTC = untreated control.

§Percent fertilizer-N response indicated in parentheses, calculated as 100 × (fertilized yield – unfertilized yield)/ unfertilized yield.

Table 3.4. Total amounts of N recovered in aboveground biomass as affected by fall NH₃ fertilization that supplied 224 kg N ha⁻¹ with no nitrification inhibitor (-NI), nitrapyrin (NP), or an experimental product (EP) with inhibitory properties.†

| 2017 | | | 2018 | | |
|-------------------------|------------|---------------------|-------------------------|------------|---------------------|
| Site no. | Treatment‡ | Total N | Site no. | Treatment‡ | Total N |
| | | kg ha ⁻¹ | | | kg ha ⁻¹ |
| 1 | -NI | 234.1 | 4 | -NI | 148.3 |
| | NP | 219.2 | | NP | 158.8 |
| | EP | 206.7 | | EP | 144.7 |
| | UTC | 185.5 | | UTC | 88.2 |
| 2 | -NI | 204.0 | 5 | -NI | 178.7 |
| | NP | 237.2 | | NP | 209.0 |
| | EP | 200.8 | | EP | 204.8 |
| | UTC | 151.1 | | UTC | 85.0 |
| 3 | -NI | 215.3 | 6 | -NI | 157.4 |
| | NP | 204.4 | | NP | 138.6 |
| | EP | 166.4 | | EP | 146.1 |
| | UTC | 128.5 | | UTC | 95.6 |
| <u>Statistics</u> | | | | | |
| Treatment | | 0.0002 | Treatment | | <0.0001 |
| Site | | 0.03 | Site | | <0.0001 |
| Treatment × site | | NS | Treatment × site | | <0.0001 |
| <u>Treatment effect</u> | | | <u>Treatment effect</u> | | |
| -NI | | 217.8ab | -NI | | 161.5a |
| NP | | 220.3a | NP | | 168.8a |
| EP | | 191.3b | EP | | 165.2a |
| UTC | | 155.0c | UTC | | 89.6b |
| <u>Site effect</u> | | | <u>Site effect</u> | | |
| 1 | | 211.4a | 4 | | 135.0b |
| 2 | | 198.3ab | 5 | | 169.4a |
| 3 | | 178.7b | 6 | | 134.4b |

†Primary data (upper nine rows) reported as a mean from triplicate plots.

‡UTC = untreated control.

Table 3.5. 2017 percentages of plant N derived from fertilizer (NDF) and soil (NDFS) after $^{15}\text{NH}_3$ was fall-applied with no nitrification inhibitor (–NI), nitrapyrin (NP), or an experimental product (EP) with inhibitory properties.†

| Site no. | Treatment | Grain | | Leaves | | Stalks | | Husks | | Total‡ | |
|-------------------------|-----------|-------|-------|--------|-------|--------|-------|-------|-------|--------|-------|
| | | NDF§ | NDFS¶ | NDF§ | NDFS¶ | NDF§ | NDFS¶ | NDF§ | NDFS¶ | NDF§ | NDFS¶ |
| % | | | | | | | | | | | |
| 1 | –NI | 30 | 70 | 30 | 70 | 23 | 77 | 28 | 72 | 29 | 71 |
| | NP | 33 | 67 | 32 | 68 | 28 | 72 | 32 | 68 | 32 | 68 |
| | EP | 24 | 76 | 23 | 77 | 19 | 81 | 23 | 77 | 23 | 77 |
| 2 | –NI | 32 | 68 | 32 | 68 | 23 | 77 | 21 | 79 | 31 | 69 |
| | NP | 31 | 69 | 30 | 70 | 24 | 76 | 30 | 70 | 30 | 70 |
| | EP | 27 | 73 | 30 | 70 | 19 | 81 | 26 | 74 | 26 | 74 |
| 3 | –NI | 35 | 65 | 35 | 65 | 25 | 75 | 32 | 68 | 33 | 67 |
| | NP | 31 | 69 | 34 | 66 | 26 | 74 | 30 | 70 | 31 | 69 |
| | EP | 31 | 69 | 32 | 68 | 24 | 76 | 28 | 72 | 30 | 70 |
| Statistics# | | | | | | | | | | | |
| Treatment | | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Site | | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Treatment × site | | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Treatment effect | | | | | | | | | | | |
| –NI | | 32 | 68 | 32 | 68 | 24 | 76 | 27 | 73 | 31 | 69 |
| NP | | 32 | 68 | 32 | 68 | 26 | 74 | 31 | 69 | 31 | 69 |
| EP | | 27 | 73 | 28 | 72 | 21 | 79 | 26 | 74 | 26 | 74 |
| Site effect | | | | | | | | | | | |
| 1 | | 29 | 71 | 28 | 72 | 23 | 77 | 28 | 72 | 28 | 72 |
| 2 | | 30 | 70 | 31 | 69 | 22 | 78 | 26 | 74 | 29 | 71 |
| 3 | | 32 | 68 | 34 | 66 | 25 | 75 | 30 | 70 | 31 | 69 |

†Primary data (upper nine rows) reported as a mean from triplicate plots.

‡Values represent total aboveground biomass (grain + leaves + stalks + husks).

§Calculated by Eq. [2].

¶Calculated as $100 - \text{NDF}$.

#NS = not significant at $P > 0.05$. At lower P levels, mean values for treatments or sites did not differ significantly when followed by the same letter.

Table 3.6. 2018 percentages of plant N derived from fertilizer (NDFE) and soil (NDFS) after $^{15}\text{NH}_3$ was fall-applied with no nitrification inhibitor (–NI), nitrapyrin (NP), or an experimental product (EP) with inhibitory properties.†

| Site no. | Treatment | Grain | | Leaves | | Stalks | | Husks | | Total‡ | |
|-------------------------|-----------|---------|---------|---------|---------|---------|---------|--------|--------|---------|---------|
| | | NDFE§ | NDFS¶ | NDFE§ | NDFS¶ | NDFE§ | NDFS¶ | NDFE§ | NDFS¶ | NDFE§ | NDFS¶ |
| % | | | | | | | | | | | |
| 4 | –NI | 24 | 76 | 26 | 74 | 20 | 80 | 31 | 69 | 24 | 76 |
| | NP | 28 | 72 | 29 | 71 | 25 | 75 | 26 | 74 | 28 | 72 |
| | EP | 24 | 76 | 27 | 73 | 22 | 78 | 25 | 75 | 24 | 76 |
| 5 | –NI | 37 | 63 | 37 | 63 | 32 | 68 | 36 | 64 | 37 | 63 |
| | NP | 46 | 54 | 43 | 57 | 41 | 59 | 43 | 57 | 45 | 55 |
| | EP | 45 | 55 | 43 | 57 | 41 | 59 | 42 | 58 | 44 | 56 |
| 6 | –NI | 32 | 68 | 36 | 64 | 32 | 68 | 38 | 62 | 32 | 68 |
| | NP | 37 | 63 | 39 | 61 | 35 | 65 | 40 | 60 | 37 | 63 |
| | EP | 32 | 68 | 35 | 65 | 30 | 70 | 33 | 67 | 32 | 68 |
| Statistics# | | | | | | | | | | | |
| Treatment | | 0.05 | 0.05 | NS | NS | NS | NS | NS | NS | 0.04 | 0.04 |
| Site | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0002 | 0.0002 | <0.0001 | <0.0001 |
| Treatment × site | | NS | NS | 0.005 | NS | NS | NS | NS | NS | NS | NS |
| Treatment effect | | | | | | | | | | | |
| –NI | | 31b | 69a | 33 | 67 | 28 | 72 | 35 | 65 | 31b | 69a |
| NP | | 37a | 63b | 37 | 63 | 34 | 66 | 36 | 64 | 37a | 63b |
| EP | | 33ab | 66ab | 35 | 65 | 31 | 69 | 33 | 67 | 33ab | 67ab |
| Site effect | | | | | | | | | | | |
| 4 | | 25c | 75a | 27c | 73a | 22c | 78a | 27b | 73a | 25c | 75a |
| 5 | | 43a | 57c | 41a | 59c | 38a | 62c | 40a | 60b | 42a | 58c |
| 6 | | 33b | 67b | 37b | 63b | 32b | 68b | 37a | 63b | 34b | 66b |

†Primary data (upper nine rows) reported as a mean from triplicate plots.

‡Values represent total aboveground biomass (grain + leaves + stalks + husks).

§Calculated by Eq. [2].

¶Calculated as $100 - \text{NDFE}$.

#NS = not significant at $P > 0.05$. At lower P levels, mean values for treatments or sites did not differ significantly when followed by the same letter.

Table 3.7. Uptake efficiency of fertilizer N applied in the fall as NH₃, determined from ¹⁵N recovery in grain, leaves, stalks, and/or husks, and from grain yield differences measured in 2017.†

| Site no. | Treatment | Fertilizer N uptake efficiency (FNUE)‡ | | | | | |
|-------------------------|-----------|--|--------------------------------|-------|--------|--------|-------|
| | | By differences§ | From ¹⁵ N recovery¶ | | | | Total |
| | | | Grain | Grain | Leaves | Stalks | |
| % | | | | | | | |
| 1 | –NI | 10 | 22 | 5 | 3 | 1 | 30 |
| | NP | 13 | 22 | 5 | 4 | 1 | 32 |
| | EP | 7 | 16 | 4 | 2 | 1 | 23 |
| 2 | –NI | 17 | 21 | 4 | 3 | 0 | 28 |
| | NP | 24 | 24 | 4 | 3 | 1 | 32 |
| | EP | 20 | 17 | 4 | 2 | 0 | 23 |
| 3 | –NI | 24 | 23 | 6 | 3 | 1 | 32 |
| | NP | 27 | 18 | 5 | 4 | 1 | 28 |
| | EP | 12 | 16 | 4 | 2 | 0 | 22 |
| Statistics# | | | | | | | |
| Treatment | | NS | NS | NS | 0.02 | NS | NS |
| Site | | 0.02 | NS | NS | NS | NS | NS |
| Treatment × site | | NS | NS | NS | NS | NS | NS |
| Treatment effect | | | | | | | |
| –NI | | 17 | 22 | 5 | 3ab | 1 | 30 |
| NP | | 21 | 21 | 5 | 4a | 1 | 31 |
| EP | | 13 | 16 | 4 | 2b | 0 | 23 |
| Site effect | | | | | | | |
| 1 | | 10b | 20 | 5 | 3 | 1 | 28 |
| 2 | | 20a | 21 | 4 | 3 | 0 | 28 |
| 3 | | 21a | 19 | 5 | 3 | 1 | 27 |

†Primary data (upper nine rows) reported as a mean from triplicate plots.

‡FNUE = fertilizer N uptake efficiency.

§Calculated by Eq. [1].

¶Calculated by Eq. [3].

#NS = not significant at $P > 0.05$. At lower P levels, mean values for treatments or sites did not differ significantly when followed by the same letter.

Table 3.8. Uptake efficiency of fertilizer N applied in the fall as NH₃, determined from ¹⁵N recovery in grain, leaves, stalks, and/or husks, and from grain yield differences measured in 2018.†

| Site no. | Treatment | Fertilizer N uptake efficiency (FNUE)‡ | | | | | |
|-------------------------|-----------|--|--------------------------------|---------|---------|-------|---------|
| | | By difference§ | From ¹⁵ N recovery¶ | | | | Total |
| | | Grain | Grain | Leaves | Stalks | Husks | |
| % | | | | | | | |
| 4 | -NI | 19 | 12 | 1 | 2 | 0 | 16 |
| | NP | 24 | 16 | 1 | 3 | 0 | 20 |
| | EP | 17 | 12 | 1 | 2 | 0 | 16 |
| 5 | -NI | 34 | 24 | 1 | 3 | 1 | 29 |
| | NP | 43 | 34 | 2 | 6 | 1 | 42 |
| | EP | 42 | 32 | 2 | 6 | 1 | 40 |
| 6 | -NI | 21 | 18 | 1 | 3 | 1 | 23 |
| | NP | 14 | 18 | 1 | 3 | 1 | 23 |
| | EP | 17 | 17 | 1 | 2 | 1 | 21 |
| Statistics# | | | | | | | |
| Treatment | | NS | 0.04 | NS | 0.05 | NS | 0.03 |
| Site | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.008 | <0.0001 |
| Treatment × site | | 0.009 | NS | 0.005 | NS | NS | NS |
| Treatment effect | | | | | | | |
| -NI | | 25 | 18b | 1 | 3b | 1 | 23b |
| NP | | 27 | 23a | 1 | 4a | 1 | 28a |
| EP | | 25 | 20ab | 1 | 3ab | 1 | 26ab |
| Site effect | | | | | | | |
| 4 | | 20b | 13c | 1c | 2b | 0b | 17c |
| 5 | | 40a | 30a | 2a | 5a | 1a | 37a |
| 6 | | 17b | 18b | 1b | 3b | 1ab | 22b |

†Primary data (upper nine rows) reported as a mean from triplicate plots.

‡FNUE = fertilizer N uptake efficiency.

§Calculated by Eq. [1].

¶Calculated by Eq. [3].

#NS = not significant at $P > 0.05$. At lower P levels, mean values for treatments or sites did not differ significantly when followed by the same letter.

CHAPTER 4: Summary and Conclusions

The dilution manifold and collection tank described allow knifed applications of $^{15}\text{NH}_3$ to research plots with a tractor-mounted tool bar. By using the complete system, a 2-yr field study using anhydrous $^{15}\text{NH}_3$ to evaluate fall N fertilization for Midwestern corn production, a typical N rate (224 kg N ha^{-1}) was applied, with or without a nitrification inhibitor, to microplots at six on-farm sites with contrasting soils under a corn-soybean rotation or continuous corn. At harvest, FNUE by ^{15}N recovery averaged 20% in grain and 27% in total aboveground biomass, while NDFE in either case never exceeded 46%. Of the two nitrification inhibitors and at only two of the six sites studied, NP but not EP was effective for significantly increasing FNUE. Site differences had a much larger effect, as FNUE was highest for the soil that was lowest in organic C and potentially mineralizable N.

Based on data obtained under typical Midwestern conditions and even with the addition of NP, the majority of the N applied in the fall as anhydrous NH_3 is not taken up by the following corn crop. If this practice is to be used, uptake efficiency can be improved by accounting for soil N mineralization.

CHAPTER 5: References

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