1-1-2013

Evaluation of Nitrification and Methods to Minimize Denitrification Loss for Rice (Oryza Sativa L.) on Mississippi Alluvial Plain Soils

Paxton Wayne Fitts

Evaluation of nitrification and methods to minimize denitrification loss for rice (Oryza sativa L.) on Mississippi alluvial plain soils

By

Paxton Wayne Fitts

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Masters of Science
in Agronomy
in the Department of Plant and Soil Sciences

Mississippi State, Mississippi

May 2013

Evaluation of nitrification and methods to minimize denitrification loss for rice (Oryza sativa L.) on Mississippi alluvial plain soils

By

Paxton Wayne Fitts

Approved:

Timothy W. Walker
Research Professor of
Plant and Soil Sciences
(Major Professor)

L. Jason Krutz
Associate Extension/Research Professor
of Plant and Soil Sciences
(Committee Member)

Bobby R. Golden
Assistant Research Professor of
Plant and Soil Sciences
(Committee Member)

Jac J. Varco
Professor of Plant and Soil Sciences
(Committee Member)

Jeffrey Gore
Assistant Research Professor of
Biochemistry, Molecular Biology,
Entomology, and Plant Pathology
(Committee Member)

William L. Kingery
Professor of Plant and Soil Sciences
(Graduate Coordinator)

J. Mike Phillips
Professor and Department Head
of Plant and Soil Sciences

George Hopper
Dean
College of Agriculture and Life Sciences

Name: Paxton Wayne Fitts

Date of Degree: May 10, 2013

Institution: Mississippi State University

Major Field: Agronomy

Major Professor: Dr. Timothy W. Walker

Title of Study: Evaluation of nitrification and methods to minimize denitrification loss for rice (Oryza sativa L.) on Mississippi alluvial plain soils

Pages in Study: 56

Candidate for Degree of Masters of Science

Minimal studies have evaluated nitrification and subsequent denitrification for soils where rice is produced in the delayed-flood system. Laboratory and field experiments were conducted at USDA-ARS and the Delta Research and Extension Center in Stoneville, MS to quantify the nitrification potential of southern USA soils, and evaluate nitrogen amendments aimed to reduce nitrification rates on clay soils. The Sharkey clay soil at Stoneville, MS was one of the soils with the greatest nitrification potential. Dicyandiamide (DCD) increased the number of days that half the total recovered inorganic–N was in the ammonium–N form (half-life) by approximately 3-fold and 18% when compared to non-amended urea in the laboratory and field, respectively. Results suggested that nitrapyrin was not an effective nitrification inhibitor in southern soil. Coated urea (43%N) applied 12 days before flood establishment (dbf) was most successful at reducing nitrification resulting in yield comparable to urea applied one dbf.

DEDICATION

I would like to dedicate this thesis to my father James W. Fitts. I would like to thank him first of all for his love for the Lord Jesus Christ, and how that influenced my relationship with Christ as well. Everyone he comes into contact with hears the gospel and how to become a part of God's family. He sets an excellent example of what it means to be a Christian and to love others. I would also like to thank him for his service to our country. He served in the United States Army from September 5, 1944 to March 16, 1946. Drafted at eighteen years old, he was a private in the 2nd armored division, 67th Armored Regiment "Hell on Wheels", E Company. He participated in campaigns in England, France, Belgium, and Germany including the Battle of the Bulge serving as a tank gunner. He received an ETO ribbon with 2 battle stars, a purple heart, and a good conduct medal. He has taught me that freedom is not free, and I thank him and all the other United States military service members who have shed blood for this great country we live in. Lastly, I would like to thank him for the example he has been in my life on how to be a husband to my wife and a father to my children. He has been married to my mother Jeannie S. Fitts for over sixty years. He has five children, sixteen grandchildren, and four great grandchildren. Thank you Dad for the life you've lived, the example you've been, and the love you have for Christ, our country, our family, and me.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my Lord and Savior Jesus Christ for His grace, mercy, and love. He has blessed me with family and friends that have helped me on this journey. Only through Him are all of my accomplishments possible. I am truly thankful for my wife Lacey Fitts for believing in me when I don't believe in myself. Thank you for your love, support, and faith in me. I could not have achieved this goal without you by my side. Thanks also to my major professor and friend, Dr. Timothy Walker, for the opportunity to work in an industry I have grown to love, and for the chance to further my education at the same time. He has been an excellent mentor, providing leadership, direction, and encouragement.

I would also like to thank my committee members Dr. Bobby Golden and Dr. Jason Krutz. Dr. Golden's statistical analysis of data has been invaluable, and his previous research forms the foundation for much of the laboratory work conducted; Dr. Krutz for the use of his laboratory and growth chamber where experiments were carried out, and for his statistical analysis of data as well. Thank you gentlemen for all the help and support you have given me through the course of my studies and research. Thank you to committee members Dr. Jac Varco, for the use of laboratory equipment and personnel to run samples and generate tissue analysis results; and Dr. Jeffrey Gore for his support in helping me to reach my goal.

I very much appreciate everyone one in the rice research crew, Mr. Scott Lanford, Mrs. Jennifer Corbin, Mr. Sanfrid Shaifer, Mr. Steve Felston, Mr. Myron Ridley, and Mr. Lee Atwill. These people have helped tremendously on completing my laboratory and field work. Furthermore, I am thankful for everyone in my family. It's truly amazing to have a family that has as much love as we share for one another. Without them, none of this would be possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Rice (*Oryza sativa* L.) is the primary food staple for over half of the world's seven billion people (U.S Census Bureau, 2012). The United States only accounts for approximately 1.5% of the world's rice production; however, it produces 14% of the world's total exports following only Thailand, Vietnam, and India (Childs, 2005; Walker et al., 2008). Rice production in the United States is concentrated in Arkansas, California, Louisiana, Mississippi, Texas, Missouri, and Florida (Street and Bollich, 2003). Mississippi ranks as the fourth largest rice producing state in the United States behind Arkansas, California, and Louisiana. Mississippi produced 10,863,000 hundredweight of rice in 2011, which had a production value of $153,000,000 (Mississippi Agricultural Factbook, 2011).

As the world population and food production demand increase, the use of nitrogen (N) fertilizers will likely increase. China is one of the largest and most important agricultural countries in the world, and accounts for roughly 30% of the world N consumption (Li et al., 2009); whereas, approximately 15% of the world ammonia consumption is due to North America (Kramer, 2004). Rice producers in the southern USA generally apply between 135 and 235 kg N ha^{-1} depending on soil texture (Snyder and Slaton, 2001). As world demand for rice increases, N utilization efficiency and management is becoming more important (Snyder and Slaton, 2001).

Nitrogen Management in Rice

In United States rice production, N is the most abundantly used and most frequently applied nutrient (Norman et al., 2003). A large amount of N is required for high yielding rice cultivars to produce acceptable grain yields. Even under best management practices, not all N fertilizer applied to the rice will be absorbed by the rice plant. Nitrification and subsequent denitrification, and ammonia volatilization are the major N loss mechanisms within flooded environments (Patrick, 1982; Mikkessen, 1987). Nitrogen loss can also occur in rice to a lesser extent through leaching and runoff (Norman et al., 2003).

There are ongoing concerns about the impact that N fertilizer use has on the environment. Eutrophication of lakes and rivers may occur when the nitrates from fertilizers cause an overabundance of plant growth and decay, which decreases the quality of the water. Also, nitrogen oxides, that act as greenhouse gases, may be formed and released into the atmosphere from N fertilizer treated fields (Bronson et al., 1997; Cai et al., 1997; Kumar et al., 2000). The increasing need for food production, high demand for N fertilizers in food production, and the rising need for environmental stewardship have made N use efficiency important throughout the world.

Dry-seeding and water-seeding are the two main methods of planting rice in the USA. A dry-seeded, delayed flood cultural system is the predominant method used in Mississippi (Walker and Street, 2003). Rice in Mississippi is drill-seeded in narrow rows (15- to 25- cm apart). After emergence, a shallow (<15 cm) permanent flood is established at the four- to five- leaf plant growth stage. It generally takes 21 to 28 d for rice to reach this maturity (Street and Bollich, 2003). Rice is grown in a flooded environment until approximately two to three weeks before harvest so that harvest can occur in dry soil conditions.

Nutrient uptake patterns for rice are similar to upland row crops such as corn (*Zea mays* L) and wheat (*Triticum aestivum* L). The flooded soil environment presents the greatest challenge for managing N fertilizer because of the coordination needed regarding the application of the appropriate N source at the appropriate time to maximize availability. Contrarily, the flooded soil can provide the most stable environment for ammonium–N, thus allowing rice to be one of the most efficient users of N relative to other upland grown crops (Norman et al., 2003).

In drill-seeded, delayed-flood production systems, an ammonium-forming N source is applied at the 5-leaf growth stage and immediately incorporated into the soil using the floodwater. If this N management strategy is performed correctly, N recovery can range from 65 to 75% (Norman et al., 2003). Nitrogen fertilizer application timing is critical to obtain optimal plant growth and subsequent grain yield (Norman et al., 1992; Norman et al., 2003; Griggs et al., 2007; Norman et al., 2009).

The forms of N utilized most by rice plants are ammonium (NH_4^+) and nitrate (NO_3^-). In aerobic situations NH_4^+ is supplied to the rice roots mainly through diffusion, and NO_3^- by mass flow and diffusion. When the rice is in an anaerobic environment, NH_4^+ is very stable and accumulates, but this environment causes the NO_3^- to become unstable and convert into N_2 gas by denitrification (Norman et al., 2003). Due to the instability of NO_3^- in the flooded soil environment, ammonium-forming fertilizers are recommended for rice fertilization (De Datta and Patrick, 1986; Griggs et al., 2007).

Urea is the source of fertilizer most utilized in southern USA rice production because of its high N content (ca. 46% N) and relatively low cost per unit of N (Bufogle et al., 1998; Griggs et al., 2007). The drawback to using urea as an N fertilizer is its rapid transformation in the soil. Therefore, a permanent flood must be established within a few days of fertilization to incorporate the urea fertilizer into the soil. This will increase nitrogen use efficiency by preventing major N losses due to ammonia volatilization and nitrification/denitrification (Griggs et al., 2007), and thus increase nitrogen use efficiency (NUE).

Nitrification / Denitrification

Nitrification is defined as the biological oxidation of NH_4^+ or ammonia (NH_3) to NO_3^-. The nitrification potential of a soil is the maximum capacity of a soil's nitrifying bacteria population to convert ammonium into nitrate (Fortuna et al., 2003). Nitrification only occurs in the presence of oxygen; therefore, most nitrification takes place in the soil before a flood is established. Nitrification in rice production is not desirable because of the susceptibility of NO_3^- to denitrification loss in the anaerobic layer of the flooded soil profile.

Nitrification is a two-step process where NH_4^+ or NH_3 is first converted to nitrite (NO_2^-) and then to NO_3^-. Conversion into NO_2^- is due mainly to the genera of bacteria known as *Nitrosomonas*, while *Nitrobacter* are responsible for most of the conversion from NO_2^- into NO_3^- (Sahrawat, 2008). Collectively, this group of bacteria is referred to as nitrobacteria (Prosser, 1989; Sahrawat, 2008). The major factors affecting nitrification in soils are moisture, oxygen, temperature, pH, the population of nitrifying organisms, and the substrate NH_4^+ concentration (Sahrawat, 2008).

Nitrification, NO_3^- formation, and NO_3^- stability in soils are primarily affected by the interaction between soil moisture and aeration. Nitrobacteria in soils produce NO_3^- in the presence of oxygen. Nitrification rate is maximized when soil oxygen levels are approximately 20% (Black, 1957; Tisdale and Nelson, 1970; Sahrawat, 2008). Nitrification increases linearly with soil water content, up to a maximum of 60% water-filled pores (WFP) in the soil, which is approximately the point where microbial activity peaks (Linn and Doran, 1984). As soil saturation increases above 60% WFP, microbial activity declines due to reduced soil aeration as most of the soil pore spaces are filled with water (Linn and Doran, 1984).

Temperature and pH also have an effect on the nitrification potential of soils. Nitrification generally follows a bell shaped curve in response to temperature, with the optimum temperature being 25-35 °C (Focht and Verstraete, 1977). Nitrification occurs when soil pH ranges between 5.5 and 10 with the optimum pH being about 8.5. However, nitrification has been reported in soils with a pH as low as 3.8 (Tisdale and Nelson, 1970; Sahrawat, 2008). The temperate climate of Mississippi with warm annual temperatures, high annual rainfall, and neutral to slightly basic soil pH, makes it an ideal environment for maximum nitrification to take place.

Denitrification occurs in an anaerobic environment when microorganisms can utilize NO_3^- in the soil as its electron acceptor, which produces gaseous oxides that are lost to the atmosphere (Norman et al., 2003). Several factors affect denitrification in a flooded soil including soil pH, organic matter content, temperature, oxygen diffusion, and the nitrification rate of the soil (Keeney and Sahrawat, 1986). Maximum denitrification rates occur when soils are 37°C (Garcia, 1974; Garcia and Tiedje, 1982), pH values are close to neutral (Nommik, 1956; Garcia and Tiedje, 1982), and available NO_3^- is plentiful (Nommik, 1956; Blackmer, 1978; Firestone, 1980; and Garcia and Tiedje, 1982). Wetselaar (1981) estimated that in an Asian wet season culture, N losses due to denitrification averaged 18.5 kg ha^{-1} yr^{-1} (Wetselaar, 1981; Garcia and Tiedje, 1982). Since denitrification occurs only in the presence of NO_3^-, the best control of N loss due to denitrification is to minimize nitrification (Keeney and Sahrawat, 1986).

If N is applied too long before flood establishment, denitrification losses can occur. The fertilizer and/or soil NH_4^+ nitrifies prior to flooding which results in rapid N loss through denitrification when the soil becomes saturated (Golden et al., 2009). If nitrification of NH_4^+–N could be restricted through the use of nitrification inhibitors (NI), pre-flood N fertilizer could be applied weeks in advance to establishing a permanent flood. Applying a stable, pre-flood N fertilizer one or more weeks in advance to establishing a permanent flood would provide producers with more flexibility in flood establishment, weed control, and capturing rain to help flood fields (Golden et al., 2009).

Nitrification Inhibitors

Nitrification inhibitors are chemical or biological products that retard the nitrification process by killing or disrupting the metabolism of *Nitrosomonas* bacteria (Nelson and Huber, 1992). This mode of action minimizes the possibility that large losses of NO_3^-–N will occur before fertilizer–N can be absorbed by the rice plant. Soil factors affecting NI effectiveness include: organic matter content, soil temperature, pH, and soil water content (Prasad and Power, 1995). Research suggests that crops may utilize urea more effectively in the presence of NIs resulting in greater yields using similar or lesser amounts of fertilizer–N (Trenkel, 1997; Pasda et al., 2001).

Previous research indicates that NIs slows the conversion of NH_4^+ to NO_3^-, which allows more flexibility with respect to N fertilizer application timing. Three, four-dimethylpyrazole phosphate (DMPP) (ENTEC®, BASF Corp., Ludwigshafen, Germany) is a NI developed by BASF in cooperation with other universities and research institutes to be effective at low concentrations (0.5-1.0 kg a.i. ha^{-1}) (Zerulla et al., 2001). Nitrapyrin [2-Chloro-6-(trichloromethyl)-pyridine] (N-Serve® and Instinct®, Dow AgroSciences, LLC., Indianapolis, IN) is labeled as a NI that's been commercially available for over 30 years (Touchton et al., 1978; Touchton et al., 1979; Shi and Norton, 2000; Franzen et al., 2011). Nitrapyrin has been reported to inhibit nitrification in soils in the mid-western and western USA (Rudert and Locascio, 1979; Huber et al, 1969); however, it tends to be inconsistent within the southeastern USA (Touchton and Boswell, 1980; Rudert and Locascio, 1979; Redmann et al., 1964).

Chen (2010) investigated the effects of nitrapyrin and DMPP on nitrification rates of a clay loam Vertisol. The soil had a pH of 8.3 and urea was used as a control. The conditions studied included 40 and 60% water-filled pore spaces (WFPS) at temperatures of 5, 15, and 25°C. Urea was applied at a rate of 80 kg N ha^{-1}. Very little nitrification took place at 5°C at either moisture level. At 15°C and 60% WFPS, nitrification rate increased, and at 25°C and 60% moisture very little NH_4^+–N remained after 14 d. When DMPP and nitrapyrin were applied at rates of 3 and 9 mg active ingredient (a.i.) kg^{-1} soil, respectively, the rate of nitrification at 40% WFPS was considerably less at all temperatures than at the greater moisture rate. Nitrapyrin and DMPP both slowed nitrification significantly at 15°C for both moisture rates for 42 d. At 25°C, DMPP slowed NH_4^+ oxidation at 40% WFPS; but was less effective at 60% WFPS by the end of the study. DMPP was effective at inhibiting nitrification at 40% WFPS for the majority of incubation time. However, with warm (25°C) moist soil (60% WFPS) conditions, the effect substantially declined after 14 d. Nitrapyrin successfully inhibited nitrification in this particular study for the duration of time under all conditions (Chen, 2010).

Dicyandiamide (DCD) is a NI that contains 67% N. In the soil, DCD decomposes to form ammonium and nitrate, which eventually becomes plant available N (Reeves and Touchton, 1986; Reider and Michaud, 1980; Amberger and Vilsmeier, 1979). Wilson et al. (1990) reported that DCD amended urea successfully inhibited nitrification for up to 28 d on Crowley silt loam (Typic Albaqualfs) soil in Arkansas. Furthermore, a review of experiments conducted throughout the southern USA rice belt in the 1980's, where DCD was applied with urea either preplant incorporated or preflood, showed that over nine site-years of data, rice grain yields were 8% greater with DCD compared to urea alone when applied preplant. However, yields were 20% greater when urea was applied preflood compared to preplant (Wells et al., 1989). Aulakh et al. (2001) evaluated DCD in upland and flooded conditions with a subtropical sandy loam soil and results indicated that DCD was effective as a NI for up to 20 days under upland conditions (Aulakh et al. 2001).

Calcium carbide (CaC_2) is a commonly available and economical material that has been shown to inhibit nitrification in soil (Banerjee and Mosier, 1989; Mohanty and Mosier, 1990). When coated in paraffin, it can supply C_2H_2 for extended periods of time (Aulakh et al., 1991; Bronson et al., 1992). Aulakh et al. (2001) conducted research using encapsulated calcium carbide (ECC) and DCD on a subtropical sandy loam soil. The soil had an initial pH of 7.9 and was studied under upland (60% WFPS) and flooded conditions (120% WFPS). Ammonium–N was applied at 100 mg kg^{-1}. Encapsulated calcium carbide was mixed with soil at 5 mg g^{-1} soil, while DCD was applied in solution at 10 mg kg^{-1} of soil. After 10 days in upland soil conditions ECC and DCD reduced nitrification of NH_4^+–N by 93 and 68%, respectively. However, after 20 d the ECC was less effective with results suggesting that 98 and 37% of the ammonium–N was nitrified with the use of ECC and DCD, respectively. Under flooded conditions, 96 and 38% of the NH_4^+–N applied remained after 20 d using ECC and DCD, respectively. The use of NIs can be very efficient at reducing the amount of nitrification/denitrification within a soil, especially under flooded conditions (Aulakh et al. 2001).

Coated urea products have also shown potential for maintaining N availability for plant uptake over longer periods of time (Carreres et al., 2003; Xu et al., 2013). Increased interest in reducing greenhouse gas emissions and N losses from inorganic fertilizers has led to the production and marketing of polymer coated urea (PCU) products for corn production in the mid-western USA, and has inspired interest for its use in rice production (Golden et al., 2009).

Slaton et al. (2009) performed a field study investigating 38% and 43% N polymer coated urea (PCU). The study was conducted on Dewitt (fine, smectitic, thermic Typic Albaqualfsand) and Calhoun (fine-silty, mixed, active, thermic Typic Glossaqualfssilt) silt loam soils as well as a Sharkey (very-fine, smectitic, thermic Chromic Epiaquerts) clay soil. The PCU fertilizers were applied at preplant which ranged from 31 to 43 days before flood establishment (dbf) and at the 2- to 3-leaf stage (12-15 dbf) of rice. Urea was applied at the 2- to 3-leaf stage and preflood (1-2 dbf). Nitrogen was applied at 67 and 134 kg N ha^{-1} on drill-seeded rice. In the Sharkey clay soil, the highest grain yield was achieved with 134 kg N ha^{-1} at preflood. However, the 38% N PCU at 134 kg N ha^{-1} applied at 2- to 3-leaf stage produced the next highest yield. In both the Dewitt and Calhoun silt loams the greatest yield was achieved with 134 kg N ha^{-1} applied at preflood. The 38% N PCU at 134 kg N ha^{-1} at preplant application timing produced yields similar to that of urea applied at preflood in both of these soil types. The results of this study indicated that the 43% N PCU tended to release N too quickly giving rise to lower rice yields compared to the other products tested (Slaton et al., 2009).

With worldwide populations increasing, improving the efficiency of N management in rice production is becoming more important. The efficient use of fertilizers, which account for up to 25% of the total annual rice production costs, saves money and reduces environmental impact. Nitrification inhibitors may provide an additional mechanism that improves N uptake efficiency. These savings are important to USA rice producers as they seek to increase yields to meet rising demands. Further research is needed to determine the best practices in efficient N use, particularly with respect to Mississippi alluvial soils and climatic conditions where rice production is extensive.

Literature Cited

Amberger, A., and K. Vilsmeier. 1979. Dicyandiamideabbau in Quarzsand und Boden. Z. Pflanzenernaehr. Bodendkd. 142:778-785.

Aulakh, M.S., J.W. Doran, and A.R. Mosier. 1991. Field evaluation of four methods for measuring denitrification. Soil Sci. Soc. Am. J. 55:1332-1338.

Aulakh, M.S., K. Singh, and J. Doran. 2001. Effects of 4-amino 1,2,4-triazole, dicyandiamide and encapsulated calcium carbide on nitrification inhibition in a subtropical soil under upland and flooded conditions. Biol. Fertil. Soils 33:258-263.

Banerjee, N.K., and A.R. Mosier. 1989. Coated calcium carbide as a nitrification inhibitor in upland and flooded soils. J. Indian Soc. Soil Sci. 37:306-313.

Black, C.A. 1957. Soil-Plant Relationships. New York: Wiley.

Blackmer, A.M. and J.M. Bremner. 1978. Inhibitory effect of nitrate on reduction of N_20 to N_2 by soil microorganisms. Soil Biol. Biochem. 10:187-191.

Bronson, K.F., A.R. Mosier, and S.R. Bishnoi. 1992. Nitrous oxide emissions in irrigated corn as affected by nitrification inhibitors. Soil Sci. Soc. Am. J. 56:161-165.

Bronson, K.F., H.U. Neue, U. Singh, E.B. Abao, Jr. 1997. Automated chamber measurement of methane and nitrous oxide flux in flooded rice soil. I. Residue, nitrogen and water management. Soil Sci. Soc. Am. J. 61:981-987.

Bufogle, A., Jr., P.K. Bollich, J.L. Kovar, C.W. Lindau, and R.E. Macchiavelli. 1998. Comparison of ammonium sulfate and urea as nitrogen sources in rice production. J. Plant Nutr. 21:1601-1614.

Cai, Z., G. Xing, X. Yan, H. Xu, H. Tsuruta, K. Yagi, and K. Minami. 1997. Methane and nitrous oxide emissions from rice paddy fields as affected by nitrogen fertilizers and water management. Plant Soil 196:7-14.

Carreres, R., J. Sendra, R. Ballesteros, E.F. Valiente, A. Quesada, D. Carrasco, F. Leganes, and J.G. Cuadra. 2003. Assessment of slow release fertilizers and nitrification inhibitors in flooded rice. Biol. Fertil. Soils. 39:80-87.

Chen, D., H.C. Suter, A. Islam, and R, Edis. 2010. Influence of nitrification inhibitors on nitrification and nitrous oxide (N_2O) emission from a clay loam soil fertilized with urea. Soil Biol. Biochem. 42:660-664.

Childs, N.W. 2005. Rice situation and outlook yearbook. USDA Economic Res. Serv., Springfield, VA.

De Datta, S.K., and W.H. Patrick, Jr. (ed.) 1986. Nitrogen economy of flooded soils. Martinus Nijhoff Publ., Dordrecht, the Netherlands.

Firestone, M.K., R.B. Firestone, and J.M. Tiedje. 1980. Nitrous oxide from soil denitrification: factors controlling its biological production. Sci. 208:749-751.

Focht, D.D., and W. Verstraete. 1977. Biochemical ecology of nitrification and denitrification. p. 135-214. *In* Alexander, M. (ed.) Adv. in Microb. Ecol. New York: Plenum Press.

Fortuna, A., R.R. Harwood, G.P. Robertson, J.W. Fisk, and E.A.Paul. 2003. Seasonal Changes in nitrification potential associated with application of N fertilizer and compost in maize systems of southwest Michigan. Agric. Ecosyst. Environ. 97:285-293.

Franzen, D., R.J. Goos, R.J. Norman, T.W. Walker, T.L. Roberts, N.A. Slaton, G. Endres, R. Ashley, J. Staricka, J. Lukach. 2011. Field and laboratory studies comparing nutrisphere-nitrogen urea with urea in North Dakota, Arkansas, and Mississippi. J. Plant Nutri. 34:1198-1222.

Garcia, J.-L. 1974. Reduction de l'oxyde nitreux dans les sols de rizieres du Senegal: mesure de l'activite denitrifiante. Soil Biol. Biochem. 6:79-84.

Garcia, J.L. and J.M. Tiedje. 1982. Denitrification in rice soils. p. 187-208. *In* Y.R. Dommergues and H.G. Diem (eds.) Microb. Trop. Soils and Plant Prod. Dr. W. Junk Publishers, Boston.

Golden, B.R., N.A. Slaton, R.J. Delong, R.J. Norman, and E.T. Maschmann. 2009. Nitrification inhibitors influence on rice grain yield and soil inorganic nitrogen fractions. p. 215-223. *In* R.J. Norman, J.F. Muellenet, K.A.K. Moldenhauer (eds.) B.R. Wells Rice Research Studies 2008. University of Arkansas, Fayetteville.

Griggs, B.R., R.J. Norman, C.E. Wilson, Jr., N.A. Slaton. 2007. Ammonia volatilization and nitrogen uptake for conventional and conservation tilled dry-seeded, delayed-flood rice. Soil Sci. Soc. Am. J. 71:745-751.

Huber, D.M., G.A. Murray, and J.M. Crane. 1969. Inhibition of nitrification as a deterrent to nitrogen loss. Soil Sci. Soc. Am. Proc. 33:975-976.

Keeney, D.R., and K.L. Sahrawat. 1986. Nitrogen transformations in flooded rice soils. Fert. Res. 9:15-38.

Kramer, D.A. 2004. Mineral commodity profiles: Nitrogen. U.S. Dept. of the Interior, USGS, 2004-1290. Washington, D. C.: Government Printing Office

Kumar, U., M.C. Jain, S. Kumar, H. Pathak, S. Kumar, and D. Majumdar. 2000. Nitrous oxide emission from different fertilizers and its mitigation by nitrification inhibitors in irrigated rice. Biol. Fertil. Soils. 32:474-478.

Li, S.X., Z.H. Wang, T.T. Hu, Y.J. Gao, and B.A. Stewart. 2009. Nitrogen in dryland soils of china and its management. Adv. Agron. 101:123-170.

Linn, D.M., and J.W. Doran. 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Sci. Soc. Am. J. 48:1267-1272.

Mikkelsen, D.S. 1987. Nitrogen budgets in flooded soils used for rice production. Plant Soil 100:71-97.

Mississippi agricultural, forestry, and natural resources. 2012. [Online] Available at http://www.dafvm.msstate.edu/factbook.pdf (verified 5 Nov. 2012).

Mohanty, S.K., and A.R. Mosier. 1990. Nitrification-denitrification in flooded rice soils. Trans 14th Internat. Cong. Soil Sci. 4:326-331.

Nelson, D.W., and D. Huber. 1992. Nitrification inhibitors for corn production. [Online] Available at http://www.extension.iastate.edu/Publications/NCH55.pdf (verified 17 Aug. 2012).

Nommik, H. 1956. Investigations on denitrification in soil. Acta Agr. Scand. 6:195-228.

Norman, R.J., D. Guindo, B.R. Wells, and C.E. Wilson, Jr. 1992. Seasonal accumulation and partitioning of nitrogen-15 in rice. Soil Sci. Soc. Am. J. 56:1521-1526.

Norman, R.J., C.E. Wilson, Jr., and N.A. Slaton. 2003. Soil Fertilization and mineral nutrition in U.S. mechanized rice culture. p. 331-411. *In* C. Wayne Smith and Robert H. Dilday (eds.) Rice: Origin, History, Technology, and Production. John Wiley & Sons, Hoboken, NJ.

Norman, R.J., C.E. Wilson Jr., N.A. Slaton, B.R. Griggs, J.T. Bushong, and E.E. Gbur. 2009. Nitrogen fertilizer sources and timing before flooding dry-seeded, delayed-flood rice. Soil Sci. Soc. Am. J. 73:2184-2190.

Pasda, G., R. Hahndel, and W. Zerulla. 2001. Effect of fertilizers with the new nitrification inhibitor DMPP (3,4-dimethylpyrazole phosphate) on yield and quality of agricultural and horticultural crops. Biol. Fertil. Soils. 34:85-97.

Patrick, W.H., Jr. 1982. Nitrogen transformations in submerged soils. p. 449-466. *In* F.J. Stevenson (ed.) Nitrogen in Agriculture Soils. ASA-CSSA-SSSA, Madison, WI.

Prasad, R. and J.F. Power. 1995. Nitrification inhibitors for agriculture, health, and the environment. Adv. Agron. 54:233-281.

Prosser, J.I. 1989. Autotrophic nitrification in bacteria. Adv. Microb. Phys. 30:125-181.

Redmann, C.T., R.W. Meikle, and J.G. Widofsky. 1964. The loss of 2-chloro-6-(trichloromethyl) pyridine from soil. J. Agric. Fd. Chem. 12(3):207-209.

Reeves, D.W., and J.T. Touchton. 1986. Relative phytotoxicity of dicyandiamide and availability of its nitrogen to cotton, corn, and grain sorghum. Soil Sci. Soc. Am. J. 50:1353-1357.

Reider, G., and H. Michaud. 1980. Improving fertilizer efficiency. The use of a dicyandiamide nitrification inhibitor. Nitrogen. 124:31-35.

Rudert, B.D., and S.J. Locascio. 1979. Differential mobility of nitrapyrin and ammonium in a sandy soil and its effect on nitrapyrin efficiency. Agron. J. 71:487-489.

Sahrawat, K.L. 2008. Factors affecting nitrification in soils. Commun. Soil Sci. and Plant Anal. 39:1436-1446.

Shi, W., and J.M. Norton. 2000. Effect of long-term, biennial, fall-applied anhydrous ammonia and nitrapyrin on soil nitrification. Soil Sci. Soc. Am. J. 64:228-234.

Slaton, N.A., B.R. Golden, and R.J. Norman. 2009. Rice response to urea and two polymer-coated urea fertilizers. p. 211-219. *In* R.J. Norman and K.A.K. Moldenhauer (eds.) B.R. Wells Rice Research Studies 2009. University of Arkansas, Fayetteville.

Snyder, C.S., and N.A. Slaton. 2001. Rice production in the United States – an overview. Better Crops. 85:3-7.

Street, Joe E., and P.K. Bollich. 2003. Rice Production. p. 271-296. *In* C. Wayne Smith And Robert H. Dilday (eds.) Rice: Origin, History, Technology, and Production. John Wiley & Sons, Hoboken, NJ.

Tisdale, S.L., and W.L. Nelson. 1970. Soil Fertility and Fertilizers, 2nd ed. The Macmillan Co., New York.

Touchton, J.T., R.G. Hoeft, and L.F. Welch. 1978. Nitrapyrin degradation and movement in soil. Agron. J. 70:811-816.

Touchton, J.T., R.G. Hoeft, L.F. Welch, D.L. Mulvaney, M.G. Oldham, and F.E. Zajicek. 1979. N uptake and corn yield as affected by applications of nitrapyrin with anhydrous ammonia. Agron. J. 71:238-242.

Touchton, J.T., and F.C. Boswell. 1980. Performance of nitrification inhibitors in the southeast. p.63-74. *In* J.J. Meisinger (ed.) Nitrification Inhibitors-Potentials and Limitations. ASA Spec. Publ. 38. ASA and CSSA, Madison, WI.

Trenkel, M. 1997. Improving fertilizer use efficiency controlled-release and stabilized fertilizers in agriculture. International Fertilizer Industry Association, Paris.

United States Census Bureau. 2012. U.S. and world population clocks. [Online] Available at http://www.census.gov/main/www/popclock.html (verified 9 May 2012).

Walker, T.W., J.A. Bond, B.V. Ottis, P.D. Gerard, and D.L. Harrell. 2008. Hybrid rice response to nitrogen fertilization for midsouthern United States rice production. Agron. J. 100:381-386.

Walker, T. W. and J. E. Street. 2003. Rice fertilization. Information Sheet No. 1341. Miss. Agric. and For. Exp. Stn. Mississippi State, MS.

Wells, B.R., P.K. Bollich, W. Ebelhar, D.S. Mikkelsen, R.J. Norman, D.M. Brandon, R.S. Helms, F.T. Turner, and M.P. Westcott. 1989. Dicyandiamide (DCD) as a nitrification inhibitor for rice culture in the United States. Comm. Soil Sci. and Plant Anal. 20:2023-2047.

Wetselaar, R. 1981. Nitrogen inputs and outputs of an unfertilized paddy field. p. 573-583. *In* F.E. Clark and T. Rosswall (eds.) Terrestrial Nitrogen Cycles. Processes, Ecosystem Strategies and Management Impacts. Ecology Bull., Stockholm.

Wilson, C.E., Jr., R.J. Norman, and B.R. Wells. 1990. Dicyandiamide influence on uptake of preplant-applied fertilizer nitrogen by rice . Soil Sci. Soc. Am. J. 54:1157-1161.

Xu, M., D. Li, J. Li, D. Qin, Y. Hosen, H. Shen, R. Cong, X. He. 2013. Polyolefin-coated urea decreases ammonia volatilization in a double rice system of southern China. Agron. J. 105:277-284.

Zerulla, W., T. Barth, J. Dressel, K. Erhardt, K.H. von Locquenghien, G. Pasda, M. Radle, A.H. Wissemeier. 2001. 3,4-Dimethylpyrazole phosphate (DMPP) - a new nitrification inhibitor for agriculture and horticulture. Biol. Fertil. Soils. 34:79-84.

CHAPTER II

A LABORATORY ASSESSMENT OF NITRIFICATION POTENTIAL OF SOILS COMMON FOR RICE PRODUCTION AND NITROGEN STABILIZERS TO REDUCE NITRIFICATION IN TWO MISSISSIPPI ALLUVIAL PLAIN CLAY SOILS

Abstract

Nitrification and subsequent denitrification losses of applied N in rice production have been studied minimally in the delayed-flood rice production system. Laboratory incubations were conducted to determine the nitrification potential of soils common to rice production in the southern USA. The study was analyzed as a completely randomized design with 7 (soils) by 6 (sample time) factorial arrangement of treatments which included 3 replications, and the experiment was duplicated in time (runs). A similar experiment was conducted to evaluate the effectiveness of two nitrification inhibitors on clay soils. Nitrapyrin (0.56, 1.12, and 2.24 kg ha^{-1}) and dicyandiamide (DCD) (5, 10, and 15% of the N rate) were applied with urea. The study was analyzed as a split plot design with soils as the whole plot and sample timing as the subplot with 3 replications for each treatment, and a total of 2 runs utilizing a completely randomized design. The fertilizer application rate for all laboratory incubations approximated adding 115 ppm N to the soil.

Total inorganic fertilizer-N recovered and N recovery as NO_3^- and NH_4^+ for the nitrification potential experiment was subjected to the Mixed Procedure. Data from both laboratory incubations were subjected to PROC NLIN for analysis and NH_4^+ disappearance was fit to a first order kinetics model. The rate constant parameter from PROC NLIN was used to determine the number of days when half the total recovered inorganic–N was in the NH_4^+–N form (half-life) for each soil and stabilizer for the nitrification potential and nitrogen stabilizer experiments, respectively.

Nitrification potential differed among soils with half-life values ranging from 3.9 to 9.2 days. One of the soils with the greatest nitrification potential was the Sharkey clay soil at Stoneville, MS. This soil produced a half-life of 3.9 d with 42 and 91% of the total N recovered at 2 and 9 days after incubation (DAI), respectively. Nitrogen stabilizer experiment results indicated that half-life values with the application of nitrapyrin and DCD were 3.8 to 4.9 and 8.7 to 15.2 d, respectively. These results indicate that DCD is much more effective than nitrapyrin at reducing nitrification on clay soils.

Introduction

As the world's population and food production demand increase, the use of nitrogen (N) fertilizers will likely increase. In United States rice production, N is the most abundantly used and most frequently applied nutrient (Norman et al., 2003). Ammonium–N forming fertilizers are recommended for rice fertilization because of the stability of ammonium (NH_4^+) compared to nitrate (NO_3^-) in the flooded soil environment (De Datta and Patrick, 1986; Griggs et al., 2007). Urea (ca. 46% N) is the N source utilized the most in southern USA rice production because of its high N content and relatively low cost per unit of N (Bufogle et al., 1998; Griggs et al., 2007).

In the drill-seeded, delayed-flood rice production system, an ammonium-forming N source is applied at the 5-leaf growth stage and is incorporated into the soil profile using the floodwater. If this N management strategy is performed correctly, N recovery can range from 65 to 75% (Norman et al., 2003). Establishing a permanent flood a few days after fertilizer application is the most effective way to limit N losses due to nitrification/denitrification (Griggs et al., 2007). However, the drawback to using urea as an N fertilizer is its rapid transformation in the soil. If rain showers incorporate N into the soil before a permanent flood can be established, N can become unavailable to the rice plant through nitrification and subsequent denitrification.

Nitrification is the biological oxidation of NH_4^+ or ammonia (NH_3) to NO_3^-. The nitrification potential of a soil is the maximum ability of a soil's nitrifying bacteria population to convert NH_4^+ into NO_3^- (Fortuna et al., 2003). Nitrification only occurs in the presence of oxygen, so most nitrification takes place in the soil before a flood is established. Nitrification in rice production is not desirable because of the susceptibility of NO_3^- to denitrification loss in the anaerobic layer of the flooded soil. Some of the major factors affecting nitrification in soils are moisture (Parker and Larson, 1962; Justice and Smith, 1962; Sahrawat, 2008), oxygen (Black, 1957; Tisdale and Nelson, 1970; Sahrawat, 2008), temperature (Sabey et al., 1956; Focht and Verstraete, 1977; Sahrawat, 2008), pH (Sahrawat et al., 1985), the population of nitrifying organisms (Todd et al., 1975; Montagnini et al., 1986; Sahrawat, 2008) and the substrate NH_4^+ concentration (Azam et al., 2004; Sahrawat, 2008). The temperate climate of Mississippi with warm annual temperatures, high annual rainfall, and neutral to slightly basic soil pH, makes it an ideal location for maximum nitrification to take place.

Denitrification occurs in a flooded environment when the microorganisms can utilize NO_3^- in the soil as its electron acceptor which produces gaseous oxides that are lost to the atmosphere (Norman et al., 2003). Maximum denitrification rates occur when soils are 37°C (Garcia, 1974; Garcia and Tiedje, 1982), pH values are close to neutral (Nommik, 1956; Garcia and Tiedje, 1982), and available NO_3^- is plentiful (Nommik, 1956; Blackmer, 1978; Firestone, 1980; and Garcia and Tiedje, 1982). In an Asian wet season culture, N losses due to denitrification averaged 18.5 kg ha^{-1} yr^{-1} (Wetselaar, 1981; Garcia and Tiedje, 1982). Because denitrification occurs only in the presence of NO_3^-, the best control of N loss due to denitrification is to minimize nitrification (Keeney and Sahrawat, 1986). If nitrification of NH_4^+–N could be restricted through the use of nitrification inhibitors (NI), this may possibly allow preflood N fertilizer to be applied weeks in advance to establishing a permanent flood. Consequently, this approach would provide rice producers more flexibility in flood establishment, weed control, and capturing rain to help flood fields (Golden et al., 2009).

Nitrification inhibitors are chemicals or biological products that slow or delay the nitrification process by killing or disrupting the metabolism of *Nitrosomonas* bacteria (Nelson and Huber, 1992). This mode of action minimizes the possibility that large losses of NO_3^-–N will occur before fertilizer–N can be taken up by the rice plant. Nitrification inhibitors may be an additional route to increasing nitrogen use efficiency (NUE) and lessening environmental impact (Kumar et al., 2000). Research suggests that crops may utilize urea more effectively in the presence of NIs resulting in greater yields using similar or lesser amounts of fertilizer–N (Trenkel, 1997; Pasda et al., 2001).

Previous research has shown that NIs can be effective at slowing the conversion of NH_4^+ to NO_3^-, and allow for more flexibility in the timing of N fertilizer application. Nitrapyrin [2-Chloro-6-(trichloromethyl)-pyridine] (N-Serve® and Instinct®, Dow AgroSciences, LLC., Indianapolis, IN) is labeled as an NI and has been investigated and commercially available for over 30 years (Touchton et al., 1978; Touchton et al., 1979; Shi and Norton, 2000; Franzen et al., 2011). Nitrapyrin has a relatively high vapor pressure, and thus has a tendency to volatilize if not incorporated into the soil immediately after application.

Chen et al. (2010) investigated the effects of nitrapyrin on nitrification rate of a clay loam textured Vertisol that included a combination of moistures and temperatures and urea application of 80 kg N ha^{-1}. The trend was that with increasing moisture and temperature, nitrification rate increased. When nitrapyrin was applied, the rate of nitrification decreased under all conditions (Chen et al., 2010). Nitrapyrin has been reported to inhibit nitrification in soils in the mid-western and western USA (Huber et al, 1969; Rudert and Locascio, 1979); however, it tends to be inconsistent within the southeastern USA (Boswell and Anderson, 1974; Boswell et al. 1974; Boswell, 1977; and Rudert and Locascio, 1979) and other areas with high soil temperatures (Ali et al., 2008).

Dicyandiamide is a nitrification inhibitor that contains 67% N. In the soil, DCD decomposes to form NH_4^+ and NO_3^-, thus eventually becomes plant available N (Amberger and Vilsmeier, 1979; Reider and Michaud, 1980; Reeves and Touchton, 1986). Aulakh et al. (2001) conducted research in upland and flooded conditions on subtropical sandy loam soil to evaluate the effects of DCD on nitrification potential. In this study, DCD was effective as a nitrification inhibitor for up to 20 d under upland conditions (Aulakh et al. 2001). In another study, DCD applied at different rates ranged from 34 to 86% effective at reducing nitrification in a sandy loam soil (Majumdar, 2002).

Empirical evidence suggested that nitrification and subsequent denitrification may be a major N loss mechanism for soils where rice is produced in Mississippi (Franzen et al., 2011). Therefore, the objectives of this laboratory research were to: 1) determine the nitrification potential of soils common for rice production in the southern USA; and 2) evaluate nitrogen stabilizers that may reduce nitrification and subsequent denitrification in two Mississippi alluvial plain clay soils.

Materials and Methods

Site Description and Cultural Practices

Nitrification potential and N stabilizer laboratory studies were conducted at USDA-ARS in Stoneville, MS, in 2010 - 2012. The soil textures were identified using the USDA-NRCS soil survey information compiled on the Web Soil Survey for each laboratory experiment (USDA-NRCS, 2012) (Table 2.1 and 2.2). Soil was collected from the upper 5- cm of corresponding field, placed into 38 L plastic totes, and stored in an air-dry condition for approximately one month before use. Soils were screened using a U.S. standard size #10 sieve. Fifteen g subsamples of the collected soil were weighed in triplicate for moisture analysis to facilitate adding soil on a dry weight basis. The subsamples were oven dried at 105°C for 24 hours and again a few hours later to confirm oven dryness. One hundred g of each soil (dry weight equivalent) was placed into 125-mL polypropylene jars (Nalgene® straight wide-mouth jars) and brought to a uniform soil moisture content of 80% field capacity using deionized water. This moisture level was maintained throughout the incubation process, with water added as needed (approximately every 3 days). Urea (46-0-0) was passed through a U.S. standard size #7 sieve. Two granules of urea (17-31 mg) were then weighed on an analytical balance where the masses were recorded and the granules placed into a scintillation vial (20 mL) until application.

Nitrification Potential Experiment

Laboratory research was conducted to determine the nitrification potential of six different soil types from seven locations. Soils were collected from areas that have a history of rice production. The soils consisted of a Commerce very fine sandy loam (fine-silty, mixed, superactive, nonacid, thermic Fluvaquentic Endoaquepts) (33°43.31.41 N and 90°90.83.08 W); Crowley silt loam (fine, smectitic, thermic Typic Albaqualfs) (30°24.74. N and 92°34.94.90 W); Dundee silty clay loam (fine-silty, mixed, active, thermic Typic Endoaqualfs) (34°05.61.61 N and 90°59.06.64 W); Dundee silt loam (fine-silty, mixed, active, thermic Typic Endoaqualfs) (33°71.80.86 N and 90°77.26.01 W); Forestdale silt loam (fine, smectitic, thermic Typic Endoaqualfs) (33°78.81.23 N and 90°77.63.73 W); and two Sharkey clays (very-fine, smectitic, thermic Chromic Epiaquerts) (33°43.12.08 N and 90°90.63.73 W) (33°63.45.53 N and 90°88.41.40 W). The soil chemical properties were determined using the Lancaster soil testing method (Cox, 2001) (Table 2.1). The study included one N source (urea), 7 soils, 6 sample timings, 3 replications, and 2 runs. Treatments with no added N were also included.

At the end of the 14 d pre-incubation period two holes approximately 1.27- 2.54-cm deep were placed in the soil within the incubation jar. One pre-weighed granule of urea was then placed into each hole (approximately 115 ppm N jar^{-1}). The topsoil was then disturbed to cover the holes making sure to incorporate the fertilizer into the soil.

Incubation jars containing the soil/fertilizer treatments were incubated at 25 °C for a total of 26 d.

Nitrogen Stabilizer Experiment

Laboratory research was conducted to determine if nitrapyrin (applied as Instinct®) and DCD could minimize nitrification/denitrification in Mississippi clay soils. Tunica (clayey over loamy, smectitic over mixed, superactive, nonacid, thermic Vertic Epiaquepts) and Sharkey clay soils were used to evaluate the nitrification inhibitors. The soil chemical properties were determined using the Lancaster soil testing method (Cox, 2001) (Table 2.2). The research included 1 N source, 2 nitrification inhibitors applied at 3 rates, 2 soils, 6 sample timings, 3 replications, and 2 runs. Treatments with no added N were also included.

At the end of the 14 d pre-incubation period two holes approximately 1.27- 2.54-cm deep were placed in the soil within the incubation jar. One pre-weighed granule of urea was then placed into each hole (approximately 115 ppm N jar^{-1}). Stock solutions were prepared using deionized water as a carrier medium to apply nitrapyrin and DCD to urea in 5- mL aliquots *in situ* at 0.56, 1.12, and 2.24 kg ha^{-1} (42, 84, and 126 µg nitrapyrin jar^{-1}) and 5, 10, and 15% N from DCD (0.86, 1.72, and 2.58 mg DCD jar^{-1}), respectively. The topsoil was then disturbed to cover the holes making sure to incorporate the fertilizer and/or nitrogen stabilizer. The jars containing all treatments were incubated at 25°C for 26 d.

Data Collection

For both laboratory experiments, a sufficient amount of 1 M KCL solution was prepared on the day of or the day before each extraction. At each sampling time, the soils contained within the incubation jars from each treatment were quantitatively transferred into pre-labeled polyethylene 1- L bottles (Nalgene® square wide-mouth bottles). Five hundred mL of 1 M KCl was added to each 1- L bottle (1:5 soil:solution). The bottles were shaken for 1 hour, and left to settle for thirty minutes. After settling, a portion of the supernatant was decanted into a glass funnel lined with Whatman #1 qualitative filter paper. The filtrate was collected in a pre-labeled 20- mL scintillation vial until analysis of NO_3^- and NH_4^+ at each sampling time. Inorganic–N recovered from the soil receiving no N was subtracted from the inorganic–N recovered from the soil receiving each N source and/or N stabilizers to account for organic–N mineralization and estimate the percentage of fertilizer–N recovery [(Net inorganic–N / total N added) × 100]. The proportion of fertilizer–N recovered as NO_3^- and NH_4^+–N was calculated and expressed as a percent of fertilizer–N recovered. Colorimetry (San+autoanalyzer, Skalar Analytical B.V., Breda, the Netherlands; Massey et al., 2011; Peters et al., 2003) was used to determine the total amount of ammonium and nitrate recovered. Samples were collected at 2, 5, 9, 15, 20, and 26 DAI for the nitrification potential experiment, and 2, 6, 9, 14, 19, and 26 DAI for the nitrogen stabilizer experiment.

Statistical Analysis

The nitrification potential experiment was analyzed as a completely randomized design with 7 (soils) by 6 (sample time) factorial arrangement of treatments which included 3 replications and 2 runs. The soils performed similarly for both runs so replications were pooled over runs for each soil type. The nitrogen stabilizer experiment was analyzed as a split plot design with soils as the whole plot and sample timing as the subplot with 3 replications for each treatment, and a total of 2 runs utilizing a completely randomized design. The urea–N and the N stabilizers in this experiment performed similarly in both soils and runs so the soils and replications were pooled over runs for each urea–N and stabilizer treatment. Total inorganic fertilizer–N (TN) recovered and N recovery as NO_3^- and NH_4^+ for the nitrification potential experiment was subjected to the Mixed Procedure (Littell et al., 1996; SAS, 2003). The data from both laboratory incubations were subjected to PROC NLIN for analysis and NH_4^+ disappearance was fit to a first order kinetics model. The rate constant parameter from PROC NLIN was used to construct half-life values for the nitrification potential, and the nitrogen stabilizer experiments. Standard errors were used to compare half-life values for each soil in the nitrification potential experiment and urea–N and N stabilizers for the N stabilizer experiment. A straight line model was used to determine the relationship between half-life values in days and the % N from DCD.

Results and Discussion

Nitrification Potential

The percent of TN recovered and the percent of TN recovery as NO_3^- and NH_4^+ was measured for each of the samples at each sample timing (Table 2.3). Samples incubated for two days resulted in the smallest % TN recovery, with a range of 17 to 44% recovered for all of the soils. This may be due to incomplete hydrolysis of the urea granule, limiting the amount of measureable N incorporated into some of the soil samples. The maximum amount of TN recovered was reached on day 9, where TN recovery was 52 - 91%. Golden et al. (2009) recovered 80-93% at 10 days after fertilizer application for the Dewitt (Typic Albaqualfs), Henry (Typic Fragiaqualfs) and Calhoun (Typic Glossaqualfs) silt loam soils incubated at the same temperature with similar N rates added. The low recovery of N may be partly attributed to immobilization. Kissel et al. (1977) conducted research on a clay (Udic Pellusterts) soil with pH values ranging from 7.6 to 8.2. Their data suggest that immobilization rate increased as temperatures rose above 22°C. The maximum rate of immobilization they found was equivalent to 19% of the applied N (Kissel et al., 1977).

Results indicated that half-life values among the different soils ranged from 3.9 to 9.2 d (Table 2.4). Total inorganic fertilizer–N recovery for the Commerce *vfsl* soil at 2 and 20 DAI was 17 and 71%, respectively. This soil had the greatest half-life value (9.2 d), but there were no differences between it and the Dundee *sicl* (8.5 d). There were also no differences observed between the Dundee *sicl* and the Crowley and Forestdale *sil* soils, which had the next greatest half-life values of 6.1 and 5.0 d, respectively. Furthermore, half-life values were no different for the Sharkey clay soil found in Cleveland, MS (4.6 d) and the Forestdale *sil*. Nitrification potential was greatest in the Sharkey clay soil at Stoneville, MS, which had a half-life value of 3.9 days, with 42 and 91% of the N recovered at 2 and 9 DAI, respectively. However, no significant differences were observed between this soil and the Forestdale *sil*, the Sharkey clay in Cleveland, MS, or the Dundee *sil*. There was not a relationship found between the nitrification rate and the physiochemical properties of the soils studied.

Feigenbaum and Hadas (1980) found similar results using sandy (Typic Rhodoxeralf) and clay (Typic Chromoxerert) soils with high pH values (7.6-8.2) fertilized with ammonium sulfate at 100 kg N ha^{-1}. Under field conditions with soil temperatures ranging from 15-20°C, ammonium half-life values were 4.7 and 5.8 d for the sandy and clay soil, respectively (Feigenbaum and Hadas, 1980). Nitrification half-life values are independent of concentration and were within the range of 0.35 to 35 d (Rao et al., 1984; Chowdary et al., 2004).

Nitrogen Stabilizers

Depletion of soil ammonium and nitrate accumulation was much more rapid with urea and urea–nitrapyrin treatments than with urea–DCD. Results from these data indicate that the half-life values of urea–N and the NIs tested ranged from 3.8 to 15.2 d (Table 2.5). All rates of nitrapyrin and urea–N alone resulted in the shortest half-life values which ranged from 3.8 to 4.9 d. Ali et al. (2008) found similar results with the use of nitrapyrin when studying a sandy clay loam incubated at 35°C. Nitrogen was applied through ammonium sulfate at 200 mg kg^{-1}, while nitrapyrin rate was 0.25 - 0.50 mg kg^{-1}. The results indicated that almost all the NH_4^+–N disappeared within 2 weeks (Ali et al., 2008). Urea–DCD half-life values tended to increase with increasing concentration of DCD ranging from 8.7 to 15.2 d resulting in a 1.6 to 3.6 fold increase when compared to urea–N treatments. These results suggest that nitrification was inhibited by DCD much more effectively than nitrapyrin. Majumdar (2002) established that DCD applied at 5, 10, and 15% of N added was between 34 and 67% effective at reducing nitrification at the same temperature and similar moisture conditions contained within this experiment. Dicyandiamide followed a linear trend (Fig 2.1) in that with every percent of N supplied from DCD, half-life values increased 0.7 days.

Conclusion

Delayed-flood rice culture can result in high N use efficiency; however, commercial-scaled production poses challenges to do so consistently. Stabilizing N fertilizer from multiple loss mechanisms such as volatilization and nitrification/denitrification would increase NUE. Laboratory experiments indicated that half-life values with the application of urea ranged from 3.9 to 9.2 d in many soils where rice is produced in the southern USA. In field settings, establishment of a permanent flood in drill-seeded, delayed-flood rice culture may take ten or more days which can render much of the applied N subject to loss. Nitrapyrin when applied from 0.56 to 2.24 kg ha^{-1} did not effectively inhibit nitrification. Dicyandiamide was effective as a nitrification inhibitor with half-life values ranging from 8.7 to 15.2 d, and tending to increase with increasing DCD rate. Further research is necessary to investigate NIs and other products that may extend the fertilizer–N half-life in the soil. Additional research will investigate two NIs and a coated urea fertilizer to determine their capability to reduce nitrification and subsequent denitrification under field conditions. Recommendations to rice producers based on this research would be the establishment of a permanent flood as soon as possible after nitrogen application to maximize NUE

Table 2.1 Soil chemical properties for nitrification potential experiment conducted in Stoneville, MS in 2010 and 2011

Soil Texture‡	Sum of Bases	pH (1:1)	OM (%)	Extractable Nutrient Levels† P	K	Ca	Mg	Zn
				--------------------mg kg^{-1}--------------------				
Commerce *vfsl*	24.4	7.5	1.04	115	324	4131	704	2.8
Crowley *sil*	9.4	6.8	1.13	25	70	1417	260	6.7
Dundee *sil*	14.9	7.5	1.27	35	122	2641	387	2.7
Dundee *sicl*	30.3	7.0	2.07	99	290	4650	1126	3.0
Forestdale *sil*	19.0	6.9	1.66	47	136	3010	538	2.7
Sharkey *c*§	36.7	7.9	1.90	136	355	5978	1228	3.6
Sharkey *c*£	30.6	6.9	2.78	92	376	4718	1048	3.7

† Lancaster soil testing method (Cox, 2001).
‡ Soil texture classification (USDA-NRCS, 2010).
§ Location 1, Stoneville, MS.
£ Location 2, Cleveland, MS.

Table 2.2 Soil chemical properties for nitrogen stabilizer experiment conducted in Stoneville, MS in 2011 and 2012

Soil Texture‡	Sum of Bases	pH	OM	Extractable Nutrients†				
				P	K	Ca	Mg	Zn
		(1:1)	(%)	--------------------mg kg^{-1}--------------------				
Sharkey clay	39.4	7.6	2.24	157	432	6570	1219	4.6
Tunica clay	28.2	7.4	1.76	140	445	4707	828	2.9

† Lancaster soil testing method (Cox, 2001).
‡ Soil texture classification (USDA-NRCS, 2010).

Table 2.3 Percent of total inorganic fertilizer–N (TN) recovery and percent of TN recovery as NO_3 and NH_4 as affected by soil and sample time, averaged across two runs, for seven soils incubated at 25°C and 80% field capacity moisture.

Soil Texture	Days after Incubation																	
	2			5			9			15			20			26		
	----% Recovery of added N¥----																	
	TN†	NO_3‡	NH_4§	TN	NO_3	NH_4	TN	NO_3	NH_4	TN	NO_3	NH_4	TN	NO_3	NH_4	TN	NO_3	NH_4
Commerce *vfsl*	17	19	81	45	22	78	69	48	52	70	72	28	71	71	29	68	95	5
Crowley *sil*	30	8	92	48	20	80	52	53	47	49	83	17	54	91	9	50	98	2
Dundee *sil*	40	12	88	48	33	67	65	69	31	56	100	0	54	99	1	46	100	0
Dundee *sicl*	37	25	75	70	24	76	80	43	57	76	74	26	81	86	14	65	95	5
Forestdale *sil*	40	22	78	61	34	66	57	66	34	44	96	4	59	98	2	43	92	8
Sharkey *c*	42	15	85	70	37	63	84	77	23	78	98	2	85	99	1	77	100	0
Sharkey *c*	44	16	84	56	34	66	68	69	31	46	95	5	64	98	2	65	100	0

† TN, Total inorganic fertilizer–N recovered as NH4 and NO3, LSD = 10.6, $\alpha < 0.05$
‡ NO3, proportion of fertilizer–N recovered as NO3, LSD = 8.3, $\alpha < 0.05$
§ NH4, proportion of fertilizer–N recovered as NH4, LSD = 8.3, $\alpha < 0.05$
¥ Mean of six replications pooled over two runs.

Table 2.4 Half-life, Y intercept, rate constant and R^2 values for each soil in the nitrification potential experiment conducted in Stoneville, MS in 2010 and 2011.

Soil Texture	Y intercept	Rate constant	Half-life¥	R^2
	----------%----------	----------d^{-1}----------	-----d-----	
Commerce *vfsl*	101.3 (6.0681)§	0.0757 (0.00802)	9.2 a	0.97
Crowley *sil*	123.7 (5.3406)	0.1144 (0.00786)	6.1 bc	0.96
Dundee *sil*	128.4 (7.8155)	0.1631(0.0145)	4.2 d	0.96
Dundee *sicl*	99.5 (6.6602)	0.0816 (0.00944)	8.5 ab	0.95
Forestdale *sil*	110.7 (10.2173)	0.1379 (0.0194)	5.0 bcd	0.97
Sharkey *c*†	126.4 (6.0915)	0.1759 (0.0121)	3.9 d	0.97
Sharkey *c*‡	120.7 (6.7988)	0.1513(0.0127)	4.6 cd	0.97

† Location 1, Stoneville, MS.
‡ Location 2 Cleveland, MS.
§ Standard error of Y intercept and rate constant with six replications pooled over two runs.
¥ Half-life, the number of days when half the total recovered inorganic–N is in the NH_4^+–N form, mean of six replications pooled over two runs.

Table 2.5 Half-life, N content, stabilizer rate, Y intercept, rate constant and R^2 values for each source in the nitrogen stabilizer experiment conducted in Stoneville, MS in 2011 and 2012.

Source	Urea†	Stabilizer Rate		Y intercept	Rate Constant	Half-life¥	R^2
	(mg)	(kg ha^{-1})	(%N w/w)	------%------	------d^{-1}------	--d--	
Urea	25			121.2 (4.674)‡	0.1631 (0.0087)	4.2 d	0.94
Nitrapyrin	25	0.56		117.2 (5.321)	0.1809 (0.0111)	3.8 d	0.93
Nitrapyrin	25	1.12		122.5 (5.445)	0.1700 (0.0103)	4.1 d	0.93
Nitrapyrin	25	2.24		119.0 (5.337)	0.1416 (0.0090)	4.9 d	0.92
DCD	25		5	110.4 (3.631)	0.0800 (0.0044)	8.7 c	0.91
DCD	25		10	109.2 (3.739)	0.0619 (0.0039)	11.2 b	0.87
DCD	25		15	108.2 (2.902)	0.0455 (0.0027)	15.2 a	0.87

† Mean weight of urea granules jar^{-1}.
‡ Standard error for Y intercept and rate constant of six replications pooled over Tunica and Sharkey clay soils and 2 runs.
¥ Half-life, the number of days when half the total recovered inorganic–N is in the NH_4^+–N form, mean of six replications pooled over Tunica and Sharkey clay soils and 2 runs.

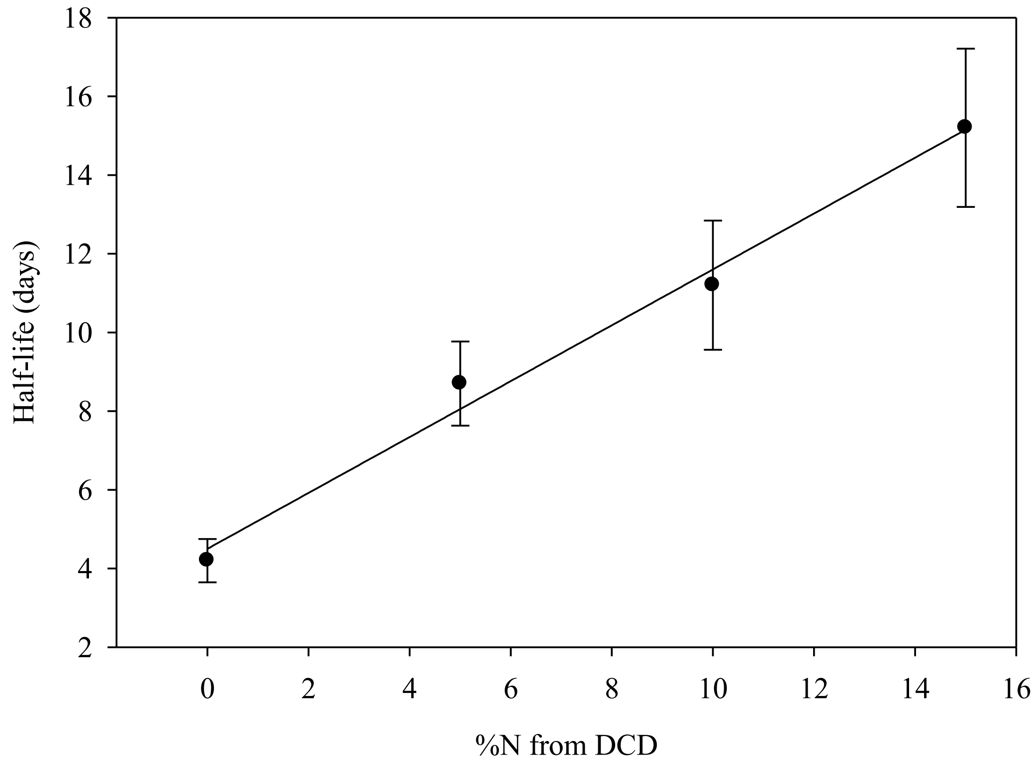

Figure 2.1 Relationship between half the total recovered inorganic–N in the NH_4^+–N form in days (Half-life) and % N from DCD with standard error bars indicating 95% confidence intervals pooled over Sharkey and Tunica clay soils and six replications in the nitrogen stabilizer experiment conducted in Stoneville, MS in 2011 and 2012. y = 0.710 (% DCD-N) + 4.50.

Literature Cited

Ali, R., J. Iqbal, G.R. Tahir, and T. Mahmood. 2008. Effect of 3,5-dimethylpyrazole and nitrapyrin on nitrification under high soil temperature. Pak. J. Bot. 40:1053-1062.

Amberger, A., and K. Vilsmeier. 1979. Dicyandiamideabbau in Quarzsand und Boden. Z. Pflanzenernaehr. Bodendkd. 142:778-785.

Aulakh, M.S., K. Singh, and J. Doran. 2001. Effects of 4-amino 1,2,4-triazole, dicyandiamide and encapsulated calcium carbide on nitrification inhibition in a subtropical soil under upland and flooded conditions. Biol. Fertil. Soils. 33:258-263.

Azam, F., S. Gill, S. Farooq, and A. Lodhi. 2004. Effect of CO_2 on nitrification and immobilization of NH_4^+–N. Biol. Fertil. Soils. 40:427-431.

Black, C.A. 1957. Soil-Plant Relationships. New York: Wiley.

Blackmer, A.M. and J.M. Bremner. 1978. Inhibitory effect of nitrate on reduction of N_20 to N_2 by soil microorganisms. Soil Biol. Biochem. 10:187-191

Boswell, F.C. 1977. Seasonal anhydrous ammonia comparison for corn with and without a nitrification inhibitor. Agron. J. 69:103-106.

Boswell, F.C., and O.E. Anderson. 1974. Nitrification inhibitor studies of soil in field-buried polyethylene bags. Soil Sci. Soc. Am. Proc. 38:851-852.

Boswell, F.C., J.G. Futral, and O.E. Anderson. 1974. Comparison of fall-applied N under formed beds with conventional spring N application for corn production. Agron. J. 66:374-377.

Bufogle, A., Jr., P.K. Bollich, J.L. Kovar, C.W. Lindau, and R.E. Macchiavelli. 1998. Comparison of ammonium sulfate and urea as nitrogen sources in rice production. J. Plant Nutr. 21:1601-1614

Chen, D., H.C. Suter, A. Islam, and R, Edis. 2010. Influence of nitrification inhibitors on nitrification and nitrous oxide (N_2O) emission from a clay loam soil fertilized with urea. Soil Biol. Biochem. 42:660-664.

Chowdary, V.M., N.H. Rao, and P.B.S. Sarma. 2004. A coupled soil water and nitrogen balance model for flooded rice fields in India. Agric. Ecosyst. Environ. 103:425-441.

Cox, M.S. 2001. The Lancaster soil test method as an alternative to the Mehlich 3 soil test method. Soil Sci. 166:484-489.

De Datta, S.K., and W.H. Patrick, Jr. (ed.) 1986. Nitrogen economy of flooded soils. Martinus Nijhoff Publ., Dordrecht, the Netherlands.

Feigenbaum, S., and A. Hadas. 1980. Utilization of fertilizer nitrogen-nitrogen-15 by field-grown alfalfa. Soil Sci. Soc. Am. J. 44:1006-1010.

Firestone, M.K., R.B. Firestone, and J.M. Tiedje. 1980. Nitrous oxide from soil denitrification: factors controlling its biological production. Sci. 208:749-751.

Focht, D.D., and W. Verstraete. 1977. Biochemical ecology of nitrification and denitrification. p. 135-214. *In* Alexander, M. (ed.) Advances in Microbial Ecology. New York: Plenum Press.

Fortuna, A., R.R. Harwood, G.P. Robertson, J.W. Fisk, and E.A.Paul. 2003. Seasonal changes in nitrification potential associated with application of N fertilizer and compost in maize systems of southwest Michigan. Agric. Ecosyst. Environ. 97:285-293.

Franzen, D., R.J. Goos, R.J. Norman, T.W. Walker, T.L. Roberts, N.A. Slaton, G. Endres, R. Ashley, J. Staricka, J. Lukach. 2011. Field and laboratory studies comparing nutrisphere-nitrogen urea with urea in North Dakota, Arkansas, and Mississippi. J. Plant Nutri. 34:1198-1222.

Garcia, J.-L. 1974. Reduction de l'oxyde nitreux dans les sols de rizieres du Senegal: mesure de l'activite denitrifiante. Soil Biol. Biochem. 6:79-84.

Garcia, J.L. and J.M. Tiedje. 1982. Denitrification in rice soils. p. 187-208. *In* Y.R. Dommergues and H.G. Diem (eds.) Microb. Trop. Soils and Plant Prod. Dr. W. Junk Publishers, Boston.

Golden, B.R., N.A. Slaton, R.J. Delong, R.J. Norman, and E.T. Maschmann. 2009. Nitrification inhibitors influence on rice grain yield and soil inorganic nitrogen fractions. p. 215-223. *In* R.J. Norman, J.F. Muellenet, K.A.K. Moldenhauer (eds.) B.R. Wells Rice Research Studies 2008. University of Arkansas, Fayetteville.

Griggs, B.R., R.J. Norman, C.E. Wilson, Jr., N.A. Slaton. 2007. Ammonia volatilization and nitrogen uptake for conventional and conservation tilled dry-seeded, delayed-flood rice. Soil Sci. Soc. Am. J. 71:745-751.

Huber, D.M., G.A. Murray, and J.M. Crane. 1969. Inhibition of nitrification as a deterrent to nitrogen loss. Soil Sci. Soc. Am. Proc. 33:975-976.

Justice, J.K., and R.L. Smith. 1962. Nitrification of ammonium sulfate in a calcareous soil as influenced by combinations of moisture, temperature, and levels of added nitrogen. Soil Sci. Soc. Am. Proc. 26:246-250.

Keeney, D.R., and K.L. Sahrawat. 1986. Nitrogen transformations in flooded rice soils. Fert. Res. 9:15-38.

Kissel, D.E., S.J. Smith, W.L. Hargrove, and D.W. Dillow. 1977. Immobilization of fertilizer nitrate applied to a swelling clay soil in the field. Soil Sci. Soc. Am. J. 41:346-349.

Kumar, U., M.C. Jain, S. Kumar, H. Pathak, and D. Majumdar. 2000. Role of nitrification inhibitors on nitrous oxide emissions in a fertilized alluvial clay loam under different moisture regimes. Curr. Sci. 79:224-228.

Littell, R.C., G.A. Milliken, W.W. Stroup, and R.D. Wolfinger. 1996. SAS system for mixed models. SAS Inst., Cary, NC.

Majumdar, D. 2002. Suppression of nitrification and N_2O emission by karanjin–a nitrification inhibitor prepared from karanja (*Pongamia glabra* Vent.). Chemosphere. 47:845-850

Massey, C.G., N.A. Slaton, R.J. Norman, E.E. Gbur, R.E.DeLong, and B.R. Golden. 2011. Bermudagrass forage yield and ammonia volatilization as affected by nitrogen fertilization. Soil Sci. Soc. Am. J. 75:638-648..

Montagnini, F.B., B. Haines, L. Boring, and W. Swank. 1986. Nitrification potential in successional black locust and in mixed hardwood forest stands in the southern Appalachians, U.S.A. Biogeochemistry. 2:197-210.

Nelson, D.W., and D. Huber. 1992. Nitrification inhibitors for corn production. [Online] Available at http://www.extension.iastate.edu/Publications/NCH55.pdf (verified 17 Aug. 2012).

Nommik, H. 1956. Investigations on denitrification in soil. Acta Agr. Scand. 6:195-228.

Norman, R.J., C.E. Wilson, Jr., and N.A. Slaton. 2003. Soil Fertilization and mineral nutrition in U.S. mechanized rice culture. P. 331-411. *In* C. Wayne Smith and Robert H. Dilday (eds.) Rice: Origin, History, Technology, and Production. John Wiley & Sons, Hoboken, NJ.

Pasda, G., R. Hahndel, and W. Zerulla. 2001. Effect of fertilizers with the new nitrification inhibitor DMPP (3,4-dimethylpyrazole phosphate) on yield and quality of agricultural and horticultural crops. Biol. Fertil. Soils. 34:85-97.

Parker, D.T., and W.E. Larson. 1962. Nitrification as affected by temperature and moisture content of mulched soils. Soil Sci. Soc. Am. Proc. 26:238-242.

Peters, J., A. Wolf, and N.Wolf. 2003. Ammonium nitrogen. p. 25-29. *In* J. Peters (ed.) Recommended Methods of Manure Analysis. Publ. A3769. Univ. of Wisconsin Ext., Madison.

Rao, P.S.C., R.E. Jessup, and K.R. Reddy. 1984. Simulation of nitrogen dynamics in flooded soils. J. Soil Sci. 138:54-62.

Reeves, D.W., and J.T. Touchton. 1986. Relative phytotoxicity of dicyandiamide and availability of its nitrogen to cotton, corn, and grain sorghum. Soil Sci. Soc. Am. J. 50:1353-1357.

Reider, G., and H. Michaud. 1980. Improving fertilizer efficiency. The use of a dicyandiamide nitrification inhibitor. Nitrogen. 124:31-35.

Rudert, B.D., and S.J. Locascio. 1979. Differential mobility of nitrapyrin and ammonium in a sandy soil and its effect on nitrapyrin efficiency. Agron. J. 71:487-489.

Sabey, B.R., W.V. Bartholomew, R. Shaw, and J.Pesek. 1956. Influence of temperature on nitrification of soils. Soil Sci. Soc. Am. Proc. 20:357-360.

Sahrawat, K.L. 2008. Factors affecting nitrification in soils. Comm. Soil Sci. and Plant Anal. 39:1436-1446.

Sahrawat, K.L., D.R. Keeney, and S.S. Adams. 1985. Rate of aerobic nitrogen transformations in six acid climax forest soils and the effect of phosphorus and CaCo3. For. Sci. 31:680-684.

Shi, W., and J.M. Norton. 2000. Effect of long-term, biennial, fall-applied anhydrous ammonia and nitrapyrin on soil nitrification. Soil Sci. Soc. Am. J. 64:228-234.

Statistical Analysis Systems (SAS). 2003. The SAS system for Windows. Release 9.1. SAS Inst., Cary, NC.

Tisdale, S.L., and W.L. Nelson. 1970. Soil Fertility and Fertilizers, 2nd ed. The Macmillan Co., New York.

Todd, R.L., W.T. Swank, J.G. Douglass, P.C. Kerr, D.L. Brockway, and C.D. Monk. 1975. The relationship between nitrate concentration in the southern Appalachian mountain streams and terrestrial nitrifiers. Agro-Ecosystems. 2:127-132.

Touchton, J.T., R.G. Hoeft, and L.F. Welch. 1978. Nitrapyrin degradation and movement in soil. Agron. J. 70:811-816.

Touchton, J.T., R.G. Hoeft, L.F. Welch, D.L. Mulvaney, M.G. Oldham, and F.E. Zajicek. 1979. N uptake and corn yield as affected by applications of nitrapyrin with anhydrous ammonia. Agron. J. 71:238-242.

Trenkel, M. 1997. Improving fertilizer use efficiency controlled-release and stabilized fertilizers in agriculture. International Fertilizer Industry Association, Paris.

USDA-NRCS. 2012. Web soil survey. Available at http://websoil-survey.nrcs.usda.gov/app/WebSoilSurvey.aspx (verified 20 October 2012). USDA-NRCS, Washington, DC.

Wetselaar, R. 1981. Nitrogen inputs and outputs of an unfertilized paddy field. p. 573-583. In F.E. Clark and T. Rosswall (eds.) Terrestrial Nitrogen Cycles. Processes, Ecosystem Strategies and Management Impacts. Ecology Bull., Stockholm.

CHAPTER III

FIELD ASSESSMENT OF NITROGEN STABILIZERS TO REDUCE NITIRIFICATION AND DENIRIFICATION IN SHARKEY CLAY SOIL

Abstract

Field research was conducted at the Delta Research and Extension Center in Stoneville, MS during 2011 and 2012. The study evaluated the effectiveness of two nitrification inhibitors (NI), nitrapyrin and dicyandiamide (DCD), and a sulfur-polymer coated urea (Agrium XCU 43% N). A factorial arrangement of treatments using combinations of nitrogen (N) sources, N stabilizer sources and rates, and application timings were arranged in a randomized complete block design and replicated four and three times in 2011 and 2012, respectively. Grain yield and total N uptake (TNU) were measured. Nitrogen (84 and 168 kg N ha^{-1}) alone and in combination with nitrapyrin (0.56, 1.12, and 2.24 kg ha^{-1}) and DCD (5, 10, and 15% of N) were dissolved in urea liquor and applied at 12 days before permanent flood establishment (dbf). Agrium was broadcasted with a custom-made granular distributor. For comparison, the same rates of urea liquor were applied 1 dbf. Treatments receiving zero N were also included. An incorporating rainfall occurred within 1 day after the 12 day application both years, thus minimizing ammonia volatilization. Data were subjected to the Mixed Procedure. Year and replications nested within year were considered random effects. Type III statistics

were used to test treatment effects, and least square means were separated at the $P < 0.05$ significance level.

Urea applied 1dbf at 168 kg N ha^{-1} provided the maximum rice grain yield and was used as the standard for comparison to all other treatments. All rates of nitrapyrin were ineffective at reducing nitrification. Increasing DCD rate to 19 kg ha^{-1} increased grain yield response although all DCD rates resulted in yields less than the standard. Agrium was most effective at reducing nitrification loss when 168 kg N ha^{-1} was applied. However, 5% yield loss still occurred relative to the standard. There was a linear relationship between grain yield and TNU. These data suggests that Agrium and some rates of DCD are effective at reducing nitrification and subsequent denitrification in Sharkey clay soil.

Introduction

Efficient use of nitrogen in rice (*Oryza sativa* L.) production is an important aspect of food security and environmental sustainability for an increasing world population. Furthermore, N fertilizer can account for 25% of the total annual rice production costs in the southern USA; therefore, there are economic advantages to improving N efficiency. In the drill-seeded, delayed-flood production system common to the southern USA, an ammonium-forming N source is applied at the 5-leaf growth stage and is incorporated into the soil profile using the floodwater. If incorporation occurs within a few days after application, N recovery can range from 65 to 75% (Norman et al., 2003). Nitrogen recovery can be greatly reduced resulting in poor yields and/or increased fertilizer and application expenses if any of the following adverse conditions exist: poorly timed N application, inadequate irrigation capacity, poor floodwater management, and soil surface moisture during application (Griggs et al., 2007). In commercial production, 7 to 10 d may be required to establish a flood. During this time, rain showers can occur which initiates the conversion of urea to ammonium and then to nitrate. Nitrate is prone to denitrification once the permanent flood is established. Stabilizing urea in the ammoniacal form would increase N recovery, even when adverse conditions are present prior to flood establishment.

Nitrification inhibitors are chemicals or biological products that delay the nitrification process by killing or disrupting the metabolism of *Nitrosomonas* bacteria (Nelson and Huber, 1992). Nitrapyrin [2-Chloro-6-(trichloromethyl)-pyridine] (N-Serve® and Instinct®, Dow AgroSciences, LLC., Indianapolis, IN) is labeled as an NI and has been investigated and commercially available for over 30 years (Touchton et al., 1978; Touchton et al., 1979; Shi and Norton, 2000; Franzen et al., 2011). Nitrapyrin inhibits nitrification in soils in the mid-western and western USA (Rudert and Locascio, 1979; Huber et al, 1969); however, it tends to be inconsistent within the southeastern USA (Touchton and Boswell, 1980; Rudert and Locascio, 1979; Redmann et al., 1964). Nitrapyrin was investigated in rice in Arkansas and Louisiana in the mid 1970's. Modest rice grain yield increases were achieved with a preplant application of nitrapyrin with ammonium fertilizer in three of four years (Touchton and Boswell, 1980; Wells, 1977). Chen et al. (2010) found that nitrapyrin was effective in decreasing nitrification over a range of soil temperatures and moisture on an alkaline clay loam Vertisol.

Dicyandiamide is a nitrification inhibitor that contains 67% N. In the soil, DCD decomposes to form ammonium and nitrate, thus eventually becomes plant available N (Reeves and Touchton, 1986; Reider and Michaud, 1980; Amberger and Vilsmeier, 1979). Wilson et al. (1990) reported nitrification inhibition with DCD amended urea in rice production in Arkansas. Furthermore, a review of experiments conducted throughout the southern USA rice belt in the 1980's in which DCD was applied with urea either preplant incorporated or preflood showed that over nine site-years of data, rice grain yields were 8% greater with DCD compared to urea alone when applied preplant. However, yields were 20% greater when urea was applied preflood compared to preplant (Wells et al., 1989). Aulakh et al. (2001) evaluated DCD in upland and flooded conditions with a subtropical sandy loam soil and results indicated that DCD was effective as a nitrification inhibitor for up to 20 days under upland conditions (Aulakh et al. 2001).

Coated urea products have also shown potential for keeping nitrogen available for plant uptake over longer periods of time (Carreres et al., 2003; Xu et al., 2013). Increased interest in reducing greenhouse gas emissions and N losses from inorganic fertilizers has led to the production and marketing of polymer coated urea (PCU) products for corn production in the mid-western USA and has inspired interest for its use in rice production (Golden et al., 2009). Slaton et al. (2009) performed research to determine the effects of a PCU (43%N) at reducing nitrification on clay and two silt loam soils. The PCU fertilizer was applied at various timings and 2 N rates. Results indicated that the PCU tended to release N too quickly to obtain optimal rice yields (Slaton et al., 2009). Research conducted in rice produced in Spain demonstrated the effectiveness of a polymer coated urea compared to urea alone when the flood was delayed 15 days after application (Carreres et al., 2003).

Each of these studies has contributed to the current understanding of nitrification inhibition under various conditions. Further research is needed to fully understand the best practices for efficient N use, particularly with respect to Mississippi alluvial plain soils and climatic conditions where rice production is extensive. The objective of this research was to evaluate products within a drill-seeded, delayed-flood rice culture that could potentially limit N loss due to nitrification and subsequent denitrification in Sharkey clay (very-fine, smectitic, thermic Chromic Epiaquerts) soil when a flood could not be established within several days after fertilizer application.

Materials and Methods

Site Description and Cultural Practices

Field experiments were conducted on a Sharkey clay soil at the Delta Research and Extension Center in Stoneville, MS, in 2011 and 2012. The soil textures were identified using the USDA-NRCS soil survey information compiled on the Web Soil Survey (USDA-NRCS, 2012) (Table 3.1). Soil samples were collected each year prior to planting and the soil chemical properties were determined using the Lancaster soil testing method (Cox, 2001) (Table 3.1). ‘CL162’ (Solomon et al., 2012) rice was drill-seeded using a Great Plains drill (Great Plains Mfg., Inc. 1525 E. North Street, Salina, KS) at a rate of 90 kg ha^{-1} and grown in a delayed-flood culture. Seeding occurred on 19 April 2011 and 20 March 2012. The earlier planting date in 2012 was due to favorable environmental conditions at that time. A flood was established at the 5- to 6-leaf growth stage and maintained until approximately 2 weeks before harvest. Experimental units (plots) consisted of eight, 4.6- m rows spaced 20- cm apart, and each replication was separated by a 1.6- m alley. The study was conducted as a factorial arrangement of treatments in a randomized complete block design. The experiment consisted of nineteen treatments that included combinations of N rates, stabilizer sources, and application timings replicated four and three times in 2011 and 2012, respectively. Urea liquor (23% N), Agrium (XCU™ 43-0-0-4S SGN 250, Agrium Advanced Technologies, Inc., Loveland, CO), nitrapyrin, and DCD were all applied with N rates of 84 and 168 kg ha^{-1}. Agrium is a slow release sulfur-polymer coated urea and was applied using a Hege 80 belt cone (Wintersteiger, Inc., Salt Lake City, UT) and a zero-max (Zero-Max, Inc., Plymouth, MN) situated onto a custom-manufactured, self-propelled distributor. All treatments other than Agrium were applied using a CO_2 backpack sprayer equipped with Teejet SJ3-04-VP nozzles. Nitrapyrin (applied as Instinct®) and DCD were applied at 0.56, 1.12, and 2.24 kg ha^{-1} and 5, 10, and 15% N w/w, respectively. The urea liquor was applied at 12 and 1 dbf. All other treatments were applied at 12 dbf. In both years, 2- cm of rainfall occurred the day after the 12 dbf application which allowed for downward movement of the urea and subsequent hydrolysis. A treatment with no added N was also included. Plots were managed according to Buehring et al. (2008) to minimize variability from pests.

Data Collection

Total above ground plant tissue was collected from the second inside row of each plot over a sampling length of 0.9 m of row when rice was at approximately 5% heading (HD). Biomass was oven dried at 60°C until a constant weight was observed. The samples were then weighed to determine the total dry matter (TDM). A Wiley Mill with a #40 screen was used to grind the samples. A dry combustion analyzer (Carlo Erba, Milan, Italy) was utilized to determine the N concentration. Total N uptake (TNU) was calculated by multiplying TDM and N concentration.

Rice plots were harvested when the range of grain moisture was between 150 and 180 g kg^{-1}. A Wintersteiger Delta combine (Wintersteiger, Inc., Salt Lake City, UT) containing a Harvest Master weighing system (Juniper Systems, Inc., Logan, UT) was used to harvest the rice plots. Grain yields were adjusted to a moisture content of 120 g kg^{-1} for analysis.

Statistical Analysis

Rice grain yield and TNU were subjected to the Mixed Procedure (Littell et al., 1996; SAS, 2003). Year and replications nested within year were considered random effects. This statistical approach has been used successfully in previous research (Carmer et al., 1989; Ottis et al., 2004; Bond et al., 2005; Walker et al., 2006). Type III statistics were used to test treatment effects, and least square means were separated at the $P < 0.05$ significance level. A straight line model was used to determine the relationship between rice grain yield and TNU.

Results and Discussion

Grain Yield

Preflood urea application at 1 dbf (Table 3.2) provided the most grain yield compared to other treatments. At both levels of N, urea application at 12 dbf resulted in approximately 1900 kg ha^{-1} less grain yield compared to 1 dbf (Table 3.3). Interestingly, yield produced from 84 kg N ha^{-1} and applied 1 dbf was similar to what was produced with 168 kg N ha^{-1} applied 12 dbf. This suggested that one-half the amount of N that contributed to grain production was lost in the system.

No combination of nitrapyrin and N rate resulted in greater yield compared to urea alone. However, grain yield tended to increase with increasing concentration of DCD when 84 kg N ha^{-1} was applied. Grain yield was 19% greater with 15% DCD compared to urea alone applied 12 dbf, and this yield was similar to that produced by 84 kg N ha^{-1} applied 1 dbf. DCD concentration was not as important when 168 kg N ha^{-1} was applied. DCD + urea applied at 12 dbf resulted in grain yields that were approximately 18% greater than urea alone. Though DCD amended urea resulted in substantially greater yields compared to urea alone applied 12 dbf, the standard 1 dbf application of 168 kg N ha^{-1} was still 8 to 11% greater than the DCD amended urea (Table 3.3).

Agrium applied 12 dbf resulted in a similar yield compared to the standard 1 dbf application of urea as well as DCD amended urea at the high N level. At the lower rate of N, Agrium also produced similar yields to the standard 1 dbf and the 15% DCD amended urea (Table 3.3).

Similar to our results, in the delayed-flood rice production system common to the southern USA, maximum rice grain yield occurs when the flood can be established within a few days after preflood N application. This practice minimizes N loss mechanisms and enhances N uptake and grain yield potential of delayed-flood rice (Griggs et al., 2007; Norman et al., 2009; Dillon et al., 2012). Boswell (1977) reported that under cool conditions nitrapyrin was effective but at greater temperatures it became ineffective due to high soil temperatures and rapid nitrapyrin breakdown (Rudert and Locascio, 1979;

Boswell, 1977). Maximum soil temperatures in our study in top 5 cm averaged 37 and 33°C for 2011 and 2012, respectively. Furthermore, the high pH and large clay content could have also minimized the effectiveness of nitrapyrin. Wells (1977) reported moderate rice yield increases with nitrapyrin for rice grown on acid silt loam soil in Arkansas. Our findings are also similar to Wells et al., (1989) in that DCD was effective at increasing grain yield when urea was applied and remained in aerobic conditions for an extended period of time; however, our grain yield response to DCD was much greater than the average of the clay soils they reported even though we allowed nitrification conditions to persist for approximately one-half the amount of time.

Previous research reported that a 40% N polymer coated urea applied basally prior to flood establishment improved grain yield and recovery efficiency compared to other N sources within the study (Carreres et al., 2003). However, Slaton et al. (2009) suggested that two PCU's similar to what we report released N too rapidly to be effective as a preplant or early post emergence application of N. The fact that we applied the PCU within 12 dbf is likely why it performed well in our experiments.

Total Nitrogen Uptake

There was a strong linear relationship between rice grain yield and TNU at heading (Figure 3.1; Table 3.3). These data confirm grain yield responses were a result of the amount of N available to the plant at the critical times for grain yield production. Increased N accumulation in the rice plant throughout the growing season improves the probability of high rice grain yields when harvested (Ntamatungiro et al., 1999). Research has shown that absorption of N during the vegetative growth stage contributes to rice development during reproductive and grain-filling stages through translocation

(Bufogle et al., 1997; Norman et al., 1992), and that the greatest TNU occurs at HD (Norman et al., 1992; Guindo et al., 1994). Nitrogen recovery has been greater 1 dbf compared to 10 dbf in other delayed-flood rice experiments (Norman et al., 2009; Dillon et al., 2012).

Conclusion

Our results confirm many others reporting rice grain yields can be maximized when an ammoniacal source of N is applied within three days before flood establishment and the field remains flooded for approximately 3 weeks. However, this seldom occurs in southern USA commercial rice production. When fertilizer is allowed to persist for several days in aerobic conditions, nitrification can occur. The Sharkey soil we investigated is prone to greater loss from nitrification and subsequent denitrification compared to ammonia volatilization. When DCD is applied between 8 and 16 kg N ha^{-1}, grain yield loss due to nitrification was substantially reduced. Additionally, a sulfur/polymer coated urea produced by Agrium was very effective as an N source within the conditions of this study. This work shows that better N efficiency can be obtained; however, economics and environmental policy will ultimately dictate the utilization of products that minimize loss of N through nitrification and denitrification. Further development of products that are effective in stabilizing urea against nitrification losses and are economically feasible are needed. Until then, growers are encouraged to manage fertilizer timing and irrigation as effectively as possible to capture the greatest N use efficiency possible.

Table 3.1 Soil chemical properties for field experiments conducted in Stoneville, MS in 2011 and 2012.

Year	Soil Texture‡	Sum of Bases	pH (1:1)	OM (%)	Extractable Nutrient Levels† P	K	Ca	Mg	Zn
					--------------------mg kg^{-1}---------------------				
2011	Sharkey clay	41.7	8.0	2.25	65	334	5350	1694	2.3
2012	Sharkey clay	32.6	7.9	2.11	79	307	4440	1148	3.2

† Lancaster soil testing method (Cox, 2001).
‡ Soil texture classification (USDA-NRCS, 2010).

Table 3.2 Dates of agronomic management and sampling events for yield and total N uptake studies conducted in Stoneville, MS in 2011 and 2012.

Event	2011	2012
Seeding†	19 April	20 March
Emergence	26 April	29 March
Preflood Treatments 12dbf‡	20 May	20 April
Preflood Treatments 1dbf	31 May	1 May
Flood establishment	1 June	2 May
5% HD§	19 July	21 June
Harvest	25 August	26 July

† Seeding, 'CL162' at 90 kg ha^{-1}.
‡ dbf, days before flood establishment.
§ HD, heading growth stage.

Table 3.3 Rice grain yield and total N uptake for all combinations of N source, stabilizer, stabilizer rate, application timing and N rate conducted on a Sharkey clay soil in Stoneville, MS in 2011 and 2012.

N Source	Stabilizer	Stabilizer Rate	dbf§	N Rate	Grain Yield¥	Total N Uptake
				--------------------kg ha^{-1}	--------------------	--------------------
Urea			1	168	9579 a	133 a
Agrium 43%			14	168	9112 ab	127 ab
Urea	DCD	15†	14	168	8794 bc	126 a-c
Urea	DCD	10	14	168	8757 bc	122 a-c
Urea	DCD	5	14	168	8498 b-d	116 a-d
Urea	Nitrapyrin	0.56‡	14	168	8079 c-e	104 c-g
Urea	Nitrapyrin	1.12	14	168	7981 de	108 b-f
Urea	Nitrapyrin	2.24	14	168	7736 e	88 f-i
Urea			14	168	7675 e	112 a-e
Urea			1	84	7360 ef	94 e-h
Urea	DCD	15	14	84	6841 fg	94 d-h
Agrium 43%			14	84	6829 fg	74 h-j
Urea	DCD	10	14	84	6217 gh	66 ij
Urea	Nitrapyrin	1.12	14	84	5979 h	64 j
Urea	Nitrapyrin	0.56	14	84	5970 h	74 h-j
Urea	Nitrapyrin	2.24	14	84	5948 h	68 ij
Urea	DCD	5	14	84	5778 h	82 g-j
Urea			14	84	5740 h	66 ij
UTC				0	4013 i	30 k

† %N from DCD

‡ Rate of nitrapyrin (kg ha^{-1})

§ dbf, days before flood establishment.

¥ Mean rice grain yield and total N uptake of seven replications pooled over two years.

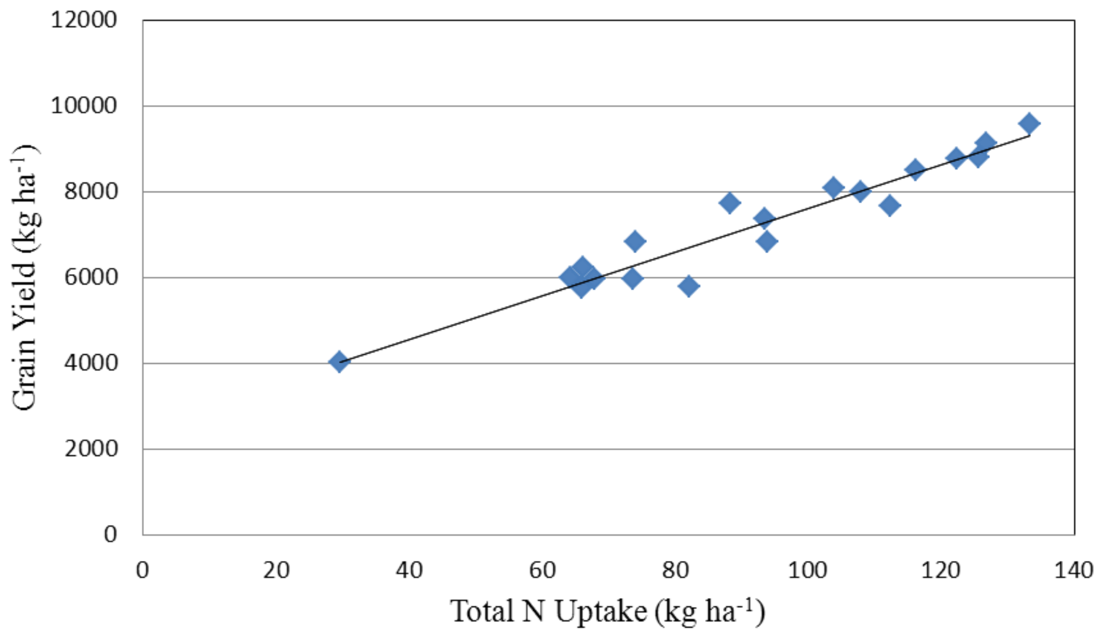

Figure 3.1 Relationship between rice grain yield and total N uptake for all combinations of N source, stabilizer, stabilizer rate, application timing and N rate conducted on a Sharkey clay soil in Stoneville, MS in 2011 and 2012. y = 50.78 (total N uptake) + 2532.

Literature Cited

Amberger, A., and K. Vilsmeier. 1979. Dicyandiamideabbau in Quarzsand und Boden. Z. Pflanzenernaehr. Bodendkd. 142:778-785.

Aulakh, M.S., K. Singh, and J. Doran. 2001. Effects of 4-amino 1,2,4-triazole, dicyandiamide and encapsulated calcium carbide on nitrification inhibition in a subtropical soil under upland and flooded conditions. Biol. Fertil. Soils 33:258-263.

Bond, J.A., T.W. Walker, P.K. Bollich, C.H. Koger, and P. Gerard. 2005. Seeding rates for stale seedbed rice production in the midsouthern united states. Agron. J. 97:1560-1563.

Boswell, F.C. 1977. Seasonal anhydrous ammonia comparison for corn with and without a nitrification inhibitor. Agron. J. 69:103-106.

Buehring, N., D. Kanter, T. Walker, J. Bond, M. Silva, L. Pringle, J. Damicone, B. Moore, J. Fox, G. Sciumbato, J. Robbins, and S. Martin. 2008. Mississippi Rice Growers' Guide. Publication 2255. Mississippi State University Extension Service, Mississippi State, MS.

Bufogle, A., P.K. Bollich, R.J. Norman J.L. Kovar, C.W. Lindau, and R.E. Macchiavelli. 1997. Rice plant growth and nitrogen accumulation in drill-seeded and water-seeded culture. Soil Sci. Soc. Am. J. 61:832-839.

Carmer, S.G., W.E. Nyquist, and W.M. Walker. 1989. Least significant differences for combined analyses of experiments with two- or three-factor treatment designs. Agron. J. 81:665-672.

Carreres, R., J. Sendra, R. Ballesteros, E.F. Valiente, A. Quesada, D. Carrasco, F. Leganes, and J.G. Cuadra. 2003. Assessment of slow release fertilizers and nitrification inhibitors in flooded rice. Biol. Fertil. Soils. 39:80-87.

Chen, D., H.C. Suter, A. Islam, and R, Edis. 2010. Influence of nitrification inhibitors on nitrification and nitrous oxide (N_2O) emission from a clay loam soil fertilized with urea. Soil Biol. Biochem. 42:660-664.

Cox, M.S. 2001. The Lancaster soil test method as an alternative to the Mehlich 3 soil test method. Soil Sci. 166:484-489

Dillon, K.A., T.W. Walker, D.L. Harrell, L.J. Krutz, J.J. Varco, C.H. Koger, and M.S. Cox. 2012. Nitrogen sources and timing effects on nitrogen loss and uptake in delayed flood rice. Agron. J. 104:466-472.

Franzen, D., R.J. Goos, R.J. Norman, T.W. Walker, T.L. Roberts, N.A. Slaton, G. Endres, R. Ashley, J. Staricka, J. Lukach. 2011. Field and laboratory studies comparing nutrisphere-nitrogen urea with urea in North Dakota, Arkansas, and Mississippi. J. Plant Nutri. 34:1198-1222.

Golden, B.R., N.A. Slaton, R.J. Delong, R.J. Norman, and E.T. Maschmann. 2009. Nitrification inhibitors influence on rice grain yield and soil inorganic nitrogen fractions. p. 215-223. *In* R.J. Norman, J.F. Muellenet, K.A.K. Moldenhauer (eds.) B.R. Wells Rice Research Studies 2008. University of Arkansas, Fayetteville.

Griggs, B.R., R.J. Norman, C.E. Wilson, Jr., N.A. Slaton. 2007. Ammonia volatilization and nitrogen uptake for conventional and conservation tilled dry-seeded, delayed-flood rice. Soil Sci. Soc. Am. J. 71:745-751.

Guindo, D., R.J. Norman, and B.R. Wells. 1994. Accumulation of fertilizer nitrogen-15 by rice at different stages of development. Soil Sci. Soc. Am. J. 58:410-415.

Huber, D.M., G.A. Murray, and J.M. Crane. 1969. Inhibition of nitrification as a deterrent to nitrogen loss. Soil Sci. Soc. Am. Proc. 33:975-976.

Littell, R.C., G.A. Milliken, W.W. Stroup, and R.D. Wolfinger. 1996. SAS system for mixed models. SAS Inst., Cary, NC.

Nelson, D.W., and D. Huber. 1992. Nitrification inhibitors for corn production. [Online] Available at http://www.extension.iastate.edu/Publications/NCH55.pdf (verified 17 Aug. 2012).

Norman, R.J., D. Guindo, B.R. Wells, and C.E. Wilson, Jr. 1992. Seasonal accumulation and partitioning of nitrogen-15 in rice. Soil Sci. Soc. Am. J. 56:1521-1526.

Norman, R.J., C.E. Wilson, Jr., and N.A. Slaton. 2003. Soil fertilization and mineral nutrition in U.S. mechanized rice culture. P. 331-411. *In* C. Wayne Smith and Robert H. Dilday (eds.) Rice: Origin, History, Technology, and Production. John Wiley & Sons, Hoboken, NJ.

Norman, R.J., C.E. Wilson, Jr., N.A. Slaton, B.R. Griggs, J.T. Bushong, and E.E. Gbur. 2009. Nitrogen fertilizer sources and timing before flooding dry-seeded, delayed-flood rice. Soil Sci. Soc. Am. J. 73:2184-2190.

Ntamatungiro, S., R.J. Norman, R.W. McNew, and B.R. Wells. 1999. Comparison of plant measurements for estimating nitrogen accumulation and grain yield by flooded rice. Agron. J. 91:676-685.

Ottis, B.V., J.H. O'Barr, G.N. McCauley, and M.J. Chandler. 2004. Imazethapyr is safe and effective for imidazolinone-tolerant rice grown on coarse-textured soils. Weed Technol. 18:1096-1100.

Redmann, C.T., R.W. Meikle, and J.G. Widofsky. 1964. The loss of 2-chloro-6-(trichloromethyl)pyridine from soil. J. Agric. Food Chem. 12(3):207-209.

Reeves, D.W., and J.T. Touchton. 1986. Relative phytotoxicity of dicyandiamide and availability of its nitrogen to cotton, corn, and grain sorghum. Soil Sci. Soc. Am. J. 50:1353-1357.

Reider, G., and H. Michaud. 1980. Improving fertilizer efficiency. The use of a dicyandiamide nitrification inhibitor. Nitrogen. 124:31-35.

Rudert, B.D., and S.J. Locascio. 1979. Differential mobility of nitrapyrin and ammonium in a sandy soil and its effect on nitrapyrin efficiency. Agron. J. 71:487-489.

Shi, W., and J.M. Norton. 2000. Effect of long-term, biennial, fall-applied anhydrous ammonia and nitrapyrin on soil nitrification. Soil Sci. Soc. Am. J. 64:228-234.

Slaton, N.A., B.R. Golden, and R.J. Norman. 2009. Rice response to urea and two polymer-coated urea fertilizers. p. 211-219. *In* R.J. Norman and K.A.K. Moldenhauer (eds.) B.R. Wells Rice Research Studies 2009. University of Arkansas, Fayetteville.

Solomon, W.L., D.G. Kanter, T.W. Walker, G.E. Baird, L.S. Lanford, S. Shaifer, and P.W. Fitts. 2012. Registration of 'CL162' long-grain rice. J. Plant Reg. 6:23-26.

Statistical Analysis Systems (SAS). 2003. The SAS system for Windows. Release 9.1. SAS Inst., Cary, NC.

Touchton, J.T., R.G. Hoeft, and L.F. Welch. 1978. Nitrapyrin degradation and movement in soil. Agron. J. 70:811-816.

Touchton, J.T., R.G. Hoeft, L.F. Welch, D.L. Mulvaney, M.G. Oldham, and F.E. Zajicek. 1979. N uptake and corn yield as affected by applications of nitrapyrin with anhydrous ammonia. Agron. J. 71:238-242.

Touchton, J.T., and F.C. Boswell. 1980. Performance of nitrification inhibitors in the southeast. p.63-74. *In* J.J. Meisinger (ed.) Nitrification Inhibitors-Potentials and Limitations. ASA Spec. Publ. 38. ASA and CSSA, Madison, WI.

USDA-NRCS. 2012. Web soil survey. Available at http://websoil-survey.nrcs.usda.gov/app/WebSoilSurvey.aspx (verified 20 October 2012). USDA-NRCS, Washington, DC.

Walker, T.W., S.W. Martin, and P.D. Gerard. 2006. Grain yield and milling quality response of two rice cultivars to top-dress nitrogen application timings. Agron. J. 98:1495-1500.

Wells, B.R. 1977. Nitrapyrin (2-chloro-6-[trichloromethyl]-pyradine) as a nitrification inhibitor for paddy rice. Down to Earth. 32:28-32.

Wells, B.R., P.K. Bollich, W. Ebelhar, D.S. Mikkelsen, R.J. Norman, D.M. Brandon, R.S. Helms, F.T. Turner, and M.P. Westcott. 1989. Dicyandiamide (DCD) as a nitrification inhibitor for rice culture in the United States. Comm. Soil Sci. and Plant Anal. 20:2023-2047.

Wilson, C.E., Jr., R.J. Norman, and B.R. Wells. 1990. Dicyandiamide influence on uptake of preplant-applied fertilizer nitrogen by rice . Soil Sci. Soc. Am. J. 54:1157-1161.

Xu, M., D. Li, J. Li, D. Qin, Y. Hosen, H. Shen, R. Cong, X. He. 2013. Polyolefin-coated urea decreases ammonia volatilization in a double rice system of southern china. Agron. J. 105:277-284.