

COVER CROP EFFECTS ON UREASE INHIBITORS

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COVER CROP EFFECTS ON UREASE INHIBITORS

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A Thesis

Submitted to

The Graduate Faculty of

Auburn University

in Partial Fulfillment of the

Requirements for the

Degree of

Master of Science.

Auburn, Alabama
August 4, 2018

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THESIS ABSTRACT
COVER CROP EFFECTS ON UREASE INHIBITORS

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Master of Science, August 4, 2018
(B.S., Auburn University, 2016)

81 Typed Pages

Directed by Elizabeth A. Guertal

Urease inhibitors continue to be introduced in the agricultural market, and thus new studies with these products are warranted. The objective of this research was to examine the utility of these inhibitors for reducing ammonia (NH_3) losses from soil (Pacolet fine sandy loam [clayey, kaolinitic, thermic, Typic Hapludults]) to which cotton (*Gossypium hirsutum* L.) had been cropped. Another soil was examined (Compass loamy sand [coarse-loamy, siliceous, thermic, Plinthic Paleudults]) to which soybeans (*Glycine max* L. Merr.) had been cropped with cover crops. For these laboratory experiments, intact (15-cm diam., 4-cm deep) cores were removed from selected plots of the Old Rotation (Auburn, AL.) and E.V. Smith Experiment Station (Shorter, AL.). Cover crop residue treatments were either none, or winter cover by either hairy vetch (*Vicia villosa*

Roth), crimson clover (*Trifolium incarnatum* L.), or cereal rye (*Secale cereal* L.). Cores were immediately removed to the laboratory and placed into glass jars for use in a 7 day incubation experiment where emitted ammonia was trapped in boric acid, with levels measured daily. Specific treatments were: 1) no residue via winter cover, or, winter cover crop residue, and, 2) untreated urea, and urea with possible urease inhibitors, including various formulations of NBPT (N-(n-butyl) thiophosphoric triamide) and maleic–itaconic acid copolymer (MIP). Treatments were arranged as a 2 x 4 factorial of residue cover and urea with/without urease inhibitors (surface applied), with 4 replications of each. The entire experiment was repeated in time, with two experiments using soil from the Old Rotation (Auburn, AL.), and three experiments with soil from E.V. Smith Research Center (Shorter, AL).

When soil was used from the Old Rotation, statistical analyses revealed a cover by inhibitor interaction on almost every sampling date. This was due to soils with a long history of green manures and 20+ years of cover cropping greatly increasing ammonia volatilization as a result of higher OM and total C levels, along with the urease inhibitor NBPT significantly reducing N losses via volatilization. When soils were used with no cover cropping history, ammonia volatilization decreased across all inhibitor treatments, but ammonia volatilization losses from those containing NBPT were still significantly lower as compared to untreated urea and urea + MIP.

Experiments using soil from E.V. Smith Research Center had different results, in that the presence of a cover crop or lack thereof, did not always affect ammonia loss like at the Old Rotation. However, a significant cover by inhibitor interaction was still significant across many sampling days. In all three experiments conducted with soil from

E.V. Smith, the inhibitor source was still highly significant ($P < 0.10$), and the use of NBPT as a urease inhibitor was effective in delaying and reducing ammonia volatilization in Days 1-2. However, cumulative N losses in Experiment 1 revealed no statistical difference across inhibitor treatments. This was most likely due to the increased soil moisture in Experiment 1- Run 1. Experiment 2 showed no significant effects from the presence of a cover crop, due to comparable soil moisture between those treatments. Use of NBPT delayed volatilization through Day 2, but cumulative losses were only significantly reduced in treatments with urea + NBPT/NPPT. In Experiment 3, average losses were greatest in the first 4 days in treatments where NBPT was not present, as compared to treatments containing NBPT. Also, cumulative losses from treatments with NBPT were only significantly lower than untreated urea when no cover was present.

Ammonia losses from urea-treated and urea + MIP were often higher from soil containing crop residue, when compared to soil with little crop residue. Use of NBPT was effective in delaying volatilization, regardless of the presence of a cover crop. Results from this laboratory study reveal that use of NBPT as a urease inhibitor may have utility in high residue cropping situations.

ACKNOWLEDGEMENTS

I owe my utmost gratitude to my Chair, Dr. Beth Guertal, for without her support and guidance this project would not have been possible. My appreciation also extends to Bill Bryce and my other laboratory colleagues, your assistance throughout this research was most valued. To my friends, thank you for your perspective and words of encouragement. Finally, my deepest thanks to my parents and sisters, for their unfailing support in whatever I pursue.

Style manual or journal used Publication Handbook and Style Manual of the American Society of Agronomy

Computer Software used Microsoft Word 2007, Microsoft Excel 2007, SAS v. 9.4

TABLE OF CONTENTS

LIST OF TABLES	x
LIST OF FIGURES.....	xi-xiii
I. LITERATURE REVIEW.....	1
Introduction.....	1
Cycling of Nitrogen in Soils.....	2
Additions of Nitrogen	5
Losses of Nitrogen	7
Inhibiting Losses from the Nitrogen Cycle.....	13
Urease Inhibitors.....	14
Factors of Nitrification.....	18
Nitrification Inhibitors.....	19
Research Objective.....	21
II. COVER CROP EFFECTS ON UREASE INHIBITORS	
Methods and Materials.....	22
Results and Discussion.....	25
Conclusion.....	34
III. SUMMARY.....	52
IV. LITERATURE CITED.....	53

LIST OF TABLES

1. Analysis of variance for main effects of cover and inhibitor, by day, location, and experiment, laboratory evaluations. Numbers represent the $P > F$36
2. Ammonia loss (percent of N applied) as a function of residue cover and urease inhibitor, Old Rotation soil. Means in columns denoted as 'Y' indicate soil with > 100 years of cover, while those with a 'N' indicate no cover.37
3. Background soil test data for the soil used for the volatilization experiments. All units are mg kg^{-1} except for organic matter (OM) and total N, which are listed in g kg^{-1} . Percent cover was determined by counting residue in a defined area with each core. This is a measure of visible cover on each core, at collection.....38
4. Soil water content (gravimetric) as determined at the beginning and end of each experiment.39
5. Ammonia loss (percent of N applied) as a function of residue cover and urease inhibitor, Experiment 1, E. V. Smith soil. Means in columns denoted as 'Y' indicate soil with 20 years of cover, while those with a 'N' indicate no cover.40
6. Ammonia loss (percent of N applied) as a function of residue cover and urease inhibitor, Experiment 2, E. V. Smith soil. Means in columns denoted as 'Y' indicate soil with 20 years of cover, while those with a 'N' indicate no cover.....41
7. Ammonia loss (percent of N applied) as a function of residue cover and urease inhibitor, Experiment 3, E. V. Smith soil. Means in columns denoted as 'Y' indicate soil with 20 years of cover, while those with a 'N' indicate no cover.42

LIST OF FIGURES

1. Diagram of the laboratory system used to measure ammonia volatilization, with jars, pump, and air scrubbers, following the procedure of O'Halloran (1993). The glass manifold is connected to an opening in each jar with silicon tubing.43
2. Cumulative N loss from soil from the Old Rotation (Auburn, AL.) as affected by inhibitor source. Losses are plotted from urea, urea with calcium salt of maleic-itaconic copolymer (urea + MIP), urea with N-(n-butyl) thiophosphoric triamide (urea +NBPT), and urea with N-(n-butyl) thiophosphoric triamide and N-(n-propyl) thiophosphoric triamide (urea + NBPT/NPPT) across a 7-day experiment. Error bars indicate standard error about the mean.44
3. Cumulative N loss from Old Rotation soil as affected by inhibitor source and a cover. Losses are plotted from urea, urea with calcium salt of maleic-itaconic copolymer (urea + MIP), urea with N-(n-butyl) thiophosphoric triamide (urea +NBPT), and urea with N-(n-butyl) thiophosphoric triamide and N-(n-propyl) thiophosphoric triamide (urea + NBPT/NPPT) across a 7-day experiment. Letters in each column represent significance ($\alpha = 0.05$) due to each treatment.45
4. Cumulative N loss as affected by the addition of a cover crop, as compared to no winter cover crop during the first experiment with soil from E.V. Smith Research Center (Shorter, AL.) Losses are plotted from urea, urea with calcium salt of maleic-itaconic copolymer (urea + MIP), urea with N-(n-butyl) thiophosphoric triamide (urea +NBPT), and urea with N-(n-butyl) thiophosphoric triamide and N-(n-propyl) thiophosphoric triamide (urea + NBPT/NPPT) across a 7 day experiment. Error bars indicate the standard error about the mean.46

5. Cumulative N loss as affected by the addition of a cover crop, as compared to no winter cover crop during the second experiment with soil from E.V. Smith Research Center (Shorter, AL.) Losses are plotted from urea, urea with calcium salt of maleic-itaconic copolymer (urea + MIP), urea with N-(n-butyl) thiophosphoric triamide (urea +NBPT), and urea with N-(n-butyl) thiophosphoric triamide and N-(n-propyl) thiophosphoric triamide (urea + NBPT/NPPT) across a 7 day experiment. Error bars indicate the standard error about the mean.....47

6. Cumulative N loss as affected by the addition of a cover crop, as compared to no winter cover crop during the third experiment with soil from E.V. Smith Research Center (Shorter, AL.) Losses are plotted from urea, urea with calcium salt of maleic-itaconic copolymer (urea + MIP), urea with N-(n-butyl) thiophosphoric triamide (urea +NBPT), and urea with N-(n-butyl) thiophosphoric triamide and N-(n-propyl) thiophosphoric triamide (urea + NBPT/NPPT) across a 7 day experiment. Error bars indicate the standard error about the mean.....48

7. Cumulative N loss from the first experiment with soil from E. V. Smith Research Center (Shorter, AL.) as affected by inhibitor source and a cover. Losses are plotted from urea, urea with calcium salt of maleic-itaconic copolymer (urea + MIP), urea with N-(n-butyl) thiophosphoric triamide (urea +NBPT), and urea with N-(n-butyl) thiophosphoric triamide and N-(n-propyl) thiophosphoric triamide (urea + NBPT/NPPT) across a 7-day experiment. Letters in each column represent significance ($\alpha = 0.05$) due to each treatment.....49

8. Cumulative N loss from the second experiment with soil from E. V. Smith Research Center (Shorter, AL.) as affected by inhibitor source and a cover. Losses are plotted from urea, urea with calcium salt of maleic-itaconic copolymer (urea + MIP), urea with N-(n-butyl) thiophosphoric triamide (urea +NBPT), and urea with N-(n-butyl) thiophosphoric triamide and N-(n-propyl) thiophosphoric triamide (urea + NBPT/NPPT) across a 7-day experiment. Letters in each column represent significance ($\alpha = 0.05$) due to each treatment.....50

9. Cumulative N loss from the third experiment with soil from E. V. Smith Research Center (Shorter, AL.) as affected by inhibitor source and a cover. Losses are plotted from urea, urea with calcium salt of maleic-itaconic copolymer (urea + MIP), urea with N-(n-butyl) thiophosphoric triamide (urea +NBPT), and urea with N-(n-butyl) thiophosphoric triamide and N-(n-propyl) thiophosphoric triamide (urea + NBPT/NPPT) across a 7-day experiment. Letters in each column represent significance ($\alpha = 0.05$) due to each treatment.51

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I. LITERATURE REVIEW

Introduction

There are two main processes by which atmospheric nitrogen (N) is fixed or made available for agricultural purposes. These processes are: 1) industrial, and, 2) biological fixation. Industrial fixation is the widespread use of the Haber-Bosch method to fix atmospheric N by using extreme temperature and pressure to produce ammonia (NH₃) (Pierzynski et al., 2005; Canfield et al., 2010). This type of N fixation can also occur naturally, via lightning activity. The vast amount of biological N fixation is a result of symbiotic relationships between crop and forage legumes and *Rhizobium* (Herridge et al., 2008). Estimated amounts of N fixed by industrial and biological processes in agricultural systems are 133 Tg and 33 Tg N annually (Canfield et al., 2010; Herridge et al., 2008).

Nitrogen from both industrial and biological N fixation procedures, when added to the soil, become part of the soil N cycle. The N cycle is a complex system of many routes for N assimilation and release from various organic and inorganic forms. Most of the various parts of the N cycle are controlled by microbial activity, with additions and losses from the N cycle often described in terms of N availability for plant uptake (nitrate-N and ammonium-N). Thus, additions and losses are discussed in reference to N as it enters or leaves this pool of soil N.

Nitrogen is added to plant available N pool in many ways: 1) fixation by lightning, 2) fertilization, 3) symbiotic N fixation, and 4) asymbiotic N fixation. Nitrogen is lost from the plant available N pool via other routes: 1) volatilization, 2) leaching, 3) plant uptake, and, 4) ammonium fixation. Many of these loss and addition routes are controlled by microbial organisms, and are also affected by temperature, soil moisture, and other factors. These will be discussed in detail within this introduction.

Efforts to manipulate the N cycle have long been an objective of crop producers. Nitrogen application procedures such as split, sidedress, or foliar applications can be used to minimize losses. *Rhizobial* inoculants may be applied to legumes to increase N fixation. Water management is used to reduce N loss from leaching or denitrification. Another tool is the use of nitrification and/or urease inhibitors, materials long used to manipulate the conversion of ammonium to nitrate, or limit the loss of ammonia to the atmosphere through volatilization.

Cycling of Nitrogen in Soils

Nitrogen is always being cycled within soils and either entering or leaving this pool of plant available N. Processes responsible for these transformations of soil N are: 1) mineralization, 2) immobilization, 3) nitrification, and, 4) denitrification. Unlike previously mentioned additions and losses of plant available N, these processes are involved in the transformation of N already within soils.

Mineralization and Nitrification

Mineralization is the conversion of organic N by microorganisms into inorganic forms. The final inorganic forms, ammonium (NH_4^+) and nitrate (NO_3^-), are the primary forms of N available for plant uptake. Mineralization consists of two processes: 1)

ammonification, and 2) nitrification. During ammonification, organic N is converted into ammonium (NH_4^+) by a wide range of microbes. First, organic N is broken down into amines and amino acids in a process called aminization. The amines and amino acids produced in aminization are used by other microbes resulting in the release of NH_4^+ as their waste product (Prasad and Power, 1997). Mineralization has been described as the sole domain of heterotrophic microorganisms, where plants must rely on microbial mineralization and/or nitrification for the production of NH_4^+ or NO_3^- (Kaye and Hart, 1997). Nitrification is the further oxidation of NH_4^+ into nitrite (NO_2^-) by *Nitrosomonas* and eventually to NO_3^- by *Nitrobacter* (Gee et al., 1990; Wrage et al., 2001). Both *Nitrosomonas* and *Nitrobacter* have slow growth rates, and their activity can be affected by various environmental conditions such as: 1) temperature, 2) pH, 3) dissolved-oxygen concentrations, and, 4) inhibitory chemicals (Gee et al., 1990).

Denitrification

Denitrification is one route by which we lose N from the N cycle. During denitrification, NO_3^- is reduced to NO_x , with the emission of the greenhouse gas N_2O as an intermediate, where it is no longer in a plant available form. This process occurs via soil residing bacteria such as *Pseudomonas*, *Bacillus*, *Thiobacillus*, *Nitrobacter*, and *Propionibacterium*. In anoxic conditions, these microbes are able to use NO_3^- as their terminal electron acceptor. Multiple enzymes are involved in the reduction of NO_3^- to N_2 including: 1) nitrate reductase, 2) nitrite reductase, 3) nitric oxide reductase, and 4) nitrous oxide reductase (Wrage et al., 2001). Other factors that influence the rate of denitrification are O_2 concentration, the availability of N and C, soil moisture, temperature, and climate and management-related factors (Tiedje, 1988; Beauchamp et

al., 1989; Dorland and Beauchamp, 1991). Of these factors, O₂ is probably the most limiting since denitrification is an anaerobic process (Tiedje, 1988). Soil pH has also been shown to influence denitrification rates, with higher rates occurring in alkaline soils as compared to acidic soils (Yamulki et al., 1997; Simek et al., 2000). It has also been noted that denitrification is sporadic throughout agricultural systems both in time and space. This is because microbial populations or activity are not consistent across operations due to varying environmental or soil conditions (Dowdell and Smith, 1974; Myrold and Tiedje, 1985). Work on spatial variability of soil denitrification suggested that there were “hot spots” of denitrification activity as a result of varying particulate soil organic carbon (SOC) concentrations throughout the soil (Parkin, 1987). A model including 336 denitrification studies suggested rates of N loss from denitrification were 87 Tg N year⁻¹ for the N-balance method in 1995, and 22 Tg N year⁻¹ for the soil core method (Hofstra and Bouwman, 2005). It was noted that the difference between these estimates is most likely due to the different methods used (Hofstra and Bouwman, 2005).

Immobilization

Immobilization is the conversion of inorganic N forms (NH₄⁺, NH₃, NO₃⁻, and NO₂⁻) to organic N, resulting in the loss of plant available and applied N by microbial processes (Jansson and Persson, 1982). Both chemical and biological processes function in the immobilization of N in soils (Trehan, 1996). High C:N ratios can lead to increased immobilization, as compared to low C:N ratios, which can lead to increased NH₄⁺ or NO₃⁻ concentrations within soils (Pierzynski et al., 2005). One study noted increasing N immobilization rates in soils with increasing SOC content or higher C:N ratios (Barrett and Burke, 2000). Corn residue containing a C:N ratio of 94:1 showed a stronger ability

to immobilize N, as compared to rice hulls and *Triticum aestivum* L. (wheat) with C:N ratios of 27:2 and 42:1, respectively (Hadas et al., 2004). Both immobilization and mineralization are major parts of the N cycle, as they provide most of the intermediates needed to recycle N throughout the environment (Jansson and Persson, 1982). Microbes are responsible for almost 50% of immobilization in soils, as they consume inorganic forms of N and convert it back to the organic form (Brookes et al., 1985).

Additions of Nitrogen

Lightning Fixation

Atmospheric N can also be fixed by lightning activity. Lightning discharges split atmospheric N (N_2), reacting with oxygen (O_2) to form nitric oxide (NO). This NO combines with O_2 to form nitrogen dioxide (NO_2), which is highly water soluble, forming nitric acid (HNO_3) and nitrous acid (HNO_2). During rainfall events, nitrite (NO_2^-) and nitrate (NO_3^-) are dissolved in water, and released for plant uptake or conversion by microbes (Noxon, 1976). Nitrogen fixation from lightning activity has been estimated at 14.4×10^6 tonnes per year (Hill et al., 1980).

Industrial Nitrogen Fixation

Fertilization is the largest addition to the N cycle, mainly due to the amount of N fertilizers used for agricultural, turf, ornamental, or home lawn purposes. The development of the Haber-Bosch process allowed N fertilizers to be industrially produced, by reducing atmospheric N_2 to NH_3 . As a result, global usage of N fertilizers has increased almost 800% from 1960 to 2000. Contributions of N from the Haber-Bosch

process are 133 Tg per year (Canfield et al., 2010). Almost 90% of the N fertilizer used globally is in the NH_4^+ form, which is eventually converted to water soluble NO_3^- during nitrification (Canfield et al., 2010). This resultant NO_3^- can be leached to groundwater resources, or lost to nearby rivers, lakes, and streams. As a result, eutrophic water bodies and hypoxic zones have been created across the globe (Canfield et al., 2010).

Biological Nitrogen Fixation

Estimates of biological N fixation range from 63×10^6 to 175×10^6 tonnes N per year (Lindstrom, 2011). Symbiotic N fixation with legume crops make up approximately 30% of this amount (Lindstrom, 2011). Some legume crops can annually contribute 55 to 140 kg N ha^{-1} , which can potentially be used for a following crop (Lindstrom, 2011). Non-symbiotic N fixation also takes place within soils via free-living organisms. Some examples of these non-symbiotic N fixers are cyanobacteria (*Anabaena*) and some heterotrophic bacteria. Non-symbiotic bacteria of importance are *Azotobacter*, *Beijerinckia*, *Azospirillum*, and *Chlostridium*. Each of these organisms use nitrogenase to catalyze the reduction of N_2 to NH_4^+ (Keuter et al., 2014). *Oryza sativa* L. (rice) production in Asia, responsible for feeding over half of the world's population, depends on cyanobacterial N fixation (Irissari et al., 2001). Free living cyanobacteria contribute an average of 20 to 30 kg N ha^{-1} per year (Vaishampayan et al., 2001).

Cycling of Organic Matter

Decaying crop residue and organic matter (OM) can also contribute additional N, especially in legume crops with low carbon to nitrogen (C:N) ratios (Li et al., 2013). As crop residue decays, mineralization of organic N to NH_4^+ can occur (Turmel et al., 2015). One study evaluated N mineralization rates from two cover crops, crimson clover

(*Trifolium incarnatum* L) and cereal rye (*Secale cereal* L.), in a conservation tillage system to which cotton (*Gossypium hirsutum* L.) had been cropped. Average soil N mineralization rates following crimson clover and rye were 0.58 kg and 0.34 kg N ha⁻¹ day⁻¹ in 1997, and 0.58 kg and 0.23 kg N ha⁻¹ day⁻¹ in 1998 (Schomberg and Endale, 2004). Cumulative soil N mineralized from this system ranged from 60 kg ha⁻¹ to 80 kg ha⁻¹ following crimson clover, and 30 kg ha⁻¹ to 50 kg ha⁻¹ following rye (Schomberg and Endale, 2004). Other estimates suggest additions to soil organic N from crop residues are 16 to 80 kg N ha⁻¹, on average (Yamoah et al., 1998). The amount of soil N mineralized is affected by temperature, pH, rate of O₂ replenishment, available water, amount and quality of plant residues, and level of other nutrients (Stanford and Smith, 1972). Other work suggests that tillage type has an effect on soil N mineralization, noting that conventional tillage can increase N mineralization rates, as compared to no-tillage (Franzluebbers et al., 1995; Aulakh et al., 1991).

Losses of Nitrogen

Ammonium Fixation

Ammonium fixation is the entrapment of NH₄⁺ in 2:1 clay minerals such as mica, smectite, and vermicullite (Barton and Karathanasis, 2002). When NH₄⁺ is applied, it can be bound in these 2:1 clay interlayers, where it is delayed in being used by the plant. This is especially common in soils having higher ammonium fixation capacities. This can also be influenced by the amount of K⁺ in these soils. Fertilizer additions of K⁺ can decrease NH₄⁺ fixation by blocking the interlayers and promoting the release of NH₄⁺ ions (Scherer et al., 2014). This process is dependent on K⁺ concentrations and fertilizer

additions of K^+ to these soils (Scherer et al., 2014). Applications of K^+ before NH_4^+ resulted in decreased NH_4^+ fixation, because the interlayer is then saturated with K^+ (Scherer et al., 2014). Other work confirmed these findings as well (Beauchamp, 1982). Typically, less than 10% of applied NH_4^+ is fixed (Drury and Beauchamp, 1991).

Nitrate Leaching

Leaching is a major pathway of loss from the N cycle, particularly in sandy soils. An end-point of microbial conversion is NO_3^- , an anion that can be readily leached from the soil profile. This results in a loss of input for producers, and possible environmental problems due to NO_3^- contamination in groundwater resources (Addiscott, 1996; Cameron et al., 1997; Spalding and Exner, 1993). Leaching is a very important aspect to consider when managing N, because the longer a fertilizer is present in a soil, the greater the chance it can be leached and unavailable for plant uptake (Addiscott, 1996). Nitrate contributions to groundwater resources have been shown to cause health problems such as methemoglobinemia (Golden and Leifert, 1999). Other studies suggest that NO_3^- in drinking water may cause stomach cancer or childhood diabetes (Addiscott, 1996). Thus, the World Health Organization (WHO) has placed standards on nitrate concentrations in drinking water to help combat these problems. These concentrations are limited to $10 \text{ mg } NO_3^- \text{-N L}^{-1}$ (World Health Organization, 1984). Nitrogen management is of utmost importance in order to protect groundwater and drinking water resources. Split applications can help to reduce the amount of $NO_3^- \text{-N}$ lost every year (Di et al., 1998). Nitrogen scavenging cover crops, like rye, can also prevent NO_3^- leaching (Dabney et al., 2001). Nitrate lost to the environment has been estimated at 49 kg and $107 \text{ kg N ha}^{-1} \text{ yr}^{-1}$

in grassland systems and conventional cropping systems (Di et al., 1998; Bjorneberg et al., 1996).

Plant Uptake

Nitrogen is temporarily removed from the soil N cycle via plant uptake. Both NH_4^+ and NO_3^- are plant available N forms, and are readily taken up and assimilated for plant use. The amount of N taken up by plants can be affected by root architecture, activity of NH_4^+ and transporters (proteins functioning in N transport), diurnal fluctuations, and temperature fluctuations (Xu et al., 2012). Nitrogen use efficiency has most recently been defined as the yield achieved per unit of available N within the soil (Hirel et al., 2011; Good et al., 2004). Some estimates suggest that 50-70% of applied N is not used by the plant and therefore lost to the environment (Hirel et al., 2011). It has been estimated that 30-40% of applied N is actually utilized by crops (Raun and Johnson, 1999). Amounts of N removed with grain have been estimated at 30% of applied N in cereal production (Raun and Johnson, 1999). Increases of NUE by only 1% could save around \$1 billion per year (Hirel et al., 2011).

Volatilization

Volatilization is another major pathway of loss from the N cycle (Hargrove, 1988; Ma et al., 2010; Frame et al., 2013). In this process NH_4^+ is converted to $\text{NH}_3(\text{g})$, which is lost to the atmosphere. This hydrolysis reaction is catalyzed by the soil bound enzyme urease, resulting in the conversion of carbamate (CH_2NO_2) to NH_4^+ , which later decomposes into bicarbonate (HCO_3^-) and ammonium NH_4^+ (Ciurli et al., 1999).

Bicarbamate can increase soil pH, converting NH_4^+ to $\text{NH}_3(\text{g})$ (Ciurli et al., 1999; Krajewska, 2009; Mobley and Hausinger, 1989).

Volatilization Factors

Volatilization can occur in both acidic and alkaline soils, and is influenced by a multitude of factors, including: 1) soil type, 2) pH, 3) soil organic matter (SOM), 4) buffering capacity, 5) temperature, 6) CEC, and 7) moisture. High soil pH, accumulation of SOM, and the presence of crop residue on the soil surface can lead to increased urease activity within soils (Hargrove, 1988; Terman, 1980). The percentage of applied N subject to volatilization is also dependent upon the N source. Urea based fertilizers, such as urea and urea-ammonium nitrate (UAN) are the most susceptible to volatilization (Pan et al., 2016). This is mainly due to the hydrolysis reaction that occurs when urea based fertilizers are applied, and the urease enzyme's activity in this reaction, promoting volatilization (Pan et al., 2016). The usage of non-urea based fertilizers such as ammonium sulfate (AS) and ammonium nitrate (AN) have shown to have lower volatilization rates compared to urea based fertilizers (Pan et al., 2016). Manure and NH_3 based fertilizers are also prone to losses from volatilization as well (Hansen et al., 1989; Somner and Christensen, 1992). One study showed volatilization losses up to 33% from poultry litter applications (Hansen et al., 1989). This is probably due to the large concentration of N within poultry litter (60-70% approximately) being uric acid or urea based (Shuler et al., 1979). Anhydrous ammonia (AA) applications have had volatilization losses of up to 50% dependent upon soil moisture levels (Somner and

Christensen, 1992). Losses of AA applications can occur as a function of injector type, depth, speed, and furrow width (Hanna et al., 2005). Use of knife injectors greatly reduce NH_3 loss compared to disc injectors (Hanna et al., 2005). This is a result of the AA being applied too shallow or failure to fully close the furrow behind the injection rig, therefore rendering AA to atmospheric volatilization.

Winter surface applications of urea to fields with snow are common in large, no-tillage wheat systems across the Northern Great Plains. When applications were made to moist soils or those soils having a high-water content, greater than 30% of surface applied N was lost (Engel et al., 2011). This study noted that producers have long used soil temperature as a guideline for urea application. However, results from this work showed the importance of soil moisture when surface-applying urea. Other work also showed high NH_3 volatilization losses where soil moisture was high at the time of application (Ni et al., 2014).

Soils with a high clay content have been shown to decrease volatilization losses, while those with a high sand content contribute to volatilization (Francisco et al., 2011). The NH_4^+ ion is more easily retained on the soil colloids of clayey soils as compared to sandy soils. This is due to the surface area and charge on these soil colloids (Francisco et al., 2011). Soils with high CEC have been shown to retain more NH_4^+ compared to those with a lower CEC, therefore leading to reduced rates of volatilization. One study using a soil with a CEC of $6.7 \text{ cmolc kg}^{-1}$, showed NH_3 volatilization losses of up to 50.9%, when compared to that from a soil with a CEC of $12.3 \text{ cmolc kg}^{-1}$, which had NH_3 losses of 18.0% (Keller and Mengel, 1986).

Soils containing higher organic C also have higher urease enzyme levels, due to greater microbial activity (Jones, 2007). The presence of OM was shown to increase volatilization losses in a study using wheat straw mulching (Francisco et al., 2011). When urea was surface applied, higher NH_3 losses due to volatilization were observed in trials with increased OM on the surface (Francisco et al., 2011).

Soil pH is another factor which affects ammonia volatilization. High soil pH favors the conversion of NH_4^+ to NH_3 (Ernst and Massey, 1960). This was confirmed in additional research in the Northern Great Plains, where soils with a pH of 8.4 had volatilization losses of up to 44.1% of applied N, as compared to 31.3% and 35.6% of applied N at two sites with a soil pH of 5.5, and 39.9% of applied N on a soil with a pH of 6.4 (Engel et al., 2011).

Increased wind speeds can also promote volatilization. This study observed that higher temperatures favored NH_3 volatilization, and that these same losses increased linearly with increased wind speeds (Watkins et al., 1972). Percent N lost as NH_3 was higher across all treatments at 18°C compared to that at 7°C (Watkins et al., 1972). These results could be attributed to the fact that increased wind speeds promoted drying conditions, therefore increasing volatilization. Others showed that a temperature increase from 20°C to 30°C increased NH_3 volatilization (Fan et al., 2011).

Rainfall following urea applications can be one of the most important aspects in controlling volatilization. The timing of rainfall is important in its effects on NH_3 volatilization. Rainfall events immediately following application can reduce and then enhance volatilization activity due to drying effects (Ma et al., 2010). Rainfall events at the middle and latter period of the volatilization process have shown some ability to

reduce losses (Ma et al., 2010). Adequate precipitation events following urea application decreased volatilization losses to less than 10% of N applied in some trials (Engel et al., 2011). Adequate rainfall following urea applications moves the fertilizer down into the soil profile where it is not directly exposed to the atmosphere and less subject to volatilization.

The method of fertilizer application can reduce volatilization as well, and banded and injected applications have been shown to reduce volatilization and boost yields compared to broadcast surface applications (Howard and Tyler, 1989). Injecting N increased N availability and decreased the potential for volatilization or immobilization from organic residues (Howard and Tyler, 1989; Mengel et al., 1982; Touchton and Hargrove, 1982). Still other work showed that injected N applications increased yields as compared to broadcast surface applications (Vetsch and Randall, 2000). Unlike broadcasted surface applications, banded and injected applications are able to apply fertilizers below the soil surface. Therefore, these subsurface applications are not readily volatilized to the atmosphere (Howard and Tyler, 1989).

Inhibiting Losses from the Nitrogen Cycle

There have been many efforts to reduce N loss through the use of various inhibitors. Both nitrification and volatilization (urease) inhibitors are commercially available, and there is a wide range of active ingredients and product names. In general, the intent is to slow the conversion of NH_4^+ to NO_3^- , or to reduce the loss of NH_4^+ to the air as NH_3 . Inhibitors can help to reduce the loss of applied N by delaying volatilization and nitrification processes. Specifically, these active ingredients are designed to block

certain enzymes and bacteria that promote volatilization or nitrification, leading to the delayed transformation of NH_4^+ to volatile NH_3 , and NH_4^+ to water soluble NO_3^- (Grant et al., 1996; Blakeley and Zerner, 1984; Chen et al., 2010; Hatch et al., 2005).

Volatilization (urease) inhibitors delay the conversion of NH_4^+ to volatile $\text{NH}_3(\text{g})$ by blocking the urease enzyme which catalyzes this hydrolysis reaction (Grant et al., 1996; Watson et al., 1994; Antisari et al., 1996). Delaying this reaction gives producers more time to receive additional rainfall, irrigate, or incorporate the urea, therefore moving the fertilizer down into the soil profile where it is not as susceptible to volatilization. If urea can dissolve and move away from the site of the application, the potential for volatilization is reduced. Use of these inhibitors in minimal or conservation tillage systems is especially common, as applied fertilizers are not incorporated (Grant et al., 1996).

Nitrification inhibitors slow the conversion of NH_4^+ to NO_3^- . The microbial oxidation of NH_4^+ to NO_2^- and eventually NO_2^- to NO_3^- is delayed by reducing the activity of *Nitrosomonas*. Soil N is more readily retained on the soil colloid when it is in the NH_4^+ form, as compared to NO_3^- . The emission of N_2O has also shown to be decreased when controlling nitrification (Chen et al., 2010 ; Hatch et al., 2005).

Urease Inhibitors

There are many different types of urease inhibitors. Multiple phosphoramidate compounds have been evaluated for urease inhibition such as: 1) N-(n-butyl) thiophosphoric triamide (NBPT), 2) N-(n-propyl) thiophosphoric triamide (NPPT), 3) phenylphosphorodiamidate (PPD), 4) thiophosphoryl triamide (TPT) , and 5) maleic-

itaconic copolymer (MIP) (Upadhyay, 2012; Franzen et al., 2011). These compounds inhibit urease by forming a chelated complex with nickel within the active site of the enzyme, therefore rendering the enzyme inactive (Blakeley and Zerner, 1984). The mode of action of other urease inhibitors is not as specific as that of the phosphoramidate compounds (Blakeley and Zerner, 1984). Other compounds such as thiourea (TU), methyl urea (MU), and ammonium thiosulfate (ATS) can serve as urease inhibitors as well (Roberts et al., 2016). However, these compounds have not been extensively studied like the phosphoramidates, nor do they have the same mode of action.

NBPT- treated urea has been shown to decrease volatilization in several field and laboratory studies. In one large scale field study, 12 field experiments were conducted across 8 different sites over 2 years. Sites contained both no-till and tilled wheat systems. Ammonia volatilization was determined from urea and NBPT-coated treatments (Engel et al., 2011). Plots fertilized with NBPT-coated urea reduced NH_3 volatilization losses across all trials, with an average of 6.9% loss of applied N, as compared to a 66% average NH_3 loss from urea treatments (Engel et al., 2011).

Other work with NBPT-treated urea was conducted with a series of six, 14-day laboratory trials, with materials applied to bare soil. Phosphoric acid was used to capture NH_3 losses. Ammonia volatilization losses ranged from 32-35% of applied N, with greatest volatilization losses measured at 12 to 24 hours following urea application. Urea coated with NBPT delayed NH_3 volatilization in all trials (Frame et al., 2012). Other work confirmed that use of NBPT delayed NH_3 volatilization (Dawar et al., 2011). Still other work, which used 3 different rates of NBPT at (0.05, 0.10, and 0.15%) found that NH_3 volatilization losses were reduced at all three rates of NBPT application (Rawluk et

al., 2001). This study also confirmed previous research that sandy soils had higher NH_3 losses than that from clay soils (Rawluk et al., 2001). A field study that focused on the effects of NBPT-coated urea in *Zea mays* L. (corn) N used 4 different N rates (56, 112, 168, and 225 kg N ha⁻¹) and found that use of NBPT increased corn ear leaf N concentrations in half of the trials, while mixed results were found with grain yields (Frame et al., 2013). A wheat and corn rotation in China used a new urease inhibitor from BASF® called Limus 2.0, which consists of both NBPT and N-(n-propyl) thiophosphoric triamide (NPPT), (75% NBPT and 25% NPPT), to help reduce NH_3 volatilization losses after urea application. Application of this NBPT/NPPT mixture reduced NH_3 volatilization losses to 0-6% of applied N, as compared to NH_3 losses of up to 25% from plots fertilized with only urea (Li et al., 2015).

Other phosphoroamide based compounds, phenylphosphorodiamidate (PPD) and thiophosphoryl triamide (TPT), have been used as urease inhibitors as well. A 1986 corn study used PPD and TPT to evaluate NH_3 volatilization losses, grain yield, and tissue N content from surface and band applied N applications. Application of granular urea resulted in higher grain yield and tissue N content, when compared to banded UAN solution. When urea prills were coated with the urease inhibitors PPD and TPT, grain yield and N content increased as compared to urea-only (Schlegel et al., 1986). It was concluded that when environmental conditions favored NH_3 volatilization, TPT was a better urease inhibitor than PPD. However, TPT has been shown to be a poor inhibitor in flooded soils (Keerthisinghe and Freney, 1994).

Some urease inhibitors are used in flooded rice fields to reduce NH_3 volatilization. A study in Thailand used PPD, NBPT and a combination of both PPD and NBPT to

examine NH_3 losses from rice paddies. It was found that the mixture of PPD and NBPT performed better than either of the two products alone. In the PPD + NBPT treatment, NH_3 losses were reduced to a fifth of that measured from the separate treatments, and grain yield increased from 3.6 to 4.1 tons ha^{-1} (Phongpan et al., 1995). Others have similarly shown the effectiveness of PPD for reducing NH_3 volatilization, as compared to untreated urea (Qui-Xiang et al., 1994).

One commercially available product called Nutrisphere[®] contains a partial calcium salt of maleic-itaconic copolymer (MIP), which is a purported urease inhibitor. However, Nutrisphere[®] is marketed as both a urease and nitrification inhibitor. Use of MIP was examined across multiple locations in a 2011 study. This experiment had eight field studies in North Dakota in a winter wheat or red wheat crop, three rice field studies on Mississippi and Arkansas, four lab experiments in North Dakota, and one lab experiment in Arkansas. Results indicated that MIP had no volatilization or nitrification inhibiting characteristics in the laboratory or field setting. Additionally, no benefit was observed when the compound was applied to spring wheat or rice in North Dakota, Arkansas or Mississippi (Franzen et al., 2011). In a 2012 study, MIP did not inhibit urea hydrolysis in three different soils (Goos, 2013). Other reports showed that MIP did not increase N efficiency in corn (*Zea mays* L.) or winter wheat (Cahill et al., 2010). These results are consistent with other findings that MIP is ineffective in inhibiting urea hydrolysis (Franzen et al., 2011; Conell et al., 2011; Goos, 2013).

Other N sources are also used to combat volatilization losses, such as AS (Hayashi et al., 2010). One study observed no NH_3 volatilization where AS was applied even though soil NH_4^+ -N content increased (Hayashi et al., 2009). Lower pH soil levels

favors the presence of NH_4^+ in the liquid phase, as compared to aqueous NH_3 , resulting in reduced NH_3 volatilization (Hayashi et al., 2009). However, use of AS in alkaline soils can promote volatilization (Schwenke et al., 2014). Increased NH_3 volatilization losses when AS was applied to a calcareous soil containing $10 \text{ g } 100\text{g}^{-1} \text{ CaCO}_3$, as compared to 60% less NH_3 volatilization from other grass paddocks containing lower concentrations of CaCO_3 ($2 \text{ g } 100\text{g}^{-1} \text{ CaCO}_3$) (Schwenke et al., 2014).

Thiosulfates can be used as urease inhibitors to reduce ammonia volatilization in urea or UAN applications. Urea containing ATS has been shown to reduce NH_3 volatilization losses up to 11% as compared to UAN-only (Sloan and Anderson, 1995). However, not all results were similar, as some work showed no yield response when ATS was added to UAN (Stecker et al., 1993; Tucker and Mengel, 2006). Conversely, corn yield increased when ATS was added to UAN, in all three years of study (Graziano and Parente, 1996). Other types of urea, such as TU, may also reduce NH_3 volatilization and greenhouse gas emissions. When 47 experiments conducted over various years were analyzed for greenhouse gas emissions, it was concluded that use of TU reduced NH_3 losses (Sylvester-Bradley et al., 2014).

Factors of Nitrification

Nitrification inhibitors can be used to block this conversion of NH_4 to NO_2 for a short period, to prevent greater nitrification losses. By delaying bacterial processes, the goal is to keep more soil N in the ammonium form in order to decrease losses to gaseous emissions, runoff, and leaching. Some studies have shown that nitrification inhibitors can delay this process for several weeks (Weiske et al., 2001; Pasda et al., 2001).

One study in a wheat and corn rotation used two different nitrification inhibitors, dicyandiamide (DCD) and 3,4-dimethylpyrazol phosphate (DMPP), to examine effects on N₂O emissions. Treatments containing DCD and DMPP reduced N₂O emissions by 35% and 38% annually compared to urea-only treatments (Liu et al., 2013). This same study noted an increase of soil inorganic N by 21% and 22% and increased soil NH₄⁺-N concentrations, when compared to urea-only treatments (Liu et al., 2013). Treatments with DCD and DMPP increased crop yield, dissolved organic carbon content (DOC), aboveground plant biomass, and N uptake compared to urea-only treatments. Crop yield and aboveground plant biomass for the DCD and DMPP treatments were 8.5-9.1% and 8.6-9.7% higher as compared to crops fertilized with the urea-only treatment (Liu et al., 2013).

Nitrification Inhibitors

Dicyandiamide (DCD) is one of the most widely used nitrification inhibitors in production agriculture today. Research has shown that it can slow nitrification and increase N content in forages, therefore allowing for both increased dry matter yields and stocking rates on winter wheat (Rao and Popham, 1999). Use of DCD also helps to reduce N₂O contributions to the atmosphere (Jumadi et al., 2008). A Chinese corn operation noted profits up to \$109.49 ha⁻¹ yr⁻¹ when using DCD (Yang et al, 2016). The ability of DCD to slow nitrification and increase NH₄⁺ concentration in soil was confirmed in other research (Liu et al., 2014). A study evaluating 3,4- dimethylpyrazole phosphate (DMPP) in both mineral and organic fertilizers found that DMPP was beneficial for inhibiting nitrification (up to 84.5%) (Florio et al., 2016). In this same

study, DMPP to reduce bacterial populations in soil (Florio et al., 2016). Nitrification was reduced by 78.3% and 84.5% after 2 and 4 weeks in this study, where temperatures reached up to 30° C (Florio et al., 2016). Another study in an Indonesian corn field compared DCD-urea to plain urea, measuring N₂O emissions and populations of nitrifying bacteria. The use of urea + DCD reduced N₂O emissions by 55.8%, and the soil became a methane (CH₄) sink. Populations of nitrifying bacteria were correlated with N₂O emissions throughout this experiment (Jumadi et al., 2008).

Another compound, 3,4- dimethylpyrazole phosphate has also been shown to reduce N₂O emissions in white clover (*Trifolium repens* L.), with no detrimental effects to earthworms (Kong et al., 2017).

Nitrapyrin is another widely used nitrification inhibitor, with multiple commercially available products available for purchase. One study used nitrapyrin to evaluate nitrification rates at elevated soil temperatures. The results showed that nitrapyrin delayed nitrification therefore keeping soil N predominately in the NH₄⁺ form, while reducing nitrification rates at temperatures up to 40°C (Fisk et al., 2015). Nitrapyrin has been noted to have a lower greenhouse gas potential, even compared to DCD, another effective nitrification inhibitor (Zhang et al., 2015).

Urease and nitrification inhibitors can be combined to reduce the losses of applied N in agricultural operations as well. Some work has shown that urea amended with NBPT and DCD increased yields and lowered N₂O emission by 37.7% (Ding et al., 2011). However, other studies suggest that combined applications of NBPT + DCD result in increased volatilization, when compared to the use of urease or nitrifications applied alone with urea. This is because there is more NH₄⁺ to be volatilized. Volatilization losses

were increased in a study when urea + NBPT + DCD was applied, compared to urea + NBPT alone (Clay et al., 1990). Still other work confirmed negative effects when using NBPT and DCD together, as compared to when inhibitors were not combined (Kawakami et al., 2013).

Adequate rainfall following fertilization is another factor to consider when applying nitrification inhibitors. One study in conventional and strip-tilled cotton fertilized with urea+ DCD had increased yields when rainfall was normal, but reduced yields when rainfall was below average, as compared to cotton yield from plots fertilized with only urea (Gordon et al., 1990).

Work with volatilization inhibitors has largely neglected the combined impact of the inhibitors use in soils with significant crop residue left as cover. Since there is evidence that accumulated organic C and crop residue can increase losses of N via volatilization, the use of inhibitors should be a topic of interest. Additionally, work with newer volatilization inhibitors that use combinations of NBPT materials is also needed. These issues are the focus of this research.

Research Objective

Continued emphasis on reduced and conservational tillage practices has created crop production systems where organic residues accumulate on the surface. This may directly affect N losses via volatilization differently from that of conventional tilled systems. As new volatilization inhibitors enter the market continued research with these products is needed, especially in the high-residue, low tillage cultivation systems of the southeastern United States. Thus, the objective of this project was to examine NH₃ loss

from cropping systems as affected by crop residue cover and presence of volatilization inhibitor.

II: COVER CROP EFFECTS ON UREASE INHIBITORS

Methods and Materials

Research was conducted as a series of multiple laboratory studies, using soil from two different locations. All experiments were repeated multiple times. Soil for the studies was collected from the Old Rotation (Auburn, AL) and the E.V. Smith Research Center, Field Crops Unit (Shorter, AL). Specific details for all experiments are provided below.

Soil Core Collection

Using a standard golf cup cutter (Par Aide Products Company, 6800 Otter Lake Road, Lino Lakes, MN. 55038-9946), intact soil cores 15-cm in diameter and 4 cm deep were collected from selected plots of the Old Rotation (Pacolet fine sandy loam [fine, kaolinitic, thermic, Typic Kanhapludult]), with cover residue treatments of either none (continuous cotton since 1896) or winter cover (since 1896, winter cover of either hairy vetch (*Vicia villosa* Roth) or crimson clover (*Trifolium incarnatum* L.). From 1896 till 1996, these winter cover treatments were grown for green manures and conventionally tilled for the potential N benefit in a continuous cotton (*Gossypium hirsutum* L.) rotation. Since 1997, plots with cover have been treated strictly as cover crops, where they are chemically terminated then strip tilled prior to planting.

For the second experiment the same sized cores were collected from a 20-year old tillage experiment located at the E.V. Smith Research Center, Shorter, AL (Compass loamy sand [coarse-loamy, siliceous, thermic, Plinthic Paleudults]) with residue treatments of either none or cereal rye (*Secale cereale* L.), to which a rotation of cotton

(*Gossypium hirsutum* L.) and soybeans (*Glycine max*) had been cropped. These plots at E.V. Smith received different tillage treatments dependent upon cover. Plots that received a winter cover crop were strip tilled prior to planting, where those with no winter cover crop were conventionally tilled. In order to best obtain uniformity of soil moisture content soil cores were collected within 2 days after rainfall events. However, there were differences in soil moisture at the time of sample collection (Table 4). Additional information about soil characteristics is provided on the following pages.

Ammonia Volatilization Experiment

Each experiment was a factorial arrangement of 4 N + urease inhibitors and 2 surface residue states (none or residue present). There were four replications of every treatment. All experiments were conducted for 7 days, and repeated at least twice. The N/urease inhibitor treatments were: 1) untreated urea (no inhibitor), 2) urea + maleic-itaconic copolymer (urea + MIP), 3) urea + N-(n-butyl) thiophosphoric triamide (urea + NBPT), 4) urea + N-(n-butyl) thiophosphoric triamide and N-(n-propyl) thiophosphoric triamide (urea + NBPT/NPPT). All products used were applied at an N rate of 112 kg ha⁻¹ to the surface of the harvested cores, with no additional water added after fertilizer application.

The volatilization system consisted of a series of 16 2-L glass canning jars, all attached to an air source via a 16-outlet manifold (Figure 1). With a total of 16 jars in each run, the jars were utilized two times to fully include the 32 treatment/rep of each experiment. Previous work with zero-N and reference urea controls have shown excellent data reproducibility from run to run, and thus empty jars and bare soil control treatments

were not included in these studies. Preliminary work demonstrated that soil-only and empty jars had NH₃ emissions of zero. All experiments were replicated in time and space.

Soil cores were placed in each jar, fertilizer/inhibitors added, and the jars sealed to begin each experiment. Ammonia loss was measured for 7 consecutive days and collected via an NH₃ trap system, following the method of O'Halloran (1993) (Figure 1). Air flow was generated by passing 100 mL min⁻¹ air stream through a 5N sulfuric acid air scrubber and across each jar, with resultant NH₃ trapped in 100 mL of 0.01 N boric acid. The boric acid trap was changed every day for 7 days, with collected samples titrated to the original pH of the boric acid using 0.01 N sulfuric acid. Mass and percent of volatilized NH₃ were calculated using the formulas below:

Equation 1: mg-N = (mL H₂SO₄ acid*Normality of H₂SO₄ acid*14 u), where 14 is the equivalent atomic weight (u) of N.

Equation 2: % N volatilized = (total mg-N for each treatment/104) *100, where 104 is the mg-N per jar.

Statistical Analyses

Analysis of variance (ANOVA) was conducted using a general linear model (GLM) function (PROC GLM) in SAS version 9.4 (SAS Institute Inc.) where data was analyzed by day. Data was first analyzed with 'Experiment Number' as a variable, to determine if data could be pooled over runs of the experiment. If 'Experiment Number' was not significant, the data was pooled over runs and reanalyzed by day. If 'Experiment Number' was significant, the data was not pooled by experiment, and results were analyzed separately. Cumulative NH₃ loss was determined by summing all NH₃ collected

over the 7 days. This cumulative loss was also analyzed via ANOVA ($P < 0.10$) to determine the presence of a significant interaction between inhibitor and cover. The effects of N source, crop residue, and the interaction of N source and crop residue were tested in the GLM model to examine differences in N volatilization among treatments. Duncan's Multiple Range Test (DMRT) was also used to compare differences between the means of both factorial groups, N source and crop residue, at $\alpha=0.05$.

Results and Discussion

Ammonia loss was analyzed each sampling day, to determine if a significant interaction was present. Initial ANOVA revealed no statistical difference between experiment runs conducted with soil from the Old Rotation, when experiments were included in the model ($P < 0.10$). However, when soil from the E.V. Smith location was used, the ANOVA revealed a significant difference between experiment runs. Analyses also revealed significant interactions between the cover crop and inhibitor at almost every sampling day for soil from the Old Rotation, and on 3 days for soil from the E.V. Smith location (Table 1). Because this cover by inhibitor interaction was more often significant than not, results are discussed by experiment, and often by day.

Soil from the Old Rotation

Data shown in Table 1 indicates that the interaction of cover and inhibitor was significant at every sampling day. Thus, results are shown as a table of interactions (Table 2). Results illustrate NH_3 loss as affected by the interaction of cover and inhibitor for the two experiments using the Old Rotation soil. When a cover crop was present, any inhibitor that contained NBPT significantly reduced NH_3 loss in the first two days, as compared to untreated urea and urea + MIP treatments. Overall greatest loss of NH_3 was

on Day 2, with an average loss of 16.4% of applied N in the urea/urea + MIP treatments, as compared to 0.9% in the treatments of urea + NBPT/ urea + NBPT/NPPT. Such substantial differences were not present in treatments which had no history of winter cover. In those treatments overall ammonia loss, by day, was less, and often not significantly affected by the presence of a urease inhibitor.

The presence of 100+ years of differences in OM tended to increase NH₃ loss via volatilization. This was especially true in the first three days of data collection, and especially for untreated urea and urea + MIP. For example, Day 2 plots that had never had cover had significantly less NH₃ loss as compared to those with cover. The only exception was in the urea + NBPT/NPPT treatment, where there was no difference due to cover, and % N volatilized was less than 0.5%, respectively (Table 2).

Significant cover by inhibitor interaction was probably due to the large difference in total C and OM concentrations between cover crop treatments at this site. Table 3 presents background data from soil collected at the Old Rotation. The winter cover treatments on these plots were established in 1896. As a result, total C in plots with long-term winter cover was nearly 3 times greater than that from plots without a winter cover crop. Organic matter concentrations from plots that receive no winter cover crop is 35% of those that do receive a winter cover crop (Table 3). Soil moisture was also always higher on average in soils that received a cover crop (Table 4).

These results were consistent with those found by Hargrove, (1988), who proposed that urease activity is increased when crop residue is present on the soil surface. Crop residues may also form a physical barrier between the N source and the soil surface (Hargrove, 1988). Still other work showed that the presence of organic crop residues

increased NH_3 volatilization from soil surfaces to which urea was applied (Francisco et al., 2011; Rochette et al., 2009). Our results were also consistent with previous work, which showed applications to a moist soil can lead to greater N loss via volatilization (Engel et al., 2011). Drying of the soil surface can also be slowed, leading to higher volatilization rates for a longer period of time (McInnes et al., 1986).

Cumulative NH_3 loss (summed over time) from the Old Rotation soil is shown in Figure 2. Greatest NH_3 loss was from soil with untreated urea applied to soil with a cover crop; a total of 40% of applied N was lost to the atmosphere. This is similar to total amounts measured by others, who found losses of 20-40% of applied N, respectively, over a similar time period (Francisco et al., 2011). Following that was the urea + MIP treatment (with cover), with approximately 30% cumulative N loss. Reductions in NH_3 loss from the use of MIP material are somewhat rare, as the majority of previous work saw no ability for this product to reduce NH_3 loss (Franzen et al., 2011). Although this work did see a reduction as compared to non-amended urea (46 versus 30% cumulative losses) loss was still significantly higher than found in treatments which received NBPT or NBPT/NPPT (cumulative losses of 20 and 8%, with crop residue, respectively) (Figure 2).

The presence of NBPT reduced NH_3 volatilization when a cover crop was present, when compared to urea without an inhibitor (Figure 3). The addition of MIP did reduce volatilization when a cover crop was present, but when no cover crop was present, MIP was ineffective in reducing loss compared to untreated urea. These results are consistent with others that showed MIP did not significantly reduce NH_3 volatilization as compared to untreated urea, but the use of NBPT as a urease inhibitor did significantly reduce

losses when compared to untreated urea (Sunderlage and Cook, 2018). Additionally, the combination of NBPT and NPPT reduced volatilization to a greater degree (5% of N applied). Others have found similar results, measuring reductions of 0-6% of applied N when this dual inhibitor was used (Li et al., 2015).

Inhibitor Performance

Average losses occurring on days 1-3 from urea + NBPT and urea +NBPT/NPPT were significantly lower compared to those from urea and urea + MIP (Table 2, Figure 2). After day 3, average NH₃ losses were less affected by the presence of an inhibitor. This was most likely due to the inhibitor being degraded and no longer able to compete with the substrate (urea), leading to increased volatilization rates in the following days (Frame et al., 2012). These results agree with other studies that urea coated with NBPT can delay, but not eliminate NH₃ volatilization (Frame et al., 2012 ; Dawar et al., 2011). Previous work showed that urea coated with NBPT delayed NH₃ loss from 0 to 96 hours, but treatments containing NBPT and untreated urea had similar levels of NH₃ loss by 312 hours (Frame et al., 2012). Still other work showed that volatilization was delayed up to 7 days in treatments containing NBPT (Dawar et al., 2011). Our work showed a significant delay for the 7-day period (Figure 2).

Ammonia losses from soil that had over 100 years of winter cover were often greater than that from treatments in which cover was never present (Figure 2). Most treatments that did not have cover had significantly less volatilization than those that did, regardless of the presence of an inhibitor. Similar research showed 10-20% reduction of NH₃ volatilization in treatments where no cover was added to the soil surface (Francisco et al., 2011). The only treatment with cover in which NH₃ losses were reduced to the

point of those without cover was in the urea + NBPT/NPPT treatment, with cumulative losses of 9%, over the 7-day period.

When no inhibitors were present, NH₃ loss in the urea fertilized plots with cover was a total of 44%, as compared to 13% loss in plots with no cover (urea only fertilized) (Figure 3). In these treatments, the addition of cover resulted in much higher volatilization. Other work showed 10-20% reduction of NH₃ volatilization in treatments where no cover was added to the soil surface (Francisco et al., 2011). This was probably due to the greater presence of the urease enzyme (Hargrove, 1988), and the fact that no inhibitor was present. Since the greatest losses (with maximum at Day 2), from treatments with untreated urea occurred over the first 4 days of these experiments, it is clear that volatilization was not delayed, and that the urea application was subject to urea hydrolysis as soon as it was applied. These results are consistent with other findings which observed high rates of NH₃ volatilization from treatments with untreated urea over the first 96 hours (4 days) (Frame et al., 2012). Differences in soil moisture between these two cover crop treatments also explain response to cover at this location (Table 4). Similar work also showed increased volatilization losses in soils with higher soil moisture contents as compared to lower soil moisture contents (Engel et al., 2011).

Soil from E. V. Smith Research Center

In contrast to experiments with the Old Rotation soil, ‘Experiment Number’ was significant when the E.V. Smith soil was used. Thus, all discussion that follows is for each separate experiment. For Experiment 1, the interaction of cover and inhibitor was significant on 3 sampling days ($P < 0.10$), and it was never significant for Experiment 2. In Experiment 3, the interaction was significant on 2 sampling days (Table 2). Given the

relative rarity in which the interaction was highly significant most discussion will center on main effects, but Tables of interaction (5-7) are provided for reference.

Unlike results from experiments with the Old Rotation soil, the lack of a cover crop typically did not result in lower levels of NH_3 loss (Tables 5, 6, and 7). While the cover crop effect was still significant across many sampling days in this experiment, the response was not as great as that observed at the Old Rotation. For example, cover had no effect on NH_3 volatilization, regardless of inhibitor, in Days 1, 3, 5, or 6 of Experiment 1. The only days in which cover did significantly affect volatilization it was on Day 2 and 4, and this occurred because soil with NBPT/NPPT had less volatilization without cover than when cover was present. In Experiment 2, the presence of cover never affected volatilization, except in two instances when soil with no cover that had received urea + MIP (Days 2 and 5) had ranging NH_3 losses (greater loss in Day 2, less in Day 5) (Table 6). In Experiment 3, results similar to both Experiments 1 and 2 were found. In Days 3 and 4, soil to which NBPT/NPPT had been added reduced volatilization when no cover was present, when compared to NBPT/NPPT in soil with cover (Table 7). Also, the addition of MIP reduced volatilization in soil that had no history of cover cropping in Days 1 and 2. This was most likely due to a smaller range in total C and OM concentrations between cover crop treatments (Table 3). In Experiment 1, cumulative losses of applied N from plots that received a winter cover crop were 34.0% for untreated urea, 34.1% for urea + MIP, 21.3% for urea + NBPT, and 23.1% for urea + NBPT/NPPT. Samples that did not receive a winter cover crop had cumulative N losses of 36.4% for untreated urea, 36.7% for urea + MIP, 26.8% for urea + NBPT, and 19.1% for urea + NBPT/NPPT (Figure 4). Even though cumulative losses were sometimes higher from

plots that did not receive a winter cover crop, statistical analyses indicated a significant cover crop effect across 5 out of 7 sampling days for Experiment 1 ($P < 0.10$) (Table 1). These differences may be due to variation in soil moisture during sample collection (Table 4). Data listed in Table 4 shows increased soil moisture in Experiment 1- Run 1 as compared to Experiment 1 – Run 2. These differences could explain why treatments that did not receive a cover crop sometimes resulted in greater volatilization losses, and the presence of NBPT as a urease inhibitor had no effect on cumulative N loss (Figure 4).

The second Experiment had significant inhibitor source effects in 6 out of 7 sampling days ($P < 0.10$) (Table 1). However, there were no significant effects from the cover crop treatments, and no significant cover by inhibitor interaction occurred throughout this entire experiment (Table 1). Cumulative N losses when no winter cover crop was present were 29.2% for untreated urea, 28.1% for urea + MIP, 17.3% for urea + NBPT, and 9.2% for urea + NBPT/NPPT. The addition of a cover crop resulted in cumulative N losses of 23.7% for untreated urea, 22.9% for urea + MIP, 15.1% for urea + NBPT, and 11.4% for urea + NBPT/NPPT (Figure 5). Losses are shown throughout this 7-day experiment in Table 6. While the effects of residue were not significant, inhibitor source was still significant with losses being lower from treatments containing the urease inhibitor NBPT and NBPT/NPPT in Days 1 and 2 (Table 6). Except for Day 1 when a cover crop was present, applied N losses were greatest in the first 4 days for treatments where the urease inhibitor NBPT was not present (Figure 5). This was due to NBPT delaying volatilization (Figure 5). Regardless of the presence of a cover crop, NBPT delayed volatilization losses in this experiment. Treatments containing NBPT/NPPT still significantly reduced cumulative volatilization losses as compared to untreated urea.

However, urea + NBPT did not significantly reduce cumulative losses in this experiment. One reason for this might be that soil moisture was much more comparable between runs and cover crop treatments, which may explain why there was no statistical difference in NH_3 loss due to the presence of a cover crop or the urea + NBPT treatment.

Results from the last experiment at E.V. Smith Research center showed a significant inhibitor source and cover effect on 2 out of 7 sampling days ($P < 0.10$) (Table 1). Average losses were also greatest during the first 4 days of this experiment in treatments where NBPT was not present, as compared to treatments containing NBPT (Table 7). Figure 6 further illustrates how losses occurred over the 7-day experiment. Cumulative N losses when no cover crop was present were 23.0% for untreated urea, 28.1% for urea + MIP, 6.9% for urea + NBPT, and 7.2% for urea + NBPT/NPPT. Treatments containing a winter cover crop showed cumulative N losses of 26.1% for untreated urea, 25.4% for urea + MIP, 20.3% for urea + NBPT, and 13.9% for urea + NBPT/NPPT (Figure 6). This differs from other work that showed 10 to 20% differences from untreated urea where cover was present, as compared to when it was not present (Francisco et al., 2011).

When compared to results from Old Rotation soil, N loss due to the presence of a cover crop was not as different from soil with no history of a cover crop. The addition of a cover crop often did not result in higher N loss via volatilization. This may be due to the fact that soil from plots receiving winter cover were did not accumulate as much OM like those at the Old Rotation. Plots at E.V. Smith that did not receive a winter cover crop are still conventionally tilled, which may have suppressed the effects of our cover treatments when using soil from this location. Differences in background soil parameters

from these two sites are further illustrated in Table 3. Differences in soil moisture between cover crop treatments from E.V. Smith were also more comparable than soil from the Old Rotation. This could be a result of varying soil types. Soil from E.V. Smith was sandier as compared to soil from the Old Rotation, which could have led to better infiltration of precipitation, regardless of the cover crop treatment. Some research also suggests that sandier soils are more prone to volatilization as compared to clayey soils (Rawluk et al., 2001). It could also be possible that soil from the Old Rotation retained the NH_4^+ ion better than soils from E.V. Smith due its CEC (Table 3), and that the presence of a cover crop and increased soil moisture between cover treatments exploited that retention, leading to increased volatilization.

Inhibitor Performance

The effects of inhibitor source at this location were similar to those observed at the Old Rotation. Average losses occurring on days 1-3 from urea + NBPT and urea +NBPT/NPPT were significantly lower as compared to those from urea and urea + MIP from the first two experiments (Tables 5 and 6). Cumulative losses from the 7 day experiments are shown in Figures 7, 8, and 9. In the third experiment, NH_3 loss from soil treated with NBPT was reduced, but by Day 3 there was no significant difference between the urea + NBPT inhibitor treatment and the control. The urease inhibitor containing NBPT/NPPT still remained effective until Day 4. Some research suggests that combining the inhibitors NBPT and NPPT is an effective way to reduce volatilization, and that products containing this formulation may provide a benefit (Li et al., 2015). After Day 3, the urease inhibitor NBPT no longer delayed volatilization, and N losses from these treatments were greater than or equal to those of the untreated urea and urea +

MIP. The addition of MIP to urea significantly reduced losses compared to untreated urea on one sampling date across all three experiments at E.V. Smith. However, cumulative losses from urea + MIP were not significantly different across all three experiments. This is consistent with other findings, which suggests MIP is ineffective in inhibiting urea hydrolysis (Connell et al., 2011; Franzen et al., 2011; Goos, 2013).

Cumulative losses from inhibitors containing NBPT/NPPT were significantly lower in experiments 2 and 3 as compared to untreated urea, but the addition of NBPT did not always significantly reduce cumulative losses (Figures 8 and 9). Use of NBPT still was effective in delaying volatilization. These results are supported by many other studies which found NBPT delayed volatilization losses as compared to untreated urea (Dawar et al., 2011; Engel et al., 2011; Frame et al., 2012).

Conclusion

Ammonia volatilization data collected from Old Rotation soil always had a significant interaction between the inhibitor and cover crop. This was because NH_3 loss via volatilization was far greater when the soil was from plots with a history of cover cropping. In this case, only the NBPT/NPPT inhibitor reduced volatilization. Our results could be attributed to many factors which are: 1) long term establishment of these plots receiving winter cover (100+ years), 2) conservational tillage for over 20 years, and 3) increased soil moisture in samples containing a cover crop as compared to those without. The presence of a cover crop in soil collected from E. V. Smith had far less of an impact on volatilization. The plots at E.V. Smith were part of a 20-year tillage experiment where both strip tillage and conventional tillage occurred. This could have resulted in better incorporation of OM and the urease enzyme leading to a suppressed effect of the cover

crop treatment at the E.V. Smith location. Previous work using surface applications on no-tilled and conventionally tilled soils found that lower volatilization rates occurred on conventionally tilled soils (Rochette et al., 2009).

Different soil types could also have been responsible for mixed effects between locations. Some research suggests that soil type is a better indicator for N loss via volatilization as compared to other background soil parameters (Rawluk et al., 2001; Francisco et al., 2011). Despite best efforts, soil moisture was not always uniform between samples after soil core collection. However, this is to be expected and is a main effect of the presence of cover, which should be measured in situ. Across both locations and all experiments completed, the inhibitor source was still highly significant over the 7-day incubation periods. Inhibitor treatments containing NBPT and or NBPT/NPPT delayed volatilization compared to untreated urea in almost every experiment. Similar work has shown that NBPT can delay and reduce volatilization as compared to untreated urea when crop residues are present (Francisco et al., 2011). However, we are unaware of previous studies using these inhibitors on soils in a cover cropping system. Thus, further evaluation of these inhibitors across various soil types, regions, and cover cropping systems is warranted.

The urease inhibitors NBPT or NBPT combined with NPPT could provide some utility in high residue and conservational tillage cropping systems. Producers should consider environmental conditions at the time of application before purchasing a urease inhibitor. Further consideration is also necessary to determine which urease inhibitor to use because the addition of MIP usually did not reduce or delay losses as compared to untreated urea.

Table 1. Analysis of variance for main effects of cover and inhibitor, by day, location, and experiment, laboratory evaluations. Numbers represent the $P > F$.

Old Rotation Soil †							
Days after application							
	1	2	3	4	5	6	7
P > F							
Inhibitor	0.01	<0.0001	<0.0001	0.01	0.22	0.47	0.03
Cover	0.0003	<0.0001	<0.0001	0.03	0.03	0.004	0.005
Cover x Inhibitor	0.01	<0.0001	0.0002	0.07	0.0003	<0.0001	<0.0001
E.V. Smith Soil							
Experiment 1							
Inhibitor	0.001	<0.0001	<0.0001	0.02	<0.0001	<0.0001	<0.0001
Cover	0.01	0.02	0.49	0.70	0.08	0.005	0.0008
Cover x Inhibitor	0.17	0.16	0.11	0.08	0.09	0.46	0.02
Experiment 2							
Inhibitor	0.002	<0.0001	<0.0001	0.008	0.15	0.005	<0.0001
Cover	0.43	0.30	0.26	0.44	0.96	0.41	0.68
Cover x Inhibitor	0.91	0.15	0.31	0.14	0.21	0.90	0.95
Experiment 3							
Inhibitor	0.002	<0.0001	0.0002	0.20	0.25	0.21	0.34
Cover	0.05	<0.0001	0.07	0.35	0.53	0.33	0.50
Cover x Inhibitor	0.40	0.02	0.19	0.01	0.36	0.34	0.49

† initial analyses with ‘Experiment Number’ indicated no significance due to that variable, and so data was pooled ($P < 0.10$).

Table 2. Ammonia loss (percent of N applied) as a function of residue cover and urease inhibitor, Old Rotation soil. Means in columns denoted as ‘Y’ indicate soil with > 100 years of winter cover, while those with a ‘N’ indicate no cover ($\alpha = 0.05$).

Days after application														
	1		2		3		4		5		6		7	
Cover														
	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N
N Source	% N Volatilized													
Untreated Urea	2.0 †Aa‡	0.01 Ab	18.6 Aa	0.4 Ab	12.7 Aa	2.2 Ab	4.8 Aa	4.5 Aa	2.2 Ba	2.8 Aa	1.6 BCa	1.8 ABa	0.8 Ba	1.2 Aa
Urea + MIP	2.0 Aa	0.00 Aa	14.2 Aa	0.2 ABb	8.4 ABa	1.3 ABb	2.8 ABa	3.1 ABa	1.2 Ba	2.5 Aa	0.8 Ca	2.0 Aa	0.5 Ba	1.4 Aa
Urea + NBPT	0.1 Ba	0.00 Ab	1.3 Ba	0.00 Bb	5.1 BCa	0.04 Bb	4.9 Aa	0.03 Bb	4.5 Aa	0.1 Bb	4.0 Aa	0.3 Bb	2.9 Aa	0.5 Ab
Urea + NBPT/NPTT	0.1 Ba	0.00 Aa	0.4 Ba	0.00 Ba	1.3 Ba	0.00 Ba	1.9 Ba	0.2 Bb	2.0 Ba	0.4 ABb	2.6 Ba	0.7 ABb	2.8 Aa	0.9 Ab

37

†: Upper case letters indicate differences due to the inhibitor, and are viewed within each column, by cover.

‡: lower case letters indicate differences due to cover (within each day and inhibitor).

Table 3. Background soil test data for the soil used for the volatilization experiments. All units are mg kg^{-1} except for organic matter (OM) and total N, which are listed in g kg^{-1} . Percent cover was determined by counting residue in a defined area with each core. This is a measure of visible cover on each core, at collection.

	pH †	P ‡	K ‡	Ca ‡	Mg ‡	Total C ¶	OM ¶ (g kg^{-1})	% Cover	CEC cmolc kg^{-1}	Total N ¶ (g kg^{-1})
mg kg^{-1}										
Soil										
Old Rotation										
No Cover	5.9	23	61	234	1551	4300	7	13	4.43	3.2
Hairy Vetch or Crimson Clover	6.2	25	57	315	1608	11400	20	53	5.35	9.1
E.V. Smith										
No Cover	6.3	12	75	48	1194	5775	9.8	16	5.12	6.4
Cereal Rye Cover	6.6	17	70	36	1057	8575	15.0	98	5.57	9.4

†: Soil pH measured in a 1:1 soil:H₂O extract

‡: Mehlich extract

¶: determined via combustion using Elementar Vario Macro CNS Analyzer, 520 Fellowship Road-Suite D-408, Mt. Laurel, NJ. 08054

Table 4. Soil water content (gravimetric) as determined at the beginning and end of each experiment.

Soil Moisture (%)							
Old Rotation Soil				E. V. Smith Soil			
		Experiment Start End				Experiment Start End	
Experiment 1 Run 1	Cover	13.3	12.3	Experiment 1 Run 1	Cover	21.9	7.2
	No Cover	5.6	2.8		No Cover	17.2	2.4
Experiment 1 Run 2	Cover	10.1	5.7	Experiment 1 Run 2	Cover	7.5	2.1
	No Cover	2.0	2.0		No Cover	3.0	3.1
Experiment 2 Run 1	Cover	8.2	7.3	Experiment 2 Run 1	Cover	2.8	1.6
	No Cover	7.5	1.6		No Cover	3.5	2.2
Experiment 2 Run 2	Cover	14.4	11.7	Experiment 2 Run 2	Cover	4.3	3.0
	No Cover	7.9	3.8		No Cover	3.8	1.7
				Experiment 3 Run 1	Cover	6.0	5.6
					No Cover	3.4	1.9
				Experiment 3 Run 2	Cover	4.0	2.5
					No Cover	2.1	1.8

Table 5. Ammonia loss (percent of N applied) as a function of residue cover and urease inhibitor, Experiment 1, E. V. Smith soil. Means in columns denoted as ‘Y’ indicate soil with 20 years of cover, while those with a ‘N’ indicate no cover ($\alpha=0.05$).

Days after application														
	1		2		3		4		5		6		7	
Cover														
	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N
N Source	% N Volatilized													
Untreated Urea	0.77 †ABa‡	0.21 Aa	10.65 Aa	5.14 Ba	11.65 Ab	14.98 Aa	6.39 Aa	10.43 Aa	2.45 BCa	3.39 BCa	1.23 Ba	1.4 Ba	0.88 BCa	0.91 Ca
Urea + MIP	0.37 Aa	0.18 Aa	12.71 Aa	9.72 Aa	11.98 Aa	15.98 Aa	5.16 Aa	3.19 Aa	1.42 Ca	2.20 Ca	1.11 Ba	1.12 Ba	0.67 Ca	0.23 Ca
Urea + NBPT	0.03 Ba	0.02 Ba	0.44 Ba	0.20 Ca	4.76 Ba	2.48 Ba	8.01 Aa	7.88 Aa	4.38 Ba	8.03 Aa	2.09 Ba	5.20 Aa	1.59 Ba	3.00 Ba
Urea + NBPT/NPPT	0.08 Ba	0.00 Ba	0.50 Ba	0.04 Cb	2.10 Ba	0.21 Ba	5.80 Aa	1.78 Bb	6.75 Aa	5.84 ABa	4.5 Aa	6.35 Aa	3.33 Ab	4.85 Aa

†: Upper case letters indicate differences due to the inhibitor, and are viewed within each column, by cover.

‡: lower case letters indicate differences due to cover (within each day and inhibitor).

Table 6. Ammonia loss (percent of N applied) as a function of residue cover and urease inhibitor, Experiment 2, E. V. Smith soil. Means in columns denoted as ‘Y’ indicate soil with 20 years of cover, while those with a ‘N’ indicate no cover ($\alpha=0.05$).

Days after application														
	1		2		3		4		5		6		7	
Cover														
	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N
N Source	% N Volatilized													
Untreated Urea	0.50 †Aa‡	0.34 Aa	5.06 Aa	4.81 Aa	9.35 Aa	13.58 Aa	4.48 ABa	6.46 Aa	1.97 Aa	2.11 ABa	1.60 Aa	1.19 BCa	0.73 Ba	0.57 Ca
Urea + MIP	0.41 Aa	0.41 Aa	3.78 ABb	7.38 Aa	9.28 Aa	5.54 Aa	4.90 Aa	3.33 ABa	2.24 Aa	0.92 Bb	1.70 Aa	0.72 Ca	0.62 Ba	0.41 Ca
Urea + NBPT	0.10 Aa	0.02 Ba	0.54 BCa	0.13 Ba	3.34 ABa	3.14 Ba	3.39 ABa	6.35 Aa	2.56 Aa	4.53 Aa	3.61 Aa	3.37 Aa	1.50 ABa	1.44 Ba
Urea + NBPT/NPPT	0.06 Aa	0.03 Ba	0.16 Ca	0.13 Ba	0.65 Ba	0.27 Ba	1.99 Ba	1.05 Ba	3.18 Aa	2.28 ABa	3.00 Aa	3.02 ABa	2.45 Aa	2.37 Aa

†: Upper case letters indicate differences due to the inhibitor, and are viewed within each column, by cover.

‡: lower case letters indicate differences due to cover (within each day and inhibitor).

Table 7. Ammonia loss (percent of N applied) as a function of residue cover and urease inhibitor, Experiment 3, E. V. Smith soil. Means in columns denoted as ‘Y’ indicate soil with 20 years of cover, while those with a ‘N’ indicate no cover ($\alpha = 0.05$).

Days after application														
	1		2		3		4		5		6		7	
Cover														
	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N
N Source	% N Volatilized													
Untreated Urea	1.18 †Aa‡	0.40 Aa	4.94 Ba	2.78 Aa	6.45 Aa	5.08 ABa	5.91 Aa	6.38 ABa	2.62 Aa	3.79 Aa	1.56 Aa	2.79 Aa	3.47 Aa	1.82 ABa
Urea + MIP	0.92 ABa	0.56 Ab	8.50 Aa	3.58 Ab	7.57 Aa	8.70 Aa	4.09 Aa	8.32 Aa	1.69 Aa	3.44 Aa	1.23 Aa	2.03 Aa	1.41 Aa	1.63 ABa
Urea + NBPT	0.14 ABa	0.01 Ba	2.12 Ca	0.13 Ba	5.91 Aa	1.18 BCa	6.95 Aa	3.32 BCa	2.24 Aa	1.15 Ba	1.31 Aa	0.60 Aa	1.60 Aa	0.52 Ba
Urea + NBPT/NPPT	0.08 Ba	0.01 Ba	0.90 Ca	0.23 Ba	2.38 Ba	0.36 Cb	5.94 Aa	1.24 Cb	1.82 Aa	1.53 ABa	1.50 Aa	1.71 Aa	1.29 Aa	2.09 Aa

†: Upper case letters indicate differences due to the inhibitor, and are viewed within each column, by cover.

‡: lower case letters indicate differences due to cover (within each day and inhibitor).

Figure 1. Diagram of the laboratory system used to measure ammonia volatilization, with jars, pump, and air scrubbers, following the procedure of O'Halloran (1993). The glass manifold is connected to an opening in each jar with silicon tubing.

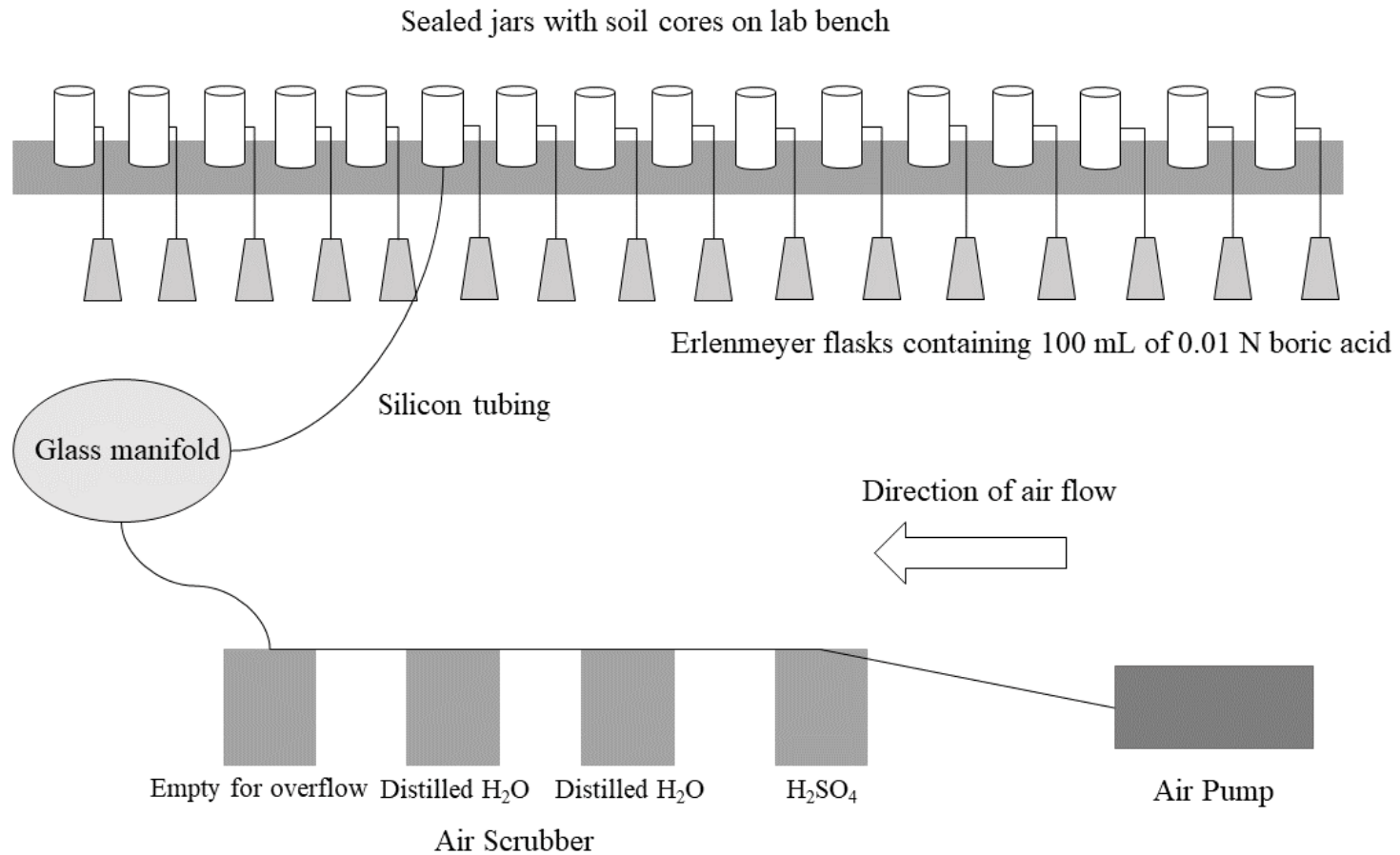


Figure 2. Cumulative N loss from soil from the Old Rotation (Auburn, AL.) as affected by inhibitor source. Losses are plotted from urea, urea with calcium salt of maleic-itaconic copolymer (Urea + MIP), urea with N-(n-butyl) thiophosphoric triamide (urea +NBPT), and urea with N-(n-butyl) thiophosphoric triamide and N-(n-propyl) thiophosphoric triamide (urea + NBPT/NPPT) across a 7-day experiment. Error bars indicate standard error about the mean.

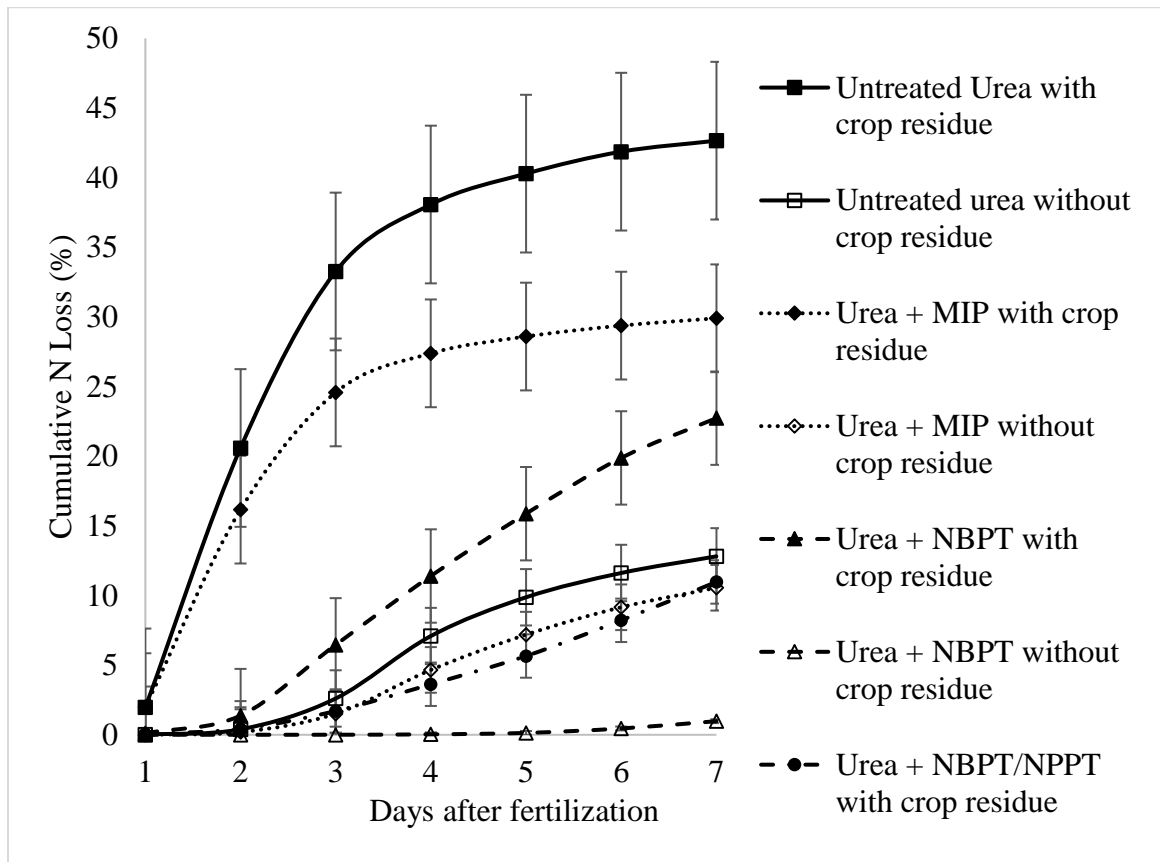


Figure 3. Cumulative N loss from Old Rotation soil as affected by inhibitor source and a cover. Losses are plotted from urea, urea with calcium salt of maleic-itaconic copolymer (Urea + MIP), urea with N-(n-butyl) thiophosphoric triamide (urea +NBPT), and urea with N-(n-butyl) thiophosphoric triamide and N-(n-propyl) thiophosphoric triamide (urea + NBPT/NPPT) across a 7-day experiment. Letters in each column represent significance ($\alpha = 0.05$) due to each treatment.

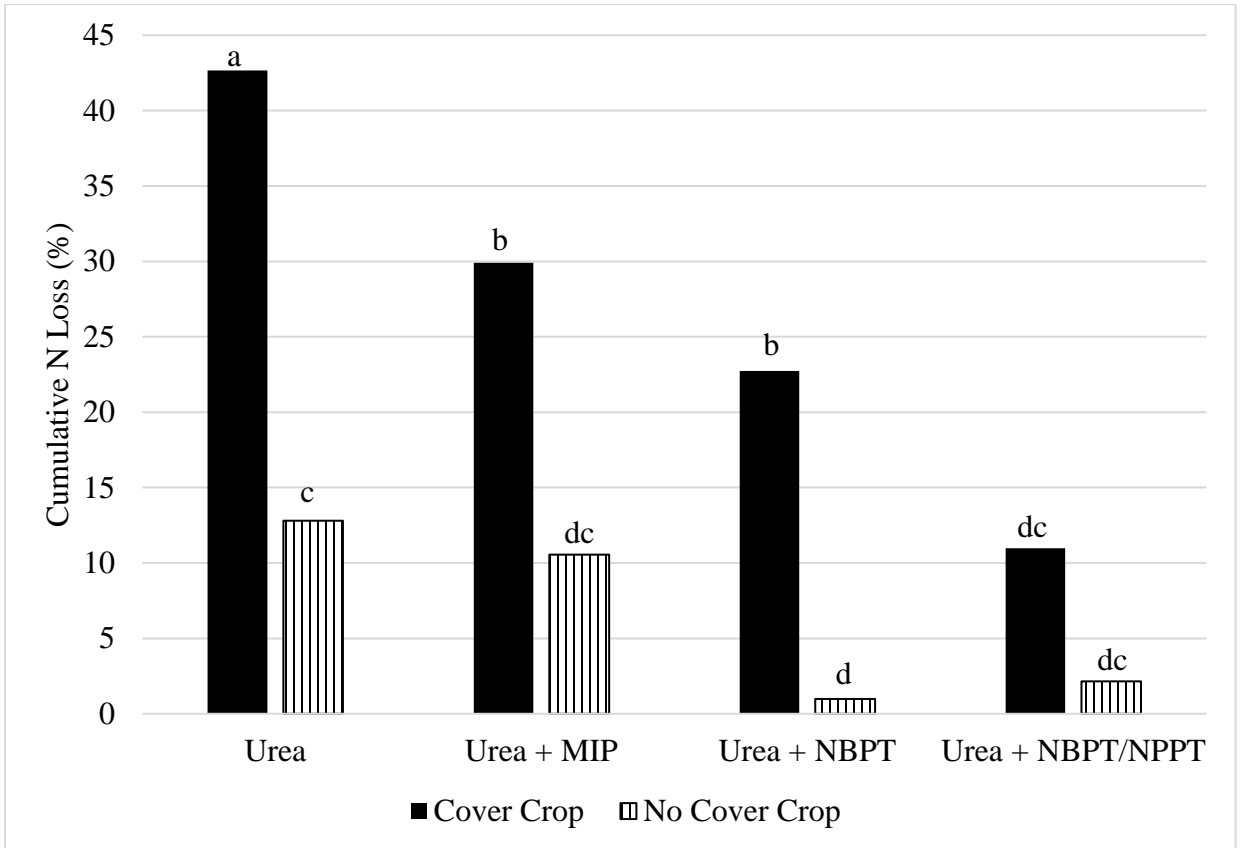


Figure 4. Cumulative N loss as affected by the addition of a cover crop, as compared to no winter cover crop during the first experiment with soil from E.V. Smith Research Center (Shorter, AL.) Losses are plotted from urea, urea with calcium salt of maleic-itaconic copolymer (Urea + MIP), urea with N-(n-butyl) thiophosphoric triamide (urea +NBPT), and urea with N-(n-butyl) thiophosphoric triamide and N-(n-propyl) thiophosphoric triamide (urea + NBPT/NPPT) across a 7 day experiment. Error bars indicate the standard error about the mean.

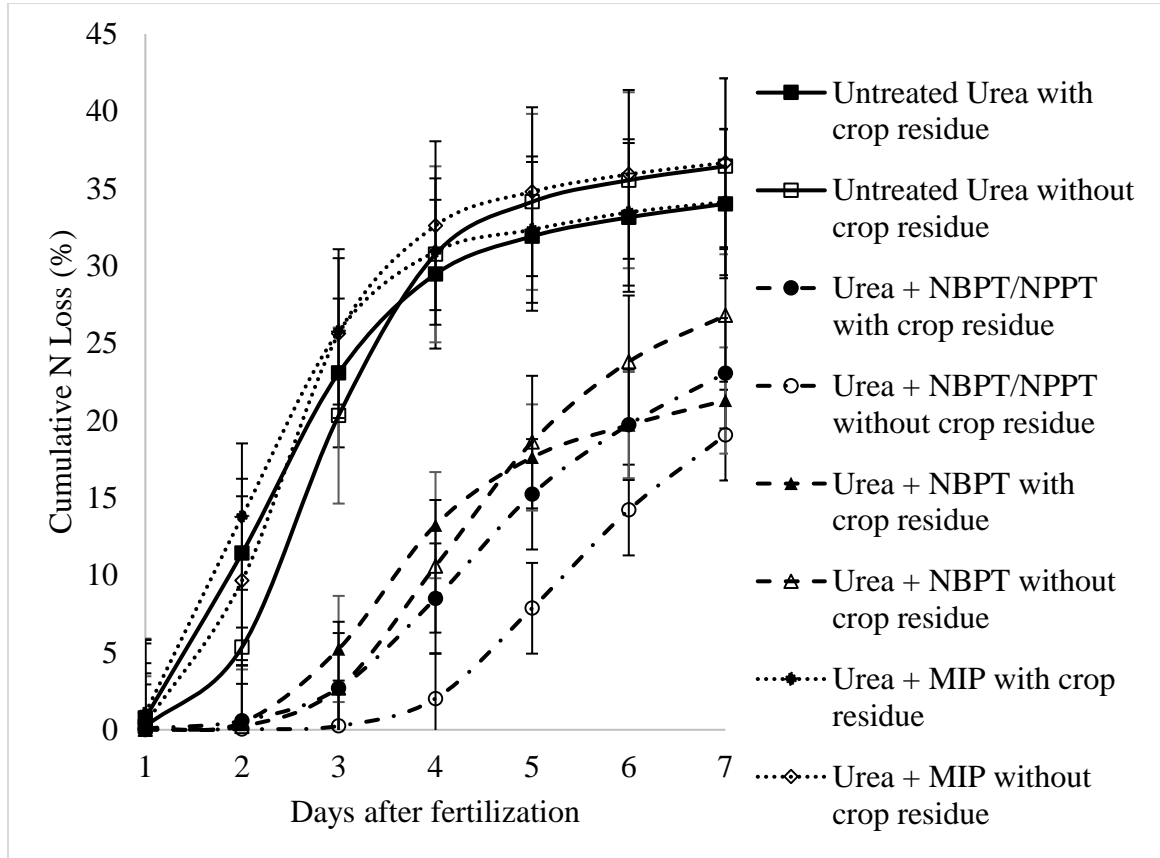


Figure 5. Cumulative N loss as affected by the addition of a cover crop, as compared to no winter cover crop during the second experiment with soil from E.V. Smith Research Center (Shorter, AL.) Losses are plotted from urea, urea with calcium salt of maleic-itaconic copolymer (Urea + MIP), urea with N-(n-butyl) thiophosphoric triamide (urea +NBPT), and urea with N-(n-butyl) thiophosphoric triamide and N-(n-propyl) thiophosphoric triamide (urea + NBPT/NPPT) across a 7 day experiment. Error bars indicate the standard error about the mean.

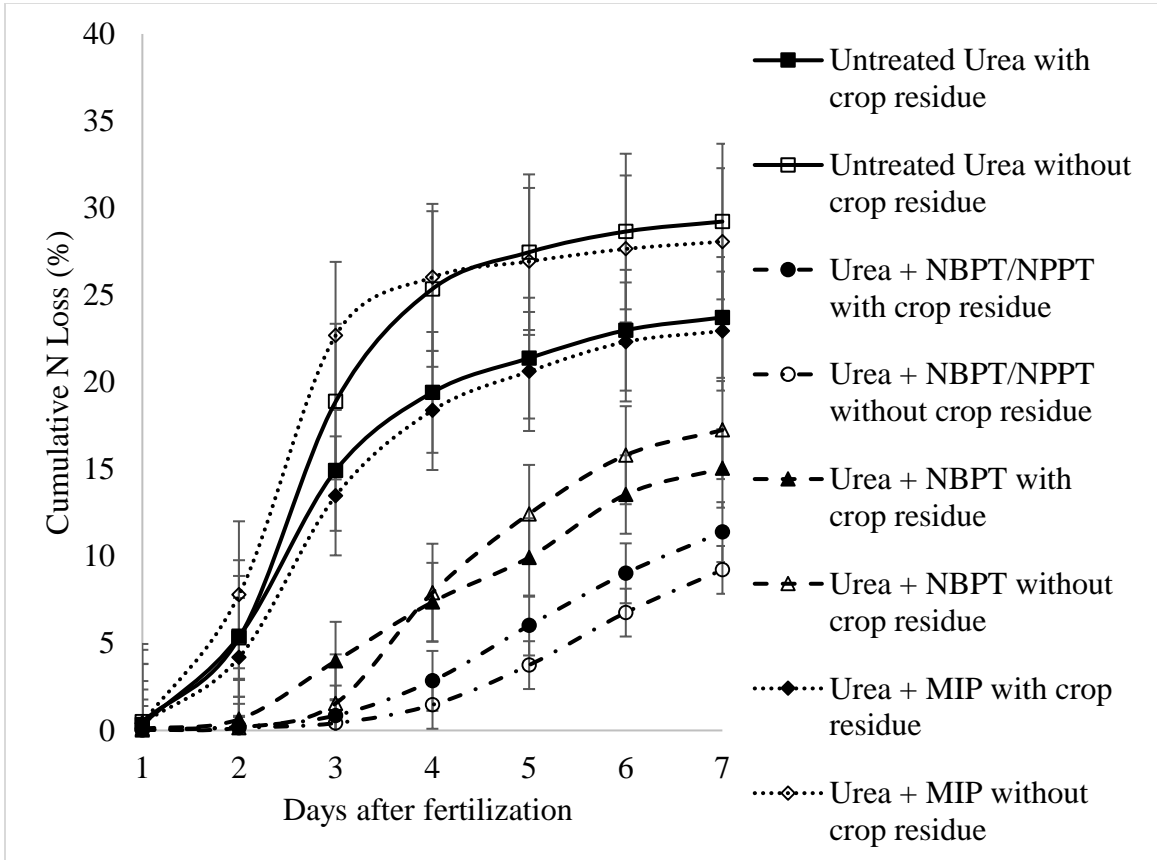


Figure 6. Cumulative N loss as affected by the addition of a cover crop, as compared to no winter cover crop during the third experiment with soil from E.V. Smith Research Center (Shorter, AL.) Losses are plotted from urea, urea with calcium salt of maleic-itaconic copolymer (Urea + MIP), urea with N-(n-butyl) thiophosphoric triamide (urea +NBPT), and urea with N-(n-butyl) thiophosphoric triamide and N-(n-propyl) thiophosphoric triamide (urea + NBPT/NPPT) across a 7 day experiment. Error bars indicate the standard error about the mean.

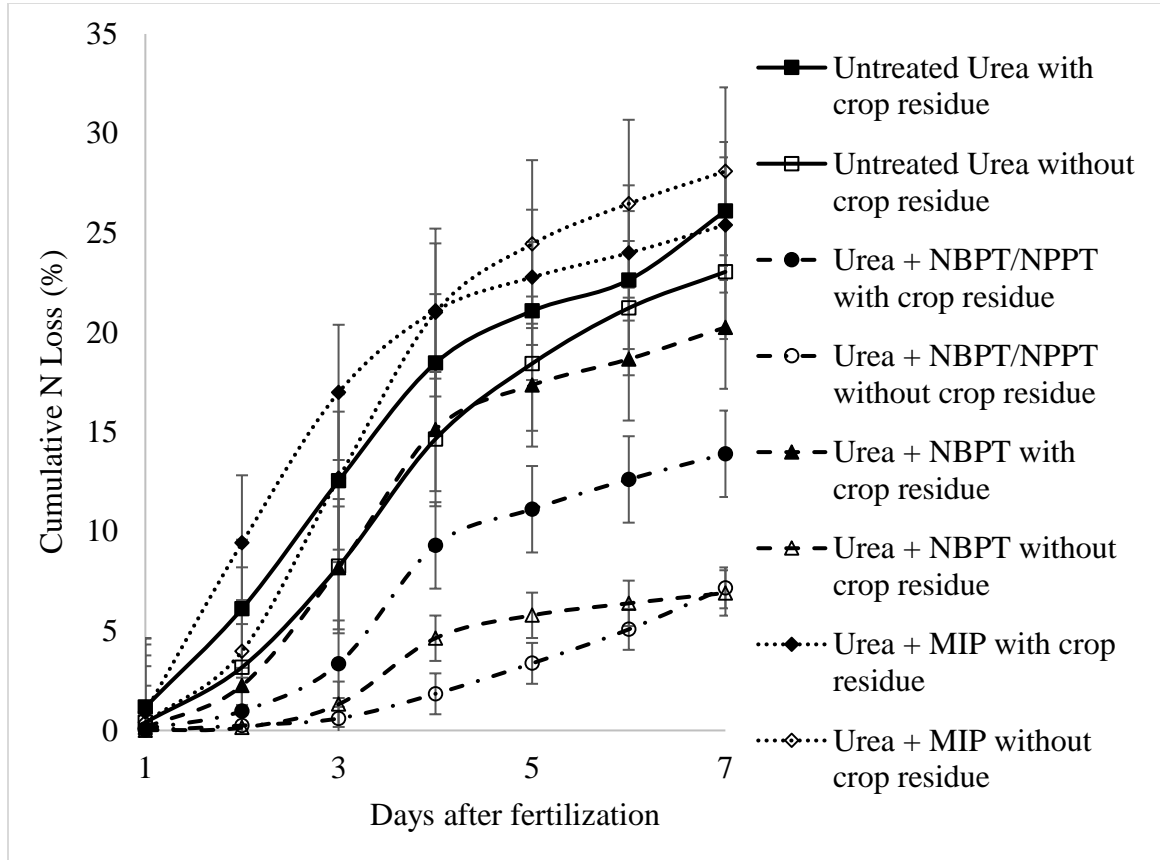


Figure 7. Cumulative N loss from the first experiment with soil from E. V. Smith Research Center (Shorter, AL.) as affected by inhibitor source and a cover. Losses are plotted from urea, urea with calcium salt of maleic-itaconic copolymer (Urea + MIP), urea with N-(n-butyl) thiophosphoric triamide (urea +NBPT), and urea with N-(n-butyl) thiophosphoric triamide and N-(n-propyl) thiophosphoric triamide (urea + NBPT/NPPT) across a 7-day experiment. Letters in each column represent significance ($\alpha = 0.05$) due to each treatment.

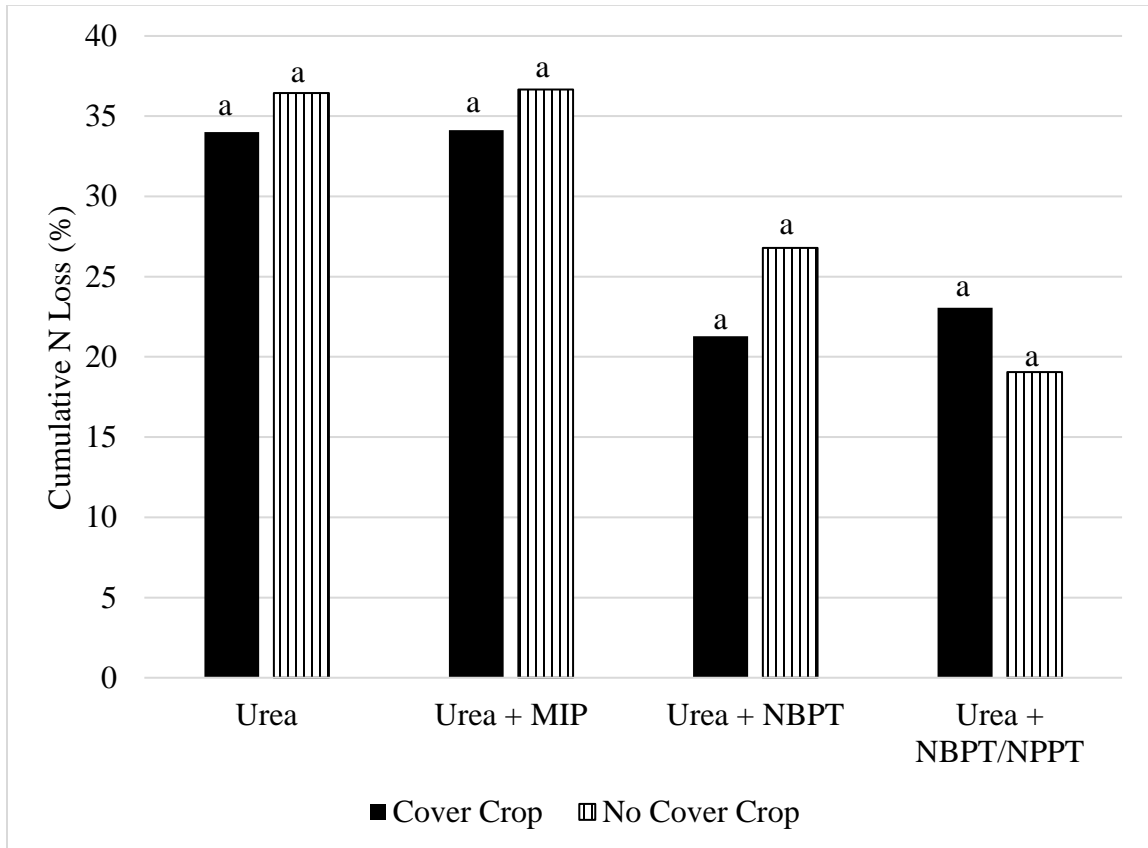


Figure 8. Cumulative N loss from the second experiment with soil from E. V. Smith Research Center (Shorter, AL.) as affected by inhibitor source and a cover. Losses are plotted from urea, urea with calcium salt of maleic-itaconic copolymer (Urea + MIP), urea with N-(n-butyl) thiophosphoric triamide (urea +NBPT), and urea with N-(n-butyl) thiophosphoric triamide and N-(n-propyl) thiophosphoric triamide (urea + NBPT/NPPT) across a 7-day experiment. Letters in each column represent significance ($\alpha = 0.05$) due to each treatment.

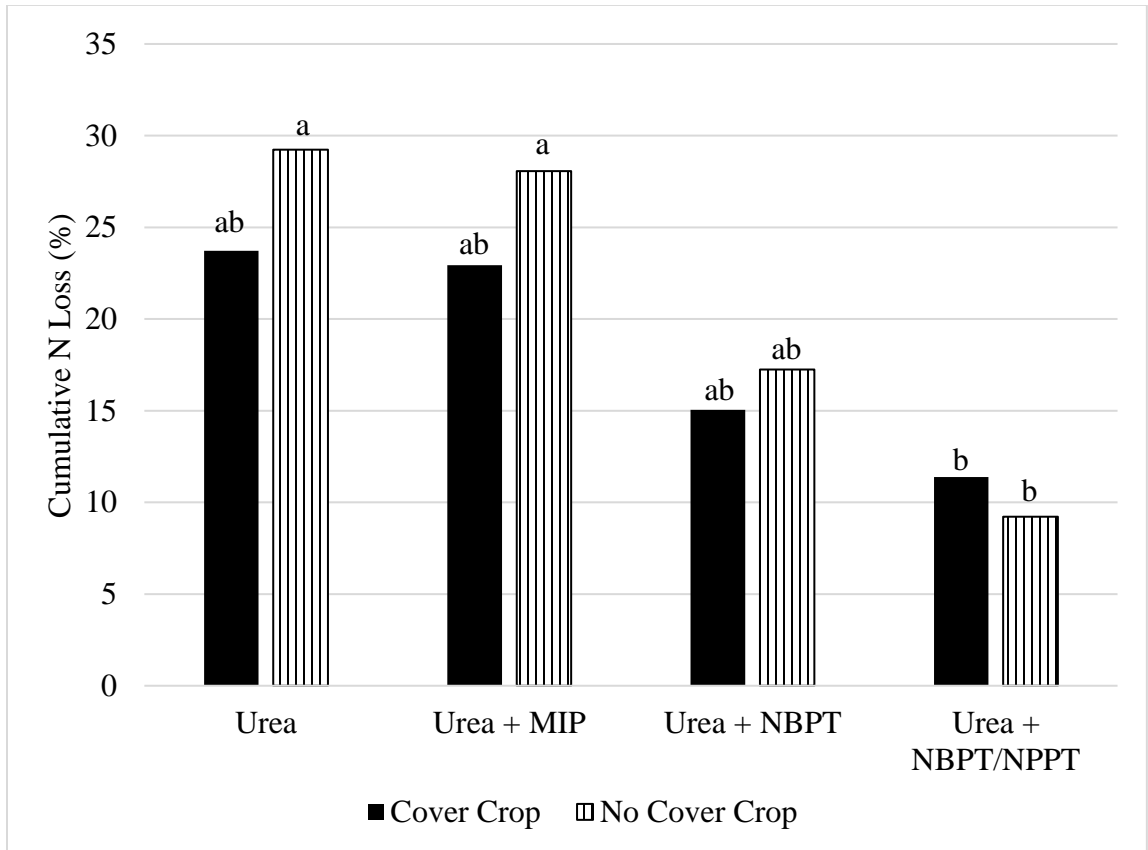
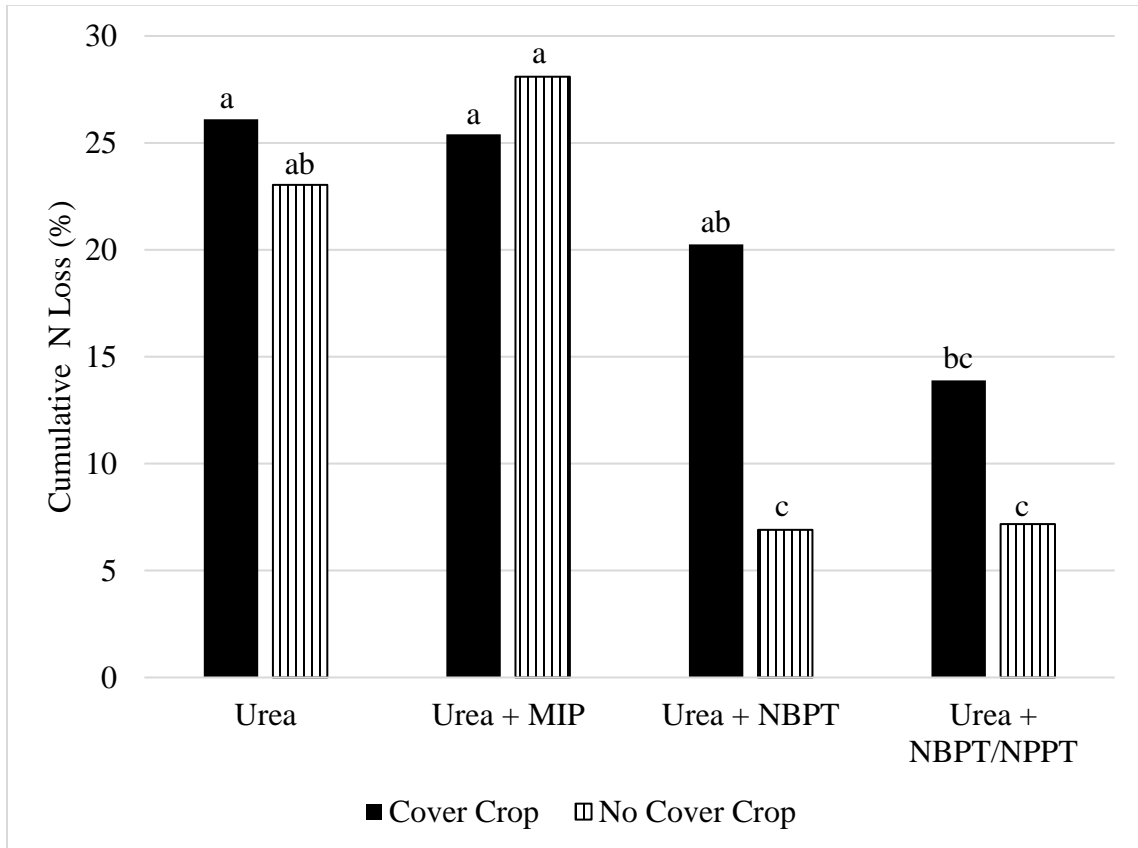


Figure 9. Cumulative N loss from the third experiment with soil from E. V. Smith Research Center (Shorter, AL.) as affected by inhibitor source and a cover. Losses are plotted from urea, urea with calcium salt of maleic-itaconic copolymer (Urea + MIP), urea with N-(n-butyl) thiophosphoric triamide (urea + NBPT), and urea with N-(n-butyl) thiophosphoric triamide and N-(n-propyl) thiophosphoric triamide (urea + NBPT/NPPT) across a 7-day experiment. Letters in each column represent significance ($\alpha = 0.05$) due to each treatment.



III. SUMMARY

This study was an examination of the effects of cover crops on urease inhibitors. Results from this laboratory study indicate that the inclusion of winter cover crops may have some effects on N loss via volatilization, and that urea applications can be subject to greater volatilization losses in soils with a long history of cover cropping. Volatilization was often delayed at least 2 days following fertilizer application in inhibitor treatments containing NBPT, and losses in these treatments were significantly lower than those from untreated urea on most sampling days. Therefore, the usage of NBPT as a urease inhibitor may provide some utility in these high residue, minimal cultivation systems. These results warrant further investigation to examine how various urease inhibitors function across various soil types, climates, and cover cropping systems. Lastly, direct measurement of urease activity in these cropping systems is warranted.

IV. LITERATURE CITED

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