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Evaluation of Enhanced Efficiency Nitrogen Fertilizers on Corn Production Systems in the Mid-South

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EVALUATION OF ENHANCED EFFICIENCY NITROGEN FERITLIZERS ON CORN PRODUCTION SYSTEMS IN THE MID-SOUTH

A Thesis

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Abstract

Nitrogen (N) is often the most yield limiting nutrient, particularly in corn (*Zea mays* L.*)* production systems. In the Mid-South, high N application rates have the potential to lead to high N loss. To minimize this loss, proper N management should focus on improving N use efficiency (NUE) while optimizing productivity. The potential to achieve both tasks can be met using enhanced efficiency N fertilizer (EENF). However, limited research has directly compared the active chemicals in EENFs for corn production in the Mid-South. A study was conducted in 2013 and 2014 at two locations in Louisiana to determine the effectiveness of EENFs on yield, grain N uptake, and NUE over varying N rates. Corn grain yield significantly increased when using EENFs compared to untreated urea (average of 1.54 Mg ha^{-1} Winnsboro, LA and 1.30 Mg ha^{-1} Saint Joseph, LA [$P < 0.0001$]). Two stabilizers paired together (NBPT and DCD) in Super U™, improved yields by nearly 3.0 Mg ha^{-1} when applied at the recommended N rate. The rate of N transformation was observed in greenhouse experiments, to determine the effectiveness of EENFs over multiple durations of time based on NH_4^+ and NO_3^- content in the soil system. While NH_4^+ concentration declined within 7 days post-application, nitrification inhibitors particularly Instinct had high NH_4^+ concentration and low NO_3^- concentration in both trials. This slower transformation minimizes the potential of N fertilizer to be lost. These results suggest crop uptake of N fertilizer would increase with higher NUE. Utilizing EENFs has the potential to increase NUE through specified conditions and time periods.

Chapter 1 Introduction

Crops grown within the Mid-South region of the United States are highly variable and influenced by many factors. As a geographically diverse region, Louisiana has benefited from its location and its proximity to water. The Mississippi River, the largest river in the United States, flows alongside the entire state from the northeastern through the southern border into the Gulf of Mexico. The state wide temperatures range by location, varying from the northern to the southern areas. Louisiana’s climate is humid subtropical allowing various crops to grow, earlier and later in its summer growing season.

Fertile soil is one of the most valuable resources to a producer. It gives structural and nutritional support to the crop. Though soil is a universal growing medium, there are various soil orders which are not equally distributed among all areas. Numerous factors affect how each of the soil orders developed, thereby affecting crop fertility. Five factors that influence the development of soils include climate, biota, parent material, topography and time (Jenny, 1941). The Mississippi River has largely contributed to influencing some of these factors. Leaving behind rich alluvial sediment, formed by the organic matter and mineral sediment from the river, which is the foundation of the soil’s parent material.

Louisiana’s soils are broadly grouped into eleven categories also known as major land resource areas (MLRAs); Arkansas River Alluvium, Eastern Gulf Coast Flatwoods, Gulf Coast Marsh, Gulf Coast Prairies, Red River Alluvium, Southern Coastal Plain, Southern Mississippi River Alluvium, Southern Mississippi River Terraces, Southern Mississippi Valley Loess, Western Coast Plain, and Western Gulf Coast Flatwoods (Weindorf, 2008). Two of these MLRAs include the soils series Sharkey and Gigger, which were used in this study. These soils are categorized separately throughout common regions in Louisiana (Weindorf, 2008).

The Sharkey soil series is located throughout Northeast Louisiana. It can be found on the Northeast Research Station in Tensas Parish, Louisiana. The area described as the Southern Mississippi River Alluvium is categorized with the soils of the Holocene plain area from northeastern Louisiana to the lower southeastern region. It is alluvial soil, above Tertiary and Cretaceous the bed rock that was deposited from the Mississippi River's runoff via flooding (Weindorf, 2008). Soils near the Mississippi River in this region are sandy to loamy to clayey in texture, though poorly drained. The soil has surface and subsurface layers that are formed from Mississippi River alluvium. The surface layer is a dark grayish brown color due to the high content of decomposed organic matter and the subsurface becomes dark gray and slightly acidic. The geographically associated soils in close proximity are Alligator, Newellton, and Commerce soils (Soil Survey Staff, 2014). Land use for the Sharkey soil is mostly cropland, though a small amount has been adopted for forest and pasture land (Weindorf, 2008). There is a high amount of shrinking and swelling in addition to high fertility rates in Sharkey soils, which is associated with the high cation exchange captacity (CEC) found with smectitic clays. The classification of the Sharkey soil series is very-fine, smectitic, Thermic Chromic Epiaquert (Soil Survey Staff, 2014)

At the Macon Ridge Research Station in Winnsboro, LA, the Gigger soil series can be found. These soils are found in Franklin and Madison Parish in Northeast Louisiana and were formed in the Southern Mississippi Valley Loessial Uplands. The soils in this MLRA are very distinct from those found in the Southern Mississippi River Alluvium. The loess parent material was formed due to wind deposition of lightweight silt particles. This wind deposited form of parent material is an eolian deposition, compared to the alluvial deposits from river activity. The area is classified as a Pleistocene terrace in relationship to the intermediate complexes of terraces and their deposits (Soil Survey Staff, 2014). The Gigger soil is a silt loam, with a yellowish to brown color. The subsoil is a darker brown color and more acidic than the surface layer. The upper surface of a typical Gigger is a Peoria Loess deposit, which is from the Late Wisconsin glacial period. Low organic matter and a low cation exchange capacity characterize the silty loess deposits. This has resulted in soils with moderate fertility and drainage, in addition to water holding capacity (Soil Survey Staff, 2014). Its primary usage is farmland and forest land. The taxonomic classification is a fine-silty, mixed, active, Thermic Typic Fragiudalf (Soil Survey Staff, 2014).

Southern parts of the United States, which have traditionally grown more cotton (*Gossypium hirsutum* L.*)*, have begun to transition more acres of land into various crops (Fannin et al., 2008). States like Louisiana have rapidly increased corn production. A recent monumental year was marked, in Louisiana during 2007 by producing more corn than cotton (Fannin et al., 2008). In 2013 state records were noted as the average amount of corn produced was 11,415 kg ha^{-1}. Over 1,369 farms in Louisiana contributed to this commodity’s increase across the state, raising feed grain harvest value in 2013 to $ 735.5 million dollars (LSU AgCenter, 2014). There were many reasons behind the switch in crops, one of which was the higher price ratio associated with the corn production. The ratio of profit for the production of cotton versus corn is an estimated $224-$448 per hectare (Fannin et al., 2008). Fannin et al. (2008) also noted cotton is becoming a smaller commodity in comparison with corn, due to the more expensive inputs required post-harvest. This is due to the requirements of the harvested cotton, which must be ginned and then packaged, while, corn needs to be air dried and then handled; cotton is also more labor intensive and larger amount of pesticides usage is required (Fannin et al., 2008). The amount of acres planted in corn throughout Louisiana has steadily increased. The state’s total grain production has also increase due to this change in crop production.

Originating in Mesoamerica, corn has now been cultivated globally for over 8,000 years all over the world (Gibson and Benson, 2002). The United States is the top producer, with nearly 14 billion acres of feed corn grown in the United States in 2013 (USDA- National Agricultural Statistics Service, 2013). Other countries with high corn yields are China, Brazil, Mexico, Russia, and Ukraine (USDA- National Agricultural Statistics Service, 2013). Corn has more indirect uses that contribute to the commodities appeal. Corn is used heavily in the United States for poultry feed that is in return used to produced meat, eggs, milk, ethanol, and in the production of various packaged foods and products.

There are 17 essential nutrients needed for optimal crop growth (Havlin et al., 1999). Large amounts of N are needed to sustain corn production (Watts et al., 2014). The nutrient is typically limited in non-leguminous irrigated cropping systems. Plants utilize N as a cornerstone for protein and enzyme synthesis. The nutrient's presence in crop production is critical, thus the high demand contributes toward N being one of the highest agronomic input costs of corn production systems. However, the complexity of the N cycle within the soil system can often make management difficult (McKenzie et al., 2010; Kitchen and Goulding, 2001; Marschner, 1995). To properly manage N in crop production systems, it is essential to understand the details of additions, transformations, and losses within the system (Hatfield and Parkin, 2014).

Nitrogen can be input into the environment through organic amendments such as residue from plant and animal decomposition, biological N fixation, atmospheric deposition, and organic mineralization (Marschner, 1995). Natural N fixation is a bacterially mediated reaction that can be carried out by free-living organisms or through symbiotic relationships with legume crops (Zuberer, 2005). The most common form of biological N fixation, occurs through, symbiotic fixation which occurs between legumes and Rhizobium bacteria. Estimates from these relationships and other biological fixation pathways range from 100 to 180 Mg N_2 year^{-1}; however determining the exact values of these relationships on a year to year basis is challenging (Havlin et al., 1999; Zuberer, 2005). Nitrogen can also be created atmospherically through N molecules in combination with oxygen (O_2) forming nitrogen oxides that form nitrates (Eq. 1) (Vlassak and Reynders, 1979).

$N_2O + 5H_2O \longrightarrow 10H + 2NO_3$ (Eq.1)

The atmosphere is comprised of about 78% of N, all of which can be utilized by plants once N fixation has occurred. Fixation is the process in which atmospheric N is converted into inorganic N for uptake or symbiosis. Animal manure, organic matter, and crop residue can also be mineralized as N sources. The microbial process in the soil decomposes this material, as the organic forms of N are converted into inorganic form for plant uptake. This microbial process is used primary by organic forms of N, occurs naturally in the global biochemical cycle through the circulation of soil, sediment, and water (Seitzinger et al., 2006). While the direct contributions of these forms of organic N into corn production systems vary, possible N accumulation from crop rotations can provide values for successive corn crops (Peterson and Varvel, 1989; Maloney et al., 1999).

While biological N fixation can provide N to corn production systems, derived N synthetic fixation, is also critical to the success of these production systems (Havlin et al., 1999). Synthetic N fixation or inorganic N is generally completed through the Haber-Bosch process (Eq. 2) (Ebbing, 1990).

$N_2 + 3H_2 \longrightarrow 2NH_3$ (Eq. 2)

Synthetic N is produced after atmospheric N_2 is converted to ammonia (NH_3^+) through high heat and pressure. This process creates inorganic N. Nitrogen fixation through this process has many advantages, including; fewer impurities and harmful chemicals, homogeneous nutrient concentrations, and increased N per unit cost (Brady and Weil, 1999). Requiring large amounts of fertilizer for production, growers heavily rely on inorganic N fertilizer to compensate for organic N limitations (Ribaudo et al., 2012). This drastic increase in the use of synthetic fertilizers on grains to meet optimum agronomic production has been a popular method for the last 50 years (Smil, 1999).

Once in the soil, N goes through several transformations. These transformations are influenced by environmental and biological factors creating uncertainty (Hartel, 1997). The major N transformation within the soil system is nitrification. Nitrification is an oxidative transformation of the reduced N form ammonium (NH_4^+) to nitrite (NO_2^-) and further to nitrate (NO_3^-) (Eq. 3) (Groffman, 1991; Havlin et al., 1999).

$2NH_3+3O_2 \longrightarrow 2NO_2+H + 2H_20$ (Eq. 3)

This reaction is a microbial mediated reaction, requiring two different bacteria to complete the reaction. The NH_4^+ aerobic oxidizing bacteria (AOB) *Nitrosomonas* aid in the conversion of NH_4^+ to NO_2^-. The second step in the process is the conversion of NO_2^- to NO_3^- through the aid of *Nitrobacter*. The bacteria utilize these transformations as a main energy source (Ryden and Lund, 1980).

While nitrification reaction occurs freely in the soil system, there are many environmental and soil conditions that influence the total reaction and reaction rate. As nitrification is a microbial mediated reaction environmental conditions influence rate of the nitrification rate; however, NH_4^+concentration will also directly affect the reaction (Robertson, 1989). Following fertilizer applications, applied NH_4^+, will often seek negatively charged particles to bind with. In most agricultural soils, the soil system has an overall negative charge.

This can often result in binding of the NH_4^+ molecule to the soil surface. However many environmental conditions can have significant influence on nitrification transformation in the soil system (Schmidt et al., 1982; Prosser, 1989; Mosier et al., 2002). In the soil the microbial activity is included these reactions. As both *Nitrosomonas* and *Nitrobacter* AOB, the lack of O_2 can dramatically decrease nitrification (Tisdale and Nelson, 1975). Miller and Johnson, (1963) explained that nitrification continued to proceed beyond these optimum values; however, evolution rates were slowed. These slowed conditions at high soil moisture values indicate sections of the soil with O_2 still present in microsites within the soil system (McKenney et al., 1994). In addition to soil moisture, soil temperature and soil pH can both play a significant role in nitrifying bacteria activity. As with most living organisms, nitrifying bacteria have optimum conditions in which productivity is highest. Outside these optimum conditions, both higher and lower, productivity can be drastically decreased. Malhi and McGill (1982) noted the optimum temperature for nitrifying bacteria in Alberta soils was 20°C. They further documented that outside of this optimum window, nitrification continued at a diminished rate. However, when temperature values were excess of 30°C, nitrification nearly ceased. In addition to warm temperatures influencing nitrification, cold temperatures can have a greater influence on nitrification rates. As with most biological reactions, reaction rates decreased with decreasing temperatures. Seifert (1961) demonstrated this concept by showing significant decreases in formation of NO_3^- at 2°C compared to 20°C. Soil pH in most natural and production based soils can vary widely. However, as nitrification is biologically mediated reaction, optimum rate occurs at near neutral soil pH. Vinther et al. (1999) documented this concept. In laboratory and field studies, they demonstrated that as soil pH was raised from 4.2 to 6.2 nitrification and potential nitrification increased. Furthermore, outside this optimum condition high and low pH can influence nitrification rates separately. Kyveryga et al. (2004) noted that when fertilizer was applied in the fall and measured again in the spring 89% of the total applied had gone through nitrification with pHs higher than 7.5; however, only 39% had been nitrified with pHs lower than 6.0.

Environmental factors coupled with inadequate management and large N fertilizer inputs in production can result in N moving into many forms and easily being lost. NH_3^+ volatilization has the potential to be a significant N loss mechanism under certain conditions (Meyer et al., 1961; Ferguson et al., 1984). Occurring naturally in the soil plant system, NH_3^+ volatilization is when NH_3^+ is released from the soil system into the atmosphere. When urea is applied to the soil system it goes through urea hydrolysis to form NH_4^+, a more stable form of N. However, if soil and environmental conditions exist that promote volatilization a significant amount applied fertilizer can be lost through NH_3^+ volatilization (Ferguson et al., 1984; Oberle and Bundy, 1987; Pimentel et al., 2005). The reaction's sources are various forms of degraded N including organic residue, manure, or urea based fertilizers that have not been adequately incorporated into the soil system. Loss from organic N is very small in comparison to inorganic, unincorporated, surface applied urea based fertilizers (Nathan and Malzer, 1994). NH_4^+ based fertilizers, like urea (CH_4N_2O) have higher risk for loss. Estimated N loss from NH_3^+ volatilization can range from 15-40% of the total applied N (Lighther et al., 1990; Grant et al., 1990).

Volatilization of NH_3^+ is influenced by many soil and environmental factors (Nathan and Malzer, 1994). As volatilization occurs within the soil system, soil factors directly contribute to the rate and amount of N volatilized. Soil factors such as pH, soil moisture and temperature, urease activity, CEC, buffering capacity and NH_3^+ /NH_4^+ concentration in solution can significantly influence volatilization (Marschner, 1995; Havlin et al., 1999; Brady and Weil, 2004). One of the most critical soil factors for volatilization potential in soil is pH. At pH of 7.5 or higher, increased amounts of NH_3^+ occur in soil solution due to disassociation of H^+ ions from NH_4^+. This occurs as an attempt to neutralize OH^- in soil solution and decrease the concentration of H^+ that is able to associate with free NH_3^+ in soil solution (Sharpe and Harper, 1995). Moisture in the soil catalyzes the volatilization of NH_3^+ on the soil surface (Demeyer, 1995). The reduction in N loss due to moisture was shown by Meyer et al. (1961), as fewer than 2 cm of precipitation could decrease volatilization within two days. As volatilization is microbial and enzyme mediated, temperature can also greatly influence the rate of the reaction. Havlin et al. (1999) discussed how volatilization rate increased with increasing temperature; this specific relationship degraded above temperatures of 45°C.

Agronomic management contributes equally and often in conjunction with soil factors affecting NH_3^+ volatilization. Demeyer et al. (1995) confirmed an inverse situation would occur with low moisture and high pH, when using urea; this resulted in the largest total NH_3^+ volatilization rates. Such findings indicated each of these reactions complexity under various field conditions. If left on the surface and not incorporated, an increase in loss between 25-75% can result from a urea based fertilizer (Schepers and Raun, 2008).

Crop and fertilizer management can also influence volatilization. Increase crop residue is associated with higher soil moisture, which induces increased urease activity. This results in higher crop residue can limit the advantages of incorporating N fertilizer into the soil system and therefore increase volatilization (Hargrove, 1988). Oberle and Bundy (1987) discussed that even partial incorporation of crop residue can significantly reduce volatilization losses.

Furthermore, the method of fertilizer application can greatly influence volatilization losses. Surface applications can result in much higher losses than subsurface incorporations (Touchton and Hargrove, 1982; Hargrove, 1988; Nathan and Malzer, 1994). Nitrogen loss can be uncertain, as numerous factors contribute toward NH_3^+ loss when using N fertilizers.

The nitrate (NO_3^-) form of N is highly mobile throughout the system. Another form of N loss is denitrification; which is also associated with the release of N into the atmosphere from the soil system, a result in the conversion of NO_3^- to gaseous forms of N. When soils are near field capacity, microbes have the ability to replace the role of O_2 as the terminal electron acceptor by substituting nitrogen oxides molecules from other species. These microbes are heterophic anaerobic bacteria such as *Pseudomonas, Bacillus, Micrococcus, and Achrombacter* (Myrold and Tiedje, 1985). Using metabolized organic carbon that has been oxidized, they function in both anaerobic and aerobic environments (Groffman, 1991). As the primary route for inorganic N to return to the atmosphere, denitrification is essential to the global biochemical N cycle (Bowden, 1986). Most of this loss in agricultural systems adversely affects the environment through the production of nitric oxide (NO) and nitrous oxide (N_2O), two components of global climate change (Wang et al., 1976; Ryden and Lund, 1980). Denitrification occurs naturally in the soil system. The majority of the conditions that allow these microbes to shift toward using a denitrifying metabolism result from an increase amount of N applied in agricultural production (MacKenzie et al., 1998). The higher the N rate applied the larger, the amount of N able to be transformed in the soil system.

Waterlogged soil containing adequate amounts of NO_3^- or NO_2^- and available C, create the optimum conditions for an increased denitrification rate in the soil system. The quantity of N gas released through denitrification is dependent upon many factors including pH, temperature, and degradation of oxygen depletion.

In suitable soils, the most active components of denitrification are denitrifiers. In aerobic soils denitrifer's activity is minimal, as the conversion of N is regulated by oxygen (Parkin and Tiedje, 1984). McKenney et al. (1994) concluded denitrification occurs as oxygen inhibits in a step down fashion moving from N_2O, to NO, then finally to NO_2^-. The onset of the saturated soil reduces oxygen, while the resulting electron flow induces denitrification. Davidson (1992) observed this occurrence in saturated soils within 15 minutes. Bremner and Shaw (1958) found that the optimum soil pH range for denitrification was from 6.0-8.0. However, Klemedtsson et al. (1978) found denitrifying activity present in both acidic and basic extremes, with pH levels of 3.5 and 11, respectively. Findings of optimal denitrification temperatures between 28-37 ˚C were reported by George and Antoine (1982), while Schanbel and Stout (1994) observed initial temperatures occurring as low as 5-7 ˚C.

Organic matter that has decomposed is a critical component, as it is a requirement for microbial denitrification of NO_3^-. Easily mineralized C sources and low C/N residue were found to increase the likelihood of higher denitrification rates (Aulakh et al., 1991). Soil with low C/N ratio has low residual N. Soils with available C from freshly incorporated residue had higher denitrification rates (Havlin et al., 1999). However, timing affected this relationship, as MacKenzie et al. (1998) noted that periods of longer time showed higher rates of denitrification in no till soil verse conventional tilled.

Soil water content above field capacity is needed to inhibit O_2 which ceases microbiological activity and enables denitrifying activity to proceed. The microbial activity function is limited in anaerobic conditions. Denitrification moves in a metabolic pathway through bacteria, removing inorganic N from the soil. Microsite characteristics such as aggregate size, soil temperature, and microbial activity with in water content and the soil affect the onset of denitrification (Myrold and Tiedje, 1985; Renault and Sierra, 1994). Sandy coarse soils lose NO_3^- much quicker than sandy coarse soils, due to the anaerobic conditions following to intense precipitation activities (Schepers and Raun, 2008). Denitrification occurs at higher rates from shorter terms of intense rainfalls (Sexston et al., 1985). Continually occurring through millions of denitrifers, the conditions required to transform N into NO_3^- can be very complex. Low levels of denitrification from the response of proper soil management and environmental conditions are critical to agronomic production.

The ability of the N source to adsorb to negatively charged soil colloids is critical for the N source to remain in the soil solution and be taken up by plants. The combination of high mobility of NO_3^- with continuous water movement increases the susceptibility of NO_3^- to become leached below the active root zone. Continuous downward movement of NO_3^- from the soil profile can result in detrimental impacts on groundwater. This downward movement of NO_3^- from the soil system into the ground water can be very environmentally harmful (Brady and Weil, 2004; Howarth et al., 2002; Pimentel et al., 2005). One of the most serious concerns is high NO_3^- in drinking water. Excess NO_3^- can create a lack of O_2 in the blood of infants resulting in a potentially fatal condition known as methemoglobinemia. Nitrate in water can also drastically decrease the quality of many economically and recreationally important surface waters (Howarth et al., 2002).

The hypoxia zones in the Chesapeake Bay and Gulf of Mexico have been attributed to excessive NO_3^- leaching deposited into large bodies of open watr (Pimentel et al., 2005).

Similar to denitrification, many factors can influence N leaching. Precipitation activities are key components that contribute to this form of N loss. Soil mineralogy is another factor which affects the rate of infiltration that occurs through the soil profile. Coarse particle soils such as sandy soils are more susceptible to loss compared to finer particles, which have the ability to hold more water (Schepers and Raun, 2008). Excess water with high N application rate resulted in higher NO_3^- concentrations in tilled soils, which have the potential to be lost through leaching or denitrification (James et al., 2001). Humidity, high temperatures, and Mediterranean climates increased the concentration of NO_3^- in the soil during spring and late fall post-harvest, in arid and semiarid soils (Beaton, 1971; Brady and Weil, 2004). Tillage and timing of N source application are influential in reducing NO_3^- leaching. The amount of increased infiltration in the soil solution can create more space for NO_3^- to easily be lost. A study showed two tillage techniques, no till and chisel plow, both had similar rates of NO_3^- loss from leaching in the soils (Zhu and Fox, 2003). Nitrate leaching will occur naturally, but a balance is needed for minimized loss because of the quantity of loss impacting productivity, biodiversity, human health, and air and water systems in the world (Ladha et al., 2005).

Although N in the soil system can easily be lost, its presence is essential for crop production. The inorganic N form is a part of the metabolic process affording the needed energy and growth that occurs within a plant**.** Kitchen and Goulding (2001) concluded that cereal crop's production systems often utilize the N source inefficiently. In addition to NUE inefficiencies, an increase in synthetic fertilizer has been seen within the last 50 years, which has contributed to the largest input into the global cycle (Smil, 1999).

Urea is a common fertilizer used for cereal crops in the Mid-South. Urea contains large concentrations of N (46%). The product's popularity is attributed to its economic accessibility, large amount of N, and safe distribution. Urea has a high susceptibility to loss as NH_3^+ when applied to the soil surface through the process of volatilization.

Plants are able to use two forms of N in the soil system: NO_3^- and NH_4^+. Both sources represent the inorganic form of N. Nitrate is the highly preferred form of N for absorption by plant, but is free and easily leached out of the soil or denitrified when saturated (Jemison and Fox, 1994). It is easier for plants to adsorb NH_4^+ from the soil as it is often taken upheld in the soil, by soil particles with a negative charge (Bronson, 2008). Additional energy is needed to convert NH_4^+ to NO_3^- by plants.

Fertilizer usage is an important aspect of agronomic management needed to maximize crop production. The relationship between corn production and fertilizer usage is based on reaching an economic profit (Burgener, 2013). Growers are competing to grow food for the world with limited resources and knowledge to maximize N efficiency. The negative impacts of fertilizer loss on resources are experienced globally and the continuation on such a large scale could continue to decrease agronomic production in the future, evident through poor nitrogen use efficiency (NUE) (Shaviv, 2000; Halvorson et al., 2014a; Watts et al., 2014).

The rise in synthetic N fertilizer use, along with global consciousness for environmental concerns has created a need for efficient management practices that ensure optimum NUE. To achieve this, management practices must pair highest plant available N in the soil with periods of rapid N uptake in the crop. Traditional management practices involve splitting N applications to coincide with periods of uptake. Despite this method, many growers have difficulty utilizing and adopting these practices into their production system. A potential solution for these agronomic problems is to increase NUE, with advanced technologies.

The use of enhanced efficiency N fertilizers (EENF) has the ability to minimize N fertilizer loss through NH_3^+ volatilization and NH_4^+ based losses. Ferguson et al. (1984) describe them as formulations, additives, or physical factors, to conventional fertilizers that contain various active chemical ingredients to increase nutrient uptake. These products were originally introduced in the 1960s and 1970s. However, advanced technology has altered the composition creating more efficient products. The majority of these products are chemically or polymer coated active ingredients applied on to granular or liquid fertilizers such as, urea (CH_4N_2O) or liquid Urea Ammonium Nitrate (UAN). Hatfield and Ventera (2014) and Shaviv (2000) noted that the increased NUE in N uptake by corn could be directly related to the release of available N from the EENF. Understanding the timing of uptake and effective agronomic management practices, using these products will increase NUE globally (Noellsch et al., 2009).

There are three main categories for these products: stabilized fertilizers, slow release, and controlled released products (Trenkel, 1997). While controlled and slow release fertilizers are essential tools for increasing NUE, the use of chemical additives, or stabilized fertilizers, has seen more wide-spread use in production agriculture fields (Trenkel, 1997). To extend the availability of N fertilizer for the cash crop, stabilizer products are chemical additives that have been combined with N fertilizers, inhibiting a natural mechanism, either of enzymes or microbes in the soil (Olson-Rutz et al., 2011; Halvorson and Bartolo, 2014). By inhibiting these enzyme and microbial processes, soil N is held in a more environmentally secure form until conditions exist for the fertilizer to be incorporated or taken up by the plant. The two major stabilizer products are urease inhibitors and nitrification inhibitors.

Urease inhibitors decrease the activity of the urease enzyme and therefore decrease urea hydrolysis (conversion of urea to NH_4^+) (McCartey et al., 1989; Rawluk, 2000; Sistani et al., 2014) Fertilizer applied to the soil surface converts through hydrolysis quickly and is highly susceptible for NH_3^+ to become volatilized into the atmosphere (McCarty et al., 1989). Some of the complex compounds in the urease products that inhibit the naturally occurring urease enzyme in soil include: inorganic salts of Hg, Ag, and Cu; dihydir phenols, N-n-butyl, and quinones each as hydroquinone, p-bensoquione, and specified substituted p-bensoquinones (McCartey et al., 1989). Some of these products are N-(n- butyl)- thiophosphoric triamide (NBPT) in the products Agrotain 20% and Agrotain Ultra, and Super U by Koch Fertilizer, LLC, Wichita, KS.

In Louisiana, corn grows in the spring throughout the summer seasons. The high temperatures in summer increase the rate of NH_3^+ volatilization (Rachhpal-Singh and Nye, 1986). Using urease inhibitors, the urease enzymes are inhibited for seven to fourteen days, allowing time for precipitation to incorporate urea into the soil system, hydrolyzing the N into NH_4^+ before NH_3^+ is volatilized from the surface (Watson, 2005). N-(n-butyl)-thiophosphoric triamide is one of the most common chemical ingredients used to formulate urease inhibitors. The ingredient NBPT reduces loss through volatilization, but this can be subjective based upon the various climatic conditions in an area (Nelson et al., 2008). Rawluk (2000) found urease inhibitors to be less effective on fine textured soil than coarser soils.

Nitrification inhibitors, a stabilized fertilizer inhibits the biological oxidation of NH_4^+ to NO_3^- through the active chemical ingredients. While some disagreements exist in the literature, the use of nitrification inhibitors has been shown to reduce leaching and denitrification losses for four to seven weeks (Nelson and Huber, 2001; Bronson, 2008; Olson- Rutz et al., 2011). The active ingredients of commercially available nitrification inhibitors differ, but the most common are DCD and Nitrapyrin. These products perform by inhibiting the *Nitrobacter* and *Nitrosomonas* in converting NH_4^+ to NO_2^- and eventually NO_3^- (Ronaghi et al., 1993; Nelson and Huber, 2001; Olson-Rutz et al., 2011). This allows the NH_4^+ to remain in a stable N source for a longer period of time and available for plant uptake. The EPA requires labeling of active chemicals for commercial use within the United States. Some of these products are: Dicyandiamide (DCD) in Super by Koch Fertilizer, LLC, Wichita, KS, (2-chloro-6-[trichloro-methy] pyrideine) Nitrapyrin in Instinct/N-Serve® by Dow Chemical Co., Midland MI., and a partial calcium salt of maleic-itaconic copolymer in the product Nutrisphere- N®, by Specialty Fertilizer Products LLC in Leawood, KS.

Research on the management factors affecting nitrification inhibitors, particularly NUE in corn production is limited, with fewer studies having been completed in the Mid-South (Burazco et al., 2014). The rate of nitrification was consistently reduced using these products in the in other regions outside the Mid-South (Touchton and Hargrove, 1982). Soil temperature, pH, organic matter, rate of diffusion, volatilization, sorption, and soil temperature were noted to have influenced the length of time for the effectiveness of nitrification inhibitors. Utilizing nitrification inhibitor can inhibit the transformation of NH_4^+ -N form of N. The timing of application for these EENF products is an essential component for effective usage.

A large time gap between nitrification inhibitor application and optimum period of uptake for the crop increases the probability of lower agronomic yields (Schepers and Raun, 2008). As temperature dependent fertilizers, the inhibitors are able to delay action in temperatures below 15˚C. Burazco et al. (2014) found it significantly increased NUE 17% using the EENF Nitrapyrin, while no effects on corn grain yield were found. They speculated that variability in response could be attributed to post application weather and the extent of the soil mineral N loss in the environment. Other studies related similar results to impacts based on weather, particularly spring application verse fall application response (Wolt, 2004; Randall and Vetsch, 2005). Only in one of the six years did Randall and Vetsch (2005) note a significant increase in grain yield. The timing of fertilizer application and the amount of precipitation can affect the environmental conditions moving N in various forms and uptake in the soil systems. The largest value of these nitrification products can be found when N was applied at or below critical values (Wolt, 2004). Significant improvements in plant N uptake were found in corn using this same EENF Nitrapyrin in a greenhouse experiment (Ronaghi et al., 1993). The nitrification inhibitors reduced N loss; however the EENFs response was inclusive in plant N uptake.

Designed to deliver soluble N at gradual rates through diffusion, controlled release products manage the amount of N present to reduce loss (Trenkel, 1997; Dinkins et al., 2011). Control release fertilizer, is typically comprised of a thin permeable polymer coated urea-aldehyde N fertilizer capsule in granular form (Baylock and Tisdale, 2006). These products have been categorized by scientists as inorganic and organic, additionally marked as low soluble compounds (Shaviv, 2000; Baylock et al., 2004). The release of these EENF products through the polymer coatings is determined by two factors; soil moisture and temperature. Moisture is the factor triggering when the fertilizer is released from each individual malleable capsules (Baylock and Tisdale, 2006). As water moves into the polymer coated capsule, the fertilizer inside is diffused and into the soil system. In addition to moisture, temperature affects the soil system microbial reaction, as another catalyst for the coated product to release the N product (Trenkle, 2000). The polymer coated urea products include Environmentally Smart Nitrogen (ESN) manufactured by Agrium Advanced Technologies in Calgary, Canada.

Sistani et al. (2014) did not find any benefit using ESN or Super U, over a three year study. The N loss was speculated to have been attributed to volatilization and leaching. In agreement with Sistani et al. (2014) findings using EENFs, Halvorson et al. (2010) and Halvorson and Jantalia (2011) noted many environmental factors influence the use of each product differently. These factors that influence agronomic production while using EENFs include soil type, infiltration, high N rate application, and management practices. Conversely, in a three year study, Halvorson and Bartolo (2014) found ESN, polymer coated inhibitor significantly increased grain yield in comparison to urea. The increase in yield was seen two of the three trial years. Hatifeld and Parkin (2014) assumed heavy precipitation resulted in significant improvement in grain yield using ESN, Super U, and Agrotain Plus during 2008-2010 under various combinations of fertilizer side dressed in continuous corn.

Enhanced Efficiency Nitrogen Fertilizers are very controversial products as the effects are not fully understood. Hatifield and Parkin et al. (2014) suggest the usage of EENFs is subjective. Various factors influence EENFs effect on grain yield, which are similar to those affecting crop growth: rate, weather, timing, and management practices (Cahill et al., 2010; Halvorson et al., 2010; McKenzie et al., 2010). Weather is one of the major catalytic factors affecting EENFs results on crop production (Halvorson and Bartolo, 2014).

Little is known on the effects of EENFs in the Mid-South, as crop production systems and management practices have changed over time. Studies in the eastern region of the United States using EENFs indicated inconsistent results in yield (Cahill et al., 2010). Other researchers including Noellsch et al. (2009) found EENFs in particular have positive responses on claypan soils. Conversely, Ebelhar et al. (2007) concluded the potential advantage of using these EENFS decreases when using rates above the recommended rate. Continued research on EENFs is needed to make recommendations, particularly within the Mid- South.

Future research should verify EENFs ability to contribute to sustainability by creating better N efficiency standards, while providing economic assets to growers. This would have an impact on N_2O emission affecting climate change. This type of environmental degradation from N loss can contaminate ground and surface water sources. The usage of EENFs has the capacity to reduce denitrification, leaching, and volatilization levels compared to untreated synthetic fertilizer (Halvorson et al., 2014b). Finding the correlation of these products, to soil types, crops, and application timing could result in improved management (Delgado and Mosier, 1996; Bolan et al., 2004; Akiyma et al., 2010). The results of using these EENF products could drastically reduce effects of N fertilizer in agriculture and improve the industry towards optimum agronomic efficiency and greater global sustainability (Halvorson et al., 2014a).

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Chapter 2 Enhanced Efficiency Nitrogen Fertilizers Influence on Corn Production in Mid-South

2.1 Introduction

Economic advantages have shifted productions systems in the Mid-South region of the United States, particularly Louisiana, from cotton (*Gossypium hirsutum* L.*)* to grain crops, specifically corn. As one of the most fertilized cereal crops, corn requires high quantities of N in the soil as it is often limited in availability (Watts et al., 2014). This has resulted in higher amounts of N fertilizer added to cropping systems to sustain yields. This drastic increase in the use of synthetic fertilizers in grain crops has been the prevalent method to meet optimum agronomic production (Smil, 2001). High inputs coupled with inadequate management of N inputs on individual production systems, can result in fertilizer being easily lost, creating agronomic, economic, and environmental problems (Spalding and Exner, 1993; Williams et al., 1998).

Globally there is an increasing need to maximize agronomic production to meet the needs of increasing population, while ensuring optimum nitrogen use efficiency(NUE) for maximum N recovery in agronomic yield (Hatfield and Parkin, 2014). However, challenges arise when soil N levels are greatly influence by not only application practices, but also environmental conditions. Louisiana's location provides a unique climatic region as well as highly variable soils, as both were influenced by the Mississippi River's depositional events; they individually contribute toward the difficulties in proper management (Beaton, 1999; Brady and Weil, 2004). High rainfall, fluctuating temperatures, and poorly drained soil commonly lead to N loss in the region through volatilization, denitrification, and leaching. To minimize these losses, high nutrient availability must coincide with periods of rapid nutrient uptake.

To overcome these challenges, past research has shown, management practices must be implemented to limit the environmental impact on available N and ensure adequate N and minimal losses have occurred prior to crop uptake (Shaviv, 2001; Halvorson et al., 2014; Watts et al., 2014).

A management practice that has the potential to maximize N availability with N demand, is enhanced efficiency nitrogen fertilizer (EENF). While interest in EENFs has increased in recent years, EENFs were introduced in the 1960s by the Association of American Plant Food Control Officials (AAPFCO, 2013). While the potential value of these products have been noted, a majority of research has focused on quantifying N losses with limited and highly variable results focusing on crop yield, especially on upland field crops (Shaviv, 2001; Halvorson et al., 2010; Blackshaw et al., 2010; Halvorson et al., 2011; Linquist et al., 2013; Burazco et al., 2014; Halvorson and Bartolo, 2014; Hatfield and Parkin, 2014; Hatfield and Veterea, 2014; Sistani et al., 2014). Hatfield and Parkin (2014) reported that while EENF (both polymer-coated and chemical inhibitors) did not increase in-season growth, significant yield increases were consistently found. While Watts et al. (2014) reported positive results from EENF; their study's response slightly differed as results were inconsistent through the trials. Particularly they concluded EENFs, namely polymer-coated, and Super U, did not significantly improved cotton lint yields compared to urea or urea- NH_4^+ sulfate. Watts et al. (2014) did not find an increase from polymer-coated urea; however Halvorson and Bartolo (2014) reported a significant increase in corn grain yields compared to untreated urea. Utilizing the product Super U in compassion to the untreated fertilizer, both Watts et al. (2014) and Halvorson and Bartolo (2014) found no significant impact. In addition to crop yield, Halvorson and Bartolo (2014) reported increased recovery efficiency, similar to NUE, of polymer-coated were improved by 19% over untreated urea, which was not found in Super U. A similar trend was seen by Burazco et al. (2014) who found a 17% increase in NUE for EENF, Nitrapyrin compared to untreated urea.

While the theory behind EENF emphasizes potential for improving N management in high loss environments, varying positive results in the literature have limited wide-spread adoption of these products. One possible explanation for the varied positive results could be the influence of soils and environmental conditions, which greatly influence N dynamics, including N losses. These findings denote a need for continued validation of these products in variable conditions. Additionally, limited research findings are currently available on the influence of these EENF on corn production systems in the Southeast, specifically the Mid-South, across N application rates. Therefore, the objectives of this study were to 1) evaluate the influence of EENF and N application rate on corn yield in two distinct systems in the Mid-South, and 2) determine the impact of EENF on corn N uptake and NUE on corn production systems.

2.2 Materials and Methods

2.2.1 Site Description

Field trials were established on Macon Ridge Research Station in Winnsboro, LA (32° 8'29.11"N and 91°42'33.80"W) on a Gigger silt loam soil (fine-silty, mixed, active, thermic Typic Fragiudalf) and the Northeast Research Station in Saint Joseph, LA (31°56'59.76"N 91°13'57.21"W) on a Sharkey clay soil (very-fine, smectitic, thermic Chromic Epiaquert) during the 2013 and 2014 seasons. Trials were not established in the same sites in consecutive years, though the soils were similar between two years.Both locations were grown under furrow irrigated conditions; however due to excess moisture at Saint Joseph, the trial was not irrigated in

2014 (Table 2.2.1). Annual temperature and precipitation for both locations are provided in Figure 2.3.1.

Table 2.2.1 Agronomic data from Winnsboro and Saint Joseph, LA during 2013 and 2014.

Site	Year	Hybrid	Planting Date	Harvest Date	Planting population (plants ha^{-1})
Saint Joseph	2013	Mycogen 2C786	04/2013	08/2013	36,960
	2014	Pioneer 1883HYR	03/2014	09/2014	36,960
Winnsboro	2013	Pioneer 1319HR	04/2013	08/2013	35,840
	2014	Pioneer 1319HR	03/2014	09/2014	35,840

2.2.2 Treatments and experimental design

Four varying EENF products and three N application rates were evaluated. The EENFs included urease inhibitors, nitrification inhibitors, and combination of urease and nitrification inhibitors. The urease inhibitor evaluated was Agrotain Ultra (NBPT [N-(*n*-butyl)thiophosphoric triamide]; Koch Fertilizer LLC, Wichita, KS). Two nitrification inhibitors evaluated included: Instinct (Nitrapyrin [2-chloro-6-(trichloromethyl) pyridine; Dow AgroScience LLC; Indianapolis, IN) and Nutrisphere (partial Ca salt of maleic-itaconic copolymer; Specialty Fertilizer Products LLC, Leawood, KS). While the EENF with both urease and nitrification inhibitor evaluated was Super U (NBPT and DCD dicyanduamide [2-Cyanoguanidine]; Koch Fertilizer LLC, Wichita, KS). All inhibitor rates were applied in accordance to individual labels. In addition, untreated urea (46%) was included as a production standard to compare evaluations.

Three N application rates were evaluated; however, the specific application rates varied by location. Application rates included current application recommendations, based on LSU

AgCenter, as well as 33.6 kg N ha above and below (87% and 112%). Thus N rates for the Winnsboro were 235, 269, and 302 kg N ha^{-1}, while Saint Joseph was 269, 302, and 336 kg N ha^{-1}. Each location included a non-fertilized treatment, used as a check plot to evaluate natural N contributions during the season. The four EENFs and three N application rates were evaluated as a complete factorial design within a randomized complete block design with six replications.

2.2.3 Trial Management

Prior to trial establishment, on a yearly basis, soil samples were collected. These baseline samples were used to guide nutrient management for the following season. The Winnsboro site year nutrients were supplied in both 2013 and 2014; however, fertilizer and amounts differed. In 2013, 19, 11, 67.2, and 67.2 kg ha^{-1} of S, Zn, P, and K were applied, respectively. In 2014, only P and K were applied at the rate of 67.2 kg ha^{-1} for both nutrients. According to soil tests, no fertilizer application was required at Saint Joseph. For Winnsboro, fertilizer was broadcast in December prior to planting. Following application, the fertilizer was incorporated by reforming the beds using a bed shaper (AMCO Manufacturing, Inc., Yazoo City, MS).

Four weeks prior to scheduled planting, all plots were chemically burned down using a tank mix of 2, 4-D (2, 4- Dichlorophenoxyacetic acid) and glyphosate (N (phosphonomethyl) glycine) at the rate of 1.25 kg ha^{-1}. At planting, plots were planted using a John Deere MaxEmerge Vacumax planter (Deere & Company, Moline, IL.). Alleys between plots were created shortly following emergence. Individual plots measured 13.7 m in length and 4 m wide with 1 m row spacing. The N application treatments were applied immediately following plot establishment. Plots were maintained weed-free throughout the growing season by manually removing weed on a weekly basis. All insect and disease management was carried out in accordance with LSU AgCenter recommendations. At harvest, plots were further shortened by 3 m (1.5 m on both front and back). This was carried out to minimize alley effect, which can create a high amount of variability. The middle two rows of the four row plots were mechanically harvested at maturity using a Massey Ferguson 8XP small-plot combine (Kincaid Equipment Manufacturing, Haven. KS.).

2.2.4 Data collection

In-season vegetative samples, for plant N analysis, were collected at two critical growth stages, 10-leaf stage (V10) and tasseling (VT) (Ritchie et al., 1997). Biomass samples were collected in a similar manner at both growth stages. Plant samples were collected from a 0.5 m section of the non-harvest rows. Samples were collected from the interior of the plot to ensure minimal alley effect of increased biomass or nutrient uptake. Plant samples at the second sampling (VT) were taken from a different non-harvest row compared to the initial sampling (V10). This was done to minimize the influence of the initial sampling on the successive sampling. Analysis for plant N uptake and NUE were only completed on 4 (replications 2-5) of the 4 row plots. Plant samples were dried at 48°C for 72-hours, weighed, and ground to pass a 1 mm sieve. Plant tissue samples were then analyzed for total N concentration using a Vario El Cube CHNS model (Elementar Americas Inc. Mountlaurel, NJ) (Colombo and Giazzi, 1982). Total N concentration paired with sample weight was used to determine N uptake. At maturity plot grain weights were mechanically determined, as noted in the previous section. Plot weights and moisture were utilized to estimate corn grain yield with moisture content adjusted 150 mg kg^{-1}. In each plot, grain subsamples were collected to analyze for grain N content. In a similar method discussed above, grain samples were dried, processed, and analyzed for total N concentration. As with the plant tissue samples, grain yield and N concentration was used to determine grain N uptake. Additionally, grain N uptake was utilized to determine NUE using the difference method (Varvel and Peterson, 1999), using the following the components (Eq. 4).

$$\mathrm{NUE} = \frac{\text{grain uptake}_N - \text{grain uptake}_0}{\text{Nitrogen rate}} \qquad \text{(Eq. 4)}$$

Where:

grain uptake$_N$= grain N uptake for the N fertilized treatments

grain uptake$_0$= grain N uptake for the unfertilized treatments (check)

Nitrogen rate= the rate of Nitrogen fertilizer applied

The NUE was determined on an individual replication and then averaged across replication

2.2.5 Data analysis

Analyses of variance was conducted using the mixed procedure (SAS 9.4, SAS Institute Cary, NC) to analyze the difference in corn grain yield, uptake, and NUE among N rate, EENF, and any interaction between N rate and EENF. Mixed procedure was used as it is more robust when models utilize both fixed and random variables. The mixed model N rate and EENF were evaluated as fixed effects while locations and replications were random effects. Post-hoc analyses for the main and interactive effects were analyzed using Tukey adjustments for protected LSD means when interactive effects were noted slice modifier was implemented. All significant comparisons were made at a 0.05 probability level.

2.3 Results and Discussion

2.3.1 Grain Yield

A significant effect by location and treatment as well as year and location interaction existed; therefore, all data was analyzed and discussed separately. Furthermore, yields from the check plot were found to be significantly lower than fertilizer applied treatments at both locations in both 2013 and 2014 (Table 2.3.1). Overall, yields from the unfertilized treatments were higher at Winnsboro compared to those from Saint Joseph (Table 2.3.1). Data from the check plots were not discussed further; however, these values were used to estimate NUE.

Yields for the N fertilized treatments were found to be highly variable across both site years in response to applied treatments (Table 2.3.1). At Winnsboro the unfertilized check treatments yielded higher compared to Saint Joseph (11.1 and 11.1 compared to 9.4 and 10.6 for Winnsboro and Saint Joseph in the 2013 and 2014 season, respectively) when averaged across all applied treatments. Lower yields in Saint Joseph during both 2013 and 2014 were potentially due to higher rainfall experienced, especially during the early months of the growing season (Fig.2.3.1). The high precipitation conditions, paired with higher clay content in the soils at Saint Joseph, potentially resulted in soil conditions being at or near saturation, especially during early growth stages. Singh and Ghildyal (1980) reported the effect of high moisture conditions on corn growth. For all site years, no significant interaction was found between N rate and EENF for corn grain yield (Table 2.3.1). Therefore, the main treatment effects N rate and EENF were analyzed and discussed separately. For the N rate main effect, a significant response was noted for all four site years; however, yield response differed between locations and years ($P \leq 0.297$).

Table 2.3.1 Corn grain yield at three specified Nitrogen rates and their responses to five Enhanced Efficiency Nitrogen Fertilizer (EENF) and untreated urea, during two trials at Winnsboro, LA and Saint Joseph, LA in 2013 and 2014.

		Winnsboro		Saint Joseph	
		2013	2014	2013	2014
Factor	Treatment				
		------------------Mg Ha^{-1}	----------------		
N rate	Check	3.5[†]	3.9	1.0	0.7
	Below recommended	10.3b[‡]	10.5b	7.5c	9.9b
	Recommended	11.4a	11.2ab	9.0b	10.6ab
	Above Recommended	11.6a	11.5a	11.7a	11.3a
EENF	Urea	10.3b[§]	9.4b	8.4c	8.9b
	Agrotain Ultra	11.9a	14.5a	9.2bc	10.5ab
	Super U	12.2a	13.9a	10.9a	11.0ab
	Nutrisphere	10.4b	8.3c	8.3c	11.0ab
	Instinct	10.8b	9.2bc	10.1ab	11.6a
ANOVA	N rate	0.0002	0.0119	<0.0001	0.0297
	EENF	<0.0001	<0.0001	<0.0001	0.0331
	N rate X EENF	0.5114	0.2705	0.2303	0.1196

†Below recommended, recommended, and above recommended N rate based upon location Winnsboro N rates 235, 269, and 302 kg N ha $^{-1}$and Saint Joseph N rates 269, 302, and 336 kg N ha $^{-1}$.

‡All check treatments were significantly lower than all fertilized treatments for both locations.

§Lower case letters within columns and factor indicates different significant difference using Tukey adjusted LSD means at α=0.05 significance level.

In 2013 at Winnsboro, a significant increase in corn grain yield was found when N rate increased from 235 kg N ha^{-1} to 269 kg N ha^{-1}, but no further significant result was found when the N rate further increased to 302 kg N ha^{-1} (Table 2.3.1). The lack of response at the higher N rates could be attributed to soybeans, the previous crop. This potential N-credit in the soil increasing corn yield following a legume, such as soybeans, has been previously noted in the literature.

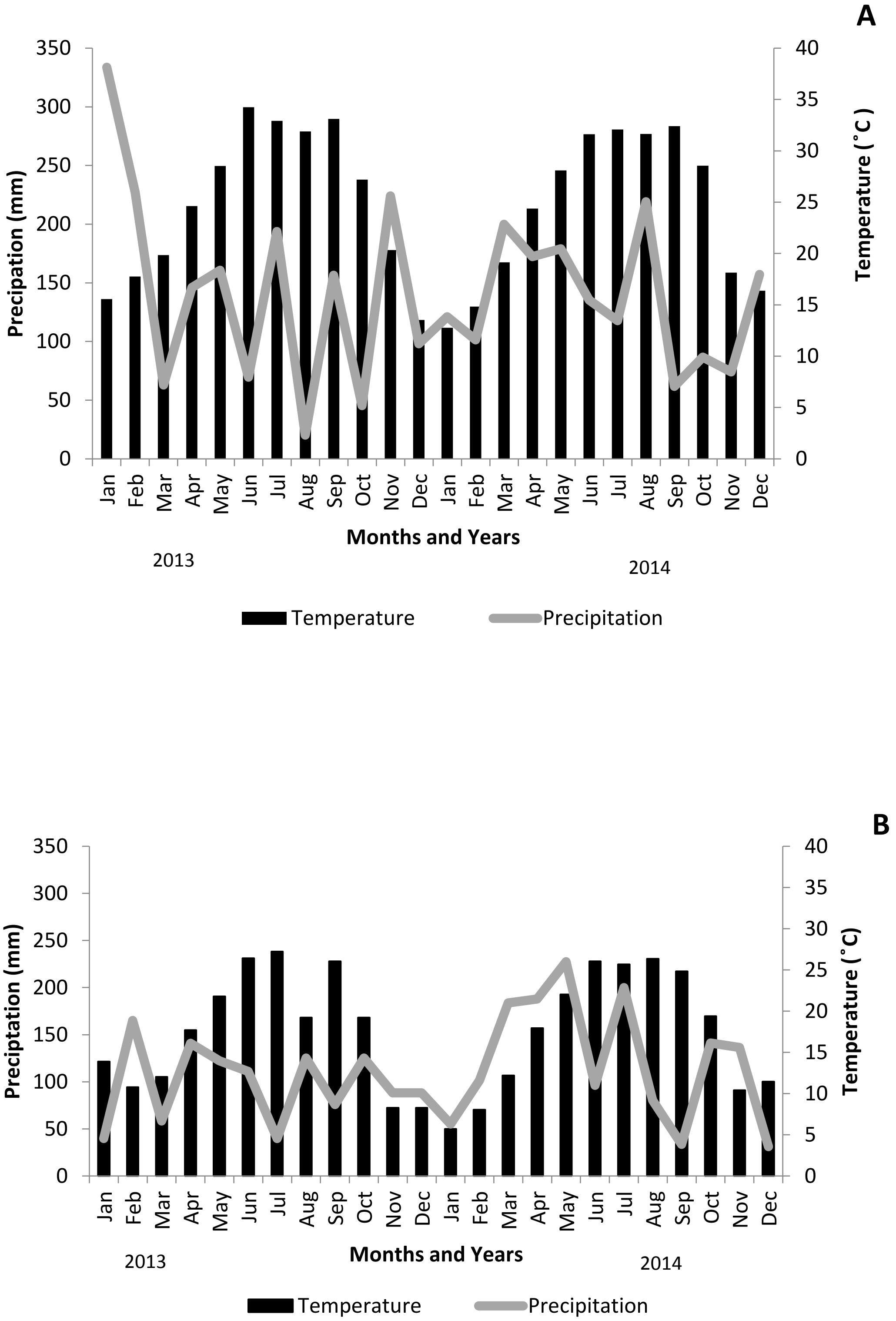

Figure 2.3.1 Monthly precipitation and mean monthly temperature at Winnsboro (A) and Saint Joseph (B), LA during 2013 and 2014.

Peterson and Voss (1984) reported that corn received an approximately 45 kg N ha^{-1} credit when grown in rotation with soybeans. This concept was emphasized by Peterson and Varvel (1989), who reported continuous corn required, on average, double the amount of N addition to achieve optimum yields, compared to rotating corn with a legume. In 2013, corn grain yields at Saint Joseph showed a linear response to continually increasing N rates, with over a 4 Mg ha^{-1} increase in yields between the low (269 kg N ha^{-1}) and high (336 kg N ha^{-1}) N rates (Table 2.3.1). As opposed to results from Winnsboro in 2013, the Saint Joseph followed a high residue grain sorghum crop, which could potentially result in in-season applied N immobilization. This would, therefore, diminish the amount of applied N available for that crop. Green and Blackmer (1995) detailed the potential increased of N immobilization following a grain crop compared to a legume crop. They emphasized that the difference in N demand following grain compared to a legume crop was more associated with the longer immobilization period for grain crops compared to legumes. In 2014, both Winnsboro and Saint Joseph responded similarly to continually added N (Table 2.3.1). Both locations resulted in a significantly increase in corn grain yield when N application increased from the low application (235 and 269 kg N ha^{-1} for Winnsboro and Saint Joseph, respectively) to the high application rate (302 and 336 kg N ha^{-1} for Winnsboro and Saint Joseph, respectively), but neither found a significant differences with the mid-application rate.

Similar to the N application rate, EENF treatments were found to have a significant response in all site years ($P<0.0005$). However, the responses, averaged over N rate, were more diverse than those found with N rates (Table 2.3.1). Additionally, the response of EENF was more consistent between locations.   For Winnsboro in 2013, significant increases in grain yield were noted for Agrotain Ultra and Super U compared to all other treatments, with over 1 Mg ha^{-1}

yield increases found (Table 2.3.1). Similarly in 2014, Winnsboro corn treated with Agrotain Ultra and Super U had significantly higher grain yields than all other treatments. However, corn yield differences between the Agrotain Ultra and Super U were much greater than those found in 2013 (with yield increases of 5.1 and 4.5 Mg ha^{-1} for Agrotain Ultra and Super U compared to untreated urea, respectively). The significant increase in 2013 and 2014 from Agrotain Ultra and Super U can be a contribution of the urease inhibitor present in both products. The gain from the urease inhibitors at Winnsboro was magnified by environmental conditions present during application along with the soil type. The soils at Winnsboro have been reported to be droughty soils with low organic matter, resulting in low water hold capacity (Selim, 1984). Additionally, a 72- (2013) and 168-hour (2014) period with no appreciable precipitation was experienced following surface application of treatments. Rawluk et al. (2001) found that a high amount of volatilization begins to occur two to five days following fertilization, depending on soil temperature. By 10 days following fertilization volatilization losses could accumulate to 10 to 25% of applied N. This could also be the reason for the higher yield gain with urease inhibitors in 2014 than 2013. Furthermore, a yield decrease was found for Instinct (numerical not significant) and Nutrisphere (significant) treated plots compared to untreated urea.

In 2013 season at Saint Joseph, Super U and Instinct yielded significantly higher than untreated urea (Table 2.3.1). A similar trend was also noted during the 2014 season, as Instinct was significantly different from the untreated urea ($P \leq 0.0331$). These findings suggest, as opposed to the W location, NO_3^- based losses (leaching and denitrification) were the dominant loss mechanisms at Saint Joseph. In 31day incubation study by Peng et al. (2015) reported relatively lower NO_3^- leaching loss among nitrification inhibitors in comparison to conventional fertilizer. Additionally, in both years the yields from Agrotain Ultra was found to be not significantly different than Nutrisphere, Instinct, or the untreated urea treatments, but was numerically higher than the untreated urea though yielded lower than both Instinct and Super U. This indicated that either minor NH_3^+ occurred or the urease inhibitor delayed the full transformation from urea to NO_3^-. However, a clear explanation for this effect was not illustrated in the results. Yeomans and Bremner (1986) found that urease inhibitors had the potential to decrease denitrification for short-term; however, they noted that this only occurred at high urease inhibitor application rate. Zhengping et al. (2007) also noted the potential of urease inhibitors to minimize denitrification for hydroquinone but not for NBPT, the active ingredient of Agrotain Ultra. For Saint Joseph in 2014, the influence from EENF on corn grain yield was not as drastic, all the EENFs showed no significant differences (Table 2.3.1). Instinct was the only EENF significantly different from the untreated urea. This overall advantage of EENF from the active ingredient, Nitrapyrin in Instinct a nitrification inhibitor noted significant effects both years. The value of nitrification inhibitors have been shown in the literature on high clay content soils, which typically have poor drainage. Randell and Vetsch (2005) reported the grain from Nitrapyrin, in a trial, on high clay content soils. Similar to the current study, they noted the advantage of a nitrification inhibitor in the years with high rainfall.

2.3.2 Biomass Uptake

Estimating crop biomass uptake not only has the potential to provide explanations for yield response to applied N rate and EENF treatments, but also to gives an indication of N availability differences between treatments.

Dharmakeerthi et al. (2006) found high N concentration in the above-ground biomass compared to the N application indicated luxury uptake and therefore high N availability.

Corn biomass N uptake collected at each of the site years was highly variable (Table 2.3.2). No significant differences were found among the N rates or an interaction between N rates and EENF applied. Consequently, a significant impact of EENFs was only noted at one location for one of the two growth stages. During the 2013 and 2014, at Winnsboro, EENF significantly affected biomass N uptake at VT ($P \leq 0.0349$). At Winnsboro in 2013, a significant difference in uptake was noted for the Super U treatments compared to untreated urea, while no other differences were found among the corn. However at Winnsboro in 2014, the following year none of the EENFs significantly differed from the untreated urea. The only significant differences were between Agrotain Ultra and Nutrisphere. The lack of plant N uptake response at various growth stages with EENF has been seen in previous research (Burazco et al., 2014; Halvorson and Bartolo, 2014; Hatfield and Parkin, 2014). Burazco et al. (2014) reported that N uptake in collected prior to side-dress application (V6; 6 true leaf stage) did not differ between Nitrapyrin and the non-treated N fertilizer. Similar findings were noted by Halvorson and Bartolo (2014). They found no significant difference in biomass N uptake between Super U and untreated fertilizer. However, they noted that the application of polymer coated urea did increase uptake compared to untreated urea. Hatfield and Parkin (2014) also reported no significant increase from EENF on biomass N uptake. They theorized that the lack of response to EENF resulted from potential in-season N mineralization from organic matter. The lack of treatment response from both EENF and N rates emphasizes a potential high available N fraction in the soil system.

Table 2.3.2 Effects of Nitrogen fertilizer treatment on plant biomass uptake at V10 and VT growth stage, grain yield, and ANOVA values during 2013 and 2014 for trials in Winnsboro and Saint Joseph, LA.

			V10		VT		Grain Uptake	
Location	Factor	Treatment	2013	2014	2013	2014	2013	2014
			kg ha^{-1}					
Winnsboro	N Rate	Check	94[†]	57	111	145	42	39
		235	196a[‡]	151a	190a	220a	124b	133a
		269	207a	150a	198a	208a	144a	153a
		302	216a	140a	208a	219a	152a	152a
	EENF	Urea	196a[§]	158a	195b	198ab	124c	114b
		Agrotain Ultra	194a	185a	203ab	233a	153ab	211a
		Super U	204a	171a	214a	208ab	162a	184a
		Nutrisphere	203a	163a	200ab	191b	123c	102b
		Instinct	196a	167a	195ab	198ab	137bc	120b
	ANOVA	N Rate	0.7359	0.6141	0.1328	0.7636	0.0067	0.4008
		EENF	0.8024	0.1790	0.0501	0.0349	0.0002	<0.0001
		N Rate x EENF	0.6797	0.6197	0.8673	0.3689	0.4491	0.1557
Saint Joseph	N Rate	Check	-	63.9	-	89.9	5.5	7.1
		236	-	149.6a	-	192.2a	74.4b	120.5b
		302	-	147.6a	-	189.9a	90.6ab	130.3ab
		336	-	153.7a	-	209.0a	109.7a	14.3a
	EENF	Urea	-	148.8a	-	198.9a	79.1b	106.5b
		Agrotain Ultra	-	158.1a	-	207.2a	85.7b	132.3ab
		Super U	-	139.3a	-	194.1a	109.5a	134.7ab
		Nutrisphere	-	152.3a	-	192.6a	83.4b	142.6ab
		Instinct	-	160.8a	-	199.8a	100.1ab	147.5a
	ANOVA	N Rate	-	0.9427[§]	-	0.6673	0.0045	0.0423
		EENF	-	0.6013	-	0.6359	0.0230	0.0582
		N Rate x EENF	-	0.6138	-	0.4384	0.8763	0.0528

† All checks were significant for both locations.

‡N rate based upon location Winnsboro N rates 235, 269, and 302 kg N ha $^{-1}$and Saint Joseph N rates 269, 302, and 336 kg N ha $^{-1}$.

§Lower case letters within columns and factors indicates different level of significance using Tukey adjusted LSD means at α=0.05.

However, unlike Hatfield and Parkin (2014), the two soils in which the study was conducted have been noted to have low OM level; therefore, the high available N could be potentially attributed to both OM mineralization and residual N levels in the soil.

2.3.3 Grain Nitrogen Uptake

While N application rate and EENF resulted in very few significant differences for biomass N uptake, these effects did significantly influence corn grain N uptake (Table 2.3.2). A significant interaction was found between EENF and N rate at Saint Joseph 2014. Based on the responses, each was separately discussed.

The effects of types of inhibitor on corn grain yields were comparable to grain N uptake across all site years. This is expected as grain yield is a main component in determination of grain N uptake. However the similar trend between corn grain N uptake and grain yielded was only seen at Saint Joseph in 2014 (Table 2.3.1. and 2.3.2). For N rate in 2013, Winnsboro resulted in a significant effect in grain N uptake when N application rate increased from 235 to 269 kg N ha^{-1} by 19.9 kg N ha^{-1} ($P < 0.0423$). While corn N uptake increased by 8.1 kg N ha^{-1} between the N rates 269 and 302 kg N ha^{-1} this was not found to be significantly different. In 2014, at Winnsboro no significant differences between any of the N rates applied were found (Table 2.3.2). The lack of response for this treatment could possibly be a result of the available N being a limited factor. The response of grain N uptake was found to be similar between 2013 and 2014 at Saint Joseph (Table 2.3.2). A significant increase in grain N uptake was found between the 269 and 336 kg N ha^{-1}; however, no other significant effects were noted. Schwab and Murdock (2010) found grain yields of corn, fertilized with EENFs were significantly higher than untreated urea at a low N application rate each year during a three year trial. They concluded the greatest increase in yield in these products would be seen in the low to middle range of N application. However, similar reports were not found in this study; the lowest N application rate 269 kg N ha^{-1} had a relatively lower grain N uptake than the other site years (Table 2.3.2).

As each of the site years were significantly influenced by EENF, results showed similarities in the type of EENF products used by location ($P \leq 0.0582$). For Winnsboro in 2013, corn grain from Super U and Agrotain Ultra resulted in significantly higher N uptake compared to the untreated urea (Table 2.3.2). However Instinct had no significant difference from Agrotain Ultra or the untreated urea in grain N uptake. In both years at Winnsboro, the usage of untreated urea resulted in a higher corn grain N uptake than Nutrisphere. Grain N uptake for the untreated urea compared to Nutrisphere in 2013 was 125 and 122 kg N ha^{-1}, respectively, as it was 114 and 102 kg N ha^{-1}, respectively, in 2014. In a six year study highlighting geographic variables including mountains, coastal, and piedmont areas Cahill et al. (2010) found Nutrisphere to be the lowest fertilizer source in percentage of grain N uptake for 50% of the site years. At Saint Joseph in 2013, grain N uptake from Super U was the only EENF that resulted in a significant difference from the untreated urea (Table 2.3.2). However Instinct was not significantly different from the other EENFs or the untreated urea (Table 2.3.2).The following year, 2014 at Winnsboro Agrotain Ultra and Super U significantly differed from the untreated urea, in addition they showed significant differences from both Instinct and Nutrisphere Winnsboro in 2014 showed uptake from. The increased grain N uptake from the urease inhibitor, present in both Super U and Agrotain Ultra on Winnsboro was illustrated in the silty loam texture. Conversely at Saint Joseph, Instinct a nitrification inhibitor was the only EENF significantly different from the untreated urea, although it had no significant difference from Super U (Table 2.3.2). Saint

Joseph validated the strength of the nitrification inhibitor's presence from the products Super U and Instinct on the silty clay soil. Super U, which contains urease and nitrification inhibitor, improved yields over untreated urea across both locations and years. Therefore significant effects in grain N uptake by location were determined based upon the other EENF products in comparison to Super U. The EENFs that had no significant difference from the product Super U, were predominantly more effect product in controlling N loss.

At the Saint Joseph in 2014 location, a significant interaction between N rate and EENF was seen in grain N uptake. Instinct significantly improved grain N uptake at 269 and 302 kg N ha^{-1} in comparison to the lowest N rate applied, 235 kg N ha $^{-1}$ (Table 2.3.2). While the corn grain N uptake from Agrotain applied at the 302 kg N ha^{-1} N rate was significantly greater than the untreated urea at 269 kg N ha^{-1}. Grain N uptake from the EENFs, Super U and Nutrisphere applications at 336 kg N ha^{-1} were significantly greater than the untreated urea at 269 kg N ha^{-1}. While Nutrisphere had greater grain uptake than untreated urea at the lowest N rate applied 269 kg N ha^{-1}. While the lowest and highest N rate applications 269 and 336 kg N ha^{-1} had a significant effect in comparison to the untreated urea while the middle N application 302 kg N ha^{-1}which had no effect.

2.3.4 Nitrogen Use Efficiency

Nitrogen use efficiency in the corn production systems was analyzed using the difference method. Our objective was to determine EENF and N application rate effectiveness based upon NUE response. Research on NUE using various EENF products is limited (Randall and Vetsch, 2004; Ciampitti and Vyn, 2012; Burzaco et al., 2014; Halvorson and Bartolo, 2014; Hatifeld and

Parkin, 2014). The multiple variables in addition to the numerous methods to determine maximize efficiency making this concept complex.

During 2013, N rate effect for Saint Joseph was the only site year in which NUE significantly responded to the varying N rates ($P \leq 0.017$). At Saint Joseph the highest N rate applied, 336 kg N ha^{-1} was significantly different from the 269 and 302 kg N ha N^{-1}rates (Table 2.3.4). However, it should be noted that NUE values for Saint Joseph in 2013 were much lower than all other site years, with NUE in 2013 was 28.1% compared to 47.3% in 2014 at the lowest N rate applied 269 kg N ha^{-1}. Winnsboro in 2013 and both site years at Saint Joseph varied from the expected NUE response, as the highest NUE should be found at the lowest N rate applied. During 2013 at Winnsboro the highest NUE response 42.6% applied at 302 kg N ha^{-1} in comparison to 30.1% and 39.1%, for 2013 and 2014 respectively at 235 kg N ha^{-1}(Table 2.3.4) This could be contributed to the previous crop's residue as sorghum grain and corn planted prior to the study. However these findings are opposite to the reports of Wortman et al. (2011) who noted utilizing agronomic optimal rates of N fertilizer rather than higher rates was a critical point of NUE and other studies. NUE response at Saint Joseph during 2013 was the only site year to show significant differences among the N applications rates applied. The highest N rate 336 kg N ha^{-1} significantly differed from the other N rates applied.

The effects of EENF on NUE for all site years were similar to the EENF product type, as seen in corn grain yield and grain N uptake. Both years, at Winnsboro, corn NUE response between Agrotain Ultra and Super U was not significantly different, though it was significantly different from the untreated urea ($P \leq 0.0003$).

Particularly in 2014, the average NUE from the EENFs, Super U and Agrotain Ultra was twice that of the other EENFs and untreated urea average (62% compared to 29.2%).

Table 2.3.4 Analysis of Nitrogen Use Efficiency responses to Nitrogen rate, Enhanced Efficiency Fertilizers (EENF), and their interaction during 2013 and 2014 trials in Winnsboro and Saint Joseph, LA.

Factor	Treatment	Winnsboro		Saint Joseph	
		2013	2014	2013	2014
		-------------------	%	-------------------	
N rate					
	Below Recommended	39.2a[†]	44.8a	28.1b	47.3a
	Recommended	42.6a	42.8a	30.1b	45.6a
	Above Recommended	40.9a	39.6a	37.5a	46.7a
EENF					
	Urea	34.8c[‡]	31.6b	27.1 c	36.4 b
	Agrotain Ultra	46.4ab	63.5a	29.5 bc	46.9 ab
	Super U	50.0a	60.5a	38.2 a	46.9 ab
	Nutrisphere	33.9c	26.5b	28.3 c	50.4 ab
	Instinct	39.3bc	29.8b	35.4 ab	52.5 a
ANOVA					
	N rate	0.4100	0.2805	0.0017	0.8298
	EENF	0.0003	<.0001	0.0003	0.0572
	N rate X EENF	0.4673	0.7024	0.5193	0.0500

† Below recommended, recommended, and above recommended N rate based upon location Winnsboro N rates 235, 269, and 302 kg N ha $^{-1}$and Saint Joseph N rates 269, 302, and 336 kg N ha $^{-1}$.

‡ Lower case letters within columns and factor indicates different level of significance using Tukey adjusted LSD means at α=0.05 level.

However NUE effects at Winnsboro showed Nutrisphere, Instinct, and the untreated urea were found to have no significant differences (Table 2.3.4). The benefit of the urease inhibitor in both products minimizing N loss through volatilization increased NUE on silt loam soil. Noted as an essential indicator, Hatfield and Parkin (2014) observed higher NUE was a direct result of greater uptake that occurs often in modern corn hybrids during the reproductive stage of plants. As previously seen in 2013 at Saint Joseph, results showed corn NUE effects from Super U were significantly different from the untreated urea (Table 2.3.4). For Saint Joseph in 2013, NUE in corn production showed Super U differed significantly from the Agrotain Ultra, Nutrisphere, and untreated urea, while Instinct was not significantly different from the Super U or Agrotain Ultra. Saint Joseph in 2014 resulted in a significant interaction between untreated urea and Instinct. Each of the EENFs had no significant difference from each other (Table 2.3.4).

2.4 Conclusions

Nitrogen management is critical to increase N efficiency and optimum crop production. Many factors affect the performance of EENFs; this study chooses to evaluate soil, climate, and N application rates on corn grain yielded, N uptake, and NUE. Varying sites with distinct soil types were clear factors that affected the performance of the EENFs over two trial years. The gain from the urease inhibitor was evident possibly due to unfavorable environmental conditions following application in the silty loam soils. While the nitrification inhibitor present increased grain yield at the clay site. Analysis during early plant growth stages was overall inclusive, however VT did indicate a critical time period of N uptake in the crop while utilizing the fertilizer. NUE in the corn increased 81% using the EENFs in comparison to untreated urea across both sites. All the findings were subject to soil types. In agreement with the current literature the active ingredients at the NBPT, Nitrapyrin, and DCD were found to be effective active ingredients. These can be utilized to increase agronomic production and efficiency of nutrients.

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Chapter 3 Enhanced Efficiency Nitrogen Fertilizer's Nitrogen Transformation in the Greenhouse

3.1 Introduction

Nitrogen has the potential to be lost as it transitions into various forms in the soil (Krajewska, 2009). Synthetic N fertilizer is often lost through volatilization (NH_4^+), denitrification (N_2, N_2O), leaching (NO_3^-), and surface runoff (Bronson et al., 2004; Mosier et al., 2006). Low prices are the main advantage for using urea, the primary source inorganic N fertilizer. While subjectively high N loss is the disadvantage of the fertilizer (FAO, 2006). A number of factors, including high N fertilizer application generally contribute to low NUE (Raun and Johnson, 1999). Soares et al. (2012) reported up to 60% of the applied N using urea can be lost to environmental conditions. Some of the many management factors that affect fertilizer movement are water management and incorporation (Rochette et al., 2001; Dawar et al., 2011). Reduced agronomic potential and economic loss are some of the major effects from these inadequacies in N fertilizer.

Effective use of urea would result in the fertilizer being rapidly converted to NH_4^+ and remaining in the soil system. Urease, a naturally occurring enzyme, catalyzes urea into carbamate which decomposes into biocarbamate and NH_4^+ (Frame et al., 2012; Ciurli et al., 1999). During this process, soil pH increases, influencing further transformation of $NH_4^+$to NH_3^+ (Cirurli et al., 1999; Kissel et al., 2008; Krajewska, 2009). The NH_3^+ left on or near the soil surface can be lost into the atmosphere due to volatilization. Factors such as high temperatures, soil texture, crop residue, and organic carbon can elevate the activity of urease which can lead to higher rates of NH_3^+ volatilization (Antisari et al., 1996). Once in the NH_4^+ form, nitrification occurs and converts NH_4^+ to NO_3^-. However the NO_3^- form is highly mobile and subject to loss through leaching and denitrification.

To address these inefficiencies and reduce N loss, chemical compounds have been formulated as addition to fertilizers to inhibit N transformation (Peng et al., 2015). These products, which can be coated on or incorporated into urea based fertilizers, are known as enhanced efficiency N fertilizers (EENF). The EENFs provide temporary control of N transformation in the soil. Composed into categories including stabilizer inhibitors, control release and, slow release fertilizers EENFs are able to increase N efficiency, therefore allowing greater crop uptake (Trenkel, 1997). One type of EENFs are urease inhibitors which work to inhibit the urease enzyme, allowing fertilizer time needed for adequate conditions for plant uptake. Another type of EENF, are nitrification inhibitors, which control and maintain the NH_4^+ form for a longer period of time reducing loss through leaching and denitrification (Burazco et al., 2014). The final category of EENFs includes slow released fertilizers which slowly, diffuse fertilizer into the soil over a period of time. While these coated products potentially minimize N loss these EENFs do not modify N transformation in the soil systems. The fertilizer in these products is urea enclosed in a polymer coating. The rate of dispersion is dependent on soil temperature and moisture (Peng et al., 2015).

Through the incorporation of EENFs, a great potential exist to decrease N loss (Bundy and Bremner, 1973; Halvorson et al., 2014). However, the advantages depend on many factors including time, water, and temperature, which create complex interactions (Keeney, 1980). Carmona et al. (1990) found in both a field and laboratory study that N-(n-butyl) phosphoric triamide (NBPT), a urease inhibitor, minimized NH_3^+ loss through volatilization by 37.3 % in comparison to the untreated urea in treatments across multiple soil textures and tillage systems.

Many other studies have confirmed that NBPT has been the most effective and most common urease inhibitor (Brynes and Amberger, 1989; Chai and Bremner, 1987; Wolt, 2004; Frame et al., 2012). However positive results have not been consistent. Antisari et al. (1996) concluded that reduction from volatilization varied by soil and the application rates applied of the product. For controlling denitrification and leachate based losses, one of the most effective nitrification inhibitors is Nitrapyrin (Wolt, 2004; Soares et al., 2012; Burazco et al., 2014). In a 31 day incubation study Peng et al. (2015) reported relatively lower NO_3-N leaching loss among nitrification inhibitors, Nitrapyrin and maleic-itaconic acid copolymer in comparison to untreated fertilizer. In a field study evaluating corn, Burazco et al. (2014) found a positive effect on grain yield using Nitrapyrin, but concluded that post- application weather was a factor for variability in other agronomic findings including plant biomass and NUE. Varying from the agronomic uses of urease and nitrification inhibitors, slow release products have primarily been used on turf. Although it has been recently shown some positive increases yield in row crops such as corn, wheat, and rice (Peng et al., 2015). In a greenhouse experiment, Mikkelsen et al. (1994) found higher NO_3^- leaching loss from the untreated fertilizer applications compared to the coated slow-release fertilizers. Similarly, Wang and Alva (1996) reported N loss on sandy soils were 58% lower using slow release fertilizer versus NH_4^+ fertilizer. Nelson et al. (2008) suggested slow release products like ESN™ (Environmentally Smart Nitrogen) showed promising results, in compared to urea. However Nelson et al. (2008) noted that conditional requirements were needed for effective results; their study indicated the polymer coated urea had no effect on corn yield the following year. However, NO_3^- concentrations had been reduced, which in turn could potentially reduce leaching (Noellsch et al., 2009). Nevertheless, EENFs as an input can maximize the NUE of fertilizer in the soil system.

The objectives of this study were 1) to evaluate the effect of different EENFs on the rate of transformation of applied N fertilizer to NH_4^+ and NO_3^- in the soil system over multiple durations of time, and 2) to evaluate the potential of these EENFs in minimizing N losses from soil.

3.2 Materials and Methods

Two separate greenhouse experiments were conducted at Louisiana State University greenhouse in Baton Rouge, LA. Greenhouse experiment 1 (G1) was a 7 day study, while the Greenhouse experiment 2 (G2) lasted a duration of 50 days. Gigger silt loam (fine-silty, mixed, active, Thermic Typic Fragiudalf) was used for both studies; the baseline samples analyzed prior to the trials showed in a pH of 6.6, and a texture consisting of 8.4% sand, 54.3% silt, and 37.3% clay. These soils were collected from the Macon Ridge Research Station in Winnsboro, LA (32˚ 8’29.11”N and 91˚ 42’33.80”W). Soils were obtained from the top 15 cm of the soil and transported to the greenhouse for preparation. Soils were air dried for two days, and sieved (2 mm). Soil was added to plastic pots (11 and 9.5 cm in diameter on the top and bottom, respectively and 9.9 cm in height). Plastic bags were used to line the inside the pots to create a close system, eliminating any leaching prior to the addition of the soil. Five EENF products were applied to the soil and evaluated. The EENFs included urease inhibitors, nitrification inhibitors, combination of urease and nitrification inhibitors, and a slow release fertilizer. The urease inhibitor evaluated was Agrotain Ultra (NBPT [N-(*n*-butyl)thiophosphoric triamide]; Koch Fertilizer LLC, Wichita, KS). Two nitrification inhibitors evaluated included: Instinct (Nitrapyrin [2-chloro-6-(trichloromethyl) pyridine; Dow AgroScience LLC; Indianapolis, IN) and Nutrisphere (partial Ca salt of maleic-itaconic copolymer; Specialty Fertilizer Products LLC, Leawood, KS). While the EENF with both urease and nitrification inhibitor evaluated was Super

U (NBPT and DCD dicyandiamide [2-Cyanoguanidine]; Koch Fertilizer LLC, Wichita, KS). The slow release fertilizer, utilized was Environmentally Smart Nitrogen (ESN); Agrium Advanced Technologies, Calgary, Canada). All inhibitor rates were applied in accordance to individual labels. In addition, untreated urea (46%) was included as a production standard to compare evaluations.

An N rate of 269 kg N ha^{-1} was used for each fertilizer product based on the LSU AgCenter's current recommendation for the location the soil was collected (LSU AgCenter, 2014). The two greenhouse trials were evaluated in a completely randomized block design with four replications for G1 and six replications for G2. No additional nutrients were added to the soils prior to fertilization or beyond N treatments. The two trials were conducted in early fall and mid spring, in the same greenhouse. During both experiments, samples were irrigated manually daily for the G1 and every other day for G2 while the greenhouse remained at 26 ˚C.

Fertilizer was broadcast on top of each of the pots prior to the first day of the experiment. For G1, soil samples were removed daily from the greenhouse for analysis, while during G2 samples were taken every 10 days. Samples were transported from the greenhouse to the laboratory in paper bags and immediately oven dried at 48˚C for 24 hours. Samples were ground to pass a 2 mm sieve and oven dried for two hours prior to analysis. The 2M KCl soil extraction method was used to analyze the amount of NH_4^+-N and NO_3^--N with a Lachat QuickChem Automated Ion Analyzer (Lachat Instruments, Loveland, CO) (Keeny, and Nelson, 1987). The samples were analyzed for total N using a Vario El Cube CHNS model (Elementar Americas Inc. Mountlaurel, NJ) (Colombo and Giazzi, 1982).

Analysis of variance was conducted using the mixed procedure (SAS, 9.4, SAS Institute Cary, NC) on the concentrations of NH_4^+, NO_3^-, and their sum using product, day, and their interaction as factors. Post-hoc analyses for the main and interactive effects were analyzed using a Tukey adjustment for protected LSD means. When interactive effects were noted, a slice modifier was implemented. All significant comparisons were made at a 0.05 probability level, while standard error was set ±1 level.

3.3 Results and Discussion

The factors in G2 which included the Day, EENF, and their interaction had significant effect on both soil NH_4^+ and NO_3^- content ($P \leq 0.0123$). In G1, a significant interactive effect between EENF and days was observed for NH_4^+, but not for the NO_3^-. For G2, a significant interaction between EENF was also observed. Furthermore, the percentage of total inorganic N for both trials (G1 and G2) were similar, showing little influence by the products used or the days analyzed. The significant interactive effects will be discussed by product and day, while the other effects will be discussed separately.

3.3.1 Greenhouse 1 (7 Day Study)

There was a significant effect for Day x EENF interaction on NH_4^+ concentration (Table 3.3.1.1). The greatest accumulation of NH_4^+ in the soil occurred on D2, D3, and D4 in four of the six products; untreated urea, Nutrisphere, Agrotain Ultra, and Super U (Figure 3.3.1.1). On D1, the first day after treatments were applied to the soil, the concentration of NH_4^+ ranged from 23-176 mg NH_4^+ kg^{-1}. It has been reported by several researches that over half of the total N loss through ammonia volatilization occurs in as few as ≤10 days following fertilizer application (Keller and Mengel, 1986; Palma et al., 1998, Faria et al., 2013; Peng et al., 2015). At D1, the

Table 3.3.1.1 Analysis of NH_4^+ concentration in greenhouse 1 (7 Day) based on the effects by day, Enhanced Efficiency Nitrogen Fertilizers (EENF), and their interaction.

Factor	Treatment	Day						
		1	2	3	4	5	6	7
		--------------------------------mg NH_4^+ kg^{-1}-----------------------------------						
EENF								
	Urea	63ab†	78a	95a	158ab	104a	113a	186a
	Agrotain Ultra	47b	79a	70a	71ab	50a	49a	65ab
	Super U	43b	76a	129a	60b	64a	72a	91ab
	Nutrisphere	50b	134a	139a	213a	83a	133a	105ab
	Instinct	176a	96a	129a	111ab	96a	92a	123 ab
	ESN	23 b	16a	22a	23b	20a	23a	26b
ANOVA								
	Day	<0.0129						
	EENF	<0.0001						
	EENF*Day	0.0044						

†Lower case letters within columns and factor indicates different level of significant interaction between EENF*Day using Tukey adjusted LSD means at α=0.05 level.

Instinct treated soil differed significantly from the other EENFs on D1, although it was not significantly different from the untreated urea (P=0.0044) (Table 3.3.1.1).The decrease in NH_4^+ in the Instinct treated soil could have been a result of rapid hydrolysis of the fertilizer increasing NH_4^+ concentration even in the presence of the nitrification inhibitor creating more readily available NH_3^+ for loss (Peng et al., 2015). Gioacchini et al. (2002) and Zaman et al. (2008) found due to the longer extent of NH_4^+ in the soil, nitrification inhibitors resulted in increased NH_3^+ loss. On D2, the soil treated with Nutrisphere drastically increased in NH_4^+ concentration surpassing the Instinct treated soil. Only the soil treated with Nutrisphere maintained the largest concentration of NH_4^+, for the next two days (D2-D4), while soils treated with the rest of the products had declined (Table 3.3.1.1)

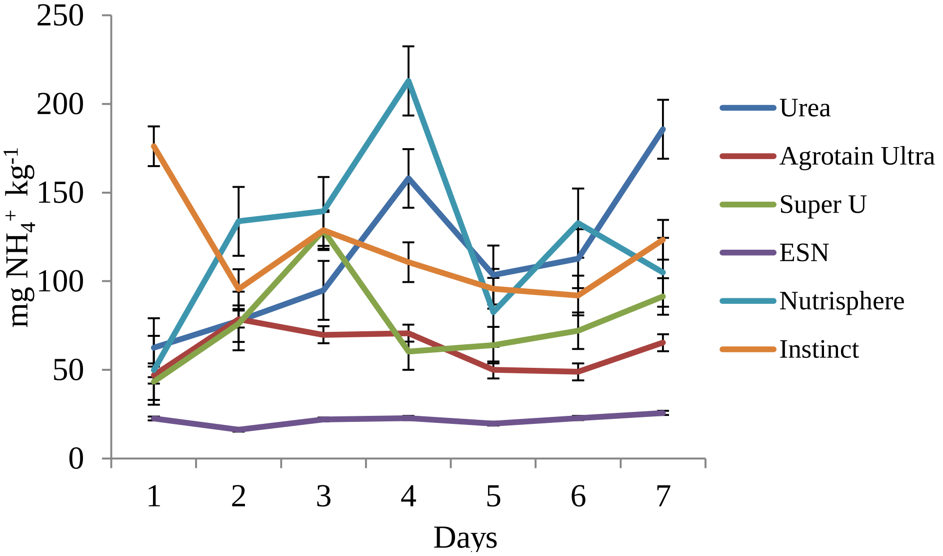

Figure 3.3.1.1 Changes on ammonium content in the soil using Enhanced Efficiency Nitrogen Fertilizers with in a 7 day greenhouse trial. Error bars are ± 1 standard error.

While no significant difference was seen in the NH_4^+ concentration during D2 or D3, on D4, a new trend emerged. At D4 the soil treated with Nutrisphere reached its peak concentration at 213 mg NH_4^+ kg^{-1}, the untreated urea similarly followed its upward trend (Figure 3.3.1.1). On D4 Nutrisphere, Instinct and the untreated urea were significantly different all other treatments, though not significantly different from each other (Table 3.3.1.1).

No significant differences were noted on D5; however the soil applied with untreated urea did begin to increase in the amount of NH_4^+ found in the soil. The movement of the untreated urea compared to the EENFs was more frequent, particularly during the final three days of the study when the soil moisture content was possibly the highest (Figure 3.3.1.1). Soil moisture was relatively high as the soils were maintained at field capacity during the entire study. This precipitation reduced the risk of NH_3^+ loss through volatilization (Harper 1983; Bouwmesster et al. 1985; Ferguson and Kissel 1986). Since moisture was a critical factor, the trial was designed to have a routine water regime in addition to consistent climatic settings in the greenhouse. Meyer et al. (1961) found moisture decreased volatilization within two days following precipitation greater than 2 cm. Although Nutrisphere treated soil surpassed the untreated urea again on D6, the untreated urea ended the study on D7 with the highest concentration of NH_4^+ present (Figure 3.3.1.1). The untreated urea was significantly higher than the ESN (Table 3.3.1.1). Throughout the entire 7 days the ESN treated soil was consistently low in NH_4^+concentration compared to the other products. Environmentally Smart Nitrogen demonstrated its potential in maintain low NH_4^+ concentration during the first critical days of N volatilization loss. The product was designed to disperse the fertilizer over a period of time in small increments to prevent accumulation of $NH_4^{+.}$

No significant interaction between product and day was found for NO_3^-. The untreated urea had the largest accumulation of NO_3^- at the beginning and end of the study (Figure 3.3.1.2). This was to be expected as the inhibitor products were intended to inhibit or slow the transformation of N. Nutrisphere had the lowest NO_3^- concentration on D1 and untreated Urea on D7. However, the effect by day showed that the NO_3^- concentrations at D5 and D7 were significantly different (Figure 3.3.1.2).

This suggests more effects from the NO_3^- could have possibly occurred over a longer period of time (Peng et al., 2015). The untreated urea differed significantly from the Nutrisphere, Agrotain Ultra and ESN (Table 3.3.1.2).

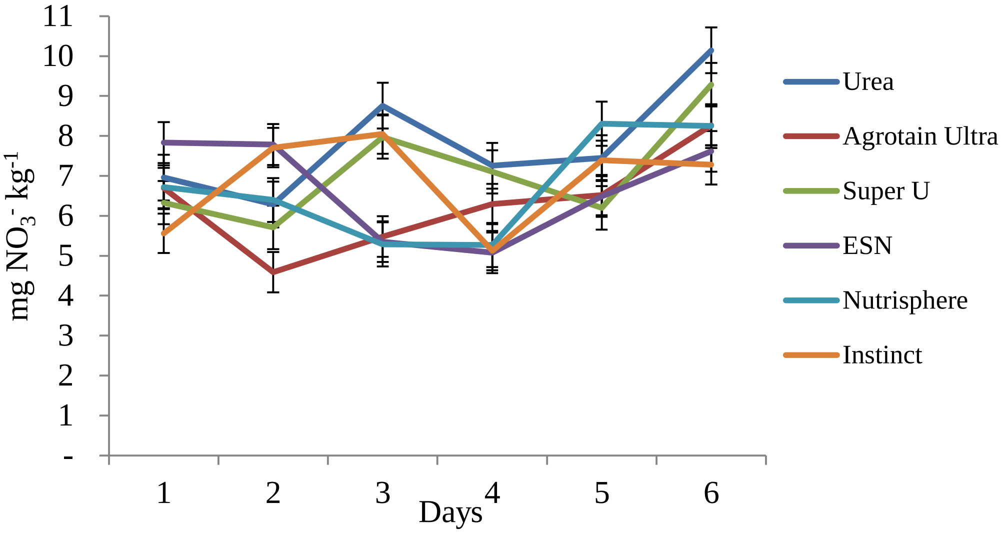

Figure 3.3.1.2 Changes on nitrate content in the soil using Enhanced Efficiency Nitrogen Fertilizers with in a 7 day greenhouse trial. Error bars are ± 1 standard error.

These stabilizer inhibitors and slow release products active ingredients are Nitrapyrin, NBPT, and a polymer coating respectively. In agreement with other studies the potential difference in fertilizer source and possible reduction in N loss shows the effectiveness of EENF products in the trial (Halvorson et al., 2014; Peng et al., 2015). Malhi and McGill (1982) noted the optimum temperature for nitrifying bacteria in Alberta soils was 20°C; when temperature values were in excess of 30°C, nitrification nearly ceased. The study kept the greenhouse setting at 26°C; however soil temperature and moisture data was not collected. Overall results showed the inhibitors significantly reduced N transformation more than the untreated urea in environmental conditions prone to loss.

Table 3.3.1.2 Analysis of NO_3^- concentration in greenhouse 1 (7 Day) based on the effects by day, Enhanced Efficiency Nitrogen Fertilizers (EENF), and their interaction.

Main Factor	Sub Factor	mg NO_3^- kg^{-1}
Day		
	1	6.5ab
	2	6.7ab
	3	6.4ab
	4	6.8ab
	5	6.0b
	6	7.1ab
	7	8.5a
	***P*-value**	**0.0147**
EENF		
	Urea	8.0a
	Agrotain Ultra	6.4b
	Super U	6.9ab
	Nutrisphere	6.3b
	Instinct	6.8ab
	ESN	6.7b
	***P*-value**	**0.0123**
EENF*Day	***P-value***	**0.2044**

†Lower case letters within column and row indicate different level of significance by Day and EENF, respectively using Tukey adjusted LSD means at α=0.05 level.

3.3.2 Greenhouse 2 (50 Day Study)

The NH_4^+ concentration in the soil from the majority of products consistently decreased; Super U, Nutrisphere, the untreated urea, and Instinct (Figure 3.3.2.1). The ESN treated soil had significantly lower concentration of NH_4^+ in comparison to the other EENFs and the untreated urea on D10 (Table 3.3.2.1). However on D20, ESN treated soil was significantly different from the Agrotain Ultra, Super U, Instinct, and untreated urea.

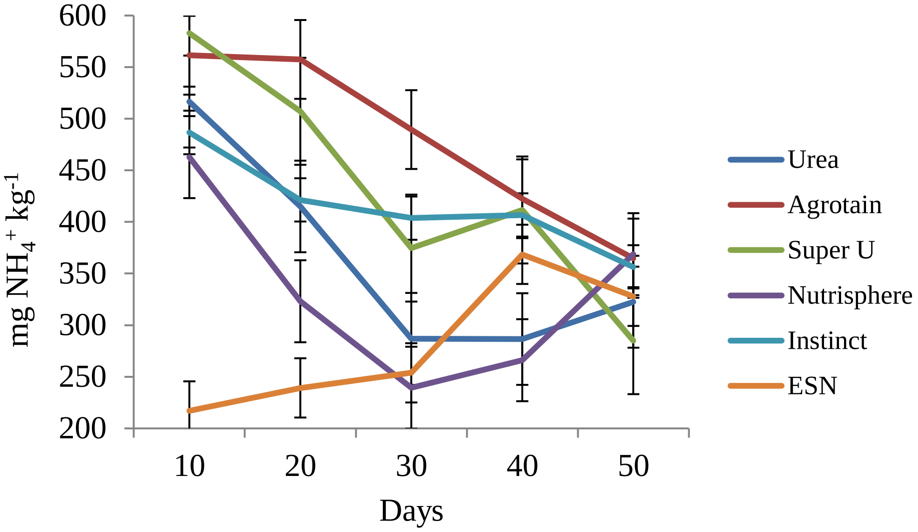

Figure 3.3.2.1 Changes on ammonium content in the soil using Enhanced Efficiency Nitrogen Fertilizers with in a 50 day greenhouse trial. Error bars are ± 1 standard error.

In a 14 day study using the active ingredient NBPT, compared to untreated urea indicated over 50% of the total N loss was directly lost from NH_3^+ volatilization within 24 hours in 26˚C laboratory conditions (Frame et al., 2012).

On D30, the soil treated with Agrotain Ultra differed significantly from those with untreated urea, ESN, and Nutrisphere, although there was no significant difference observed from Instinct or Super U (Table 3.3.2.1). Frame et al. (2012) quantified NH_3^+ loss using the EENFs Agrotain and Arborite Ag, these were found to be the highest on D4 and D5 at 19% of the applied N of a 14 day study. The last twenty days of the analysis showed more NH_4^+ transformation began to occur among the products. The means for each of the product's soil NH_4^+concentration became closer together as the days progressed. No significant differences were found for D40 and D50.

Table 3.3.2.1 Analysis of NH_4^+ concentration in greenhouse 2 (50 Day) based on the effects by day, Enhanced Efficiency Nitrogen Fertilizers (EENF), and their interaction.

Factor	Treatment	Day				
		10	20	30	40	50
		---------------------------------mg NH_4^+ kg^{-1}-----------------------------				
EENF						
	Urea	517a†	415ab	286bc	287a	323a
	Agrotain Ultra	562a	557a	489a	422a	365a
	Super U	583a	507a	374ab	412a	285a
	Nutrisphere	462a	323bc	239b	266a	369a
	Instinct	486a	421ab	403ab	407a	356a
	ESN	217b	239c	254b	369a	328a
ANOVA						
	Day	<0.0001				
	EENF	<0.0001				
	EENF*Day	<0.0001				

†Lower case letters within columns and factor indicates different level of significant interaction between EENF*Day using Tukey adjusted LSD means (0.05) level.

Day was noted in G2 as an effect, and no significant differences on NO_3^- concentration among the EENFs were noted for the first thirty days (Table 3.3.2.2). By D40, the soil treated with Instinct was significantly different from the other EENF products. The final analysis conducted on D50 showed Agrotain Ultra treated soil had the highest concentration of NO_3^- and was significantly different from Instinct and Super U nitrification inhibitors. Soil moisture was further from field capacity during the beginning of the study, which could be the cause of the lack of response.

Table 3.3.2.2 Analysis of NO_3^- concentration in greenhouse 2 (50 Day) based on the effects by day, Enhanced Efficiency Nitrogen Fertilizers (EENF), and their interaction.

Factor	Treatment	Day				
		10	20	30	40	50
		------------------------------mg NO_3^- kg^{-1}---------------------------------				
EENF						
	Urea	159a†	322a	303a	388a	394ab
	Agrotain Ultra	216a	223a	296a	423a	507a
	Super U	180a	251a	265a	306a	352b
	Nutrisphere	219a	361a	310a	439a	378ab
	Instinct	161a	214a	282a	291b	347b
	ESN	165a	253a	278a	380a	377ab
ANOVA						
	Day	<0.0001				
	EENF	<0.0001				
	EENF*Day	0.0123				

†Lower case letters within columns and factor indicates different level of significant interaction between EENF*Day using Tukey adjusted LSD means (0.05) level.

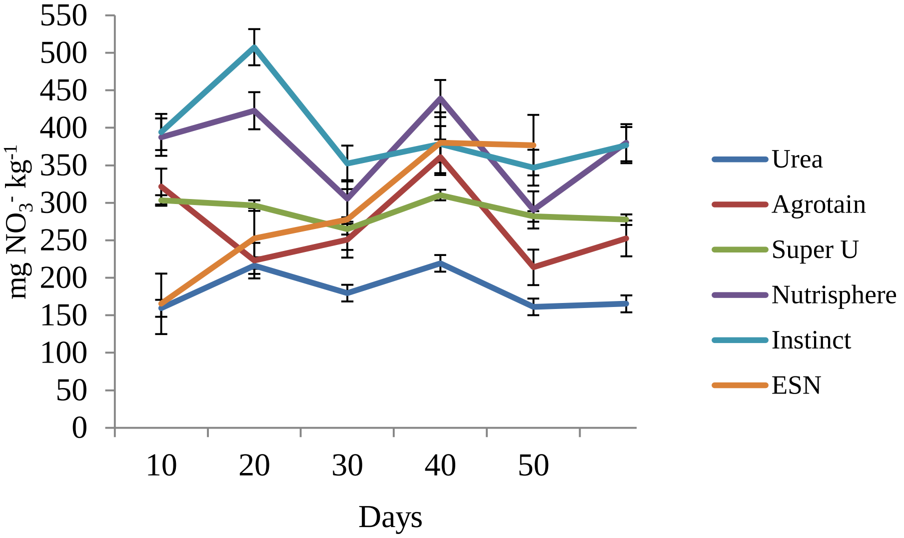

Figure 3.3.2.2 Changes on nitrate content in the soil using Enhanced Efficiency Nitrogen Fertilizers with in a 50 day greenhouse trial. Error bars are ± 1 standard error.

Maharjan et al. (2014) found that a critical factor in some of the NO_3^- based losses were the timing and intensity of irrigation. The water regime was every other day over a 50 day period in humid and hot temperatures. Soil moisture was associated by Malhi and Mc Gill (1982) for being conducive to microbial nitrification. The accumulation of precipitation over the 30 days could be the reason behind the lack of response during the beginning of the study.

3.3.3 Total Inorganic Nitrogen

For both G1 and G2 the effect of EENFs, day, and their interaction on total inorganic N content of the soil was not significant. Saninju et al. (2014) associated higher soil total inorganic N concentration to the greater NH_4^+ content present in an irrigated experiment for conservation reserve program.

3.4 Conclusions

In the 50 day study (G2) results were much clearer in comparison to the seven day study (G1) for NO_3^-. Both experiments were used to analyze the transformation of EENFs in the soil. A majority of the soil treated with the EENFs maintained low NH_4^+concentrations within the 7-day period. Water and a controlled setting were environmental factors that influenced the experiments effects possibly dispersing the fertilizer’s through the soil system. The NO_3^- concentration in the soil measured in G1 suggests that some EENFs rapidly converted NH_4^+ to NO_3^- which was easily lost via denitrification. However in G2, the EENFs delayed the accumulation of NO_3^- during the initial few days of the study. Only after forty days post application did NO_3^- concentration increased. In a field set up, these products particularly Instinct, Agrotain Ultra, and Super U had adequate transformation time allowing plant available N to move within the soil system and eventually taken up by the plant. The findings present reasonable data that the products are beneficial at maximizing N forms desirable to increase NUE in the soil systems.

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Chapter 4 Conclusions

The objective of the two studies was to evaluate the effectiveness of multiple EENF products on corn productivity and N management in the Mid-South. Using various soil textures and N rates, trials emulated agronomic management practices. Previous research has shown the advantages of these EENFs have been inconsistent. Studies have tested the EENFs on a number of crops throughout multiple trials. The results have varied as environmental factors and agronomic management influence their increase or decrease in yield. Some findings have shown no significant gain or loss using the products in comparison to urea. However, our findings differed on a wide range of parameters.

Yield, N uptake, and NUE increased from using EENFs. Soil texture and environmental conditions seemed to be some of the factors influencing the type of EENF product that was beneficial for the system. On the silty loam soil, the urease inhibitor products, targeted to minimizing volatilization showed great potential in corn production. While the nitrification inhibitor showed the most increase in yield on the clay soils as the products reduce N loss through denitrification and leaching. Corn grain yield was significantly increased using EENFs compared to untreated urea (average of 1.54 Mg ha^{-1} Winnsboro, LA and 1.30 Mg ha^{-1} Saint Joseph, LA). When applied at the recommended N rate, Super U, a urease and nitrification inhibitor , improved yields by nearly 3.0 Mg ha^{-1}. Despite inconclusive responses in uptake during the mid to late growth stages (V10-R1), significant effects were found among EENF and the interaction between N rate and EENF at one location. This indicated, unlike with corn yields, grain N uptake response to N rate varied with different EENFs at a single location. It was also concluded using these products at the recommended middle N application rate had optimal effects on corn grain yield.

The greenhouse study illustrated another concept behind reduced N loss using the EENFs. Based upon the N concentration over a period of time, the value of slower transformation of the N, specifically into NH_4^+ and NO_3^- was evident. This was validated in the G1 study, as the urease inhibitor and slow release products had the lowest accumulation of NH_4^+ during the 7 day study. The water added to the pots in the 50 day study indicated the effects of larger water regimes drastically increased the NO_3^-, specifically for the nitrification inhibitor products. The slow release product, ESN had the lowest accumulation of NH_4^+ during the first 30 days of the 50 day study. While Instinct, a nitrification inhibitor, reduced NO_3^- concentrations and Agrotain Ultra a urease inhibitor maintained low NH_4^+ concentration compared to the other products. Based on these studies, EENFs have the potential to be effective in increasing production, while reducing N loss and therefore increasing NUE in corn production. Further research can be drawn from this study, particularly expanding on many environmental effects on EENFs effectiveness. The amount of N lost can be limited by proper understanding of the timing of N uptake and the factors affecting N loss.

Appendix

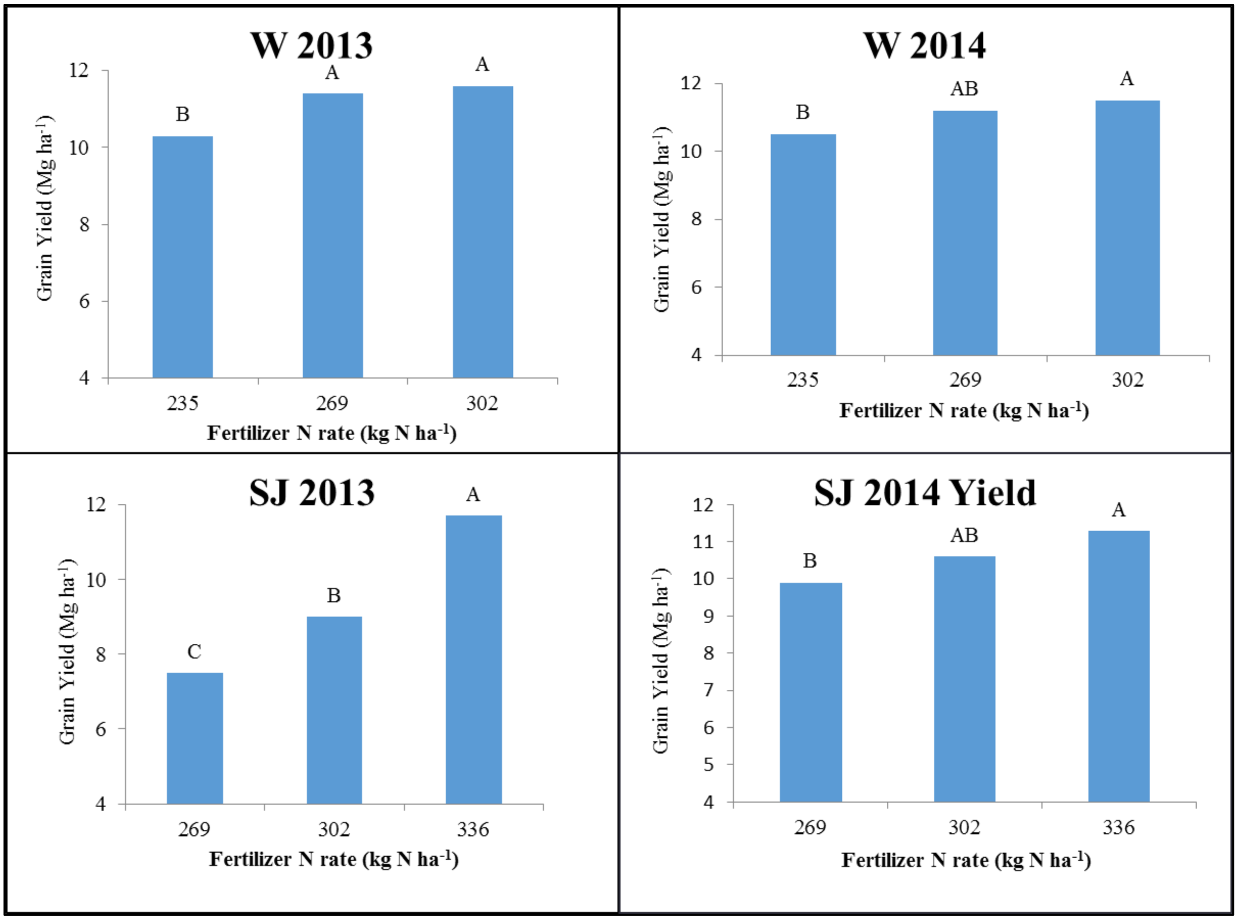

Figure A.1Corn grain yield response to three specified nitrogen rates applied at Winnsboro and St. Joseph, LA in 2013 and 2014, with letters representing upper case letter indicate different levels of significance using Tukey adjusted LSD means (0.05) level.

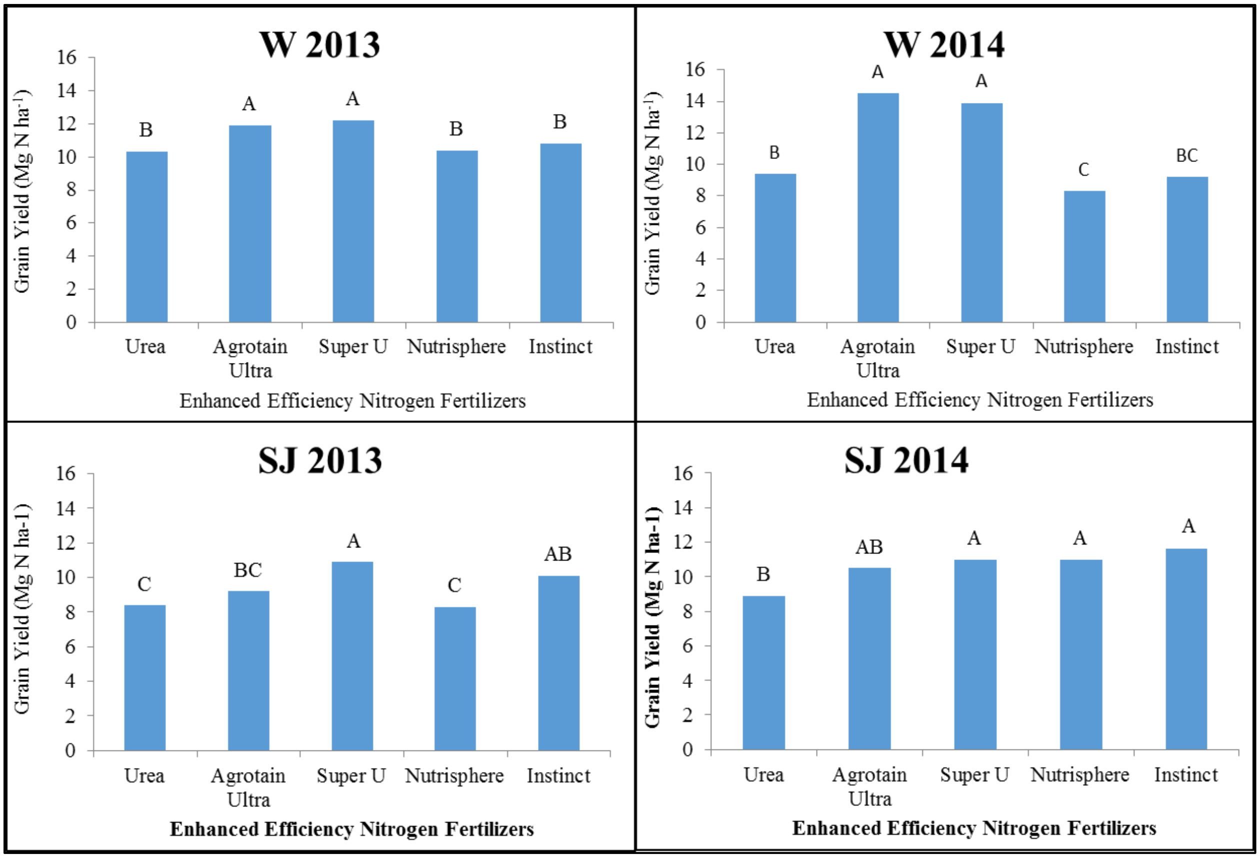

Figure A.2 Corn grain yield response to Enhanced Efficiency Nitrogen Fertilizer (EENF) at three specified Nitrogen rates at Winnsboro and St. Joseph, LA in 2013 and 2014, with letters representing upper case letter indicate different levels of significance using Tukey adjusted LSD means (0.05) level.

Vita

Shanice Jones was born in Georgia. Upon graduating high school she attended Tuskegee University, in Tuskegee Alabama. In May 2013 she obtained her bachelors of Science degree in Environmental Science. June 2013 Ms. Jones began her tenure at Louisiana State University in the School of Plant, Environmental and Soil Sciences studying in the agronomy department.