Fertilizer Nitrogen Uptake Efficiency in Soft Red Winter Wheat and the Ability of N-STaR to Detect Alkaline Hydrolyzable Nitrogen in Crop Residues

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Fertilizer Nitrogen Uptake Efficiency in Soft Red Winter Wheat and the Ability of N-STaR to Detect Alkaline Hydrolyzable Nitrogen in Crop Residues

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Crop, Soil, and Environmental Sciences

By

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Auburn University
Bachelor of Science in Animal Sciences, 2010

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This thesis is approved for recommendation to the Graduate Council.
ABSTRACT

Soil testing methods such as the Illinois Soil Nitrogen Test (ISNT) and Direct Steam Distillation (DSD) have been developed which measure alkaline hydrolyzable-N (AH-N) as a means of estimating potentially mineralizable-N. Crop residues play an important role in N cycling. However, the ability of the ISNT and DSD methods to determine AH-N within crop residues is unknown. Therefore, the first objective of this study was to determine the ability of the ISNT and DSD to quantify potentially mineralizable-N within five different crop residues common to Arkansas. Corn (Zea mays L.), soybean (Glycine max, L.), wheat, rice (Oryza sativa, L.), and grain sorghum (Sorghum bicolor, L.) residues were labeled with $^{15}$N using 10 atom% $^{15}$N labeled-urea. A 0.2 g subsample of residue was subjected to both the DSD and ISNT. Hydrolyzed-N was captured and analyzed for atom % $^{15}$N to compare fertilizer atom % $^{15}$N to that of the original residue. Total N was quantified to establish percent recovery. Analysis of variance for percent N recovery showed a significant residue by method interaction (p<0.0001) indicating that the two methods recovered varying amounts of N based on the type of residue. Atom % $^{15}$N recovered from the soybean residue as AH-N was significantly lower than what was quantified in the plant tissue. Conversely, atom % $^{15}$N recovered from the rice residue as AH-N was significantly greater than that which was quantified in the original plant tissue. Comparison of atom % $^{15}$N in the residue and recovered AH-N suggested that certain crop species partition fertilizer N differently. The final objective of this study was to determine the influence of N rate and application time on fertilizer N uptake efficiency (FNUE) for winter wheat on a poorly-drained silt loam soil. Six different fertilizer N-rates were applied by hand ranging from 0 to 225 kg N ha$^{-1}$ at three different times: Early-single, Late-single, and Split applications in 1.5 x 1.74 m microplots using 2.65 atom% $^{15}$N-labeled urea. There was a significant application time by rate
interaction (p<0.0408). The greatest FNUE was achieved with the Early-single and Split applications at the 90 kg N ha\(^{-1}\) rate, and were 80.1% and 83.1%, respectively. The minimum yield-maximizing, N-rate was determined to be 135 kg N ha\(^{-1}\) applied as an Early-single or Split application. The Late-single application across all N-rates resulted in lower FNUE and yield. Soil N uptake was not significantly different for any of the treatments that received fertilizer regardless of N rate or timing of application, but were significantly higher than soil N uptake where no fertilizer was applied. Total N uptake by the wheat was directly related to fertilizer N uptake with the Early-single and Split application tending to have higher TN uptake than the Late-single application. Results of both the TN uptake and FNUE support the yield data obtained in these trials and indicate that current N rate recommendations for wheat produced in the delta region of Arkansas optimize fertilizer N inputs while maintaining high yields. These results highlight the importance of proper rate and application time for maximizing FNUE and yield in winter wheat production.
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I would also like to express my appreciation to Chester Greub, Stephanie Williamson, and Carri Scott. Your assistance both in field work and in lab work have been monumentally helpful. Your have all gone above and beyond what was required of you for my sake, and I cannot say thank you enough. Nevertheless, I am even more grateful for your friendship. It is a rare thing to enjoy the workplace as much as I have, but I have truly cherished being a part of the N-STaR family.
DEDICATION

I would like to dedicate this thesis to my loving husband, Clayton Clark. You are the picture of sacrificial love to me. Thank you for being the servant leader that you are. Jesus reveals His character more to me every day through you. These past couple of years would have been impossible without you. I love you forever.

Also, to my wonderful daughter, Camella. You bring so much joy into my life. I hope one day you see the value of education and the blessing it is to be offered the opportunity to pursue it. I pray you are known for your character, hard work, and integrity that you may bring glory to Jesus in whatever avenue you choose to pursue in life. I love you more than you can imagine.
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CHAPTER ONE

Introduction and Literature Review
INTRODUCTION

United States agriculture is an extremely important industry contributing to almost one percent of the domestic GDP and the U.S. is the world’s largest agricultural exporter. Wheat (*Triticum aestivum* L.) is ranked as the third largest field crop, in terms of total acreage, with corn (*Zea mays* L.) and soybeans (*Glycine max* L.) holding the first and second rankings. In 2011 alone, 18,490,000 ha of wheat were produced in the U.S. and approximately half was exported to countries around the world. With worldwide populations growing and diminishing farmland, due to the development of arable land, producers are faced with the challenge of growing more food on less cropland. Over the past 50 years, corn yields have tripled and wheat yields have doubled, despite fewer acres being farmed. Although wheat has continued to hold a considerable portion of the agricultural industry, its production has continued to decline due to changes in government policies that allow farmers more freedom in planting, increased corn planting for ethanol, and lower profits compared to other crops (Economic Research Service, 2012).

Mississippi, Louisiana, and Arkansas play a significant role in U.S. wheat production. Although rice (*Oryza sativa* L.) is a dominant crop in Arkansas, over 210,000 ha of wheat were harvested in Arkansas in 2011 with a five-year average wheat harvest of 215,000 ha (National Agricultural Statistics Service, 2012). Southeastern wheat production is mainly located on poorly-drained silt-loam to clayey-textured soils. This presents no problem for rice production, but wheat is best suited for production on soils with adequate internal drainage and does not perform well under water-logged conditions. Typically, the seasons of wheat dormancy and growth (winter and spring) have greater total rainfall than summer or fall. Average seasonal rainfall in the Arkansas Delta Region, as reported by the National Oceanic and Atmospheric
Administration, for winter and spring seasons are 33.4 cm and 38.8 cm, respectively. Low temperatures combined with small plants and heavy precipitation allows for little evapotranspiration to alleviate potentially saturated soils of the excess moisture. Other problems associated with poorly drained soils are the buildup of Fe$^{2+}$ and Mn$^{2+}$ in the soil and wheat plant resulting in concentrations that may be toxic and severely limit wheat yields (Carver et al., 1995). Wheat is successful grown on these poorly drained soils by placing the wheat on raised beds or through the use of drainage ditches to allow drainage of excess water from the field.

In addition to dealing with less than optimal growing conditions, agriculture production costs continue to rise. Nitrogen (N) fertilizer represents one of the greatest costs of production agriculture, and prices of fertilizer N continue to rise as demand and manufacturing costs increase. With fossil fuel prices rising, there seems to be no decrease in fertilizer N prices in the foreseeable future, which raises the stakes for implementing greater fertilizer N uptake efficiency (FNUE) and management practices. World-wide, N is the most heavily applied nutrient in production agriculture, both in terms of tonnage and acreage. For cereal crops N is the most limiting nutrient to growth and therefore must be applied to most fields to maximize yield and, correspondingly, profit. In 2012 fertilizer N represented 31% ($1.50 \text{ kg}^{-1}$) of the input production costs associated with Arkansas wheat production, and this fraction is larger than any other row crop produced within the state (University of Arkansas Cooperative Extension Service, 2012). Due to the large portion of input costs devoted to N it is imperative that FNUE is maximized in order for producers to remain profitable and stay in business.

The majority of Arkansas wheat research to date has been conducted on well-drained soils, and a study is needed that establishes the fertilizer N needs and efficiency of wheat produced on poorly-drained soils, where most of the wheat in Arkansas is predominantly
planted. Incorporating a study using the stable isotope N$^{15}$, will allow direct quantification of the FNUE. Marginal cost has become vital for producer profitability in wheat. With so little room for error, it is essential that maximum FNUE is achieved.

Current averages reported by the National Agricultural Statistics Service (2011) for winter wheat fertilizer N are based on yield goal in bushels acre$^{-1}$. Current Nitrogen rate recommendations can range from 85-225 kg N ha$^{-1}$, but do not take into account the current levels of potentially mineralizable soil-N which may differ greatly from one location to the next. Over-application of fertilizer N can lead to yield decreases, and profit losses by both increased cost and decreased yield. Current fertilizer N recommendations for Arkansas wheat grain production range from 100 to 135 kg N ha$^{-1}$ on loamy-textured soils following crops other than fallow (less N) or rice (more N). According to the 2011 Wheat Verification Program, producers participating in the program were applying approximately 135 to 145 kg N ha$^{-1}$, which is slightly above the recommendation guidelines.

The objective of the literature review is to give a brief overview of the previous research in wheat which relates to fertilizer N uptake and utilization on poorly-drained soils, and to summarize the research status which pertains to how crop residue type and placement affects its N content and the ability to quantify it by different chemical methods.
Wheat N uptake experiments in Arkansas using the stable isotope $^{15}$N were conducted by Bashir et al. (1997) on a Roxanna silt loam near Kibler, AR. Nitrogen fertilizer was applied at tillering in two applications at a rate of 112 kg ha$^{-1}$. Bashir et al. (1997) concluded that a maximum fertilizer N accumulation of 74.4% occurred in the plant at flag leaf emergence then declined until maturity. This indicates that plant sampling should be conducted at the flag leaf emergence stage for accurate fertilizer N uptake measurements. Approximately 86% of applied fertilizer was accounted for in the plant and the soil. A study by Daigger et al. (1976) showed that as the amount of fertilizer N applied increased, N losses became increasingly greater. A possible means of applied N loss is thought to be ammonia volatilization through the leaves and spikes of the wheat plants themselves. Parton et al. (1998) and Wetselaar and Farquhar (1980) showed that plants with larger leaf surface area have greater N losses (and lower NUE) than those with less leaf area. Hence, stomatal conductance, which is increased by high light intensity, high temperature, high N level, and plentiful moisture, increase N losses. Although a greater understanding of N assimilation was obtained, Bashir’s experiment was carried out on well-drained soil, which does not give an accurate representation of typical soils used for wheat production in Arkansas. This poses a problem when trying to give recommendations to producers or base further research decisions off of the previously conducted ones. Hence, a study conducted on poorly drained soils more commonly used for wheat production needs to be initiated.
Nitrogen is generally the most growth limiting nutrient and essential to crop quality because protein content in crops is directly related to N supply. Ecologically, efficient use of fertilizer N is important to long-term sustainability, ground water quality, greenhouse gas emission, and global warming due to NO$_3^-$ leaching and N$_2$O emissions (Grant, 2002). Nitrogen management plays a substantial role in improving quality and yield in crops, ensuring environmental safety, and maximizing production economics (Campbell, 1993).

**Crop Residue Effect on Mineralization**

Legume and cereal crop residue plays a significant role in soil N cycling. Type of residue, placement, level of incorporation, and water management can dictate potentially mineralizable soil-N. Many researchers have devoted studies to how crop residues affect denitrification and N mineralization in the soil. These studies report wide variations in mineralized-N associated between incorporated and surface-applied crop residues. In some cases, N immobilization actually increased (Doran, 1987; Aulakh, 1991). Aulakh et al. (1991) researched the effect of vetch (*Vicia villosa*), soybean, corn, and wheat crop residue on N mineralization and found that in crops possessing wide C:N ratios, a net N immobilization occurred, whereas crops with low C:N ratios (vetch, soybean) can increase N mineralization in the soil. Drury et al. concluded in 1991 that cover crop varies by type in amount of readily available C, and that this is related to denitrifying microbial activity and N mineralization later in the season.

**Illinois Soil Nitrogen Test**

In order to make accurate fertilizer N recommendations, the proper soil-N fraction must be measured. Because of the dynamic nature of soil NO$_3^-$ concentrations, measuring the organic
soil N supply that mineralizes to feed the plant would be ideal rather than measuring only NO$_3^-$
Mulvaney et al. (2001) identified amino sugar-N as this ideal measurable soil N fraction. He and his colleague then developed the Illinois Soil Nitrogen Test (Kahn et al., 2001) now referred to as the ISNT. Though the ISNT is able to identify soils cropped to corn with an amino sugar-N concentration above a critical level as nonresponsive to N-fertilizer applications (>250 mg kg$^{-1}$) or responsive (<200 mg kg$^{-1}$), it is unable to give N-fertilizer rate recommendations based on these results.

Previous techniques to determine potential N mineralization are based on anaerobic and aerobic incubation of the soil, and although these are consistent and relatively accurate, they do not lend themselves to routine laboratory analysis due to the ~ 14 d analysis time. Bushong et al. (2008) determined that the ISNT was a comparable N-testing technique that was more conducive for routine soil analysis due to its simplicity and rather quick analysis time.

Wall et al. (2010) and Steckler et al. (2008) both concluded that further ISNT studies were needed to calibrate site-specific recommendations. Wall et al. (2010) inferred that because the ISNT measures the microbial fraction of soil N, variability in microbial populations among regions would make it impossible to apply the same ISNT-based fertilizer recommendations across a variety of soils. They stated that site-specific calibrations with defined sampling periods should be further investigated because of soil ISNT-N differences attributed to sampling depth, tillage, previous crop, soil texture, etc. Steckler et al. (2008) added to Wall’s investigations by concluding that ISNT results were also influenced by landscape positions. His study was able to use the ISNT to broadly categorize soils as either having high or low N fertility, but they found no relationship on a field-by-field basis. Some of his results attributed landscape position as a
possible culprit for ISNT-N variability due to differences in N loss mechanisms according to these positions.

**Direct Steam Distillation (DSD)**

Roberts et al. (2009) identified Direct Steam Distillation (DSD) as a less variable and more time/resource efficient method than the ISNT. Although the ISNT recovers more amino sugar-N as a whole, DSD has less sample to sample variability and recovers a larger percentage of soil-N. The ISNT detects amino sugar-N and NH$_4$-N, but does not measure any additional amino acid-N as the DSD does. The DSD method is also a more rapid analytical method, requiring only 6 to 7 min per sample, as opposed to a 5 h incubation with the ISNT. This time savings gives laboratories the ability to analyze a greater volume of samples in a given period of time. The original ISNT method was conducted on hot plates in an open environment which was prone to quantifying different levels of potentially mineralizable soil N, based on changing environmental laboratory conditions. The DSD also gives laboratories the ability to better control the analytical environment therefore decreasing sample to sample variability.

**N-STaR: Nitrogen Soil Test for Rice**

Roberts et al. (2009) took DSD a step further, by investigating the relationship between N mineralization and soil depth. They concluded that soil sampling depth was variable across both sites and depths and showed the importance of calibration based on soil texture and cropping system. From this, N-STaR was developed using correlation and calibration procedures, which made fertilizer N predictions possible for rice. Initially, N-STaR was based on calibration studies and DSD techniques for rice on silt loam soils. Similar studies have been conducted with wheat on silt loam soils, although the data have not been published. By analyzing crop residues using
N-STaR, conclusions can be drawn to further expand the capabilities of this technology and determine how much potentially-available N the N-STaR method is able to detect in plant material, as opposed to soil samples. After determining the amount of AH-N contained within these residues, further research will be able to determine the amount of potentially mineralizable-N credited to soils through crop residues, and make N-STaR recommendations to producers accordingly. Therefore, the objective of this study was to compare the quantity of total nitrogen (TN) and atom % $^{15}$N recovered by the ISNT and DSD methods from crop residues commonly grown in Arkansas and to determine how the N proportions from crop residues as measured by AH-N methods compare with traditional crop residue analysis.

**SUMMARY**

Correct application of fertilizer N is crucial for maximizing yield and minimizing environmental N loss. Most wheat production in Arkansas is located on the poorly-drained loamy and clayey textured soils of the Mississippi Delta Region. To date, most literature on FNUE is based on experiments conducted on well-drained soils. By conducting a study on a poorly-drained silt loam soil, determination of the most efficient N rate and application time can be made which relate directly to the majority soils used for Arkansas wheat production. Additionally, including a lab study which defines the ability of ISNT and DSD to measure AH-N will allow N-STaR to make more accurate potentially mineralizable-N estimations and appropriate fertilizer N recommendations to producers in regards to crop residue management. Thus, the first objective of this study is to determine how soft red winter wheat yield and FNUE are influenced by N-rate and time of application on poorly-drained silt loam soils using $^{15}$N-labeled urea. The second objective of this study is to establish to what degree the ISNT and DSD measure N in crop residues themselves, and determine if C:N ratio affects the ability to quantify
amino sugar-N. From there, further decisions can be made of what management practices, recommendations, and soil sampling instructions should be given to farmers who include crop residues as part of their production practices.
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CHAPTER TWO

Quantifying Alkaline Hydrolyzable-N in Crop Residues
ABSTRACT

Crop residues play a significant role in soil nitrogen (N) cycling. The type of residue, C:N ratio, tillage, and soil moisture influence potentially mineralizable soil-N. This study was established to estimate the N mineralization potential of various crop residues using Direct Steam Distillation (DSD) and the Illinois Soil Nitrogen Test (ISNT). Corn (Zea mays L.), soybean (Glycine max, L.), wheat (Triticum aestivum, L.), rice (Oryza sativa, L.), and grain sorghum (Sorghum bicolor, L.) residues were labeled with $^{15}$N using 10 atom% $^{15}$N labeled-urea. To assess the N mineralization potential of various crop residues, 0.2g of residue was subjected to both the DSD and ISNT. Hydrolyzed-N was captured and analyzed for atom % $^{15}$N to compare fertilizer atom % $^{15}$N to that of the original residue. Total N was quantified to establish percent recovery. For percent N recovery there was a significant residue by method interaction (p<0.0001) indicating that the two methods recovered varying amounts of N based on the type of residue. Atom % $^{15}$N recovered from the soybean residue as alkaline hydrolyzable-N (AH-N) was significantly lower than what was quantified in the plant tissue. Conversely, atom % $^{15}$N recovered from the rice residue as AH-N was significantly greater than that which was quantified in the original plant tissue. Comparison of atom % $^{15}$N in the residue and recovered AH-N suggested that certain crop species partition fertilizer N differently. Specific estimation of N mineralization potential of crop residues could aid producers in determining fertilizer N needs and encourage the development and implementation of soil-based N tests.
INTRODUCTION

Legume and cereal crop residue play a significant role in soil nitrogen (N) cycling. Type of residue, placement depth, level of incorporation, and water management can influence residue decomposition and potentially mineralizable soil-N. Many researchers have assessed how crop residues affect N mineralization and denitrification in the soil. These studies found wide variations associated with incorporated and surface-applied crop residues. Aulakh et al. (1991) researched the effect of vetch (*Vicia villosa*), soybean, corn, and wheat crop residue on N mineralization and found that crops possessing wide C:N ratios resulted in net N immobilization, whereas crops with narrow C:N ratios (vetch, soybean) can result in net N mineralization in the soil. Those in which a net N mineralization occurs provide readily available N for plant uptake, thus potentially decreasing fertilizer N needs. However, net N immobilization, due to the N required by the microbes to decompose the residue, could possibly increase fertilizer N needs in order to maximize crop yield. Drury et al. (1991) concluded that cover crops vary widely in the amount of readily available C, and this is related to denitrifying microbial activity and N mineralization later in the season. If the soils where crop residues are incorporated experience net N mineralization during the season, the successive crop’s N needs are supplemented by the incorporated crop residues. Thus, fertilizer N needs are reduced by crop residues in which a net N mineralization occurs.

Much research has been conducted to determine a chemical soil analysis procedure that could correctly quantify or estimate potentially mineralizable soil-N and in turn be calibrated to predict crop fertilizer N needs. Mulvaney et al., (2001) identified a soil organic-N fraction that was reportedly able to determine corn responsiveness to fertilizer N. A subsequent publication outlined a simpler version of the soil organic-N test, which was coined the Illinois Soil Nitrogen
Test (ISNT) (Kahn et al., 2001). The ISNT estimates potentially mineralizable soil-N based on alkaline hydrolysis which has been referred to as alkaline hydrolyzable-N (AH-N). Although the ISNT has been reported to be able to accurately predict N responsive versus N nonresponsive soils (Khan et al., 2001), Barker et al. (2006) concluded it was unable to provide fertilizer N rate recommendations for the responsive soils. The premise for ISNT is that it estimates potentially mineralizable soil-N by mimicking microbial N mineralization via estimating amino sugar-N, NH$_4$-N, and some amino acid-N (Khan et al., 2001). However, it has been shown to suffer from sample analysis variability due to its susceptibility to environmental laboratory conditions and extensive analysis time (Bushong et al., 2008; Spargo and Alley, 2008). In response to the issues of sample variability and time requirement, a direct steam distillation (DSD) technique was developed by Bushong et al. (2008). The DSD method is a modified alkali distillation procedure which also measures AH-N, but reduces sample variability and analysis time from 5 hr to approximately 7 min. Later, the N-Soil Test for Rice (N-STaR) was developed to correlate soil AH-N concentrations obtained by DSD to specific fertilizer N recommended rates for rice produced on silt loam soils (Roberts et al., 2011).

Understanding how crop residues interact with chemical estimates of N mineralization of potentially mineralizable-N compounds found in common crop residues will be important for the implementation of these new N soil test methods. It is known that crop residues contribute to N cycling in the soil and successive crop N needs. Traditionally, crop residue N availability has been estimated by means of incubation, but these are often untimely and laborious. The ISNT and DSD have shown to correlate well with N mineralization estimates from soil incubation studies (Bushong et al., 2008). However, it is unknown if AH-N methods correlate with crop residue incubations in a similar manner. Therefore, the objective of this study was to compare
the quantity of total nitrogen (TN) and atom % $^{15}$N recovered by the ISNT and DSD methods from crop residues commonly grown in Arkansas and to determine how the N proportions from crop residues as measured by AH-N methods compare with traditional TN analysis of crop residue.

**METHODS AND MATERIALS**

**Labeling Crop Residues with $^{15}$N**

A greenhouse study was initiated to label crop residues with $^{15}$N for a series of laboratory studies to quantify the potentially mineralizable-N contained in the residues. Corn, soybean, rice, wheat and grain sorghum are the primary row crop species produced in Arkansas and thus, were selected as they represent the crop residues that would most likely influence N mineralization/immobilization and ultimately the successive crop’s fertilizer N needs. Pots were filled with air dried Captina (fine-silty, siliceous, active, mesic, typic fraguidults) silt loam soil that was obtained from the Arkansas Agricultural Experiment Station located in Fayetteville, AR. Routine soil analysis indicated a pH of 6.8 and although all nutrients were within the acceptable range for the crops species selected, a nutrient solution based on the work of Yoshida (1998) was used weekly throughout the growing season to ensure that nutrients other than N were non-limiting. Urea labeled with 10 atom% $^{15}$N was obtained from Sigma Aldrich (Miamisburg, OH, USA) and used as the primary N source for all species including soybean. Fertilizer N additions were applied to each crop based on growth stages outlined by University of Arkansas recommendations for wheat, grain sorghum, corn, and rice (Johnson, 1992; Espinoza and Kelley, 2003; Espinoza and Ross 2003; Hardke, 2013) in the respective crop production handbooks. Crops were allowed to grow until physiological maturity, after which time the grain
was removed and the remaining crop biomass or residue was collected and oven-dried at 60˚C until a constant moisture level was achieved. Following drying, the remaining plant biomass of the various crops were weighed and then ground to pass a 2-mm screen, and a subsample of 0.1 g was analyzed for total C (TC) and TN using an Elementar vario Max (Elementar Analysensysteme GmbH, Hanau, Germany). Atom % $^{15}$N of the plant biomass of the various crops were determined by the UC Davis Stable Isotope Facility (Davis, CA), using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (Europa, Sercon, Ltd., Cheshire, UK).

**Quantification of Potentially Mineralizable Nitrogen**

Potentially mineralizable-N was determined using the DSD procedure developed by Bushong et al. (2008). Dried and ground crop residues were weighed (0.2 g) and placed in a micro-Kjeldahl flask (250 mL) and attached to the steam distillation apparatus. The addition of 10 mL of 10 mol L$^{-1}$ NaOH was added through the sample cup to prevent any loss of N prior to attachment of the flask to the steam distillation unit. Distillation proceeded for approximately 5 min at a rate of 7 mL min$^{-1}$, or until 35 mL of distillate was collected in 5 mL of 4% w/v H$_3$BO$_3$ acid indicator solution. An acidimetric titration technique was used to quantify the amount of NH$_4^+$ captured. Sequential distillations were performed on all samples containing $^{15}$N to minimize cross-contamination error much like the duplicate aliquot technique described by Mulvaney (1986). That is, the first distillation conditioned the still and was collected in H$_3$BO$_3$-indicator solution for quantification of NH$_4^+$ and the second distillation was collected in 0.1 mol L$^{-1}$ H$_2$SO$_4$ and used for $^{15}$N analysis.

Crop residues were also analyzed according to the modified ISNT described by Spargo et al. (2008) for comparison. A 0.2 g oven-dried plant sample was placed into a corresponding 473
mL modified Ball jar (Broomfield, CO). The lid was pre-fit with cable ties and machine screws to suspend a 60-mm diameter Pyrex petri dish above the ground plant sample. Ten mL of 2 mol L\(^{-1}\) NaOH solution was added to the sample and mixed thoroughly. Immediately following NaOH addition, 5.0 mL of 4% w/v H\(_3\)BO\(_3\) solution was added to the petri dish and apparatus was assembled to the jar and fastened securely with a metal band. Samples were then transferred to a Precision Model 815 low-temperature incubator (Thermo Fisher Scientific, Waltham, MA) set to 50ºC for 15 h. Following incubation, 5.0 mL of deionized water was added and titration was completed to determine N content. Samples analyzed for atom % \(^{15}\)N were titrated and then treated according to the procedure outlined by Khan et al. (2001) where the H\(_3\)BO\(_3\) was removed using methanol and the resulting (NH\(_4\))\(_2\)SO\(_4\) solvated using deionized water and prepared for \(^{15}\)N analysis.

As described above, following the DSD and ISNT procedures, AH-N was captured and analyzed for atom % \(^{15}\)N to compare to the atom % \(^{15}\)N of the recovered AH-N to that of the original crop residue. Isotope analysis for the recovery and specificity tests were determined at the University of Illinois on a Nuclide/MAAS 3-60-RMS double collector mass spectrometer (Nuclide Corp., Bellefont, PA) using an automated Rittenburg system (Mulvaney et al., 1990).

**Statistical Analysis**

Percent recovery of the TN from the crop residues data were analyzed as a split-plot with analytical method (ISNT and DSD) representing the main plot and crop residue representing the sub-plot. Crop residue following the greenhouse study had varying levels of \(^{15}\)N enrichment due to differences in plant uptake of labeled fertilizers and varying growth habits. Therefore, there was no comparison of crop residues, but rather a comparison of atom % \(^{15}\)N recovery based on
A simple one-way ANOVA was used to compare atom % $^{15}$N quantification by method within a given crop residue and means were separated using Fishers protected LSD at $\alpha=0.05$ level. Statistical analyses were conducted using JMP Pro 9 (SAS Institute Inc., Cary, NC).

**RESULTS AND DISCUSSION**

The C:N ratio of crop residues plays an important role in determining whether there will be net mineralization or net immobilization of soil N. Percent C, N, and C:N ratio of each of the crop residues is presented in Table 1 and indicates a wide range of values based on the different crop species used in this study. The N percentages obtained in this study were much higher than what would be typical of cereal crop residues, and consequently, the C:N ratios were much narrower than the 80:1 estimate suggested in previous literature (Stevenson and Cole, 1999). It is unknown how the relatively narrow C:N ratio of the cereal crops used in this study influenced the N recovery and estimate of potentially mineralizable-N. Also, because soybean were provided with fertilizer N for $^{15}$N labeling purposes, soybean crop residue TN was larger than what would be found in the field following harvest for a typical production setting. Therefore, residue composition and AH-N values for this study are not necessarily indicative of what would occur in a conventional field setting.

The percent recovery of N as AH-N from the crop residues was significantly influenced by the crop residue $\times$ AH-N method interaction ($p <0.0001$) indicating that the methods varied in their ability to hydrolyze N from these crop residues. Although the magnitude of difference between methods varied across crop residues, ISNT consistently recovered greater percentages of N as AH-N than did DSD (Table 3). The order of percent N recovery from the crop residues by the ISNT and DSD methods from highest to lowest was grain sorghum > rice > soybean >
wheat > corn (Table 2). The methods recovered just a little more N from grain sorghum compared to rice, 1 to > 2% of the N recovered for rice compared to soybean and wheat, and about twice as much from grain sorghum and rice as compared to corn. Regardless of AH-N method, ISNT or DSD, the amount of TN recovered as AH-N was for all crop residues. Bushong et al. (2008) also reported that the ISNT recovered slightly greater amounts of glucosamine-N than did the DSD method. Additionally, Bushong et al. (2008) compared the ISNT and DSD methods in recovering glucosamine-N in soil and measured percent recoveries much greater (85-94%) than those we measured from our crop residues presented here (4.39-11.70%). Recovery of N from these crop residues by the methods as AH-N or potentially mineralizable-N is very low. Results by Norman et al. (1990) would support these results for rice and soybean which reported 3% and 11% of the N in 15N-labeled rice and soybean residue was accumulated in the subsequent rice crop, however, roughly 37% of the N was recovered in the rice crop from the 15N-labeled wheat residue which would oppose the results of this study for wheat residue.

The atom % 15N recovery as TN and AH-N by the ISNT and DSD methods were determined for each of the crop residues (Table 3). The atom % 15N recovered by the TN method represents the atom % 15N label of all N compounds contained within the residue. Contrastingly, the atom % 15N values obtained using the ISNT and DSD represent only the atom % 15N label of the specific N compounds measured by the two AH-N methods. Unlike TN, the AH-N methods do not quantify all of the N compounds contained in the crop residue as evidenced by the percent N recoveries of the crop residues. The ISNT and DSD only quantify elemental N compounds such as NH4+, amino sugars and some amino acids that can be hydrolyzed with alkaline solutions (Kahn et al., 2001; Roberts et al., 2009). It is believed that the atom % 15N label of the crop
residue was influenced by the way the crops took up and assimilated the fertilizer N in the greenhouse. There was a range of initial $^{15}$N enrichments based on crop species, and was from 0.67 atom % $^{15}$N for rice to 3.73 atom % $^{15}$N for soybean (Table 4). Due to these differences in $^{15}$N enrichment there will be no comparison of crop residues, but rather a comparison of the atom % $^{15}$N recoveries by each method within a given crop residue.

When comparing the $^{15}$N recovery as influenced by each method, the greatest differences were seen in rice, which had the lowest atom % $^{15}$N enrichment of all residues, but resulted in the highest atom % $^{15}$N quantified by the ISNT and DSD. Conversely, soybean had the highest atom % $^{15}$N enrichment, but the ISNT and DSD recovered the lowest atom % $^{15}$N labels from the soybean residue. Although soybean residue had the greatest numerical total atom % $^{15}$N enrichment, both the ISNT and DSD were unable to hydrolyze as much as with the other crop residues that possessed a lower $^{15}$N label. One potential explanation for this difference is the fertilizer N allocation by each specific crop. In legume or N$_2$-fixing plants, such as soybean, a N compound known as a ureide (predominantly allantoin or allantoic acid) is produced in response to stress (King and Purcell, 2005). Typically the stressor is drought condition. However, other factors contributing to stress levels in plants could be elevated in plants confined to pots in greenhouse experiments, such as in this study. Soybeans use N$_2$ fixation as a primary N source under low soil-N conditions (80-94%) but even under fertile conditions, 25-50% of the TN is a result of N$_2$ fixation, especially during seed development, (Harper, 1987). Although many of the ureides stored in the soybean plant are remobilized during seed fill, greater amounts of leaf N are redistributed to the seeds than stem N (Purcell et al., 1998; Purcell et al., 2012). Because well fertilized soybean residues can have a high stem N concentration, one would assume greater amounts of stem ureide concentration to affect the recovery of N by the ISNT and DSD methods.
The potential preferential allocation of $^{15}$N fertilizer N into ureides may have significantly influenced the ability of the ISNT and DSD to quantify the fertilizer N that was assimilated by the soybean plant. Although no previous studies have been conducted to determine the ability of the ISNT and DSD methods to quantify ureides such as allantoin, work by Mulvaney et al. (2001) and Roberts et al. (2009) suggest that these methods would not be able to hydrolyze much of the ureide-N due to the relatively low recovery of N from urea (<5% for ISNT and 11% for DSD). Based on strength of the cyclic structure of ureides, it is unlikely that the N in these compounds are readily mineralized in the soil.

Contrastingly, in rice, the ISNT and DSD both recovered high numerical atom % $^{15}$N amounts of AH-N despite much lower initial enrichment. The N metabolism of rice is quite different than that of soybean. Literature suggests that a significant portion of N storage in rice plants is in the form of amino acids, NH$_4^+$, and some NO$_3^-$ depending on the form of fertilizer-N applied (Marwaha and Juliano, 1976). The ISNT quantifies roughly 100% of NH$_4^+$ and 50% of transition amino acid-N (asparagine and glutamine), whereas DSD is able to quantify approximately 100% of the NH$_4^+$ and 30% of the transition amino acid-N. Neither ISNT nor DSD quantify NO$_3^-$ (Roberts et al., 2009). Therefore, the rice preferentially allocated the fertilizer N applied into compounds with a greater degree of quantification by the AH-N methods than those into which soybean allocated the fertilizer N. This would explain the differences between the soybean and rice crop residue extremes in atom % $^{15}$N results. Of the TN contained within the other residues, wheat, corn, and grain sorghum had similar atom % $^{15}$N recoveries intermediate between rice and soybean. This could be explained by the similarities in production systems. Although they are cereal crops like rice, they are upland crops and even if irrigated, are certainly not produced under continuous flooding conditions. The fertilizer-N source used in this
study was urea, and although it is an NH$_4^+$ forming fertilizer, the management practices following fertilizer-N application could have a significant impact on the amount and ratio of NH$_4^+$ and NO$_3^-$ taken up by the various crops and ultimately how they are partitioned in the plant (Buerkert et al., 1995). Direct-seeded, delayed-flood rice production uses urea as a fertilizer-N source, but the addition of a permanent flood immediately following fertilizer application minimizes nitrification and the majority of the N taken up by the rice crop is in the NH$_4^+$ form. Upland crops such as corn, grain sorghum and wheat are more likely to take up a combination of both NH$_4^+$ and NO$_3^-$ as the urea has ample time to hydrolyze and nitrify prior to crop uptake. Therefore, cereal crops that are grown under upland conditions appear to assimilate both NH$_4^+$ and NO$_3^-$ in equal proportions of which the portion which remains in the $^{15}$N labeled NH$_4^+$ form can only be quantified by AH-N methods.

**CONCLUSIONS**

The results of this study indicate that the ISNT and DSD are similar in AH-N recovery N from crop residues though the magnitude of difference between methods among crop residues is different. Both methods recovered <12% of N from the crop residues used in this study. Each crop allocates fertilizer N differently, and AH-N methods measure these N compounds according to N metabolism of the specific crop. Although these methods generally quantified <10% of the N within crop residues, the N that is quantified is potentially mineralizable-N, which should be available for the subsequent crop. Due to the narrow C:N ratios of the cereal crop residues used in this study, it was thought that a greater proportion of potentially mineralizable-N would be recovered than what would be recovered from crop residues in the field. However, the low AH-N recoveries indicated that this was not the case.
No studies have been conducted on ISNT or DSD’s ability to quantify ureides because the goal of these methods is to measure potentially mineralizable-N, which is in the form of AH-N. The presence and persistence of compounds such as ureides in the soil has not been documented and their influence on N cycling is not known. It is thought that the ISNT primarily measures NH$_4^+$, amino sugar-N, and to a lesser extent, transition amino acids, and urea. The DSD procedure measures NH$_4^+$, yet quantifies slightly less amino sugar-N, but greater amounts of amino acid-N and some urea-type compounds. With the chemical structure of ureides being drastically different than that of amides, more research needs to be conducted to determine how much, if any, is quantifiable using the ISNT or DSD as this could influence N rate recommendations using N-STaR where soybean is grown in the crop rotation.

Further research is needed to identify the ability of ISNT and DSD to quantify the N contained in crop residues when incorporated into the soil. Data concerning the correct time to soil sample following crop residue incorporation will be essential to ensure that the proper N credits or deficiencies are accounted for and the correct N rate recommendation is made using AH-N soil analysis methods such as N-STaR.
LITERATURE CITED


Table 1. Percent total C (TC), total N (TN) and C:N ratio for the five crops used in this study.

<table>
<thead>
<tr>
<th>Crop Residue</th>
<th>TC</th>
<th>TN</th>
<th>C:N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>37.43</td>
<td>0.73</td>
<td>51:1</td>
</tr>
<tr>
<td>Corn</td>
<td>41.14</td>
<td>2.09</td>
<td>20:1</td>
</tr>
<tr>
<td>Soybean</td>
<td>42.65</td>
<td>3.79</td>
<td>11:1</td>
</tr>
<tr>
<td>Grain Sorghum</td>
<td>41.11</td>
<td>2.48</td>
<td>17:1</td>
</tr>
<tr>
<td>Wheat</td>
<td>39.91</td>
<td>2.26</td>
<td>18:1</td>
</tr>
</tbody>
</table>

Table 2. Comparison of percent nitrogen (N) recovery from crop residues by either the Illinois Soil Nitrogen Test (ISNT) or Direct Steam Distillation (DSD) analysis techniques.

<table>
<thead>
<tr>
<th>Crop Residue</th>
<th>ISNT</th>
<th>DSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>11.54</td>
<td>9.59</td>
</tr>
<tr>
<td>Corn</td>
<td>5.58</td>
<td>4.39</td>
</tr>
<tr>
<td>Soybean</td>
<td>9.20</td>
<td>8.32</td>
</tr>
<tr>
<td>Grain Sorghum</td>
<td>11.7</td>
<td>10.09</td>
</tr>
<tr>
<td>Wheat</td>
<td>9.14</td>
<td>7.17</td>
</tr>
</tbody>
</table>

LSD$_{0.05}$ to compare % N recovery based on method and crop residue
- Same method across crop residues: 0.06%
- Same crop residue across methods: 0.12%
Table 3. Comparison of atom % $^{15}$N recovered by total N (TN), Illinois Soil Nitrogen Test (ISNT), and Direct Steam Distillation (DSD) methods for each crop residue.

<table>
<thead>
<tr>
<th>Crop Residue</th>
<th>TN</th>
<th>ISNT</th>
<th>DSD</th>
<th>LSD&lt;sub&gt;0.05&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>0.67</td>
<td>5.20</td>
<td>5.43</td>
<td>0.18</td>
</tr>
<tr>
<td>Corn</td>
<td>2.09</td>
<td>2.99</td>
<td>3.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Soybean</td>
<td>3.73</td>
<td>0.86</td>
<td>0.86</td>
<td>0.04</td>
</tr>
<tr>
<td>Grain Sorghum</td>
<td>2.50</td>
<td>2.52</td>
<td>2.58</td>
<td>0.09</td>
</tr>
<tr>
<td>Wheat</td>
<td>2.26</td>
<td>2.39</td>
<td>2.41</td>
<td>0.03</td>
</tr>
</tbody>
</table>
CHAPTER THREE

The Influence of Fertilizer $^{15}$N Rate and Application Time on Soft Red Winter Wheat Yield and Fertilizer N Uptake Efficiency
ABSTRACT

Nitrogen represents 31% of the input costs associated with Arkansas wheat (*Triticum aestivum* L.) production. The only $^{15}$N wheat research to date in Arkansas has been conducted on well-drained soils, but the majority of production is located on poorly-drained silt loam soils. Therefore, a study was conducted during the 2011-2012 and 2012-2013 growing seasons to help establish the fertilizer N uptake efficiency (FNUE) of wheat produced on poorly-drained soils using the stable isotope $^{15}$N. Trials were conducted at the Pine Tree Research Station in Colt, Arkansas on a silt loam soil. Six different fertilizer N-rates were applied by hand ranging from 0 to 224 kg N ha$^{-1}$ at three different times: Early-single, Late-single, and Split applications in 1.5 x 1.74 m microplots using 2.65 atom% $^{15}$N-labeled urea. There was a significant application time by rate interaction ($p<0.0408$). The greatest FNUE was achieved with the Early-single and Split applications at the 90 kg N ha$^{-1}$ rate, and were 80.1% and 83.1%, respectively. Minimum yield-maximizing N rate was determined to be 135 kg N ha$^{-1}$ applied as an Early-single or Split application. The Late-single application across all N-rates resulted in lower FNUE and yield. Soil N uptake was not significantly different for any of the treatments that received fertilizer regardless of rate or timing of application, but were significantly higher than soil N uptake where no fertilizer was applied. Total N uptake by the wheat was directly related to fertilizer N uptake with the Early-single and Split application tending to have higher TN uptake than the Late-single application. Results of both the TN uptake and FNUE support the yield data obtained in these trials and indicate that current N-rate recommendations for wheat produced in the delta region of Arkansas optimize fertilizer N inputs while maintaining high yields. These results highlight the importance of proper rate and application time for maximizing FNUE and yield in winter wheat production.
INTRODUCTION

Over 182,000 hectares of wheat were harvested in Arkansas in 2012, and that number increased to almost 249,000 hectares in 2013 (National Agricultural Statistics Service, 2013). Although wheat is best suited for well-drained soils, a significant amount of wheat is produced on Arkansas’ poorly-drained loamy and clayey-textured soils. Without adequate drainage, soil oxygen depletion can exceed diffusion of oxygen into the soil causing soil microbe populations to shift from aerobic microbe populations to facultative and anaerobic microbes. These microbes, which thrive under reduced conditions, use oxidized forms of plant nutrient elements as alternatives to oxygen as electron acceptors (Inglett, Reddy, and Corstanje, 2005). Thus, increased concentrations of Fe$^{2+}$ and Mn$^{2+}$ can lead to toxicity and reduce wheat yields (Carver and Ownby, 1995). Typically, the seasons of wheat dormancy and growth (winter and spring) have greater total rainfall than summer or fall. Average seasonal rainfall in the Arkansas Delta Region, as reported by the National Oceanic and Atmospheric Administration, for winter (December-March) and spring (March-June) seasons are 33.4 cm and 38.8 cm, respectively. Meager plant biomass during these months combined with low temperatures provide for little evapotranspiration which perpetuates elevated soil moisture. Compensation for these challenges is achieved by planting wheat on raised beds or incorporating drainage ditches to prevent extended periods of topsoil saturation due to low evapotranspiration.

In addition to dealing with less than optimal growing conditions, agriculture production costs continue to rise. Nitrogen (N) fertilizer represents one of the greatest costs associated with production agriculture. As fossil fuel prices rise, there is a greater need for informed management practices which maximize fertilizer N uptake efficiency (FNUE). The majority of fertilizer consumption in the world is in the form of N fertilizer (International Fertilizer Industry
For cereal crops N is the most limiting nutrient for growth and therefore must be applied to most fields to maximize yield. Nitrogen fertilizer costs producers approximately $1.50 kg N\textsuperscript{-1} or $33.0 ha\textsuperscript{-1} (135 kg N ha\textsuperscript{-1}, current recommendation for the majority of Arkansas wheat production area), which accounts for 31% of total input costs associated with Arkansas wheat production (University of Arkansas Cooperative Extension Service, 2012). Due to the large portion of input costs devoted to N it is imperative that FNUE is maximized in order for wheat production to remain profitable.

A prior study found maximum rate of N uptake by wheat to occur shortly after the plant breaks dormancy to resume growth in the spring (Baethgen and Alley, 1989a). In this study, Baethgen and Alley used the Zadoks scale for plant growth and attributed GS 30 to the period of rapid N uptake (Zadoks, et al., 1974). Current studies tend to utilize the Feekes scale for wheat growth stages, with Feekes 3 corresponding to Zadoks’ GS 26, and Feekes 6 corresponding to GS 31. Baethgen and Alley (1989) concluded that GS 30 immediately preceded a period of rapid N uptake and utilization due to the high yield of the single GS 30 application treatments.

A study in England found a fertilizer N rate (100 kg N ha\textsuperscript{-1}) split into two applications, at Feekes 2-3 and Feekes 6, increased grain yield and decreased lodging over a single application of 200 kg N ha\textsuperscript{-1} at Feekes 2-3 (Dilz, 1971). A similar study by Dilz et al. (1982) later found an increase in grain yield when a fertilizer N application of 100 kg N ha\textsuperscript{-1} was split at Feekes 6 and Feekes 9 compared to a single application at Feekes 2-3 at the same rate. Bashir et al. (1997) found that dry matter accumulation increased from 1,001.5 g m\textsuperscript{2} at Feekes 10.1 to 1,514.0 at 11.1-11.2 where fertilizer N accumulation decreased from 7.38 g m\textsuperscript{2} at Feekes 10.1 to 6.52 g m\textsuperscript{2} at Feekes 11.1-11.2. Seeing that the plant dry matter accumulation increase (51.2%) during this time was inversely proportional to the fertilizer N accumulation (-11.7%), it could be assumed
that the plant continued to take up fertilizer N in decreasing amounts even past Feekes 10.1. Another study conducted on a poorly-drained clayey soil found that fertilizer N contributed to higher yield even when applied at Feekes 9 and 10. Fertilizer N at Feekes 9 was reported to contribute to increased kernels per spike and greater kernel weight whereas fertilizer N at Feekes 10 was found to increase kernels per spike only (Mascagni and Sabbe, 1991). However, Bashir et al., (1997) showed that the greatest total N (TN) and fertilizer N accumulation increases happened between Feekes 4-5 and Feekes 6-7. Consequently, a fertilizer N application past Feekes 6-7 should have a lower efficiency than at Feekes 3. Sabbe (1978) found wheat yields to be greatest when applying fertilizer N in mid- to late-winter as dormancy broke. Baethgen and Alley (1989b) found that winter wheat response to early and late fertilizer N was dependent upon tiller density at the time of application. Greater tiller density at Feekes 2-3 responded in higher yields when fertilizer N was applied later (Feekes 4-5), but yield was negatively impacted in treatments with high tiller density where fertilizer N was applied early (Feekes 2-3). Conversely, treatments where less tillers were documented at Feekes 2-3 responded to early and split N treatments by creating more tillers and producing high grain yield.

Both over- and under-application of fertilizer N can lead to yield decreases and profit losses with over-application contributing to lodging and increased disease pressure resulting in decreased yields. Current fertilizer N recommendations for Arkansas wheat grain production range from 100 to 135 kg N ha⁻¹ on loamy-textured soils following crops other than fallow (less N) or rice (more N). According to the 2011 Wheat Verification Program, producers participating in the program were applying approximately 135-145 kg N ha⁻¹, which is slightly above the recommendation guidelines (Grimes et al., 2011).
Previous research in Arkansas conducted on a Roxana sandy loam (coarse-silty, mixed, nonacidic, thermic Typic Udifluvent) showed a maximum fertilizer N accumulation of 74.4% when plant samples were taken at Feekes 8-9, and $^{15}$N-enriched fertilizer was applied in a single application at Feekes 2-3 (Bashir et al., 1997). This research was limited in scope having only a single N application time and a single N rate. Results were directed at determining how fertilizer N accumulated in the wheat plant throughout the course of the growing season and was not necessarily directed at maximizing FNUE by optimizing N rate and time or method of application. Currently, wheat production in Arkansas has been greatest in areas that are also typically cropped to rice and therefore are often produced on soils that are poorly-drained and have restricting soil horizons in the upper 30 cm of the soil profile. Research similar to Bashir et al., (1997) is needed on poorly-drained soil to determine if soil saturation would contribute to greater N loss in a single fertilizer N application as opposed to a split fertilizer N application. The cost of production associated with N fertilization coupled with environmental concerns increases the need for research identifying the yield-maximizing N-rate and application strategy. Therefore, the objective of this study was to determine how soft red winter wheat yield and FNUE is influenced by N-rate and time of application on poorly-drained silt loam soils using $^{15}$N-labeled urea.

**METHODS AND MATERIALS**

Two field experiments, one in 2012 and one in 2013, were conducted to evaluate the responsiveness of wheat to fertilizer N rate and time of application. Trials took place at the Pine Tree Research Station (PTRS) near Colt, Arkansas on a Calloway silt loam (Fine-silty, mixed, active, thermic Aquic Fraglossudalfs) in 2012 and a Calhoun silt loam (Fine-silty, mixed, active, thermic Typic Glossaqualfs) in 2013. Soil series and classification were defined by Web Soil
Survey, by the Natural Resources Conservation Service (Soil Survey Staff, 2010). The Calloway and Calhoun soil series are both classified as poorly-drained soils and are representative of the standard production setting for wheat produced on poorly-drained silt loam soils in the Eastern Arkansas Delta Region.

Soil samples were collected to a 10-cm depth prior to planting and submitted to the University of Arkansas Diagnostic Lab (Fayetteville, AR). Samples were subjected to Mehlich-3 extractable nutrients analysis (Helmke and Sparks, 1996) to ensure P, K, S, and other micronutrients were not limiting to wheat growth (Table 1). Prior to planting 29 kg P ha\(^{-1}\) and 83 kg K ha\(^{-1}\) were broadcast and incorporated at each location. Weeds, insects, and diseases were controlled using best management practices according to University of Arkansas wheat production recommendations (Johnson, 1992). The wheat cultivar Ricochet was drill-seeded at a rate of 118 kg ha\(^{-1}\) with 19 cm row spacing.

Three different fertilizer-N application times for each rate were carried out as follows: Early-single (Feekes 3), Late-single (Feekes 6), and Split application (one-half of the N applied at Feekes 3 followed by one-half of the N applied at Feekes 6). Feekes 3 was selected for this study as the first fertilizer N application time because it is the period immediately following the resumption of growth from winter dormancy and prior to active tillering, and Feekes 6 was selected as the second fertilizer N application time to determine to what extent fertilizer N is able to be taken up by wheat past the optimum application time.

The yield study was conducted in 4.88 m long by 1.74 m wide plots that received six different fertilizer N-rates ranging from 0-225 kg N ha\(^{-1}\) using urea (460 g N kg\(^{-1}\)) as the fertilizer N source. The FNUE study was conducted in separate 1.74 m wide by 1.5 m long microplots
which received the same rates as the yield component of this study. Urea fertilizer N used in the FNUE trial was obtained from Isotec (Miamisburg, OH) and was enriched to 2.65 atom% $^{15}$N. Adequate border spacing (1 m) was established to ensure no cross-contamination of N-rates. Fertilizer treatments were treated with the urease inhibitor n-(n-butyl) thiophosphoric triamide (NPBT), trade name Agrotain Ultra (Koch Fertilizer LLC, Wichita, KS), by mixing 1 kg of prilled urea with 4.2 mL Agrotain, consisting of 260 g NBPT kg$^{-1}$ in order to reduce ammonia volatilization loss potential. Fertilizer treatments for both the yield and FNUE trials were applied by hand at the designated growth stages. Fertilizer application times were based on wheat growth stage and differed in both years due to differences in soil and climatic conditions (Table 2).

Plant samples were collected to determine the TN uptake of wheat by removing the aboveground portion of a 1.83 m section of a bordered row from every plot. Plant samples that were used to calculate TN uptake were taken at Feekes 8-9 (Flag Leaf Emergence), which was identified by Bashir et al., (1997) as the growth stage where maximum TN uptake occurred. Plant samples were oven dried at 60° C, ground to pass a 2-mm screen, and a subsample of 0.1 g was analyzed for TN using an Elementar vario Macro (Elementar Analysensysteme GmbH, Hanau, Germany). The TN uptake was determined as the product of TN concentration and biomass. Atom % $^{15}$N was determined by the University of California Davis Stable Isotope Facility (Davis, CA), using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (Europa, Sercon, Ltd., Cheshire, UK). Fertilizer enrichment within the plant was calculated from atom % $^{15}$N change according to the equation:

$$F = TN(x-y/z-y)$$

Where F is the amount of fertilizer N taken up by the plant (kg N ha$^{-1}$), TN is the total N uptake (kg N ha$^{-1}$), x is the atom percent $^{15}$N measured in the plant, y is the average atom percent $^{15}$N
measured in the untreated control, and \( z \) is the atom percent \(^{15}\text{N} \) of the enriched urea fertilizer applied. The percent FNUE was calculated based on the equation:

\[
\text{FNUE} = \left( \frac{F}{A} \right) \times 100
\]

Where \( F \) is the amount of fertilizer N taken up by the plant (kg N ha\(^{-1}\)), and \( A \) is the fertilizer N application rate (kg N ha\(^{-1}\)).

Analysis of variance (ANOVA) was carried out using JMP PRO 9.0 (SAS Institute, Inc., Cary, NC). Statistical analysis of yield, TN uptake and soil N uptake was arranged as a randomized complete block design with a three (application time) by six (N rate) factorial treatment structure with four replications and year included as a random effect. Analysis of variance for the FNUE portion of the study was also arranged as a randomized complete block design with a three (application time) by five (N-rate) factorial treatment structure with four replications and year included as a random effect. Means were separated where appropriate using the least significant difference (LSD) test, assessing significance at \( p<0.05 \).

**RESULTS AND DISCUSSION**

**Total Nitrogen Uptake**

Total N uptake was significantly affected by the interaction between N-rate and application time \( (p=0.0014) \) (Table 3). Total N (Table 4) uptake consistently and significantly increased as N-rate increased for all Early-single and Split treatments, while the Late-single application seemed to reach a plateau in TN uptake around 135 kg N ha\(^{-1}\). There were no significant differences observed between the Early-single and Split applications within a given N-rate. No differences existed among any treatment until 135 kg N ha\(^{-1}\), which was numerically but not statistically lower than the Early-single or Split applications (10 and 20 kg ha\(^{-1}\) less,
respectively). However, for every rate above 135 kg N ha\textsuperscript{-1} the Late-single application produced less TN uptake than the Early-single or Split applications. The Late-single applications were also not significantly different from one another among the 135 kg ha\textsuperscript{-1}, 180 kg ha\textsuperscript{-1}, and 225 kg ha\textsuperscript{-1} N-rates. Additionally, the 90 kg ha\textsuperscript{-1} and 180 kg ha\textsuperscript{-1} Late-single treatments were not different from one another, suggesting that TN uptake was less influenced by N rates greater than and equal to 90 kg ha\textsuperscript{-1} for the Late-single application than the Early-single or Split treatments were.

Reduced biomass could have attributed to inhibition of further N uptake for plants which received only Late-single fertilizer N applications. Bashir (1997) reported TN uptake to reach maximum accumulation at Feekes GS 8-9 with no significant change in TN uptake through maturity when fertilizer N was applied in a single application during tillering. Contrastingly, fertilizer-N uptake was shown to peak at Feekes GS 8-9 then decline through maturity. Natural senescence of the wheat plant could contribute to top growth fertilizer N loss through ammonia volatilization, predominantly in leaf tissue. Although N uptake continues through maturity, the rate of additional N uptake slows in response to the decreasing rate of biomass accumulation. Thus, the concentration of fertilizer N would decrease even as TN concentration remains constant, according to Bashir et al., (1997) which only had an early single application. In contrast, for Late-single treatments fertilizer N uptake results (TN uptake – soil N uptake) might have been greater if more time had been allowed between fertilizer N application and plant sampling. However, it is likely that TN uptake results would not have drastically changed, since N loss through stomatal conductance would still occur influencing only the ratio of the fertilizer N concentration to TN concentration at maturity.
Soil Nitrogen Uptake

An analysis of variance (Table 3) indicated that the only factor affecting soil N uptake was N rate (<0.0001). Soil N uptake significantly increased from the 0 kg N ha\(^{-1}\) N-rate to the 45 kg N ha\(^{-1}\) N-rate then remained relatively constant as N rate increased for all the application strategies. There were no differences among any treatments within a given N rate. Contrastingly, fertilizer N uptake mirrored the trends of TN uptake for every N-rate and application time. Thus, the differences in TN uptake for this study can be attributed to the magnitude of difference in fertilizer N uptake as affected by N rate and application time alone, not soil N uptake. Research on wheat grown in a wheat-rice cropping system as compared to a wheat-corn cropping system has shown root mass in the wheat-rice cropping system to be reduced by up to 48% compared to the wheat-corn system. Researchers attributed this difference in part to the presence of a plow pan in the wheat-rice system. It is thought that the majority of the wheat root system was restricted to the top 5 cm of soil, though wheat roots are reported to penetrate nearly 180 cm in other cropping systems on light-textures soils (Jalota et al., 1980; Sur et al., 1980). It is likely, in this study, that fertilizer N encouraged development of a more extensive root system compared to treatments receiving no fertilizer N, but soil physical properties could have inhibited the full potential of the root systems for treatments receiving fertilizer N.

Wheat Yield

The ANOVA (Table 3) indicated that there was a significant N application time by rate interaction \((p=0.0058)\). Overall, the minimum yield-maximizing N-rate and application method was 135 kg N ha\(^{-1}\) applied as an Early-single or Split application (Table 5). Yield tended to increase as N-rate increased within the Split-application treatments until N-rate reached 135 kg
at which time grain yield reached a plateau and declined when N-rate exceeded 180 kg N ha$^{-1}$. Wheat receiving N as the Split application had similar yields as the equivalent amount of N applied as an Early-single, but the Late-single N application produced yields that were numerically and sometimes statistically lower for each N-rate >45 kg N ha$^{-1}$. Over-fertilization with N can have an adverse effect on grain yield due to increased lodging, delayed maturity, and increased disease (Wells et al., 1995). Split application of N-rates greater than 180 kg N ha$^{-1}$ reduce wheat yield. For the Early-single application, yield tended to increase as N-rate increased until yield reached a plateau at rates of 135-225 kg N ha$^{-1}$. Although this study indicated that the Early-single N application timing could produce similar yields to the Split application at rates of 90-180 kg N ha$^{-1}$, N from the Early-single application could suffer substantial loss in years with greater rainfall increasing the risk associated with applying all the N prior to the Feekes 3 growth stage.

For the Late-single application, the soil inorganic-N content was too low to produce significant tillering before fertilizer N was applied, and the fertilizer N was applied late enough that the wheat could not regain all of the yield potential exhibited by the treatments that received at least a portion of the N prior to the Feekes 6 growth stage. Except for the 45 and 90 kg N ha$^{-1}$ rates, wheat yields for the Late-single application were statistically lower within a N-rate than wheat yields from either the Early-single or Split application. The greatest yields for the Late-single application were not achieved until 180 kg N ha$^{-1}$ was applied, and even then grain yield was ~800 kg ha$^{-1}$ lower than the maximum yields attained with the Early-single and Split treatments. However, it is surprising that the Late-single applications were able to provide sufficient N to achieve the yields that they did. Previous work on a silty clay soil has shown that fertilizer N applied as late as Feekes stage 10 can significantly increase wheat yield (Mascagni et
al., 1990). In light of these findings, it might be deduced that wheat yield is able to overcome inadequate tillering and recover some yield potential through other yield components (number of spikes per m$^{-1}$, number of kernels per spike, and kernel weight) (Mascagni and Sabbe, 1991).

**Fertilizer Nitrogen Uptake Efficiency**

The ANOVA (Table 6) showed a significant rate by application time interaction ($p=0.0408$). No statistical differences existed among the Split treatments from 45 kg N ha$^{-1}$ to 180 kg N ha$^{-1}$, although the highest N rate of 225 kg N ha$^{-1}$ (72.0%) was significantly lower than the 90 kg N ha$^{-1}$ rate (83.1%) (Table 7). Likewise, for all Early-single treatments, no significant differences existed among the 45 to 180 kg N ha$^{-1}$, with the only difference existing in 90 kg N ha$^{-1}$ (80.1%) being greater than the 225 kg N ha$^{-1}$ (68.8%) treatment. Unlike the other application times, Late-single applications had several significant differences within the treatment group, and all Late-single treatments had inferior FNUE compared within a rate to the other application times, with 45 kg N ha$^{-1}$ being the only exception. For Late-single applications FNUE was greatest at the lowest N-rate (78.7%) and decreased as N-rate increased.

Numerically, the Early-single and Split fertilizer N treatments had greater FNUE than the Late-single application. For the same reason, Late-single treatments tended to have inferior FNUE because plants at Feekes 6 which had not received any fertilizer N to contribute to growth were smaller than the Early-single or Split treatments which had received fertilizer N prior to the period of rapid growth. Also, Late-single treatments were allotted less time (3-4 wk) in between fertilizer N application (Feekes 6) and plant sampling (Feekes 8-9), which did not allow adequate time for fertilizer N uptake. Thus, Late-single application FNUE decreased with increasing N-
rate. Split treatments received the benefit of early N application, which allowed the plants to take up the fertilizer N and utilize it during the period of rapid growth.

Most treatments exhibited exceptional FNUE values with 10 of the 15 treatment combinations resulting in FNUE values of >75%. Timely rainfall (Figures 1 and 2) allowed for incorporation of fertilizer N, yet no exorbitant rainfall events occurred immediately following application. This allowed the urea to be incorporated into the soil, lessening N loss through ammonia volatilization, and avoiding N loss through denitrification. Additionally, lower temperatures at the time of fertilizer application and non-basic soils contributed to conservation of NH$_4^+$ in the soil for plant uptake throughout the growing season (Stevenson and Cole, 1999).

**CONCLUSIONS**

Current Arkansas winter wheat recommendations (100-135 kg N ha$^{-1}$ applied as a Split application) are based on N response trials closely associated with production settings, whereas, the only $^{15}$N research conducted in Arkansas were on soils which are not necessarily indicative of actual production settings. As a result, this study was established to verify the current recommendations on a poorly-drained soil. Wheat grain yields were maximized by application of 135 to 225 kg N ha$^{-1}$ as an Early-single application or 135 to 180 kg N ha$^{-1}$ Split application. The Early-single fertilizer N application method is perhaps a less economically sound decision due to the potential for significant N loss in one or multiple events following application of all of the fertilizer N. Years with greater rainfall pose the greatest threat to increased fertilizer N loss through denitrification, runoff, and/or leaching. Although the results averaged across two years of research do not show clear differences between the Early-single and Split application N-fertilization methods, applying the total N-rate in two splits may increase N recovery and reduce N loss compared to an Early-single application with little additional cost.
The results also support previous research (Baethgen and Alley, 1989a) which suggests that the initial fertilizer N application should be applied no later than Feekes stage 5. The Single-late fertilizer N application method does not provide enough N to optimize early plant development on N-deficient soils. Though decreased spring tillering can be compensated for in other yield components, maximum yields require adequate tillering.

Soil N uptake was consistent across all N-rates and application times for all treatments receiving fertilizer N. Therefore, differences in TN uptake was dictated by N-rate and application time alone and not affected by native soil N. Many soils in the Mississippi Delta region are traditionally cropped to rice, which can create a relatively restrictive plow pan. Soil physical properties could limit root growth potential, and therefore the ability of the wheat plant to fully exploit native soil N.

Overall, FNUE was greatest for the Split application treatments, with maximum FNUE being achieved at 90 kg N ha\(^{-1}\) (83.1\%). Although FNUE values for the Early-single application were slightly lower than the Split application treatment, this may not hold true in years with greater than average rainfall occurring early in the growing season shortly after wheat breaks winter dormancy. Possible reasons for such high FNUE values of this study are given by precipitation data for 2012 and 2013. All treatments received some amount of rainfall within two days of fertilizer application. This allowed for incorporation of urea, lessening the loss of N through ammonia volatilization and providing prolonged N availability for plant uptake. However, no excessive rainfall events occurred after application which would have resulted in fertilizer N loss through denitrification. Results of both yield and FNUE components of the study indicate that the 135 kg N ha\(^{-1}\) Split fertilizer application supplies adequate N for maximum yield while minimizing environmental N-loss risk.
LITERATURE CITED


Table 1. Selected soil chemical property means from 0-10 cm deep soil samples ($n=4$) collected from N-fertilization trials located at the Pine Tree Research Station (PTRS) near Colt, AR during 2012 and 2013 growing seasons.

<table>
<thead>
<tr>
<th>Soil Series</th>
<th>Soil OM$^a$</th>
<th>Soil pH</th>
<th>Mehlich-3 soil nutrients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td></td>
<td>P</td>
</tr>
<tr>
<td>Calloway</td>
<td>2.8</td>
<td>7.7</td>
<td>35</td>
</tr>
<tr>
<td>Calhoun</td>
<td>2.6</td>
<td>7.1</td>
<td>29</td>
</tr>
</tbody>
</table>

$^a$ OM, Organic matter

Table 2. Timing of Early-single, Late-Single and Split applications to wheat based on growth stage on a poorly-drained silt loam at the Pine Tree Research Station (PTRS) near Colt, AR during the 2012 and 2013 growing seasons.

<table>
<thead>
<tr>
<th>Year</th>
<th>Planting Date</th>
<th>Application Time†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Early-single and</td>
</tr>
<tr>
<td></td>
<td></td>
<td>First Split</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Late-single and</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Second Split</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Days Between</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Applications</td>
</tr>
<tr>
<td>2011-12</td>
<td>Oct. 7, 2011</td>
<td>February 21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>March 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21</td>
</tr>
<tr>
<td>2012-13</td>
<td>Oct. 22, 2012</td>
<td>March 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>April 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
</tr>
</tbody>
</table>

$^†$ N treatments were applied 100% early at the tillering stage (Feekes 3), 100% late at the first visible node (Feekes 6) or a split application of 50% at Feekes 3 and 50% at Feekes 6.
Table 3. Analysis of variance for total N (TN) uptake, soil N uptake, and wheat yield as affected by N rate and application time.

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of Freedom</th>
<th>TN uptake p value</th>
<th>Soil N uptake p value</th>
<th>Yield p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N Rate (NR)</td>
<td>5</td>
<td>&lt;0.0001</td>
<td>0.3077</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Application Time (AT)</td>
<td>2</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NR x AT</td>
<td>10</td>
<td>0.0014</td>
<td>0.1896</td>
<td>0.0058</td>
</tr>
</tbody>
</table>

Table 4. Total N uptake as influenced by fertilizer-N rate and application time, soil N uptake as influenced by the main effect of N-rate from samples taken at the Feekes 8-9 at the Pine Tree Research Station during the 2011-12 and 2012-2013 growing seasons.

<table>
<thead>
<tr>
<th>Application time</th>
<th>Total N Uptake</th>
<th>Soil N Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>N rate</td>
<td>Early-single</td>
<td>Late-single</td>
</tr>
<tr>
<td>0</td>
<td>38.1</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>80.2</td>
<td>84.6</td>
</tr>
<tr>
<td>90</td>
<td>120.8</td>
<td>117.8</td>
</tr>
<tr>
<td>135</td>
<td>157.7</td>
<td>146.3</td>
</tr>
<tr>
<td>180</td>
<td>197.6</td>
<td>129.4</td>
</tr>
<tr>
<td>225</td>
<td>229.7</td>
<td>147.8</td>
</tr>
</tbody>
</table>

LSD\textsubscript{0.05} = 27 kg N ha\textsuperscript{-1} \quad LSD\textsubscript{0.05} = 7.7 kg N ha\textsuperscript{-1}

\( ^a \) Single early applied at Feekes stage 3; Single late applied at Feekes stage 6; and Split involved applying one-half of the N at Feekes stage 3 followed by one-half of the N applied at Feekes stage 6.

\( ^b \) 0 kg N ha\textsuperscript{-1} treatment TN uptake reported as an average across all applications.
Table 5. Winter wheat yield means, averaged across years, as influenced by the fertilizer N-rate and application time interaction at the Pine Tree Research Station (PTRS) near Colt, AR during the 2012 and 2013 growing seasons.

<table>
<thead>
<tr>
<th>N rate (kg N ha(^{-1}))</th>
<th>Application time(^{a})</th>
<th>Early-single</th>
<th>Late-single</th>
<th>Split</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>-------------</td>
<td>-------------</td>
<td>-------</td>
</tr>
<tr>
<td>0</td>
<td>3105(^{b})</td>
<td>-------------</td>
<td>-------------</td>
<td>-------</td>
</tr>
<tr>
<td>45</td>
<td>3734</td>
<td>3864</td>
<td>4390</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>4902</td>
<td>4775</td>
<td>4913</td>
<td></td>
</tr>
<tr>
<td>135</td>
<td>6193</td>
<td>5110</td>
<td>6590</td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>6061</td>
<td>5777</td>
<td>6493</td>
<td></td>
</tr>
<tr>
<td>225</td>
<td>6592</td>
<td>5576</td>
<td>5843</td>
<td></td>
</tr>
</tbody>
</table>

LSD\(_{0.05}\) = 623 kg ha\(^{-1}\)

\(^{a}\) Single early applied at Feekes stage 3; Single late applied at Feekes stage 6; and Split involved applying one-half of the N at Feekes stage 3 followed by one-half of the N applied at Feekes stage 6.

\(^{b}\) 0 kg N ha\(^{-1}\) treatment yields reported as an average across all applications.

Table 6. Analysis of variance P-values for fertilizer nitrogen uptake efficiency (FNUE) of wheat as affected by N-rate and N application time.

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of Freedom</th>
<th>FNUE</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0018</td>
</tr>
<tr>
<td>N Rate (NR)</td>
<td>4</td>
<td></td>
<td>0.0018</td>
</tr>
<tr>
<td>Application Time (AT)</td>
<td>2</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NR x AT</td>
<td>8</td>
<td></td>
<td>0.0408</td>
</tr>
</tbody>
</table>
Table 7. Fertilizer N uptake efficiency, averaged across years, as influenced by the fertilizer N rate and application time interaction at the Pine Tree Research Station (PTRS), near Colt, AR during the 2011-12 and 2012-2013 growing seasons.

<table>
<thead>
<tr>
<th>N rate kg N ha⁻¹</th>
<th>Application timeᵃ</th>
<th>FNUET</th>
<th>Late-single</th>
<th>Split</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early-single</td>
<td>70.8</td>
<td>78.7</td>
<td>77.6</td>
</tr>
<tr>
<td>45</td>
<td></td>
<td>80.1</td>
<td>69.6</td>
<td>83.1</td>
</tr>
<tr>
<td>90</td>
<td></td>
<td>78.1</td>
<td>65.6</td>
<td>82.5</td>
</tr>
<tr>
<td>135</td>
<td></td>
<td>77.7</td>
<td>47.4</td>
<td>76.8</td>
</tr>
<tr>
<td>180</td>
<td></td>
<td>68.8</td>
<td>45.6</td>
<td>72.0</td>
</tr>
<tr>
<td>225</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD₀.₀₅ = 10.8%

ᵃ Single early applied at Feekes stage 3; Single late applied at Feekes stage 6; and Split involved applying one-half of the N at Feekes stage 3 followed by one-half of the N applied at Feekes stage 6.
Figure 1. Daily precipitation in mm and fertilizer application days, reported from February 1, 2012 as measured by the National Oceanic and Atmospheric Administration (NOAA, 2014) weather station in Forrest City, AR.

Figure 2. Daily precipitation in mm and fertilizer application days, reported from February 1, 2013 as measured by the National Oceanic and Atmospheric Administration, (NOAA, 2014) weather station in Forrest City, AR.
The purpose of these studies was to determine appropriate fertilizer N recommendations for producers in Arkansas in regard to both soil sampling with crop residues and fertilizer N rate and application time in soft red winter wheat production. The results indicated that the ISNT and DSD recover similarly low amounts of AH-N within a crop residue, though magnitude of difference varied between methods among crop residues. Due to each crop partitioning fertilizer N differently according to the different N metabolism for the crop, the magnitude of difference among crop residue fertilizer N recovery varied. Though little N was quantified by these methods, the amount of N which was quantified was potentially mineralizable-N, which should be available for the subsequent crop. Optimum results for potentially mineralizable-N were expected due to the nature of the greenhouse study. The C:N ratios of the cereal crops grown were much narrowed than would be anticipated from typical field settings, thus it was expected that a greater proportion of potentially mineralizable-N would be recovered, but the low AH-N recoveries indicated that this was not the case. Further research is needed to determine the ability of the ISNT and DSD to quantify crop residue N when incorporated into the soil. It is essential that correct soil sampling time is achieved after incorporation of crop residues in order for N credits or deficiencies to be accounted for when AH-N soil testing methods, such as N-STaR are used.

Results from the field portion of this study determined that the current Arkansas fertilizer N recommendation of 135 kg N ha\(^{-1}\) applied as a split application to be the minimum yield-maximizing N-rate. Overall, Split applications achieved the greatest FNUE. Early-single and Split applications were not significantly different from one another based on yield results as well as FNUE. The Early-single application time is thought to be a less economical decision due to its potential for substantial N-loss in years with greater rainfall. Late-single applications produced
inferior yields and FNUE overall. Total N uptake was found to be directly related to fertilizer N uptake due to no differences in soil N uptake in any treatments receiving fertilizer N. This supports previous research that initial fertilizer N should be applied no later than Feekes stage 5. This results indicate that the 135 kg N ha\textsuperscript{-1} Split fertilizer application minimizes environmental N-loss and supplies sufficient N for maximum yield.