

# **Nitrogen Cycling from Fall Applications of Biosolids to Winter Small Grains**

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## Abstract

Environmental concerns about winter nitrogen (N) leaching loss limit the amount of biosolids applied to winter small grains in Virginia. Ten field studies were established 2012-2014 in Virginia to determine the agronomic and environmental feasibility of fall biosolids applications to soft red winter wheat (*Triticum aestivum* L.). Eight studies were located in the Coastal Plain physiographic province and two in the Ridge & Valley physiographic province. The effects of eight biosolids and urea N treatments on 1) biomass production at Zadoks growth stage (GS) 25-30, 2) soil inorganic N at GS 25-30, 3) soil mineralizable N at GS 25-30, 4) N use efficiency (NUE) at GS 58, 5) grain yield, 6) end-of-season soil inorganic N, and 7) estimated N recovery were studied. Anaerobically digested (AD) and lime stabilized (LS) biosolids were fall applied at estimated plant available N (PAN) rates of 100 kg N ha<sup>-1</sup> and 50 kg N ha<sup>-1</sup>. The 50 kg N ha<sup>-1</sup> biosolids treatments were supplemented with 50 kg N ha<sup>-1</sup> as urea in spring. Urea N was split applied at 0, 50, 100 and 150 kg N ha<sup>-1</sup>, with 1/3 applied in fall and 2/3 in spring. Biomass at GS 25-30 increased with urea N rate and biosolids always resulted in equal or greater biomass than urea. Soil mineralizable N at GS 25-30 rarely responded to fall urea or biosolids N rate, regardless of biosolids type. Biosolids and urea applied at the agronomic N rate resulted in equal grain yield and estimated N recovery in soils where N leaching loss risk was low, regardless of biosolids type or application strategy. Lime stabilized biosolids and biosolids/urea split N application increased grain yield and estimated N recovery in soils with high or moderate N leaching loss risk. Therefore, AD and LS biosolids can be fall-applied to winter wheat at the full

agronomic N rate in soils with low N leaching loss risk, while LS biosolids could be applied to winter wheat at the full agronomic N rate in soils with moderate or high N leaching loss risk.

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## Attribution

Several colleagues aided in the writing and research for all chapters except chapter 4 presented as parts of this thesis. A brief description of their contributions is included here.

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## Abbreviations

AD = anaerobically digested biosolids

C = carbon

C:N = carbon to nitrogen ratio

CO<sub>2</sub> = carbon dioxide

cm = centimeter

fert. = fertilizer

g = gram

GS = Zadoks winter wheat growth stage

ha = hectare

K = potassium

kg = kilogram

LS = lime stabilized biosolids

Mg = megagram or metric ton

m = meter

m<sup>2</sup> = square meter

mg = milligram

mm = millimeter

N = nitrogen

NH<sub>4</sub><sup>+</sup> = ammonium

NH<sub>4</sub>-N = ammonium-nitrogen

NO<sub>3</sub><sup>-</sup> = nitrate

NO<sub>3</sub>-N = nitrate-nitrogen

NUE = nitrogen use efficiency

P = phosphorous

p = critical value

PAN = plant available nitrogen

pH = negative log of proton concentration

$R^2$  = coefficient of determination

x = independent continuous variable

y = dependent (or response) continuous variable

yr = year

## **Chapter 1: Introduction**

### **1.1: A brief history of biosolids in the United States**

The enactment of the Clean Water Act in 1972 necessitated greater levels of wastewater treatment and pollution removal, which led to the generation of more residual sewage sludge. The amount of sewage sludge generated by wastewater treatment doubled in the first twenty years following the enactment of the Clean Water Act (USEPA, 1993). There were several disposal options for the increased amount of sludge, including incineration, placement in landfills, ocean dumping, and application to land as a fertilizer. Sewage sludge was quickly utilized as a valuable fertilizer because it was known to contain all plant essential nutrients, including the macronutrients nitrogen (N) and phosphorous (P). Prior to a comprehensive set of regulations on the disposal and use of sewage sludge, all of the aforementioned disposal options were practiced and the effects were researched.

By the mid-1980s, the increased generation, use and disposal of sewage sludge motivated the US Congress to enact specific legislation. Section 405 of the Clean Water Act was originally aimed at preventing the disposal of sewage sludge into navigable waters, but subsequent amendment shifted its focus increasingly toward reducing harmful pollution from sewage sludge disposal and maximizing beneficial use (USEPA, 1993). To this end, the USEPA examined the potential effects of sewage sludge disposal on air quality, water quality, plants and public health in order to develop “The Standards for the Use and Disposal of Sewage Sludge” (Title 40 of the Code of Regulations Part 503). These standards became effective on March 22, 1993 and continue to regulate sewage sludge at a national level. The term “biosolids” was then applied to

sewage sludge that had been treated and processed. By 1998, 41% of biosolids were being land applied, 22% were being incinerated, 17% were being surface or landfill disposed, and the remaining 20% went to advanced treatment (12%), other beneficial use (7%) or other disposal options (1%) (USEPA, 1999). Surface disposal and incineration had declined by 1998 due to high costs associated with meeting water and air quality standards (USEPA 1999).

## **1.2: Rationale for further study of biosolids applications in Virginia**

On a state level, the regulations on biosolids must be equally stringent or more stringent than the federal regulation and are connected to the specific environmental concerns of the state. Accordingly, no jurisdictions in USEPA Water Region 3 have applied for program authorization of federal part 503 because all jurisdictions in this region have their own sewage sludge programs (USEPA, 2010). In Virginia, the use of biosolids is important in agriculture for application to pastures, hay fields, row crops, and winter cover crops. In 2006, approximately 239,000 dry tons (Mg) of biosolids were applied to nearly 23,000 hectares (ha) of permitted land (Virginia Biosolids Council, 2011). The Virginia Department of Environmental Quality (VADEQ) must approve a requested permit for the land application of biosolids. The permit includes a Nutrient Management Plan (NMP) that must be approved by the Virginia Department of Conservation and Recreation (VADCR).

Biosolids are typically provided at no cost to agricultural producers and can replace inorganic fertilizers as a nutrient source for crops. Current regulations allow the application of biosolids to meet the full agronomic N needs of a crop once every three years or 60% of the agronomic N need every year (VADCR, 2011). Many agricultural producers in the high intensity row crop region of eastern Virginia manage their land in a 2 year corn (*Zea mays* L.) – winter

wheat – soybean (*Glycine max* L.) rotation (Movafaghi et al., 2013). Of these three crops, corn is the most logical choice for biosolids applications because it requires almost double the amount of N as wheat (VADCR, 2005). Therefore, producers offset input costs most by applying biosolids to spring corn once every three years to meet its full agronomic N need. The demand for biosolids for spring corn can result in low availability of biosolids in the spring. There are also producers who manage their land under wheat and soybean rotations, and biosolids are of less value to leguminous, N-fixing soybeans (Currie et al., 2003). In either situation, there is an opportunity to apply biosolids at full agronomic N rates to winter small grains such as winter wheat. This opportunity is not taken because biosolids applications at full agronomic N rates to winter small grains are currently prohibited by VADCR (VADCR, 2011). Biosolids for winter small grains must be fall applied, and there is regulatory concern that full agronomic N rate applications would result in N leaching losses and water quality impairment. Therefore, biosolids may only be fall applied to winter small grains to meet half the agronomic N need. Loading rates that would supply 50% of winter small grain N need are too small to be economically or mechanically feasible for biosolids applicators. Biosolids are therefore rarely applied to winter small grains in Virginia.

One of the major environmental concerns linked to the regulation of agricultural biosolids use in Virginia is cultural eutrophication in the Chesapeake Bay Watershed, which has increased with increasing N and P loads (Boesch et al., 2001). Along with parts of New York, Pennsylvania, Maryland, Delaware, West Virginia and the District of Columbia, much of Virginia is in the Chesapeake Bay Watershed. Virginia is the top source of P and sediment and is the second leading source of N in the Chesapeake Bay. Virginia contributes 27% of the total N in the Bay and 20% of the N from agriculture in the Bay (USEPA, 2010). The USEPA identifies

both biosolids and chemical fertilizers as sources of the agricultural N entering the Chesapeake Bay, noting that chemical fertilizer sales have consistently risen from 1982 to 2007 (USEPA, 2010).

The detrimental effects resulting from N loss from soil to ground and surface water have been well documented. Excess N loads to estuarine and marine environments result in eutrophication, which in turn causes algal blooms (Ryther and Dunstan, 1971). When the algae die, the biological oxygen demand of the water increases and dissolved oxygen is removed from the water via microbial respiration (Diaz, 2001). Many marine species have minimum levels of required dissolved oxygen below which they cannot survive (Kramer, 1987). Once these levels are reached, the marine species dies and ecosystem composition and function suffers (Diaz, 2001). Humans are also harmed by N leaching losses to ground and surface water (Ward et al., 2005). Well water can accumulate  $\text{NO}_3\text{-N}$ , which will inhibit the oxygenation of blood by hemoglobin and convert it into methemoglobin (Greer and Shannon, 2005). The result is known as methemoglobinemia or “blue baby syndrome” because small children are particularly susceptible (Greer and Shannon, 2005). There is also a growing body of evidence for the association of  $\text{NO}_2\text{-N}$  intake with cancer and tumors in humans (Mirvish, 1995; Roediger et al., 1990).

The potential agronomic and economic benefits of fall biosolids applications to winter small grains are counterbalanced by fear and uncertainty regarding environmental impacts. It is therefore necessary to quantify the agronomic and environmental effects of fall biosolids applications to winter small grains.

### 1.3: Previous research

#### Agronomic considerations

##### Winter wheat N need

Grain production of winter wheat depends heavily on plant available nitrogen (PAN) in the soil. Residual soil N and N mineralizing from soil organic matter in agricultural soils is rarely sufficient to maximize winter wheat grain yields. Therefore, addition of supplemental N is often necessary to support maximum grain yields. Delogu et al. (1998) observed that winter wheat grain yield increased by 9.2 kg kg N<sup>-1</sup> added. Proper N fertilization of winter wheat is essential to support optimal yields and subsequent economic crop value.

Table 1.1: Ranges of optimal N fertilization rate (kg N ha<sup>-1</sup>) for winter wheat production recommended by various investigators.

Investigators	Location of Investigation	Optimal N rate (kg N ha <sup>-1</sup> )
Alley et al. (1993)	Virginia Coastal Plain	85 – 102
Correll et al. (1997)	Central Arkansas	100 – 135
Cui et al. (2006)	North China Plain	71 – 170

Both insufficient N and excessive N can lead to decreases in grain yield (Alley et al., 1993; Correll et al., 1997). Insufficient N limits N uptake, which in turn limits biomass and grain production. Excessive N increases susceptibility to lodging and disease, which often lead to decreased yields (Alley et al., 1993). Correll et al. also observed that excessive N fertilization of winter wheat can also decrease grain density, which decreases the value of grain produced. Also, the amount of PAN in soil prior to planting influences the response of winter wheat grain yield to additions of supplemental N. Sowers et al. (1994) observed limited grain yield response to



added N in soils with relatively high pre-plant N. Cui et al. (2006) found that winter wheat grain yield did not respond to added N when soil contained 212 kg PAN ha<sup>-1</sup> prior to planting.

### **Timing of winter wheat N fertilization**

Winter wheat N uptake is not uniform throughout the growing season. Plant N uptake is relatively low from planting in October/November through tillering in late winter/early spring, highest immediately after stem elongation, and very low after anthesis (Baethgen and Alley 1989; Alley et al., 1993). Austin et al. (1977) found that winter wheat plants contained 83% of total plant N present at maturity at anthesis. Varying winter wheat N uptake over the growing season has prompted many studies on the optimal timing of N fertilization. Fall/spring split application of <sup>15</sup>N-labelled fertilizer to winter wheat totaling 112 kg N ha<sup>-1</sup> resulted in higher fertilizer N recovery than an all-fall application of <sup>15</sup>N-labelled fertilizer at the same rate (Sowers et al., 1994). Split applied and all-fall applied <sup>15</sup>N-labelled fertilizer supplied 32% and 20% of total plant tissue N, respectively (Sowers et al., 1994). Boman et al. (1995) applied urea-ammonium nitrate at various rates to winter wheat in November, December, January, February or March and concluded that timing of N application had minimal influence on grain yield. Alley et al. (1993) recommend pre-plant N application of 17-34 kg N ha<sup>-1</sup> in fall and split spring application not to exceed 135 kg N ha<sup>-1</sup>. The recommended amount of spring N is based on tiller density at Zadoks growth stage (GS) 25 and either plant tissue N or soil N at Zadoks GS 30 (Alley et al., 1993). Chen et al. (2006) observed equal winter wheat grain yields resulting from a 300 kg N ha<sup>-1</sup> 50% fall/50% spring application and a single spring application of 60 kg N ha<sup>-1</sup>. Correll et al. (1997) and Ellen and Spiertz (1980) observed optimal winter wheat grain yields with no fall N application and spring N applications of 100 – 135 kg N ha<sup>-1</sup> and 120 kg N ha<sup>-1</sup>, respectively.

## **Biosolids as an N fertilizer**

### **N cycling**

A few key processes and relationships between applied biosolids, the soils on which they are applied and the plants they nurture determine N cycling in biosolids amended soils. Biosolids contain N mostly in organic forms that are not immediately plant available. Soil microbes (particularly nitrobacter and nitrosomona) mineralize the organic N, which first undergoes ammonification and then nitrification ( $\text{Org-N} \rightarrow \text{NH}_3 \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^-$ ). The mineralized N and N already present in the biosolids as  $\text{NH}_4\text{-N}$  is plant available. Since most soils have a net negative charge and  $\text{NH}_4^+$  is positively charged,  $\text{NH}_4^+$  can be retained on the soil's cation exchange sites. The fate of negatively charged  $\text{NO}_3\text{-N}$  is plant uptake, denitrification or leaching with infiltrating precipitation. Sukreeyapongse et al. (2001) note that  $\text{NO}_3^-$  in leachate from biosolids amended soils was 25 times more concentrated than  $\text{NH}_4^+$ . A simplified description of the fate of mineralized N from biosolids is plant uptake, retention in soil, or loss from soil. The use of biosolids as an N fertilizer must be viewed in the context of the complex dynamics of biosolids that contribute to N cycling.

### **Biosolids N forms and availability**

Biosolids are used as fertilizer because they contain all plant essential nutrients, especially the macronutrients N and P. The composition of biosolids varies according to the composition of the wastewater from which they are generated. The forms of N present in biosolids at application affects the availability of N. Gasiunas (2001) observed that N in fresh biosolids is dominated by organic N and  $\text{NH}_4\text{-N}$  but that biosolids release N mostly as  $\text{NO}_3\text{-N}$  after land application. Scherer et al. (1990) studied two types of biosolids, one in which the majority of total N was organic N and another in which the majority of total N was  $\text{NH}_4\text{-N}$ . The study found that application of the biosolids with more organic N resulted in more plant N

uptake than biosolids with more  $\text{NH}_4\text{-N}$ . The reason for Scherer's observations was likely the more rapid conversion of  $\text{NH}_4\text{-N}$  than organic N to soluble and leachable  $\text{NO}_3\text{-N}$ . Gilmour et al. (2003) observed PAN supplied from biosolids applications to agronomic crops (*Zea mays*, *Festuca arundinacea*, and *Sorghum bicolor*) was approximately 19 kg PAN Mg biosolids<sup>-1</sup> applied or 37% of total biosolids N. Cogger et al. (2004) studied biosolids from 14 sources produced from differing treatment and dewatering/drying processes and found that the mean PAN released during the first year after application to tall fescue (*Festuca arundinacea* Shreb) was 37±5% of total biosolids N.

### **Biosolids organic N mineralization**

Availability of biosolids N for plant uptake begins with mineralization of organic N, so it is important to accurately estimate how much organic N in a given biosolids treatment will mineralize. Many field and incubation studies have provided empirical amounts of biosolids organic N mineralization that can be used to estimate organic N mineralization from subsequent biosolids applications. Estimates of biosolids N mineralization must be made carefully because there is not widespread agreement among the results of previous studies. He et al. (2000) observed 48% organic N mineralization in a 1 yr incubation study of biosolids applied to a sandy soil (sandy, siliceous, hyperthermic Alfic Arenic Haplaquod). Gilmour et al. (2003) observed that 37% of biosolids organic N was mineralized to PAN during the first year after application, with the majority mineralized during the growing season. Wang et al. (2003) studied aerobically and anaerobically digested biosolids and observed 32% and 15% organic N mineralization, respectively. Wang's observations show that biosolids treatment processes can influence organic N mineralization. When applying three granulated biosolids treatments of 12, 24 and 48 Mg ha<sup>-1</sup> and one dewatered biosolids treatment of 22 Mg ha<sup>-1</sup>, Eldridge et al. (2008) assumed that approximately 25% of the organic N in each treatment would mineralize. However,

mineralization rates ranged from 45 – 54%, with mineralization rates inversely related to application rates (Eldridge et al., 2008). Boeira (2009) observed a similar trend of higher mineralization fractions at lower application rates. Both biosolids in the study had been anaerobically digested, but one had mineralization fractions ranging from 14 – 26% and the other had fractions ranging from 27 – 43% (Boeira, 2009). Boeira's observations show that biosolids undergoing the same treatment processes from different sources can differ in organic N mineralization.

### **The Influence of biosolids treatment process on N recommendations**

Sewage sludge treatment processes are used to reduce pathogens in biosolids to decrease the risk of detrimental effects on human health. Research has shown both little and substantial effects of sewage treatment practices on N forms and mineralization of organic N in biosolids. Rigby et al. (2009) studied mineral N release from dewatered raw sewage sludge, dewatered anaerobically digested biosolids, thermally dried anaerobically digested biosolids and lime-treated unstabilized sludge cake applied to silty clay and sandy loam soils. Initial differences in N release related to biosolids treatment process and soil type were observed, but overall N release was similar on both soil types for all biosolids (Rigby et al., 2009). Rigby et al. (2009) concluded that biosolids treatment processes and soil texture do not need to be taken into account when making biosolids N recommendations for agronomic crops in temperate areas. Cooper et al. (2005) applied digested biosolids and lime stabilized biosolids to wheat and triticale and concluded that the crops benefitted equally from both biosolids types. However, Wang et al. (2003) observed 32% organic N mineralization from aerobically digested biosolids and 15% organic N mineralization from anaerobically digested biosolids, which suggests biosolids N rates should be recommended based on differing expected N release amounts resulting from the differing treatment processes. Correa et al. (2012) studied PAN release from five biosolids types

(digested, limed, composted, heat dried and solar irradiated) when incubated alone, in a clayey oxisol and in a sandy spodosol. All biosolids types released N equally when incubated alone but released N differently when incubated in each soil type (Correa et al., 2012). The relationship between N release and biosolids type was different for each soil, i.e. irradiated > digested > heat dried > composted > limed for the N release in the oxisol, and limed > digested > composted > irradiated > heat dried for N release in the spodosol (greatest to least) (Correa et al., 2012). Correa et al. (2012) concluded that biosolids N recommendations must account for the interaction between biosolids type and soil type.

### **Influence of biosolids on grain yield**

One economic advantage of use of biosolids as a fertilizer in the United States is that they (particularly, Class B treated by a Process to Significantly Reduce Pathogens or PSRP) are free to farmers. However, biosolids are only ultimately beneficial if their use results in yields equal to or greater than those achieved with other fertilizers. Koenig et al. (2011) compared winter wheat grain yields resulting from the application of equal N rates of inorganic N fertilizer and two types of biosolids (Class A dewatered digested and Class A heat-dried). Biosolids resulted in 0 – 1.4 Mg ha<sup>-1</sup> (0 – 47%) greater grain yields than inorganic N fertilizer and never resulted in lower grain yields than inorganic N fertilizer (Koenig et al., 2011). Cooper et al. (2005) applied dewatered digested biosolids at rates of 0, 6, 12, 24 dry Mg ha<sup>-1</sup> and lime-amended biosolids at rates of 0, 1.5, 3, 4.5 dry Mg ha<sup>-1</sup> to wheat and triticale and observed 50% or greater yield increases compared to local averages. Dewatered digested biosolids applied at 24 dry Mg ha<sup>-1</sup> resulted in the greatest yield increases (Cooper et al., 2005).

## **Environmental considerations**

### **Soil residual N after winter wheat harvest**

It is important to provide as closely as possible the N needs of a winter wheat crop to optimize yield and prevent reactive N loss to air and water. Winter wheat fertilized at recommended N rates will not completely deplete the soil of inorganic N. Soil residual inorganic N can be used by crops following winter wheat or can be lost from the soil by  $\text{NH}_3$  volatilization, denitrification or  $\text{NO}_3^-$  leaching.

The distribution of inorganic N in soil immediately after winter wheat harvest partially determines the potential for its loss by leaching. Chaney (1990) observed that on average 50% of soil residual  $\text{NO}_3\text{-N}$  was present in the 0 – 30 cm depth, 30% in the 30 – 60 cm depth and 20% in the 60 – 90 cm depth. Soil inorganic N increased with N rate, slowly at rates below the agronomic N rate and steeply at rates above the agronomic N rate (Chaney, 1990). Westerman et al. (1994) observed that applications of N fertilizer up to  $90 \text{ kg N ha}^{-1}$  did not increase soil residual inorganic N over zero N control. However, N fertilizer rates greater than  $90 \text{ kg N ha}^{-1}$  increased  $\text{NH}_4\text{-N}$  at 0 – 15 cm and  $\text{NO}_3\text{-N}$  at 0 – 15 cm and below 30 cm. Olson and Swallow (1984) applied  $^{15}\text{N}$ -labelled fertilizer to winter wheat at rates of 50 and  $100 \text{ kg N ha}^{-1}$  for five consecutive years and observed that 71 – 77% of residual fertilizer N was in the 0 – 10 cm depth of the soil. In a similar study, Powlson et al. (1992) applied  $^{15}\text{N}$ -labelled fertilizer to winter wheat at rates up to  $234 \text{ kg N ha}^{-1}$  at 9 locations over 4 years and observed that 84 – 88% of residual fertilizer N was in the 0 – 23 cm depth of the soil.

### **The risk of $\text{NO}_3^-$ leaching from winter wheat fields**

Winter wheat does not take up N uniformly throughout the growing season, so fluxes of  $\text{NO}_3^-$  leaching potential are not equal at all stages of winter wheat growth (Liang et al., 2011). The following order of  $\text{NO}_3^-$  leaching potential at various winter wheat stages was observed by

Liang et al. (2011) from greatest to least to be tillering > harvest > booting > seeding. It is important to note that some studies have found winter wheat fields to be more susceptible to  $\text{NO}_3^-$  leaching loss than other winter management systems. Thomsen et al. (1993) identified winter wheat fields both continuous and in rotation treated with organic amendments as leading to more  $\text{NO}_3\text{-N}$  leaching than various rotations of ryegrass and barley. Only fallow fields that received organic amendments resulted in more  $\text{NO}_3^-$  leaching than continuous winter wheat (Thomsen et al., 1993). Francis et al. (1995) observed that winter wheat did not reduce  $\text{NO}_3^-$  leaching losses compared to fallow fields. Some environmental conditions promote  $\text{NO}_3^-$  leaching regardless of management. For example, Goulding et al. (2000) observed that when winter rains follow a dry summer and autumn, even land that has not received N fertilizer for more than 150 yr will drain water with an  $\text{NO}_3\text{-N}$  concentration greater than the European Union water quality standard of 11.3 mg/L (European Commission, 1998).

### **The risk of $\text{NO}_3^-$ leaching from use of biosolids as an N fertilizer**

#### **Residual soil PAN and N loss**

Biosolids N content is initially dominated by organic N, which is not soluble or mobile, but  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  will form through mineralization and nitrification after application. Any  $\text{NO}_3\text{-N}$  released to the soil can be taken up by plants, lost to the atmosphere through denitrification, or leached with infiltrating precipitation. Numerous investigators have studied soil residual PAN and N loss resulting from use of biosolids as an N fertilizer and have recognized the risk of  $\text{NO}_3^-$  leaching. Cogger et al. (2001) studied the effects of 7 consecutive yr of Class A biosolids applications to tall fescue at mean annual biosolids rates of 290, 580 and 870 kg total N  $\text{ha}^{-1}$  per yr. Residual soil  $\text{NO}_3\text{-N}$  remained below 25 kg  $\text{ha}^{-1}$  for the first 3 yr of the study but significantly increased in yr 4 beginning with the 870 kg total N  $\text{ha}^{-1}$  biosolids treatment (Cogger et al., 2001). Barbarick et al. (1996) applied biosolids at rates of 6.7 and 26.8

dry Mg ha<sup>-1</sup> to winter wheat fields for 11 consecutive years to determine the fate of applied biosolids N. The 6.7 dry Mg ha<sup>-1</sup> biosolids applications resulted in the following average N distributions: 54% soil residual, 9% grain removal, 38% unaccounted, and the 26.8 dry Mg ha<sup>-1</sup> biosolids applications resulted in the following average N distributions: 35% soil residual, 2% grain removal, 63% unaccounted (Barbarick et al., 1996). Luczkiewicz (2006) applied biosolids to coarse and medium grained sand in a column study and found NH<sub>4</sub>-N and NO<sub>3</sub>-N originating from the biosolids at depths greater than 80 cm. Oliveira et al. (2001) studied the movement of biosolids N through sugarcane soils by measuring soil solution N and soil residual N. Greater concentrations of NO<sub>3</sub><sup>-</sup> resulting from biosolids application than from zero N control were found in soil solution at depths of 30, 60 and 90 cm (Oliveira et al., 2001). Greater concentrations of soil residual NH<sub>4</sub>-N and NO<sub>3</sub>-N resulting from biosolids application than from zero N control were found in the 90 – 120 cm soil depth (Oliveira et al., 2001). Observed increases of soil NO<sub>3</sub>-N, soil solution NO<sub>3</sub><sup>-</sup> and unaccounted for biosolids N clearly demonstrate that use of biosolids as an N fertilizer can increase the risk of NO<sub>3</sub><sup>-</sup> leaching.

#### **Influence of timing of biosolids application on NO<sub>3</sub><sup>-</sup> leaching risk**

Another important aspect of biosolids application linked with NO<sub>3</sub><sup>-</sup> leaching risk is the timing of application. Two early studies point to fall application of liquid biosolids leading to increased NO<sub>3</sub><sup>-</sup> leaching. In a study that compared fields treated in the fall of 1971 and 1973 with pulverized municipal refuse, liquid biosolids and the two combined, King et al. (1977) observed that NO<sub>3</sub><sup>-</sup> leaching losses were increased with sludge. In a study on two consecutive fall biosolids applications in 1974 and 1975 to loess soils and sandy soils, Fleige et al. (1980) observed NO<sub>3</sub><sup>-</sup> losses as high as 517 kg ha<sup>-1</sup> on the loess soils and 819 kg ha<sup>-1</sup> on the sandy soils and also recommended that fall application should have a volumetric limit of 100 m<sup>3</sup> ha<sup>-1</sup>. Shepherd later concluded that liquid biosolids should not be applied in the fall at all due to significant N loss,



but dewatered cakes greatly reduced N loss and therefore required no temporal application restrictions (Shepherd, 1996). Dewatering biosolids reduces  $\text{NO}_3\text{-N}$  formation in biosolids amended soils by the removal of soluble  $\text{NH}_4\text{-N}$  ions that could undergo nitrification (Smith et al., 1998). ). However, in a field experiment on fall and winter applications of biosolids to corn plots, Michelin et al. (1990) found that the timing of the application had no significant effect on crop N uptake but that risk of  $\text{NO}_3^-$  leaching was higher for fall applications due to organic N mineralization and nitrification of  $\text{NH}_4\text{-N}$  before winter rains. Evanylo (2003) concluded that winter application of biosolids on coarse-textured, but not necessarily fine-textured, soils in the Virginia Coastal Plain physiographic region resulted in more  $\text{NO}_3^-$  leaching than spring applications.

#### **Comparison of $\text{NO}_3^-$ leaching risk from biosolids and inorganic N fertilizer applications**

It is important to draw a comparison between biosolids and inorganic N fertilizers because inorganic N fertilizers are the most common source of N for small grains. Inorganic N fertilizers are also subject to volatilization, denitrification and leaching. Numerous studies have compared  $\text{NO}_3^-$  loss from soils amended with biosolids and inorganic N fertilizer but have not generated consensus. After incubation trials comparing biosolids and conventional fertilizers, Niekerk et al. (2005) concluded that inorganic N fertilizers create a greater risk for nitrate leaching because they are composed of reactive N, whereas biosolids slowly release reactive N through mineralization over time. Carneiro et al. (2012) compared  $\text{NO}_3^-$  leaching from inorganic N fertilizers and organic amendments (including biosolids) on a double cropped corn-oats system. The study found that compared with inorganic N fertilizers, properly managed organic residues can reduce  $\text{NO}_3^-$  leaching except in the case of split applications of biosolids, which led to  $\text{NO}_3^-$  leaching comparable with that from inorganic N fertilizers (Carneiro et al., 2012).

Esteller et al. (2009) applied biosolids and inorganic N fertilizer to corn and observed equal ranges of  $\text{NO}_3^-$  in soil solution: 0.7 – 64 mg  $\text{NO}_3\text{-N L}^{-1}$  resulting from biosolids and 1 – 61 mg  $\text{NO}_3\text{-N L}^{-1}$  resulting from inorganic N fertilizer. Barbarick et al. (2012) observed that biosolids resulted in more soil residual  $\text{NO}_3\text{-N}$  than inorganic N fertilizer for a wheat-fallow rotation at soil depths of 30 – 60 cm and 60 – 90 cm and for a wheat-corn-fallow rotation in all soil depths except for 5 – 10 cm and 120 – 150 cm.

### **Factors mitigating risk of $\text{NO}_3^-$ loss from winter wheat fields**

#### ***Depth of winter wheat roots***

Knoch et al. (1957) observed winter wheat roots as deep as 4 m when moisture conditions were favorable. Thorup-Kristensen et al. (2009) observed that winter wheat roots grow twice as deep as spring wheat roots. Soil residual PAN at 1 – 2.5 m depth was on average 81 kg  $\text{ha}^{-1}$  less under winter wheat than spring wheat after grain harvest (Thorup-Kristensen et al., 2009). Winter wheat roots twice as deep as corn, which partly accounts for reduced soil  $\text{NO}_3\text{-N}$  observed at a depth of 1.4 – 2 m under wheat than under corn following harvest of each (Zhou et al. 2008). Winter wheat roots more deeply than spring crops and can therefore intercept soil PAN that may otherwise be lost to leaching.

#### ***Soil texture***

Correa et al. (2012) showed that there can be an interaction between soil texture and N fertilizer source, which has implications for N loss potential. Simmelsgaard (1998) studied  $\text{NO}_3^-$  concentrations in tile drain water and suction cup lysimeter soil water from fields planted with winter cereals after spring cereals and observed an inverse relationship between soil clay content and drainage/soil water  $\text{NO}_3^-$  concentration. Soil with 5, 12 and 20% clay in the 0 – 25 cm depth leached on average 68, 44 and 26 kg  $\text{NO}_3\text{-ha}^{-1}$  per yr, respectively (Simmelsgaard, 1998). Lee (2004) concluded that, although a single 50 dry Mg  $\text{ha}^{-1}$  biosolids application to a clayey soil

supplies more  $\text{NO}_3\text{-N}$  than wheat can consume in 2 yr, there is no increased risk of  $\text{NO}_3^-$  leaching due to clayey soil texture.

### ***No-till management***

Conventional tillage helps control weeds and creates a less dense seed bed but also introduces more oxygen into the surface soil, which in turn leads to more rapid loss and oxidation of soil organic matter (SOM). No-till management has become increasingly popular because it preserves SOM, which in turn increases soil fertility and decreases N fertilizer need (Rice and Smith, 1992). No-till management can also curb  $\text{NO}_3^-$  leaching loss because it promotes denitrification. Soils under no-till management retain more moisture and have overall higher populations of anaerobic microbes than soils under conventional tillage (Linn and Doran, 1984). Switching an agricultural field from conventional or minimum tillage to continuous no-till management will result in lower soil  $\text{NO}_3\text{-N}$  after 3 yr (Halvorson et al., 2001).

## 1.4: Hypotheses

1.  $H_0$ : Biosolids applied prior to wheat planting at 100% of the crop's N needs will result in N use as efficient as with split applications of inorganic N.

$H_a$ : Biosolids will be a less agronomically and environmentally sound N source for winter wheat than carefully split inorganic fertilizer applications.

2.  $H_0$ : Nitrogen use efficiency will be greater and N leaching will be less from LS biosolids than from AD biosolids because of the higher ratio of organic N:NH<sub>4</sub>-N in LS than in AD.

$H_a$ : There will be no difference between the N use efficiency and N leaching between LS and AD biosolids.

3.  $H_0$ : Nitrogen use efficiency will be greater and N leaching will be less for 50% fall biosolids PAN + 50% late winter inorganic fertilizer than for 100% fall biosolids PAN as coarseness of soil texture increases.

$H_a$ : There will be no differences in N use efficiency and N leaching between 50% fall biosolids PAN + 50% late winter inorganic fertilizer and 100% fall biosolids PAN due to soil texture.

### **1.5: Objectives**

1. To compare the effects of AD and LS biosolids and inorganic fertilizer N application rates and timing and soil texture on winter wheat biomass, grain yield, N use efficiency, and soil inorganic N distribution.
2. To test the utility of the Haney-Brinton CO<sub>2</sub>-burst method in quantifying the response of potential soil N mineralization to different types and fall N rates of biosolids applied to winter small grains.

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## **Chapter 2: The effects of biosolids type and soil texture on appropriate biosolids application rate and timing to winter wheat**

**Kevin W. Bamber, Gregory K. Evanylo, Wade E. Thomason**

### **Abstract**

Environmental concerns about winter nitrogen (N) leaching loss limit the amount of biosolids applied to winter wheat in Virginia. Ten field studies were established 2012-2014 in Virginia to determine the agronomic and environmental feasibility of fall biosolids applications to soft red winter wheat (*Triticum aestivum* L.). Eight studies were located in the Coastal Plain physiographic province and two in the Ridge & Valley physiographic province. The effects of eight biosolids and urea N treatments on six parameters related to winter wheat production and environmental N dynamics was studied: 1) biomass production at Zadoks growth stage (GS) 25-30, 2) soil inorganic N at Zadoks GS 25-30, 3) N use efficiency (NUE) at GS 58, 4) grain yield, 5) end-of-season soil inorganic N, and 6) estimated N recovery. Anaerobically digested (AD) and lime stabilized (LS) biosolids were fall applied at estimated plant available N (PAN) rates of 100 kg N ha<sup>-1</sup> and 50 kg N ha<sup>-1</sup>. The 50 kg N ha<sup>-1</sup> biosolids treatments were supplemented with 50 kg N ha<sup>-1</sup> as urea in spring. Urea N was split applied at 0, 50, 100 and 150 kg N ha<sup>-1</sup>, with 1/3 applied in fall and 2/3 in spring. The LS biosolids resulted in greater NUE at GS 58 than AD biosolids and agronomic N rate urea in coarse textured soils only. Biosolids and urea applied at the agronomic N rate resulted in equal grain yield and estimated N recovery in fine textured soils where N leaching loss risk was low, regardless of biosolids type or application strategy. Biosolids type and application strategy influenced grain yield and N loss in soils with high or



moderate N leaching loss risk, with yield and N recovery being higher with LS biosolids and with the 50% biosolids + 50% urea application strategy. To achieve the ideal balance among N use efficiency, yield and N leaching, AD and LS biosolids can be fall applied to winter wheat at the full agronomic N rate in soils with low N leaching loss risk, but only LS biosolids should be applied to winter wheat at the full agronomic N rate in soils with moderate or high N leaching loss risk.

## **2.1: Introduction**

Wheat is a good crop for application of biosolids in corn-wheat-soybean crop rotations commonly planted throughout the U.S. mid-Atlantic states due to its crop N needs. Current Virginia Department of Conservation and Recreation (VADCR, 2011) regulations permit fall applications of biosolids to supply starter N requirements, which typically meets only half the agronomic N need of winter small grains. Fall application of biosolids at the full agronomic N rate is prohibited to reduce the risk of winter N loss; however, the biosolids loading rates that supply 50% of winter small grains N needs are too low to be mechanically or economically feasible for biosolids applicators (Personal communications; Susan Trumbo, Recyc Systems; Mary Powell, NutriBlend; Allen Guillams, Synagro). Thus, wheat fertilization is dependent on inorganic fertilizer as the primary N source in Virginia even where biosolids use is permitted.

The replacement of inorganic N fertilizers with biosolids as an N source can be justified on economic and agronomic grounds. Biosolids are largely free to producers and, therefore, can reduce wheat production costs. Biosolids can produce equal or greater grain yields as inorganic

fertilizer applied at equal PAN rates (Koenig et al., 2011; Cooper, 2005). Economic and agronomic justification is counterbalanced by the environmental concern of increased N leaching loss. Inorganic N fertilizer application to winter wheat is split between periods of maximum N uptake to increase N use efficiency and reduce N loss (Alley et al., 1993). Once biosolids are applied, the interaction between treatment-dependent biosolids properties and soil properties, especially texture, determine the rate and timing of inorganic N release via mineralization (Correa et al., 2012). Fall applied biosolids may supply more PAN than winter small grains can assimilate, which increases the risk of N leaching loss. This is significant in the Mid-Atlantic Coastal Plain physiographic province, where local or site-specific assessment of N leaching loss potential was deemed necessary due to high variability of soil texture (Spalding and Exner, 1993). Accordingly, all soils in Virginia are rated for environmental sensitivity and leaching loss potential is a key parameter of consideration (VADCR, 2005).

The rate and timing of biosolids PAN release cannot always be accurately measured in the field (Abril et al., 2001). Comparing the responses of plant and soil inorganic N to biosolids and inorganic fertilizer N can assess whether or not rate and timing of biosolids PAN release optimize agronomic performance and minimize environmental risk (Barbarick et al., 1996, 2010 and 2012). This is significant because biosolids derived from various treatment processes typically release similar amounts of PAN via mineralization over the course of the year they are land applied (Gilmour and Skinner, 1998; Cogger et al., 2004). Based on research by Gilmour et al. (2003), Virginia Nutrient Management Standards and Criteria (VADCR, 2005) employ a fixed coefficient of application year organic N mineralization (i.e., 30%) to all biosolids types, except compost, commonly applied in Virginia.

The potential agronomic benefits and environmental risks resulting from fall biosolids applications to winter wheat require evaluation. The amounts of PAN supplied by biosolids in the year of application are reasonably well understood. However, the interaction between timing of biosolids PAN supply and soil texture make it uncertain whether 100% fall biosolids application are appropriate for winter wheat production and environmental protection. Our objectives were 1) to compare the effects of fall biosolids applications and fall/spring urea split applications on winter wheat biomass, grain yield and soil inorganic N, and 2) to compare the effects of biosolids type and soil texture on appropriate biosolids application rate and timing for winter small grains.

## **2.2: Materials and methods**

### **Locations, experimental design, and treatments**

Four field studies were established in the Virginia Coastal Plain and Valley & Ridge physiographic provinces in fall 2012, and six were established in the Virginia Coastal Plain physiographic region in fall 2013. Description of the sites, including county, soil series, soil family and leaching risk are presented in Table 2.1.

The field studies consisted of eight treatments replicated four times in a randomized complete block design (RCBD). Each study site was 37.8 m long x 24 m wide, with individual plots measuring 27 m<sup>2</sup>.

The treatments included four rates of inorganic N fertilizer and two rates each of two types of biosolids. All treatments were applied on the basis of agronomic PAN rate for winter wheat. Biosolids used were anaerobically digested (AD) and lime stabilized (LS). The source of the dewatered AD biosolids was Alexandria (VA) Renew Enterprises ([www.alexrenew.com](http://www.alexrenew.com)) for both 2012-2013 and 2013-2014 seasons. The sources of the LS biosolids were Blue Plains

(District of Columbia) Advanced Wastewater Treatment Plant ([www.dcwater.com/waste](http://www.dcwater.com/waste)) for both seasons and Arlington (VA) Wastewater Treatment Authority ([water.arlingtonva.us/sewer/wastewater-treatment/](http://water.arlingtonva.us/sewer/wastewater-treatment/)) for the 2012-2013 season. The LS biosolids from Blue Plains were applied at sites in the Virginia Coastal Plain and LS biosolids from Arlington were applied at sites in the Virginia Valley & Ridge Province. Both anaerobically digested and lime stabilized biosolids were applied to each study area in the fall at full and half agronomic N rates, which are 100 and 50 kg PAN ha<sup>-1</sup> (VADCR, 2005). The 50 kg PAN ha<sup>-1</sup> biosolids treatments were supplemented with 50 kg N ha<sup>-1</sup> as urea in spring. There were also four inorganic N fertilizer treatments in the form of urea (CO(NH<sub>2</sub>)<sub>2</sub>, 46% N): 0, 50, 100, and 150 kg N ha<sup>-1</sup>. Urea N was split applied at, with 1/3 applied in fall and 2/3 in spring. The application timing of the treatments is presented in Table 2.2. All treatments were surface applied without incorporation.

Biosolids rates were calculated to provide PAN based on mean analytical N composition from the previous year, as described in Table 2.3. We calculated the actual estimated PAN rates from analyses of the biosolids collected at the time of application. Therefore, the amount of estimated PAN applied was not exactly 50 kg N ha<sup>-1</sup> for 0.5 x agronomic N rate nor exactly 100 kg N ha<sup>-1</sup> for 1.0 x agronomic N rate. Biosolids samples were analyzed by A&L Eastern Laboratories ([www.al-labs-eastern.com/](http://www.al-labs-eastern.com/)) for total Kjeldahl N (Clesceri et al., 2012), NH<sub>4</sub>-N (Clesceri et al., 2012), organic N (Clesceri et al., 2012), total phosphorous (P) (USEPA, 2009), total potassium (K) (USEPA, 2009), solids (Clesceri et al., 2012), and pH (USEPA, 2009), and the results are presented in Table 2.4. The analyses were used to determine actual N, P and K applied in the biosolids (Table 2.5) and also to determine side-dress N rates for plots treated with 0.5 x agronomic N rate as biosolids in the fall.

All plots received K prior to planting in the form of muriate of potash (0 – 0 – 60) according to soil test K determined by the Virginia Tech Soil Testing Laboratory (Maguire and Heckendorn, 2011) and Virginia Department of Conservation and Recreation nutrient recommendations (VADCR, 2005). Plots treated with urea also received P in the form of triple superphosphate (0 – 45 – 0) according to soil test recommendations.

### **Site management, planting and harvest**

All locations were managed under continuous no-till practice in a 2 year, 3 crop rotation of corn-winter wheat-double crop soybean. Soft red winter wheat (*Triticum aestivum* L.) was planted at all locations October 6-20 following harvest of corn (*Zea mays*,L.). The seeding rate was 69 seedlings per row meter, and rows were spaced 19 cm apart. Wheat variety planted at each location is presented in Table 2.6 along with county and soil series. Herbicide, pesticide and fungicide use decisions were made by each farmer. Grain harvest was performed June 21, 2013 and June 24-27, 2014 at Coastal Plain study locations and July 18, 2013 at Ridge & Valley study locations.

### **Data collection, sampling, processing and analysis**

#### **Weather data**

Daily temperature and precipitation data from 2012-2014 were obtained from the National Oceanic and Atmospheric Administration monitoring station at Richmond International Airport in Sandston, Virginia (NOAA, 2014) to approximate weather conditions at all study sites located in the Coastal Plain physiographic province. Mean monthly temperature and precipitation data from 2012-2013 was obtained from the National Oceanic and Atmospheric Administration monitoring stations at Middle River Wastewater Treatment Plant in Staunton, Virginia and the Blacksburg Weather Forecast Office in Blacksburg, Virginia (NOAA, 2014) to approximate weather conditions at each study site located in the Ridge & Valley physiographic province.

Observed mean monthly temperatures and precipitation amounts from each growing season were overlaid with expected mean temperatures and precipitation amounts for comparison (Figures 2.1a-c and 2.2a-c). Temperature and precipitation data were used to make inferences about winter organic N mineralization and soil inorganic N loss.

### **Soil sampling and processing**

Soil samples were collected at three times during the 2012-2013 and 2013-2014 seasons: prior to planting and treatment application (September 25-October 5), mid-season prior to side-dress N application (March 10-17, April 18 in Frederick & Vertrees only), and after harvest (June 24-July 1 in Coastal Plain soils, July 18-21 in Ridge & Valley soils). Sample collection was delayed in Frederick & Vertrees because the wheat reached GS 25-30 about one month later than at all other locations.

Approximately 20 samples were randomly collected throughout each site with push probes prior to planting and amendment application. Each sample was taken to a depth of 30 cm and divided into 0-15 cm and 15-30 cm increments. The soil samples from each depth increment were bulk-blended to create bulk samples used to characterize the sites before amendment application and planting.

Four to five soil samples were collected randomly from the center of each plot with push probes prior to side-dress N application. Each sample was taken to a depth of 30 cm and samples taken within the same plot were bulk-blended to characterize the plot.

Two soil samples were collected near the center of each plot 2-4 days after wheat harvest. Post-harvest soil sampling was performed in 2013 with a Giddings probe in 43 of 128 plots sampled, and bucket augers were used to collect the remaining soil samples due to equipment malfunction. Soil samples were collected in 20 cm depth increments in 2013. Altavista and Bojac

were sampled to 80 cm, and Frederick-Christian and Frederick & Vertrees were sampled to 60 cm due to subsurface restrictive layers of coarse fragments. Soil samples were collected in 30 cm depth increments in 2014. Altavista, Bojac, Emporia and Kempsville were sampled to 90 cm, Roanoke was sampled to 60 cm due to restrictive layers of coarse fragments, and State was sampled to 30 cm due to the presence of a stone layer at 35 cm. All but 21 of 192 plots were soil sampled with bucket augers in 2014. Eleven of 32 plots on the State soil were sampled with bucket augers and the remaining 21 were sampled with push probe. All samples were promptly air dried, ground, and sieved through a No. 10 mesh (2mm equivalent spherical diameter).

### **Soil analyses**

Routine analyses were performed on pre-plant soil samples by the Virginia Tech Soil Testing Laboratory for P, K, Ca and Mg by the Mehlich I method and for pH by 1:1 method (Maguire and Heckendorn, 2011). Total C and N were analyzed by combustion with a Vario MAX CNS macro elemental analyzer (Elementar, Hanau, Germany). Inorganic N was extracted from soils with 2 M potassium chloride (KCl), with soil and KCl combined in a ratio of 1 g of soil to 10 mL of KCl. The combined soil and KCl was shaken mechanically for 30 minutes. The resulting supernatant was filtered through 0.45 micron filter paper to give a filtrate free of suspended solids. The filtrate was then run through a Lachat 8500 Flow Injection Analyzer to determine NO<sub>3</sub>-N by QuikChem Method 12-107-04-1-B (Knepel, 2001) and to determine NH<sub>4</sub>-N by QuikChem Method 12-107-06-2-A (Hofer, 2001). The midseason and post-harvest soil samples were analyzed for inorganic N by flow injection analysis, as described above. Bulk density estimates obtained from Web Soil Survey ([websoilsurvey.nrcs.usda.gov/](http://websoilsurvey.nrcs.usda.gov/)) were used to convert soil concentrations from analytical units of mg L<sup>-1</sup> to field scale units of kg ha<sup>-1</sup>.

### **Plant sampling**

Aboveground plant tissue was sampled from 1 m of a representative row in each plot when the wheat was at Zadoks growth stage 25-30 (tillering; Zadoks et al., 1974) during the 2012-2013 season. Plant tissue was collected twice during the 2013-2014 season: from 1 m of row when the wheat was at Zadoks growth stage 25-30 (tillering) and from 1 m<sup>2</sup> when the wheat was at Zadoks growth stage 58 (beginning of anthesis; Zadoks et al., 1974) in each plot. Grain samples were collected with a Kincaid 8XP small plot combine. Grain samples from the site in Augusta County VA were collected manually from 1 m<sup>2</sup> quadrants due to lodging, late season weed pressure and slope of the plots.

### **Plant processing and analysis**

Above-ground plant tissue collected at GS 25-30 from both seasons was weighed moist, dried at 60°C and ground in a Wiley mill to pass a 1 mm screen. Plant tissue collected at GS 25-30 from the 2013-2014 growing season was also weighed immediately after drying to estimate biomass per unit area. Plant tissue collected at GS 58 from the 2013-2014 season was weighed moist in the field before a representative subsample was taken and weighed in the field. The subsamples of plant tissue taken at GS 58 were dried at 60°C and weighed to estimate biomass per unit area. All plant tissue samples from the 2013-2014 season were analyzed for total N by combustion with a Vario MAX CNS macro elemental analyzer (Elementar, Hanau, Germany). Grain harvested from each plot was weighed by an onboard computer in the Kincaid 8XP small plot combine. Grain samples were analyzed for grain density and moisture using a Dickey-John GAC2000 grain sampler (DICKEY-john, Auburn, IL) and were only dried at 60°C when moisture content was greater than 16%. Grain yields were reported on a 13.5% moisture basis.



**Calculation of N use efficiency (NUE), estimation of soil organic N mineralization, and estimation of N recovery**

**NUE:** N uptake data from the 2013-2014 growing season was used to calculate NUE by the following equation:

$$\text{NUE} = [\text{N uptake in fertilized plots} - \text{N uptake in zero N plots}] / [\text{fertilizer PAN rate}]$$

**Estimation of soil organic N mineralization:** The relationship between pre-plant soil inorganic N ( $N_{i0}$ ), end-of-season soil inorganic N ( $N_{ir}$ ) and N uptake in plots that received no N fertilizer was used to estimate soil organic N mineralization (Est.  $N_{min}$ ) during the 2013-2014 winter wheat growing season by the following equation:

$$\text{Est. } N_{min} = \text{N uptake} + N_{ir} - N_{i0}$$

**Estimation of N recovery:** We estimated N recovery by calculating fertilizer inorganic N present in plant tissue at GS 58 and soil inorganic N after grain harvest. Mean N uptake and soil inorganic N in zero N plots ( $N_{upt,zN}$  and  $N_{ir,zN}$ ) was subtracted from mean N uptake and soil inorganic N in fertilized plots ( $N_{upt,f}$  and  $N_{ir,f}$ ) to determine fertilizer N uptake and fertilizer N residual in soil. The sum of fertilizer N uptake and fertilizer N residual in soil was divided by fertilizer N rate. Estimated N recovery was calculated with the following equation:

$$\text{Estimated N recovery} = [\text{fert. N uptake} + \text{fert. N residual in soil}] / [\text{fert. N rate}]$$

$$\text{Percent N recovery} = \text{estimated N recovery} \times 100\%$$

NUE calculations for biosolids and estimated N loss from biosolids amended plots rely on the assumption that the factors used to calculate biosolids PAN (Table 2.3) were accurate. The estimation of growing season soil organic N mineralization relies on the assumption that plots that received no N fertilizer did not lose any N via volatilization or leaching.

### Statistical analysis

All statistical analysis was performed with JMP®Pro 10.0.2 statistical software (SAS Institute Inc., 2012). The responses of biomass at GS 25-30 and grain yield to urea-N rate were measured with linear regression. We fit the increase in biomass at GS 25-30 with the increase in each urea, AD biosolids, and LS biosolids PAN applied in fall. We fit the increase of grain yield with the increase in total urea-N rate. Grain yields resulting from biosolids treatments were overlaid on the regressions resulting from urea in order to compare responses from biosolids-N with responses from urea-N. The distribution of observed urea response residuals was compared to the distribution of observed biosolids response residuals. Biosolids response residuals were calculated by subtracting the responses predicted by the urea regression line from observed biosolids responses. The urea response residuals were tested for normal distribution using the Shapiro-Wilk test. Urea residual distributions were compared to biosolids residual distributions with analysis of variance (ANOVA) when urea residuals were normally distributed and with the Wilcoxon/Kruskal-Wallis rank sums test when urea residuals were not normally distributed.

Mean separation was also used to compare responses of biomass, N uptake, grain yield, soil inorganic N distribution and estimated N recovery. Repeated measures multiple analysis of variance (MANOVA) was used to compare soil inorganic N distribution resulting from biosolids treatments and urea applied at 100 kg PAN ha<sup>-1</sup>. All data sets were tested for normal distribution with the Shapiro-Wilk test. Mean responses from treatments were compared by ANOVA and Student's t-test when data was normally distributed. Mean responses from treatments were compared by the Wilcoxon/Kruskal-Wallis rank sums and Wilcoxon multiple comparison when data was not normally distributed. The significance level of all tests was set to 0.05.

## **2.3: Results and discussion**

### **Growing season weather**

Mean monthly temperatures and precipitation in the Coastal Plain physiographic province are presented in Figures 2.1a and 2.2a. Mean monthly temperatures and precipitation in the Ridge & Valley physiographic province are presented in Figures 2.1b-c and 2.2b-c. Winter temperatures were above-average during the 2012-2013 growing season and below-average during the 2013-2014 growing season. Precipitation in the 2012-2013 growing season was erratic, with months alternating between below- and above-average. Precipitation in the 2013-2014 growing season was consistently above-average.

### **Soil drainage properties and pre-planting soil conditions**

Every Coastal Plain soil studied was susceptible to either N leaching or denitrification losses due to drainage properties (Gaines and Gaines, 1994; VADCR, 2005; Hofstra and Bouwman, 2005). Altavista and Roanoke, both of which have aquic properties at different levels of classification, are susceptible to denitrification losses (Aulakh et al., 1992; Hofstra and Bouwman, 2005). We encountered redoximorphic features in the top 30 cm of Roanoke, which indicates reduction and loss of iron (Fe) and manganese (Mn) from the soil. Soil microbes use  $\text{NO}_3\text{-N}$  as a terminal electron acceptor before Fe or Mn (Gambrell et al., 1991; Peters and Conrad, 1996). Therefore, the presence of redoximorphic features is strong evidence that significant reduction and loss of  $\text{NO}_3\text{-N}$  also occurs in Roanoke (Gambrell et al., 1991; Peters and Conrad, 1996). We did not encounter redoximorphic features in Altavista. The Coastal Plain soils we studied without aquic properties are classified as having moderate or high risk for N leaching losses (VADCR, 2005). Bojac has a high N leaching loss risk due to coarse-loamy overall texture, and Emporia, Kempsville, and State have moderate leaching loss risk due to fine-

loamy overall texture (VADCR, 2005). Soils studied in the Ridge & Valley were fine textured, sloped 7 – 15% and rated low in leaching loss risk.

Soil conditions prior to planting and amendment application, including pH, total N, inorganic N, P, K, Ca, and Mg are presented in Tables 2.7 and 2.8. Soils in the Ridge & Valley physiographic province had greater total and inorganic N than soils in the Coastal Plain, likely as a consequence of their finer soil textures and land application history of soy milk sludge (Frederick-Christian soil) and animal manure (Frederick & Vertrees soil). The Coastal Plain Kempsville soil site had a history of biosolids applications and contained greater N than other Coastal Plain site soils but less N than the Ridge & Valley soils. Although pre-plant soil N was similar among Coastal Plain soils, the coarsest textured soil always had the least soil N.

### **Biomass at GS 25-30**

The responses of biomass to fall-applied urea- and biosolids-N are presented in Figures 2.3a-j and are shown with pre-plant soil inorganic N in Table 2.9. Biomass increased with N rate regardless of N source. Biomass response as determined by linear slope coefficient was typically greatest in the soils with the least pre-plant soil inorganic N. Soil inorganic N significantly contributes to biomass production and increases in soil inorganic N decrease plant response to fertilizer (Sowers et., al 1994; Cui et al., 2006). This pattern was observed in the 2012-2013 growing season but was confounded in the 2013-2014 growing season by environmental factors. Below-average winter temperatures in the winter of 2013-2014 reduced PAN formation and consistently above-average precipitation promoted N loss. Wheat N uptake response to urea- and biosolids-N followed the same pattern as biomass, owing to the strong correlation observed between N uptake and biomass production (Austin et al., 1977).

Biomass increased as much or more with fall biosolids, regardless of type, as with urea-N (Figures 2.3a-j). Biomass response as determined by linear slope coefficient typically followed the order AD biosolids > LS biosolids > urea. Nitrogen available to the wheat from the fall-applied biosolids by this growth stage was likely greater from AD than LS because a higher portion of total N is initially in the plant-available form in AD than in LS biosolids (Tables 2.3 and 2.4).

### **Soil Inorganic N at GS 25-30**

There was no relationship between N source, N rate, and soil inorganic N at GS 25-30 (Table 2.10). Soil inorganic N was greater in spring 2013 than in spring 2014, likely owing to environmental factors. The winter of 2012-2013 had above-average temperatures (Figures 2.1a-c) and below average precipitation, which promoted greater inorganic N formation and less N loss (Rustad et al., 2001; Wang et al., 2003; Di and Cameron, 2002). The winter of 2013-2014 had below-average temperatures and above-average precipitation, which promoted less inorganic N formation and greater N loss (Cassman and Munns, 1980; Di and Cameron, 2002). One of the four soils on which studies were established in 2012-2013 is susceptible to N leaching losses, whereas four of the six soils on which studies were established in 2013-2014 are susceptible to N leaching losses (VADCR, 2005).

### **Nitrogen Use Efficiency at GS 58**

Nitrogen uptake at GS 58 increased linearly with urea-N in 2013-2014. Nitrogen assimilated by winter wheat is mobilized from tissue to grain beginning at anthesis, and the amount of tissue N at anthesis should be correlated to grain yield. Nitrogen use efficiency (NUE) calculated for the 2013-2014 season resulting from biosolids and urea applied at the agronomic N rate were not different in the studies on Altavista, Kempsville, Roanoke and State soils, which had surface soil textures of sandy loam or finer (Table 2.11). The LS biosolids resulted in greater

NUE than AD biosolids and urea in the studies on Bojac and Emporia soils, which had surface soil textures of loamy sand. The NUE calculations for biosolids would have been unreliable if estimated biosolids PAN was inaccurate.

### **Grain Yield**

The slopes of grain yield response to urea-N were similar for all soils except Frederick-Christian in 2012-2013 and all soils except for Bojac in 2013-2014 (Figures 2.4a-j). We expected the slope of yield response to urea-N to be inversely related to soil pre-plant N just as biomass at GS 25-30 had been in 2012-2013. The intercept of the urea N yield regression generally increased with soil pre-plant inorganic N in each year, esp. for the 2012-13 year when fall and winter rainfall was less than 2013-14 (Table 2.12), demonstrating that fall pre-plant N could be a reliable indicator of residual PAN. Finer textured soils and/or those with a history of organic amendment had higher intercepts than coarser textured soils and/or those that had not received organic amendments. Excess N uptake resulted in lodging in Frederick-Christian in 2012-2013, explaining the lack of grain yield response to urea- and biosolids-N. Lodging increased with urea-N rate, and only plots without added N had no lodging (Figure 2.5).

The comparison of residuals calculated by subtracting predicted grain yield response to urea-N rate from observed grain yield responses to urea- and biosolids-N is presented in Table 2.13. Biosolids resulted in grain yields 0 – 1.2 Mg ha<sup>-1</sup> greater than those predicted by the linear response grain yield to urea-N rate (Table 2.13, Figures 2.4a-j). These results agree with those of Koenig et al. (2011), who observed greater yield response to biosolids than inorganic N fertilizer by 0 – 1.4 Mg ha<sup>-1</sup>. There are two possible explanations for biosolids consistently resulting in greater yield than urea applied at the agronomic N rate. The factors used to estimate biosolids PAN (Table 2.3) may have been inaccurate, and biosolids PAN may have been underestimated

(Eldridge et al., 2008). Biosolids resulted in more biomass at GS 25-30 than was predicted by the regression of biomass response to urea-N. Increasing fall/winter tiller development (that is, biomass at GS 25-30) results in greater spring growth and increased kernels per head (Alley et al., 1993), which in turn increases yield. This explanation can be drawn only by association because we could not find a strong quantitative relationship between biomass at GS 25-30 and grain yield.

There were no effects of biosolids treatment type or application timing on grain yield in fine and fine-loamy textured soils in 2012-2013 (Table 2.13). Fall agronomic N rate LS biosolids resulted in higher grain yields than fall agronomic N rate AD biosolids in fine-loamy soils with moderate N leaching loss risk studied in 2013-2014 (Table 2.13). Above-average precipitation in March and April (Figure 2.2a) may have caused N loss in these soils. Lime stabilized biosolids supply PAN more slowly than AD biosolids due to their higher proportion of organic N and expected subsequent slower formation of  $\text{NH}_4^+$  via mineralization and  $\text{NO}_3^-$  via nitrification. Such biosolids type N dynamics may have resulted in LS biosolids providing PAN following the period of greatest  $\text{NO}_3\text{-N}$  leaching loss and increasing NUE (Alley et al., 1993). It is also possible that the factors used to estimate biosolids PAN (Table 2.3) were inaccurate, underestimating LS and/or overestimating AD organic N mineralization (Eldridge et al., 2008).

The interaction between biosolids type, fall N rate and weather explain the response of grain yield to biosolids treatments in coarse textured Coastal Plain soils studied in 2012-2014. Biosolids type did not influence grain yield in 2012-2013, but fall biosolids/spring urea split N rate resulted in higher grain yield than fall full N rate biosolids (Table 2.12). Perhaps above-average winter temperatures (Figure 2.1a-c) caused the biosolids to release more PAN than the wheat could assimilate, and the excess PAN was lost to leaching. Therefore, the split urea-N

application with the 50 kg PAN ha<sup>-1</sup> biosolids fall treatment improved NUE. Fall biosolids N rate and timing did not affect grain yield in coarse textured soils in 2013-2014, when below-average winter temperatures (Figure 2.1a) may have reduced biosolids organic N mineralization and prevented the release of more PAN than wheat could assimilate. The LS biosolids resulted in higher grain yield than AD biosolids in coarse textured soils in 2013-2014 (Table 2.12), likely for one or more of the same reasons described above in the discussion of fine-loamy soils.

### **End-of-season soil inorganic N and estimated N recovery**

End-of-season inorganic N decreased with depth in all soils except State in 2012-2014. State was only sampled at one depth interval due to restrictive layers of coarse fragments, so the changes in end-of-season inorganic N with depth could not be assessed. In all other soils, NH<sub>4</sub>-N was evenly distributed with depth and treatment effects on NH<sub>4</sub>-N were rare, especially in 2013-2014. NO<sub>3</sub>-N in all soils was highest in the surface horizon (top 20 cm in 2012-2013 and top 30 cm in 2013-2014) and then decreased sharply with depth. The fraction of NO<sub>3</sub>-N remaining in the surface horizon of the total NO<sub>3</sub>-N measured in the soil profile ranged from 0.55 to 0.90. Our observations were consistent with those of Chaney (1990) and Powlson et al. (1992), who observed 50% and 86% of total post-harvest NO<sub>3</sub>-N in surface horizons, respectively. Treatment effects on end-of-season soil NO<sub>3</sub>-N were significant only for the surface horizon. The LS biosolids, regardless of application timing resulted in more end-of-season soil inorganic N than nearly all of the urea and AD treatments (Table 2.14). This was likely a result of LS biosolids supplying inorganic N to soils at a lower rate than AD biosolids and urea after land application. Comparisons of end-of-season soil inorganic N distribution between biosolids and agronomic N rate urea are presented in Figures 2.6a-e and 2.7 and are grouped by VADCR leaching loss risk, physiographic province and year.



End-of-season soil inorganic N did not increase with urea-N rate and in any soil but Frederick-Christian 2012-2014. Erratic precipitation in spring/early summer 2013 and above average precipitation in spring 2014 (Figures 2.2a-c) likely confounded the response of end-of-season soil inorganic N to urea-N rate.

Fall biosolids applications never resulted in less estimated N recovery than agronomic N rate urea in 2013-2014, regardless of biosolids type or application timing (Figures 2.8a-f). Since N uptake and NUE was largely the same among agronomic N rate urea and all biosolids treatments, soil retention of inorganic N was the main factor that determined N recovery. The LS biosolids typically resulted in the most end-of-season inorganic N and, therefore, gave the highest N recovery.

## **2.4: Conclusions**

The timing of N application and availability is less important than N rate in increasing winter wheat grain yield and minimizing estimated N loss in fine and fine-loamy textured soils where N leaching loss risk is low. Therefore, AD and LS biosolids can be fall applied at full agronomic N rates to soils with low N leaching loss risk in the Virginia Coastal Plain and Ridge & Valley physiographic provinces. Nitrogen rate, timing of N application, and timing of N availability are critical in increasing grain yield and minimizing estimated N loss in coarser textured soils where risk of leaching loss is moderate or high. The likely relatively slow rates of LS biosolids PAN formation increased yield and slowed the rate of N loss in these soils. Virginia Coastal Plain soils with moderate or high leaching loss risk should only receive fall full agronomic N rate applications of LS biosolids. Splitting the agronomic N rate between fall biosolids and spring urea benefits grain yield in coarse-textured Coastal Plain soils. Fall

applications of full agronomic N rate LS biosolids are most beneficial to soft red winter wheat producers and production in Virginia for increasing N use efficiency, increasing grain yield, and reducing N leaching. Both AD and LS biosolids fall-applied at the full agronomic N rate are at least as efficient as split urea applications for increasing N use efficiency, increasing grain yield, and reducing N leaching.

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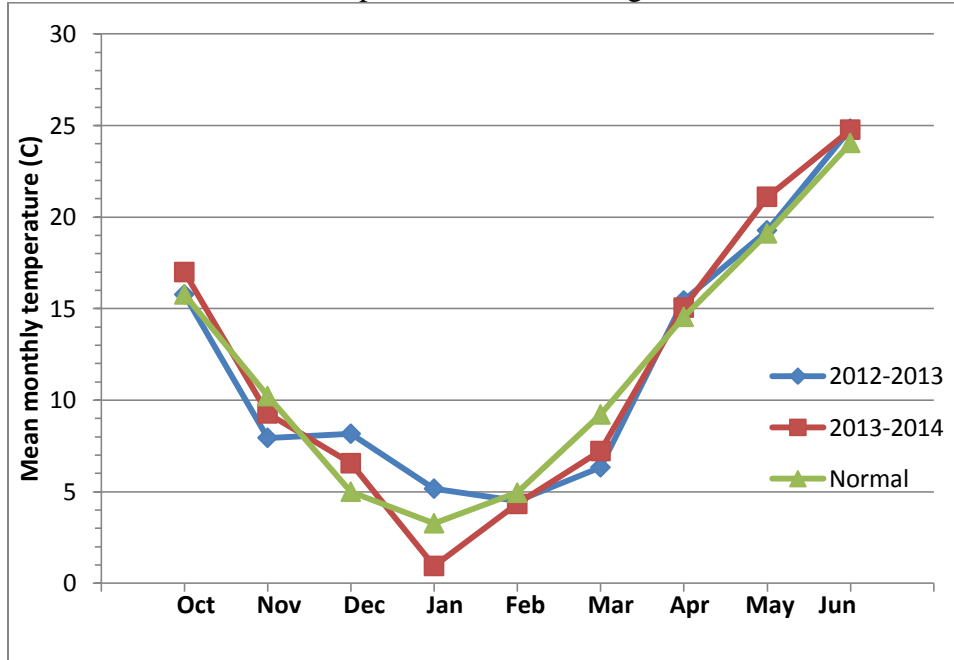


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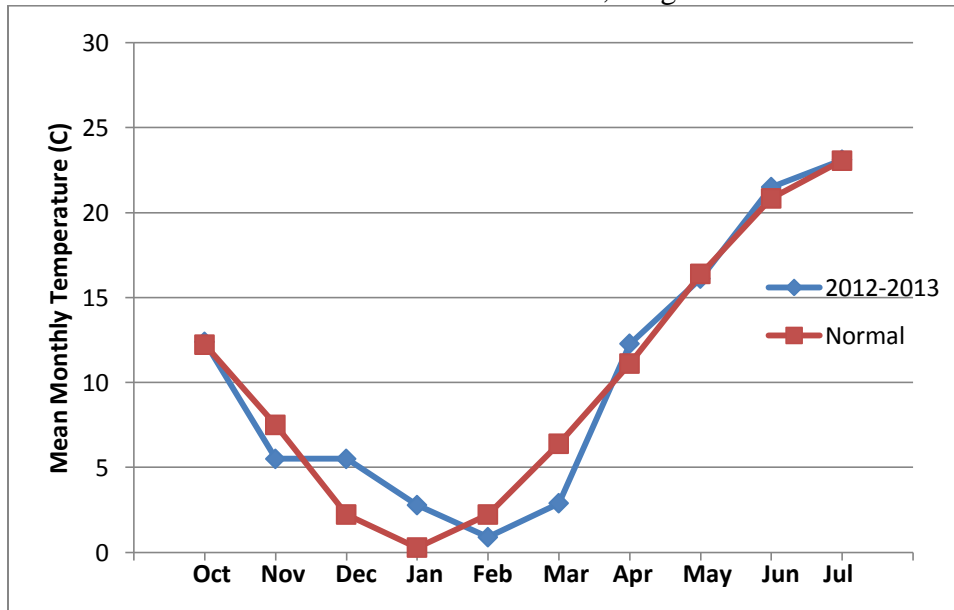


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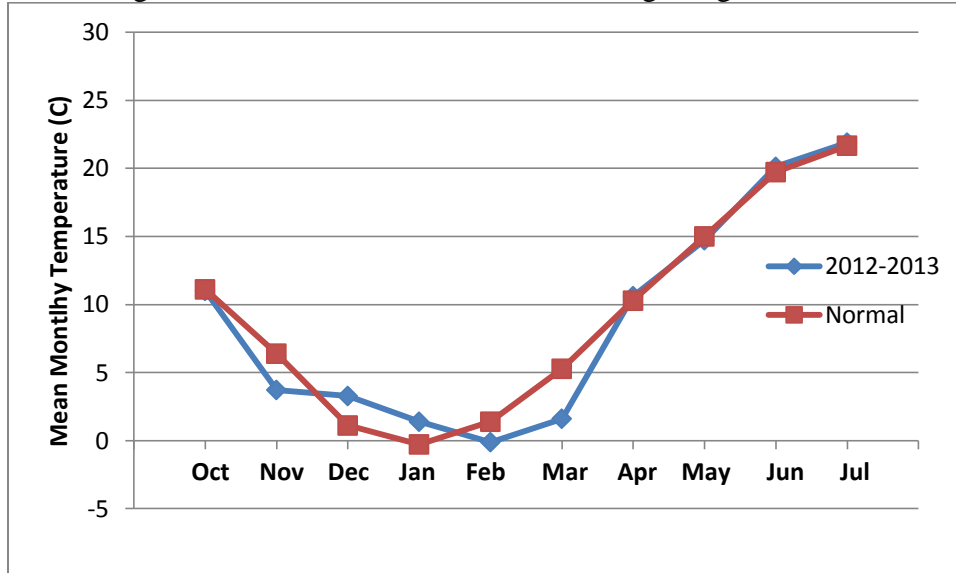


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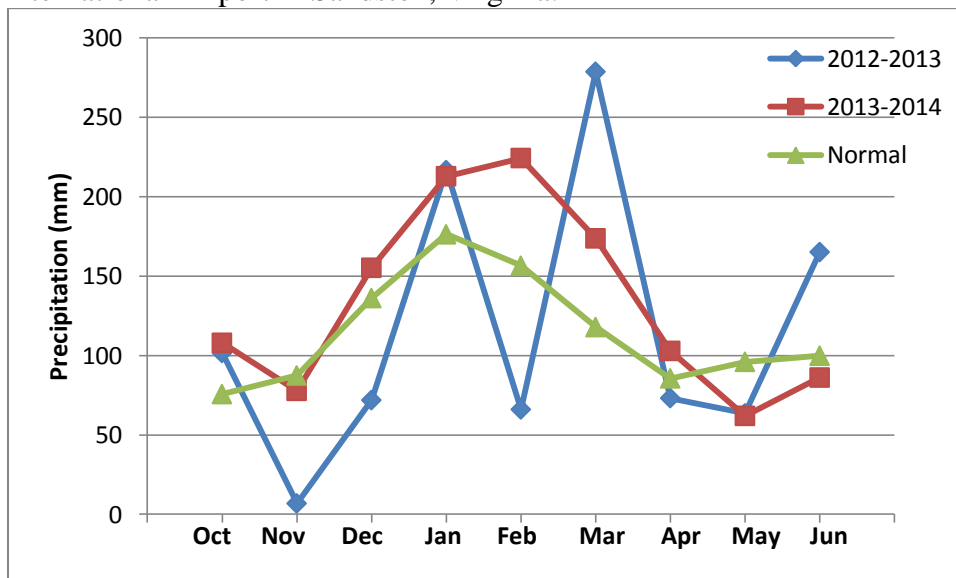




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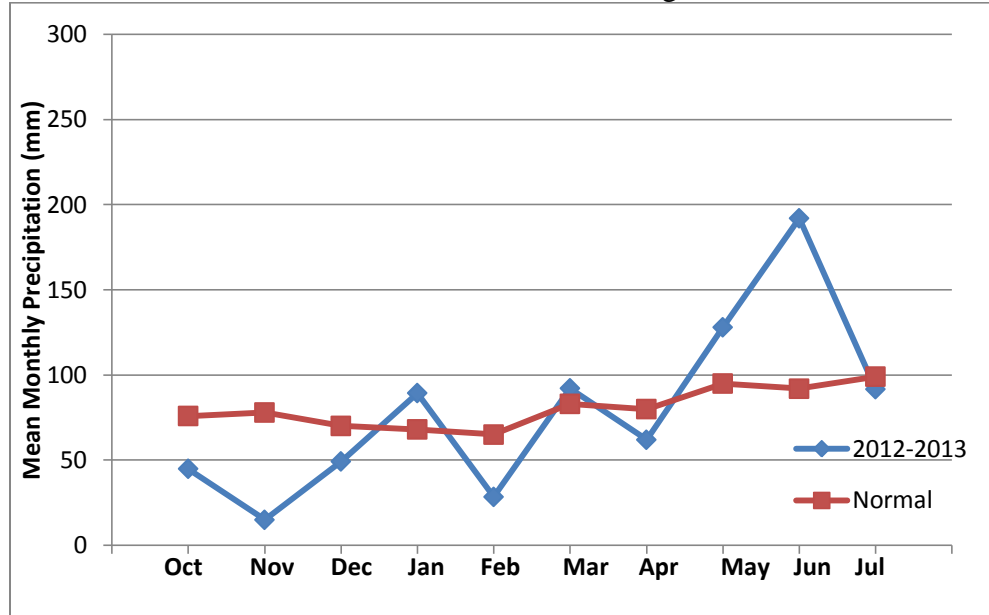


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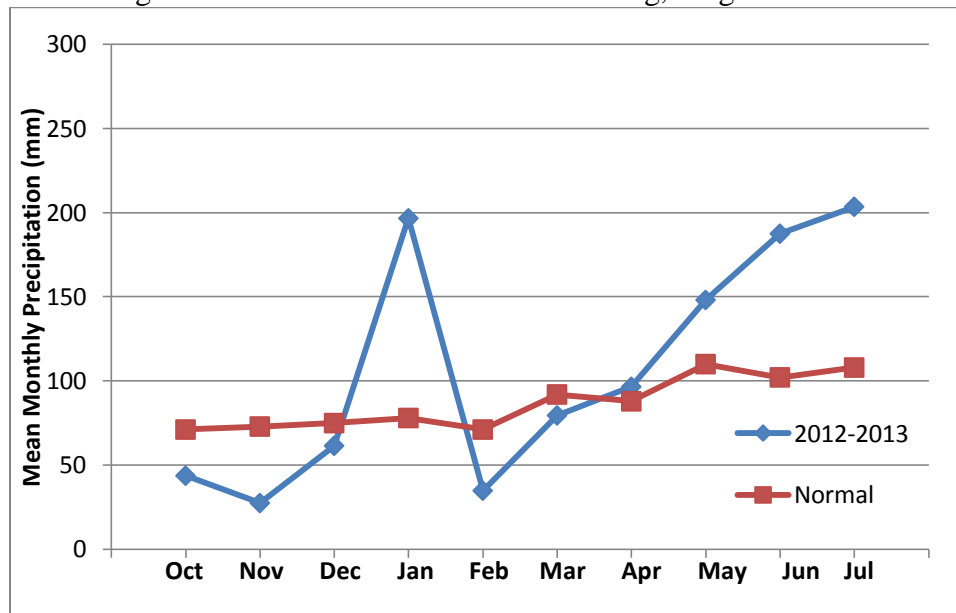


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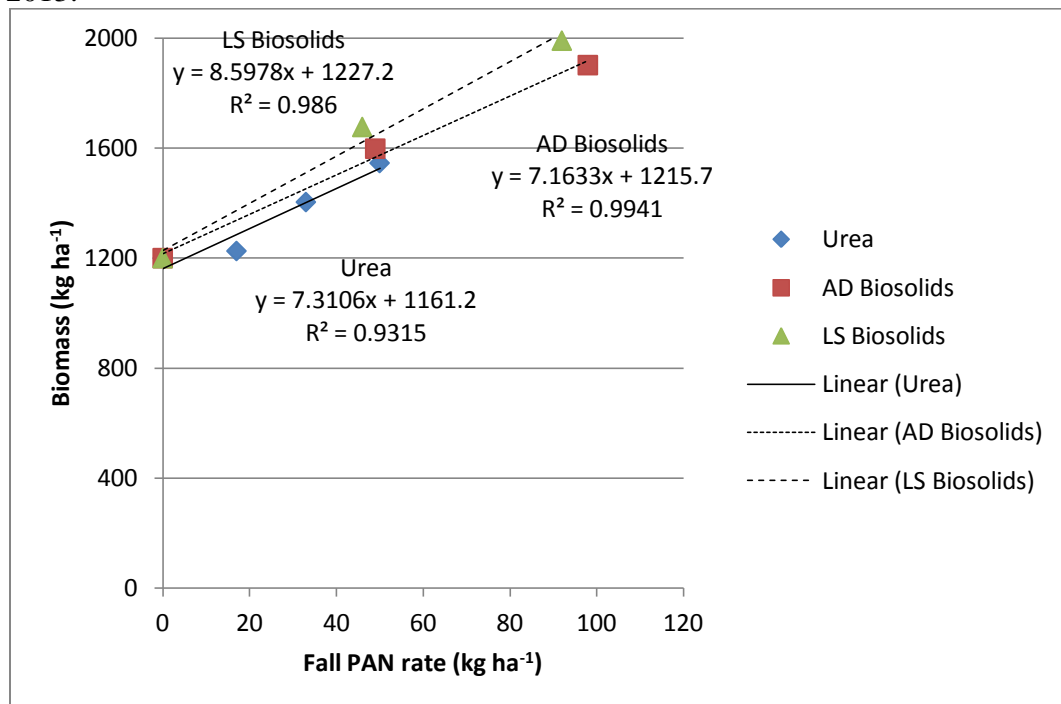


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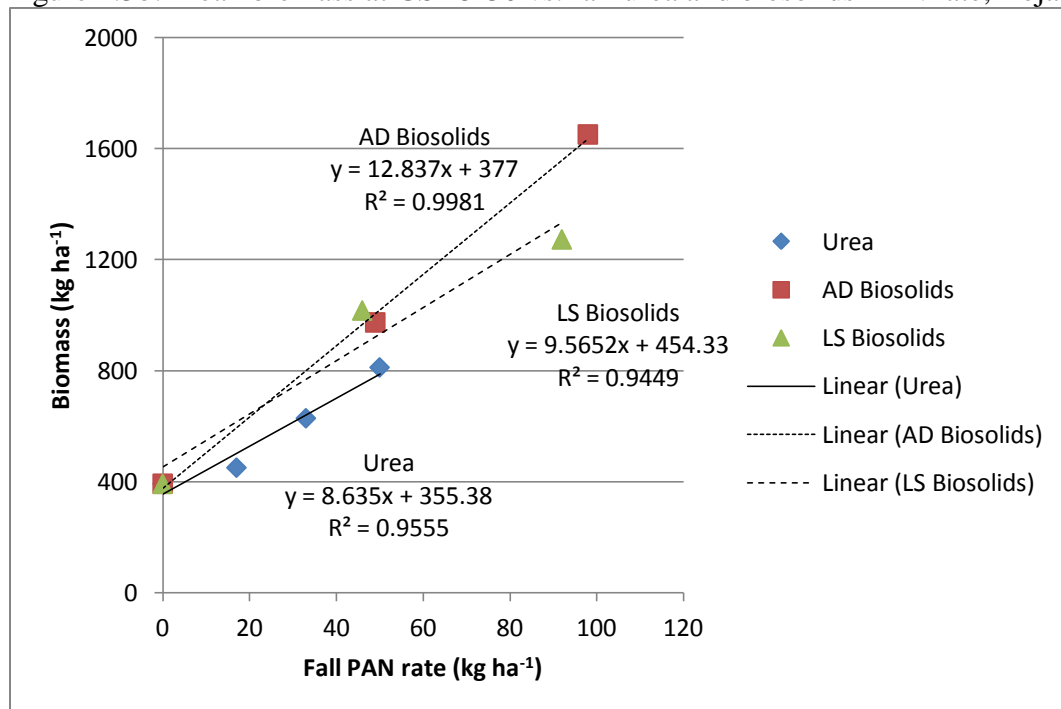


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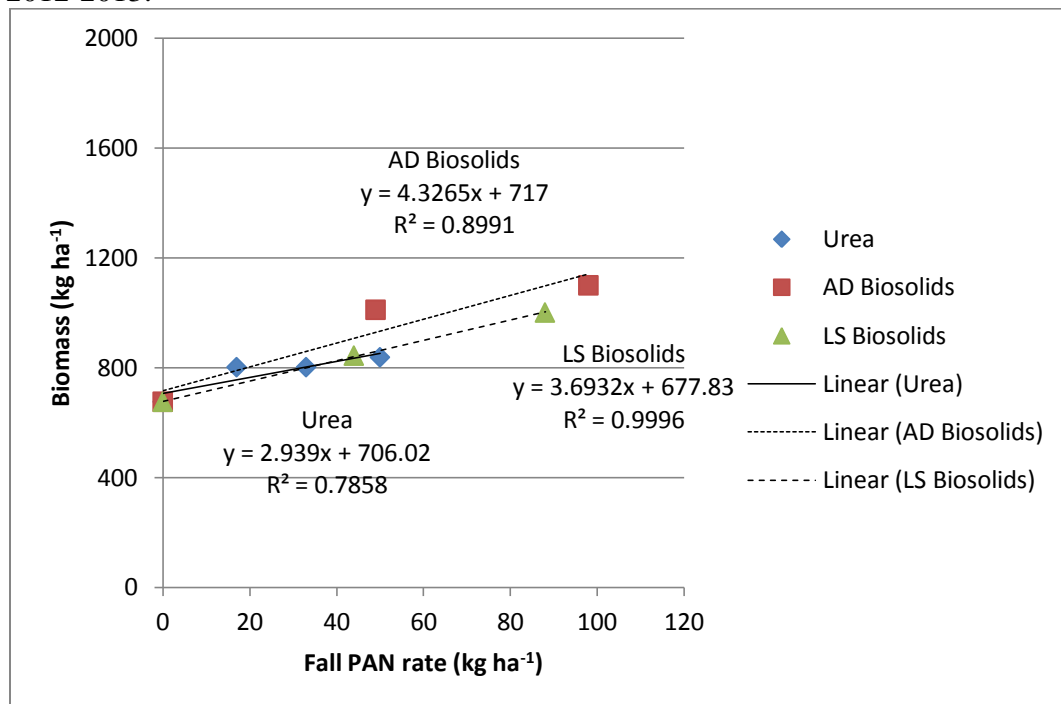


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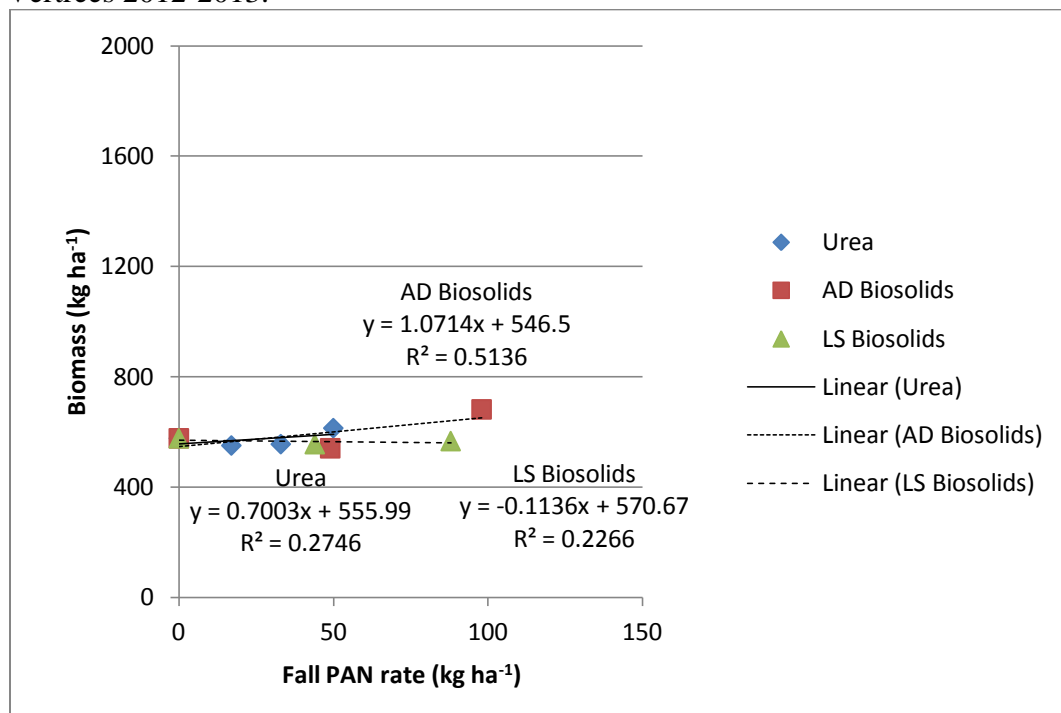


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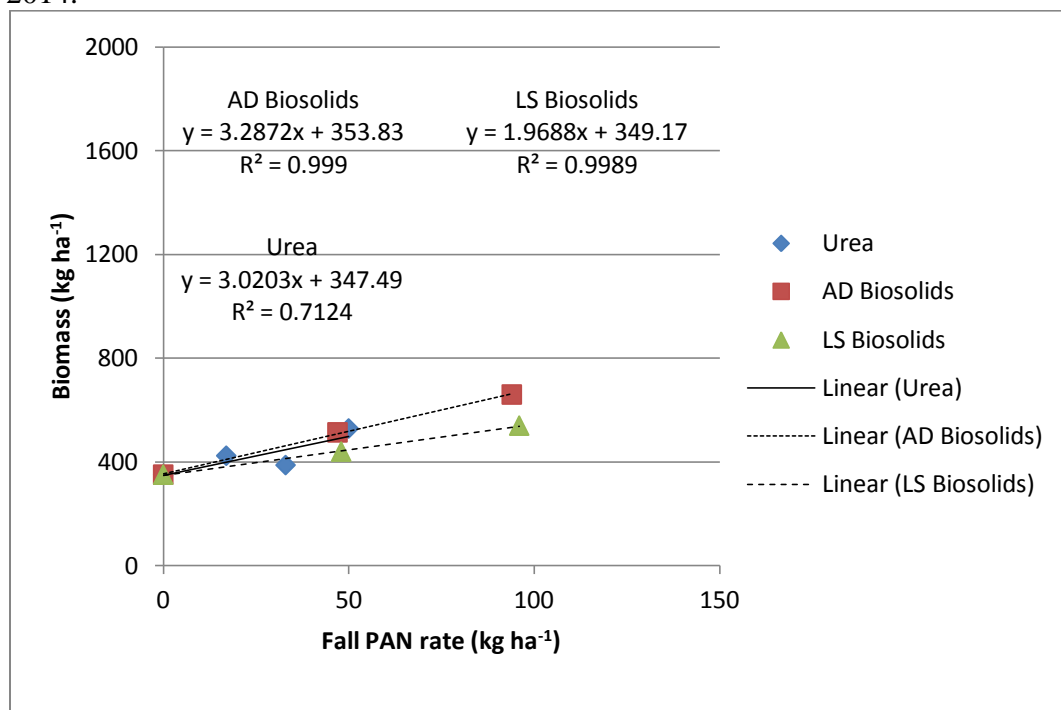


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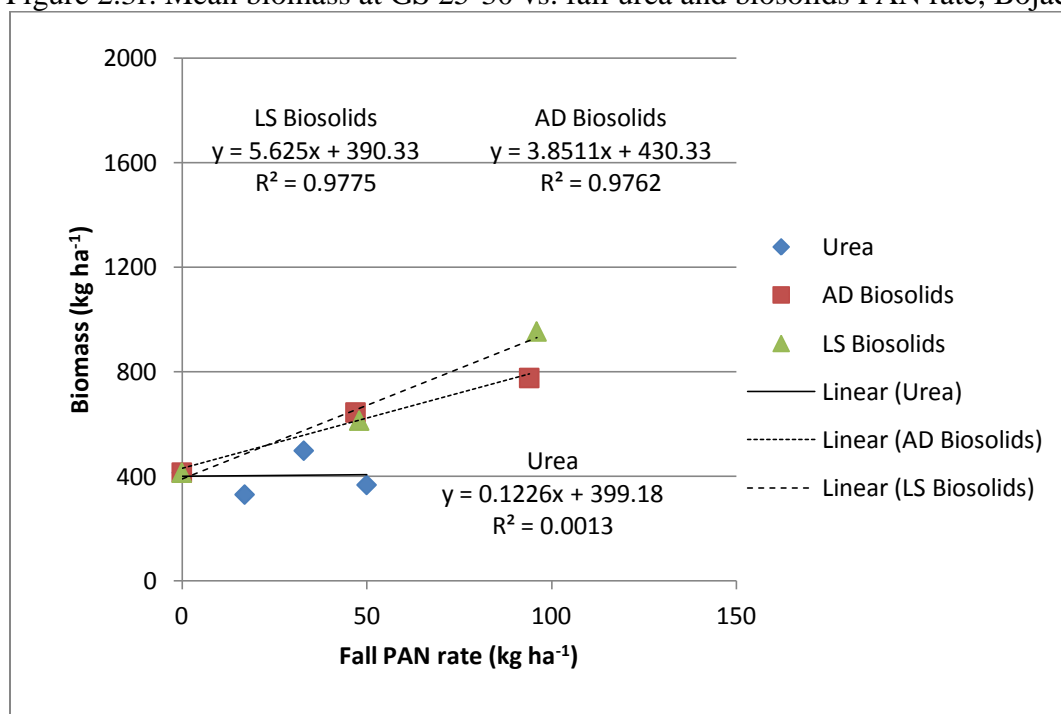


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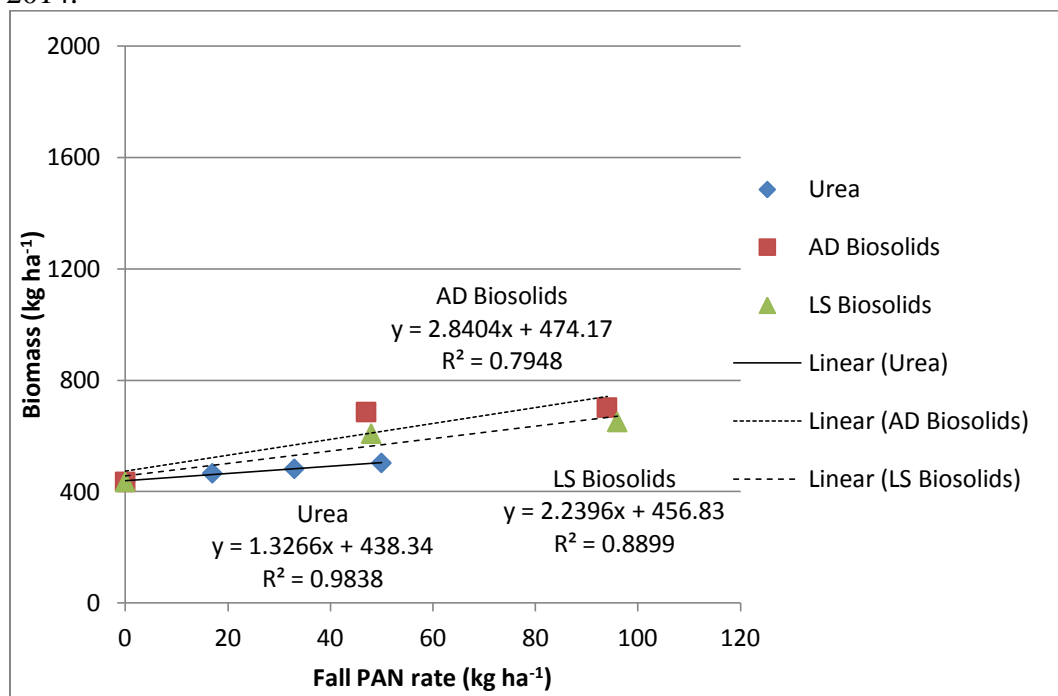


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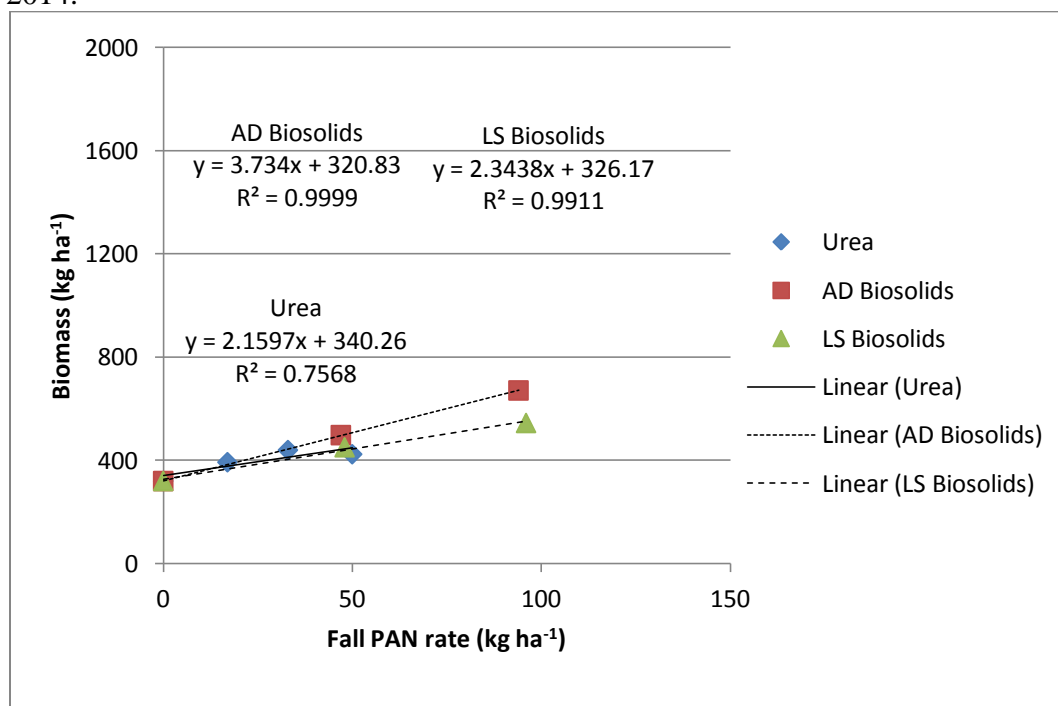


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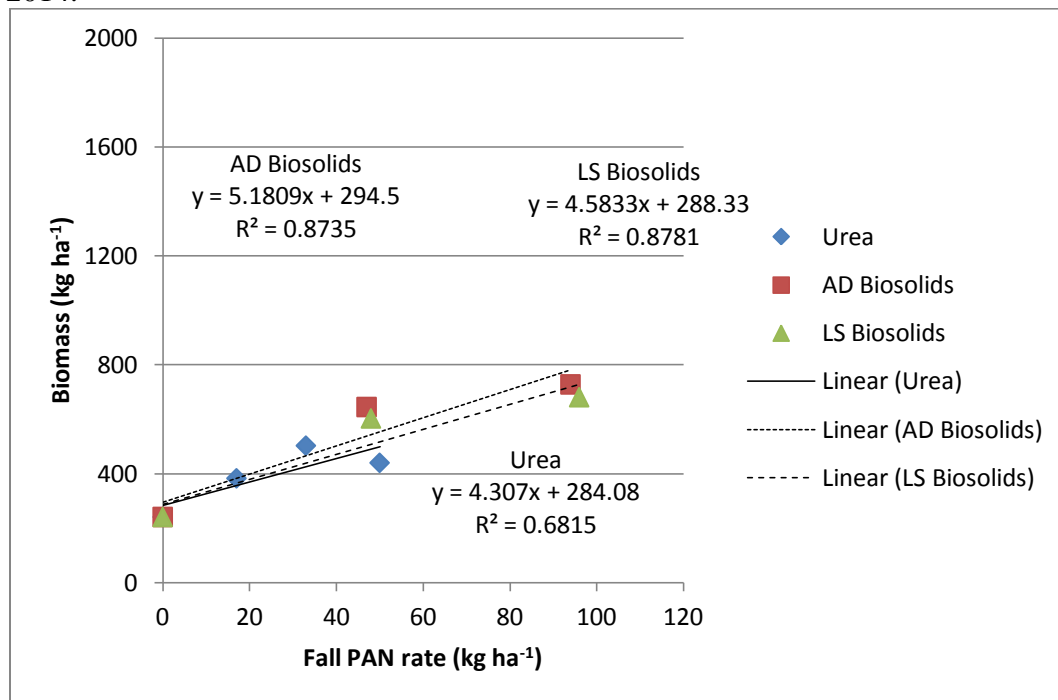


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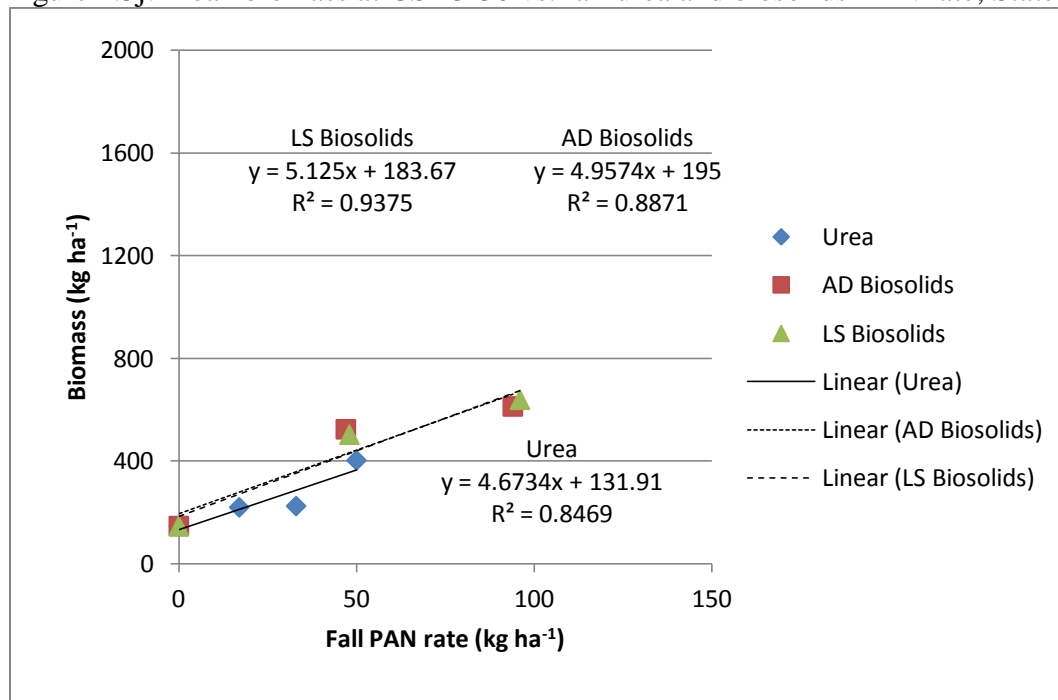


Figure 2.4a: Response of mean grain yield to urea-N, and biosolids treatments overlaid for comparison, Altavista 2012-2013.

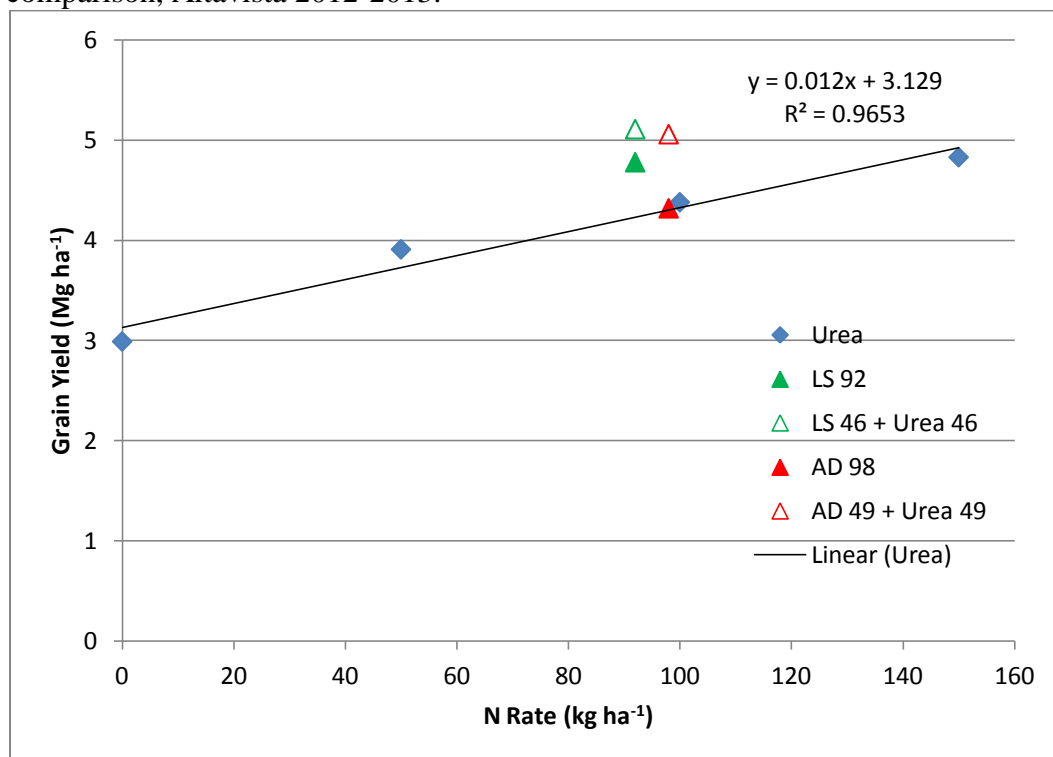


Figure 2.4b: Response of mean grain yield to urea-N, and biosolids treatments overlaid for comparison, Bojac 2012-2013.

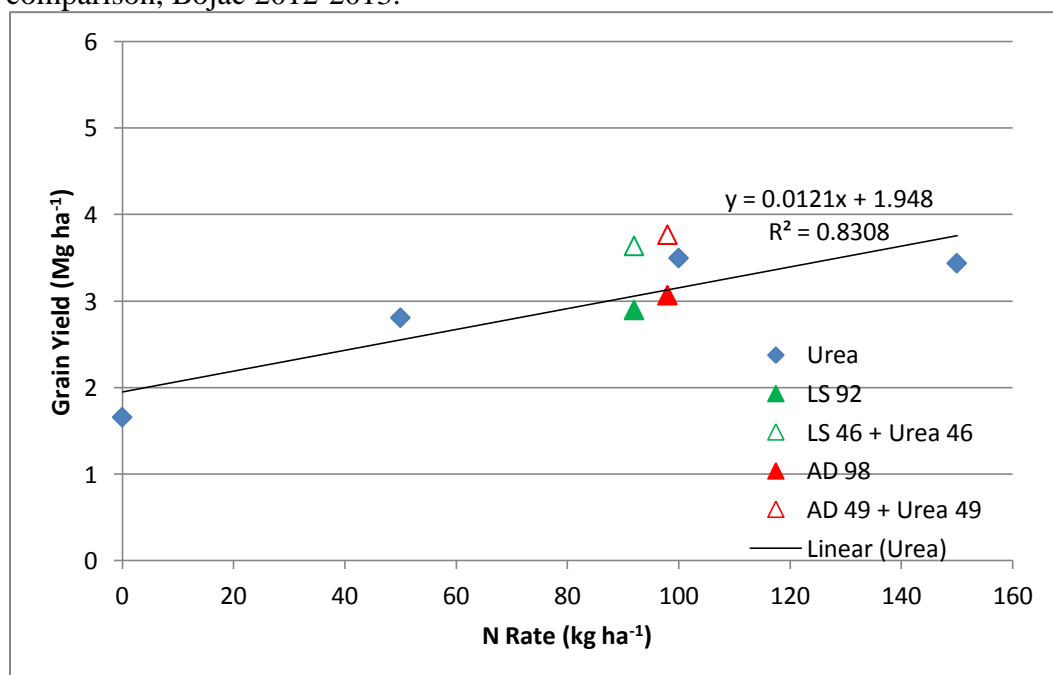


Figure 2.4c: Response of mean grain yield to urea-N, and biosolids treatments overlaid for comparison, Frederick-Christian 2012-2013.

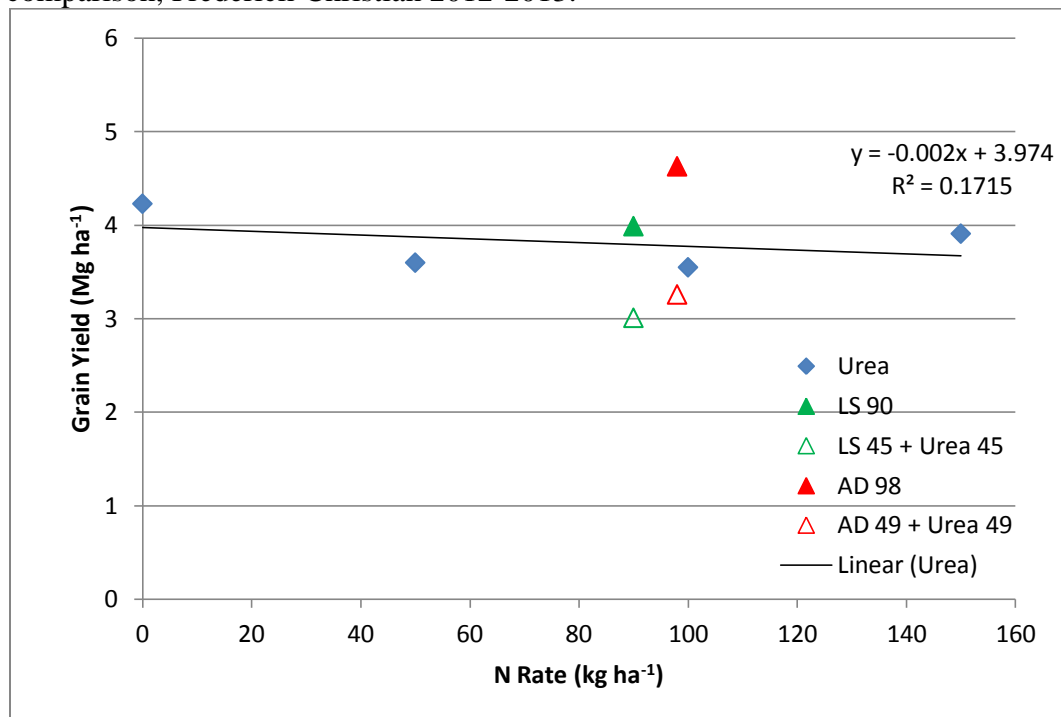


Figure 2.4d: Response of mean grain yield to urea-N, and biosolids treatments overlaid for comparison, Frederick & Vertrees 2012-2013.

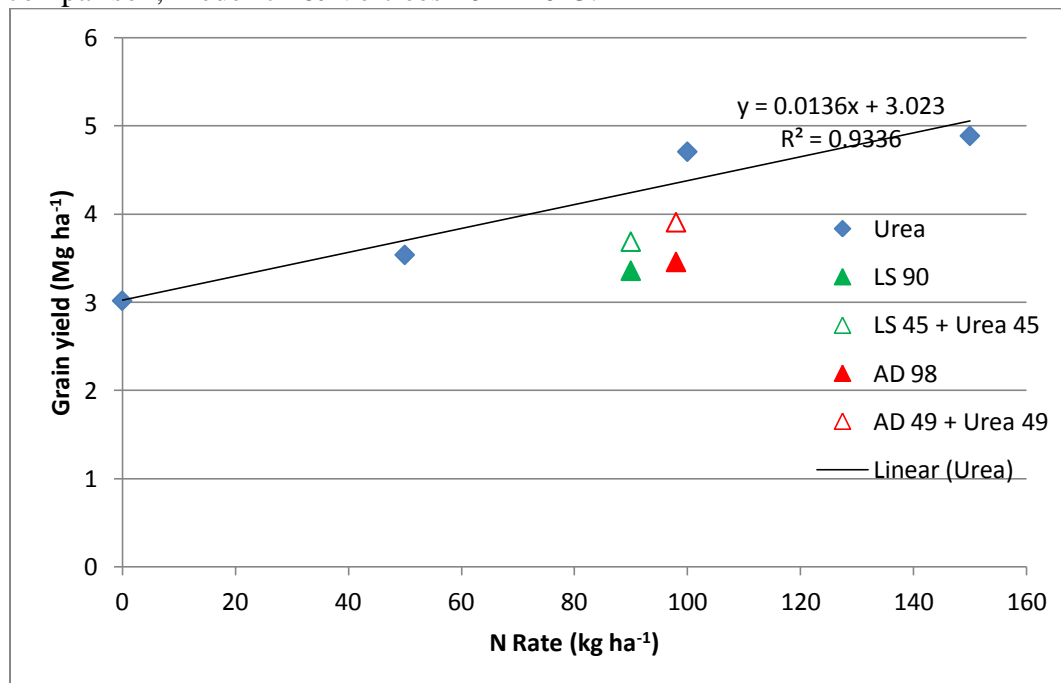




Figure 2.4e: Response of mean grain yield to urea-N, and biosolids treatments overlaid for comparison, Altavista 2013-2014.

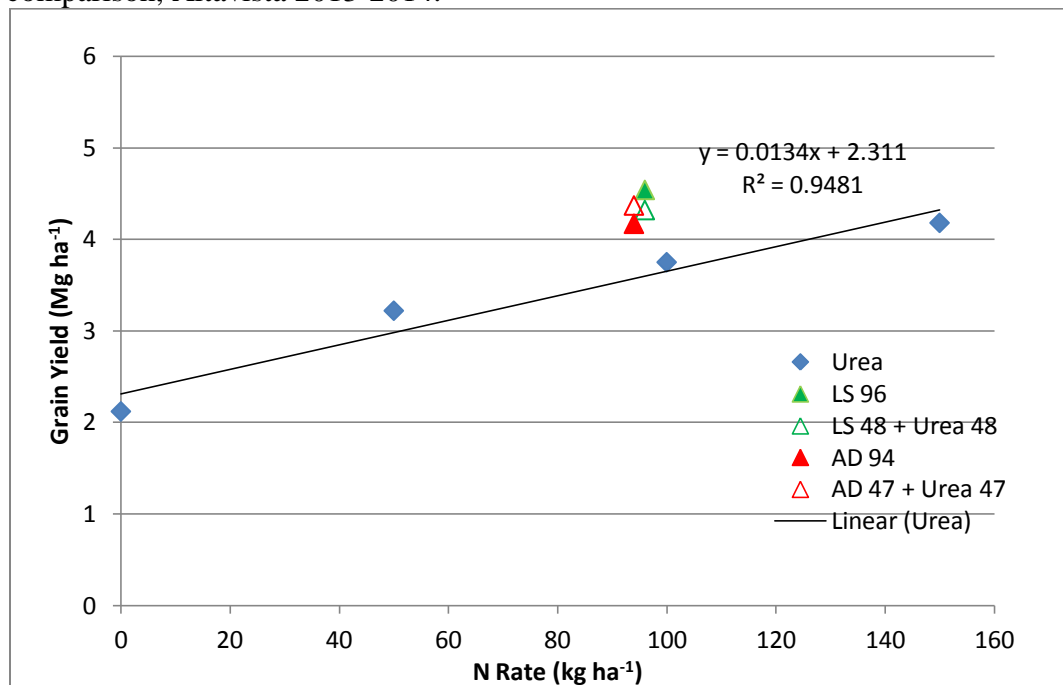


Figure 2.4f: Response of mean grain yield to urea-N, and biosolids treatments overlaid for comparison, Bojac 2013-2014.

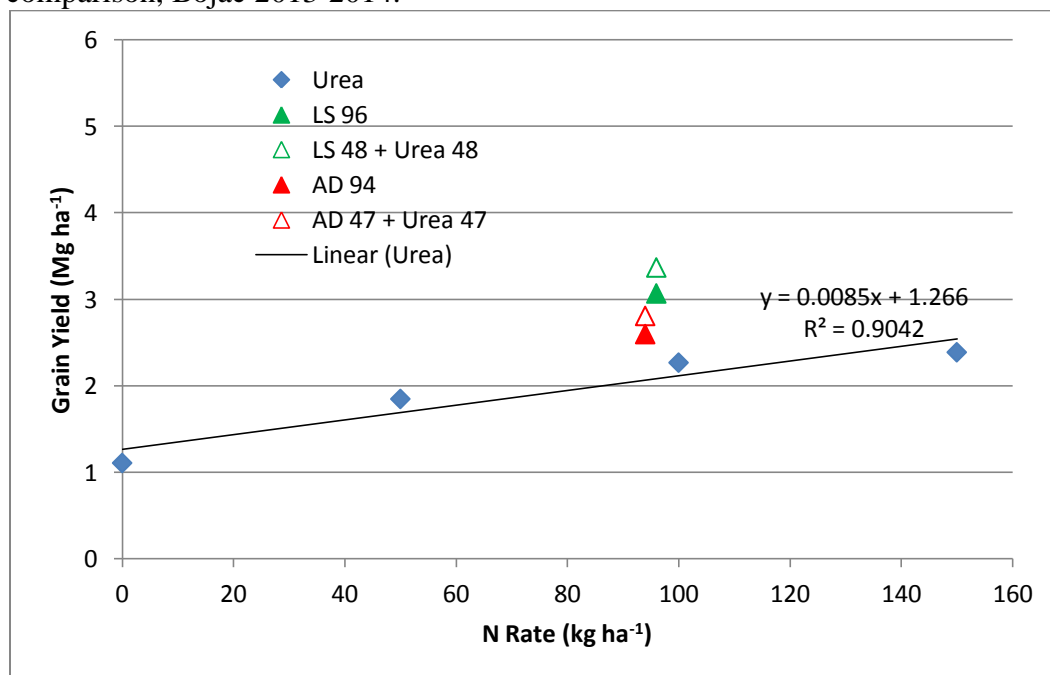


Figure 2.4g: Response of mean grain yield to urea-N, and biosolids treatments overlaid for comparison, Emporia 2013-2014.

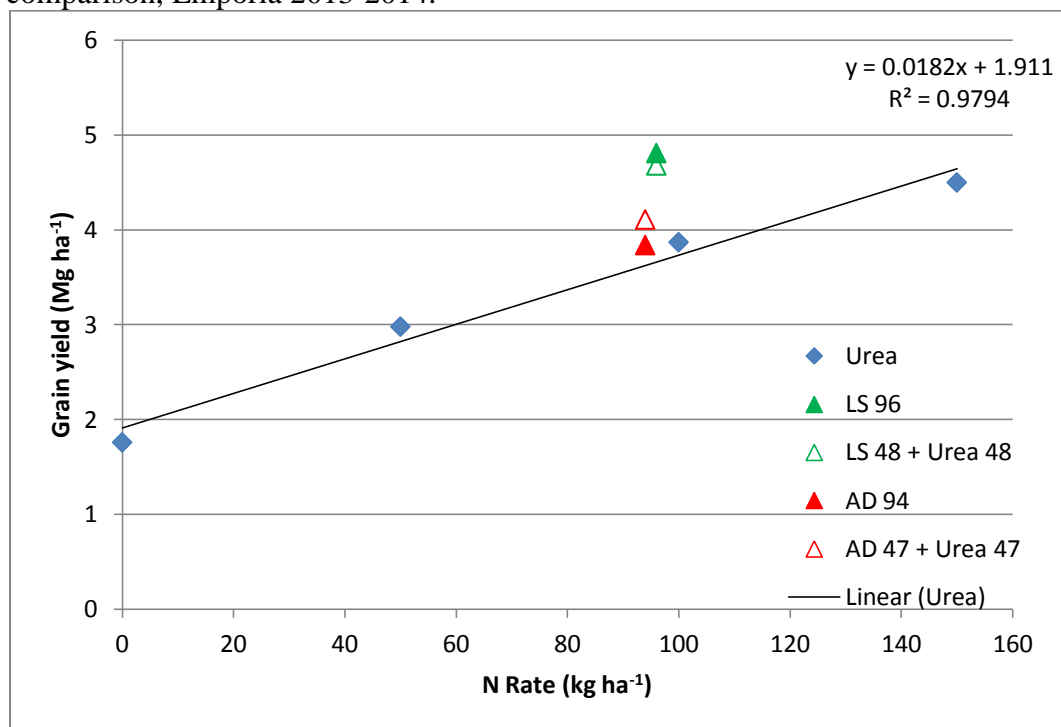


Figure 2.4h: Response of mean grain yield to urea-N, and biosolids treatments overlaid for comparison, Kempsville 2013-2014.

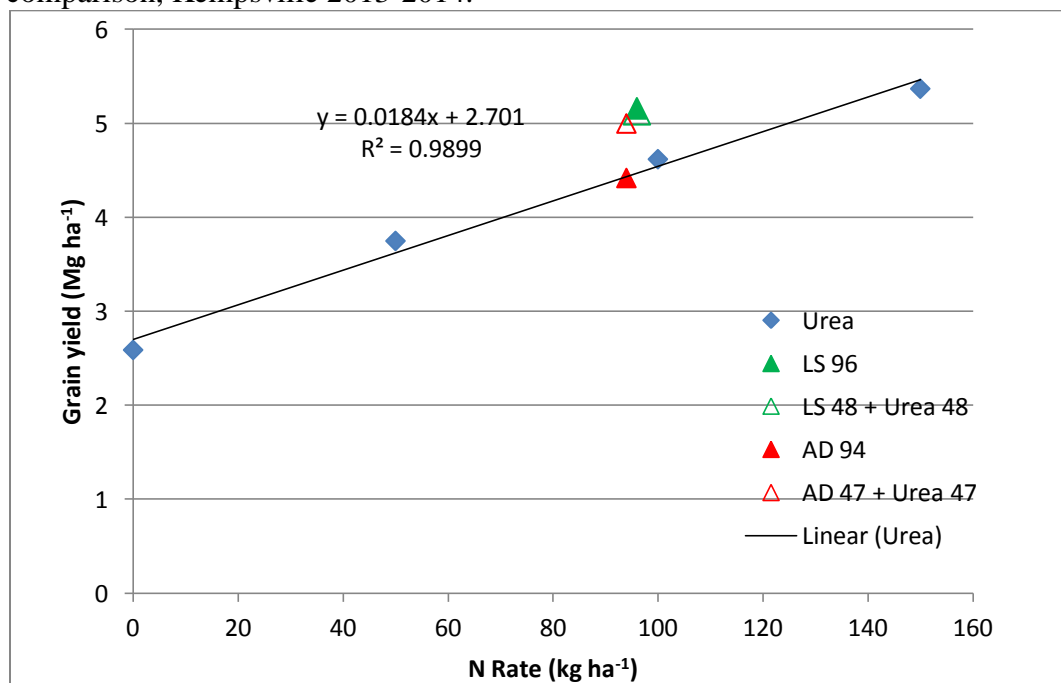


Figure 2.4i: Response of mean grain yield to urea-N, and biosolids treatments overlaid for comparison, Roanoke 2013-2014.

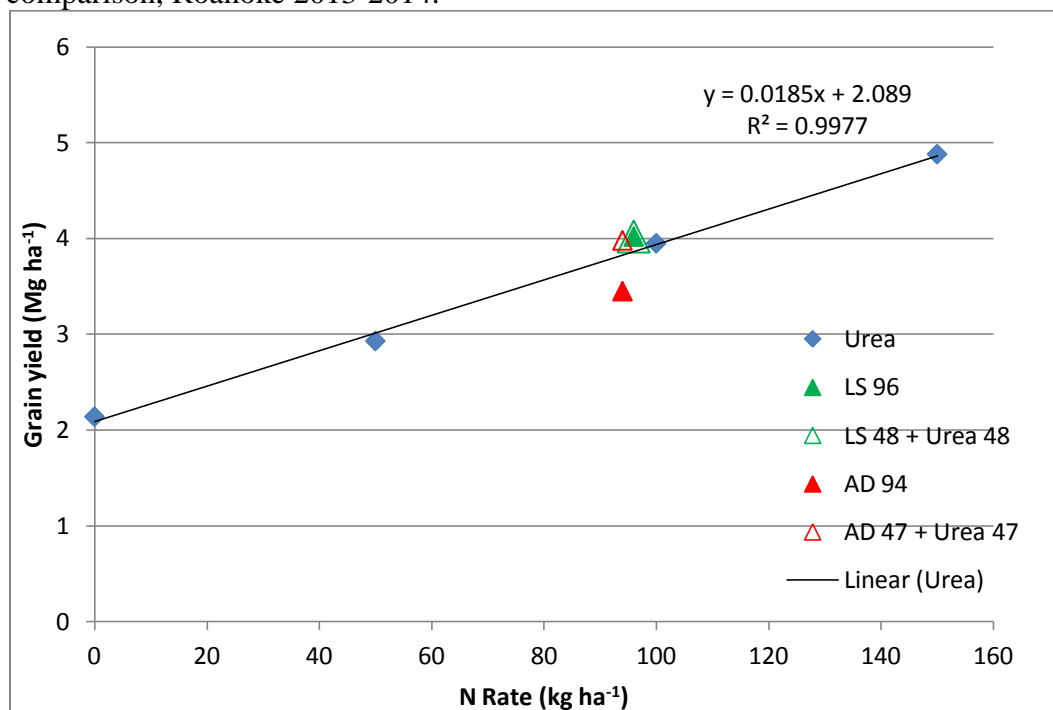


Figure 2.4j: Response of mean grain yield to urea-N, and biosolids treatments overlaid for comparison, State 2013-2014.

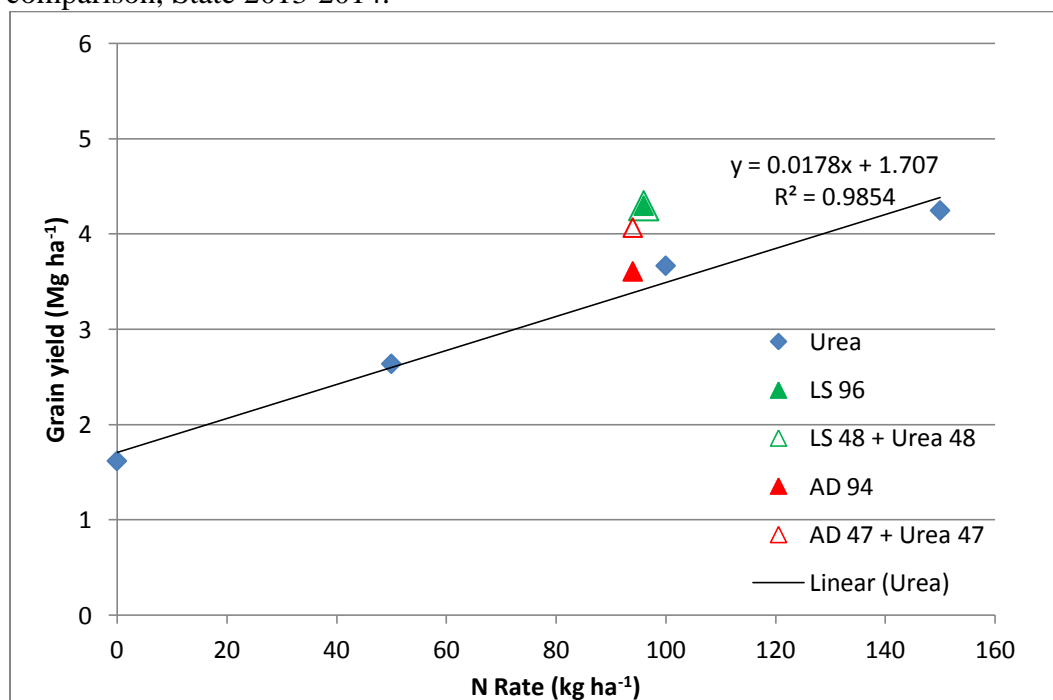


Figure 2.5: The increase of mean percentage of wheat lodging with urea-N rate, and biosolids treatments overlaid for comparison, Frederick-Christian 2012-2013.

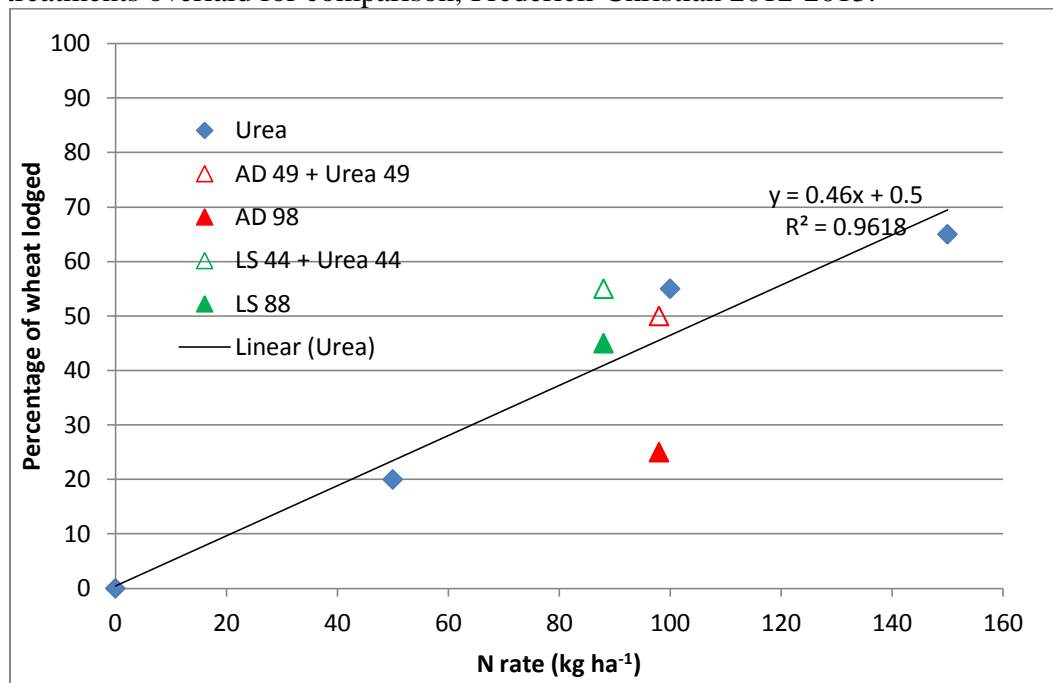
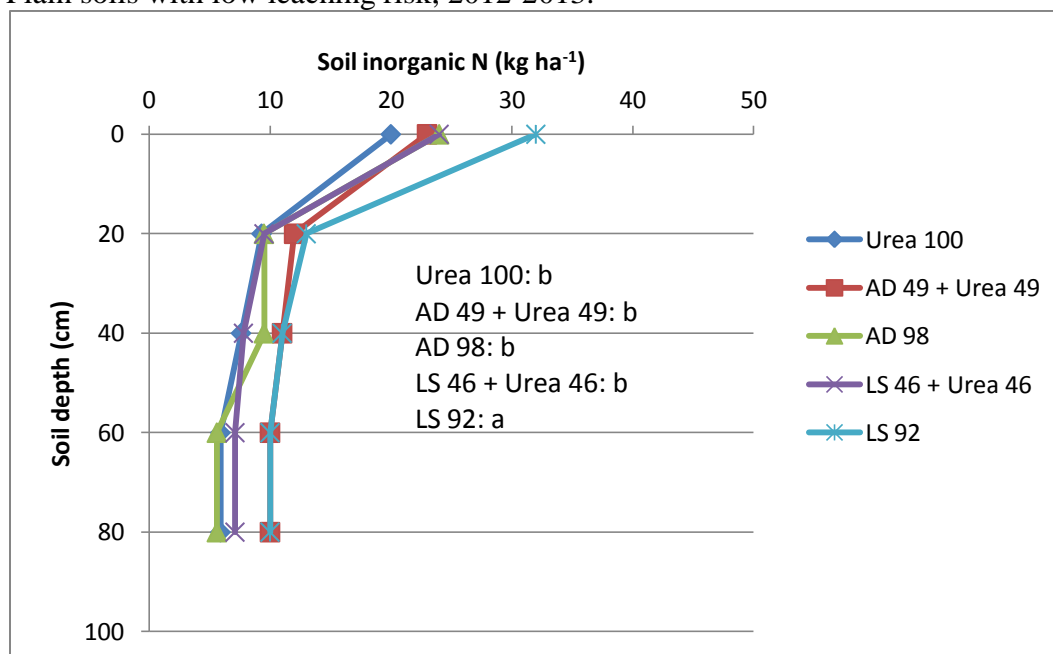
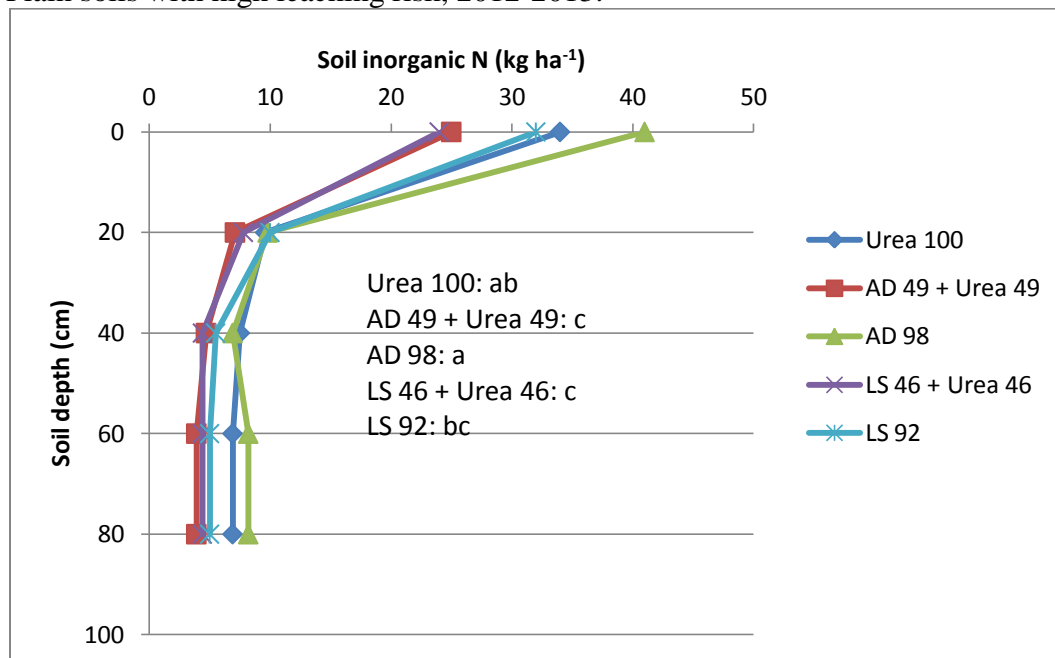


Figure 2.6a: End-of-season mean 2 M KCl extractable soil inorganic N distribution in Coastal Plain soils with low leaching risk, 2012-2013.



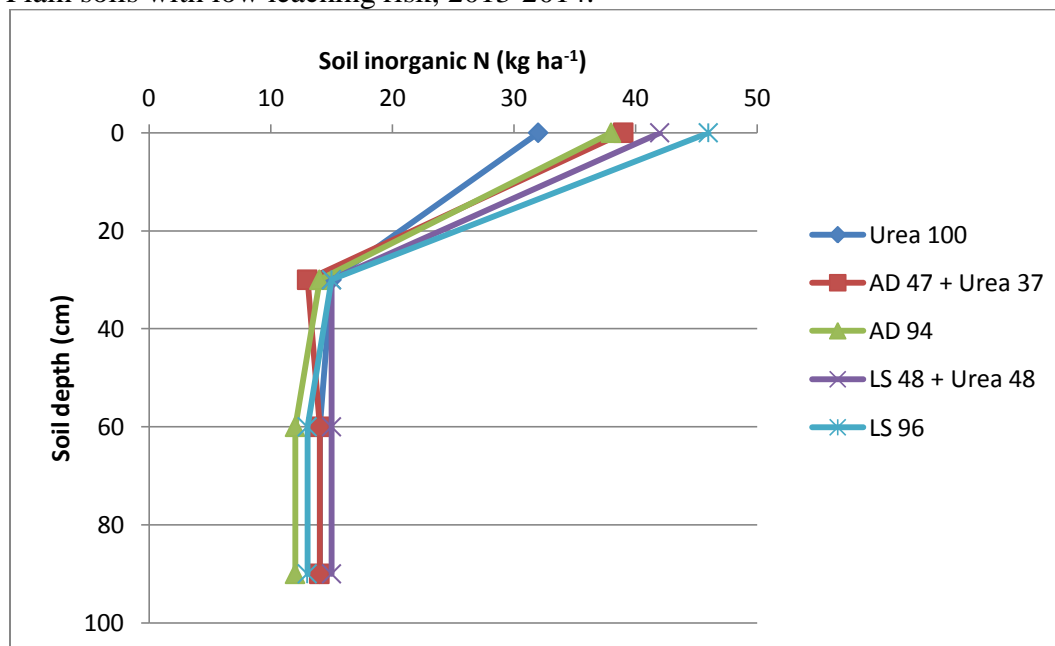
Treatments followed by the same letter resulted in equal soil inorganic N distribution at the 0.05 probability level.

Figure 2.6b: End-of-season mean 2 M KCl extractable soil inorganic N distribution in Coastal Plain soils with high leaching risk, 2012-2013.



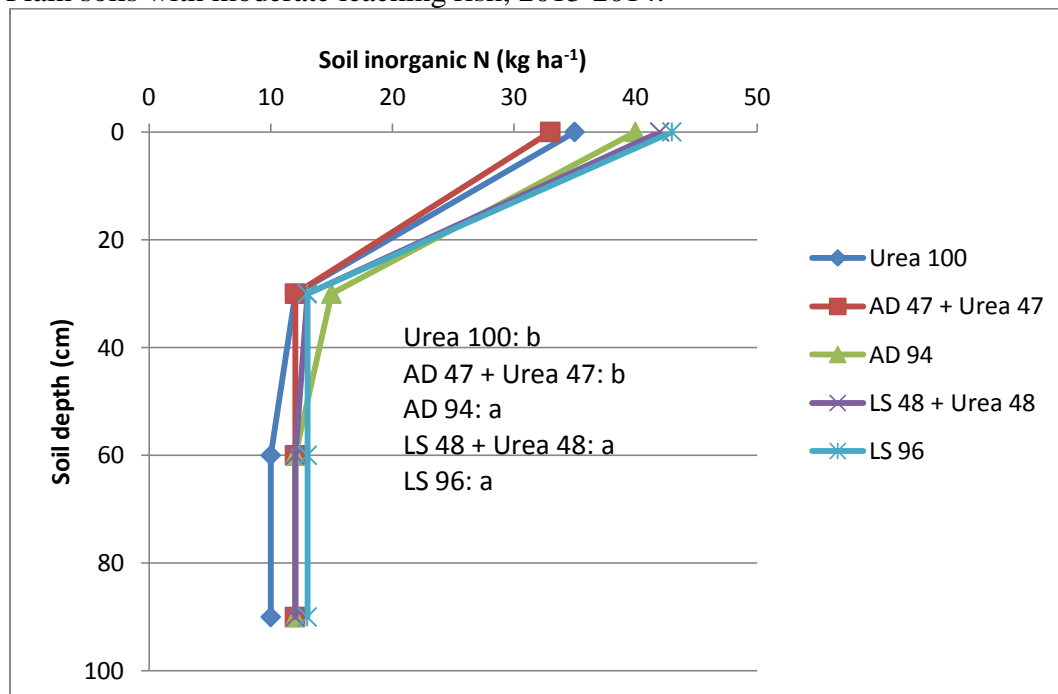
Treatments followed by the same letter resulted in equal soil inorganic N distribution at the 0.05 probability level.

Figure 2.6c: End-of-season mean 2 M KCl extractable soil inorganic N distribution in Coastal Plain soils with low leaching risk, 2013-2014.



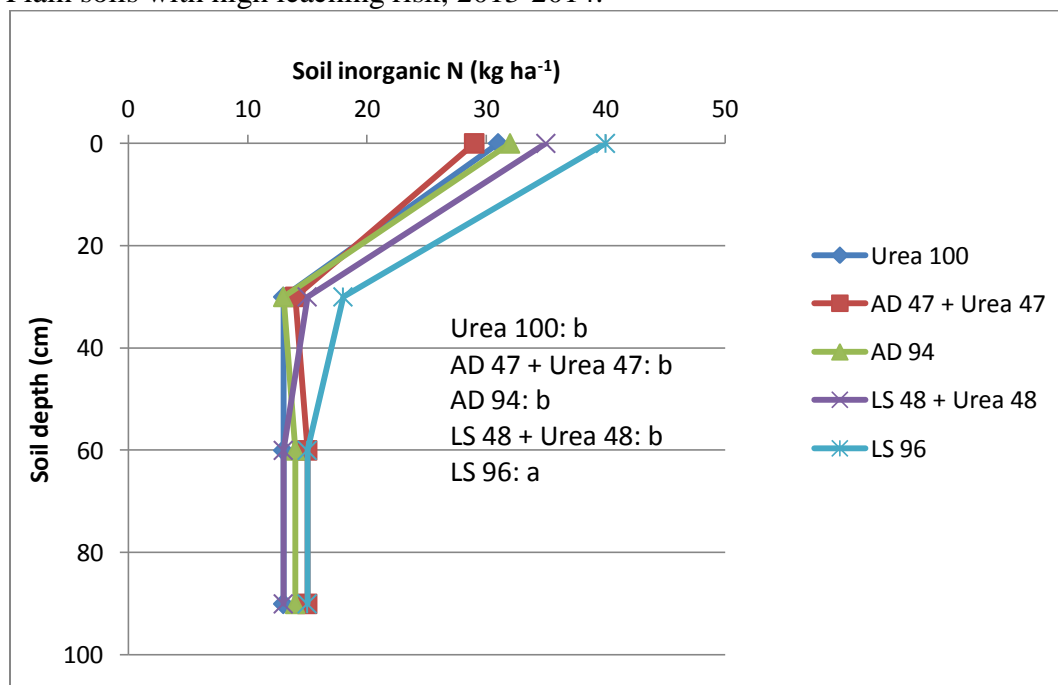
Treatments followed by the same letter resulted in equal soil inorganic N distribution at the 0.05 probability level.

Figure 2.6d: End-of-season mean 2 M KCl extractable soil inorganic N distribution in Coastal Plain soils with moderate leaching risk, 2013-2014.



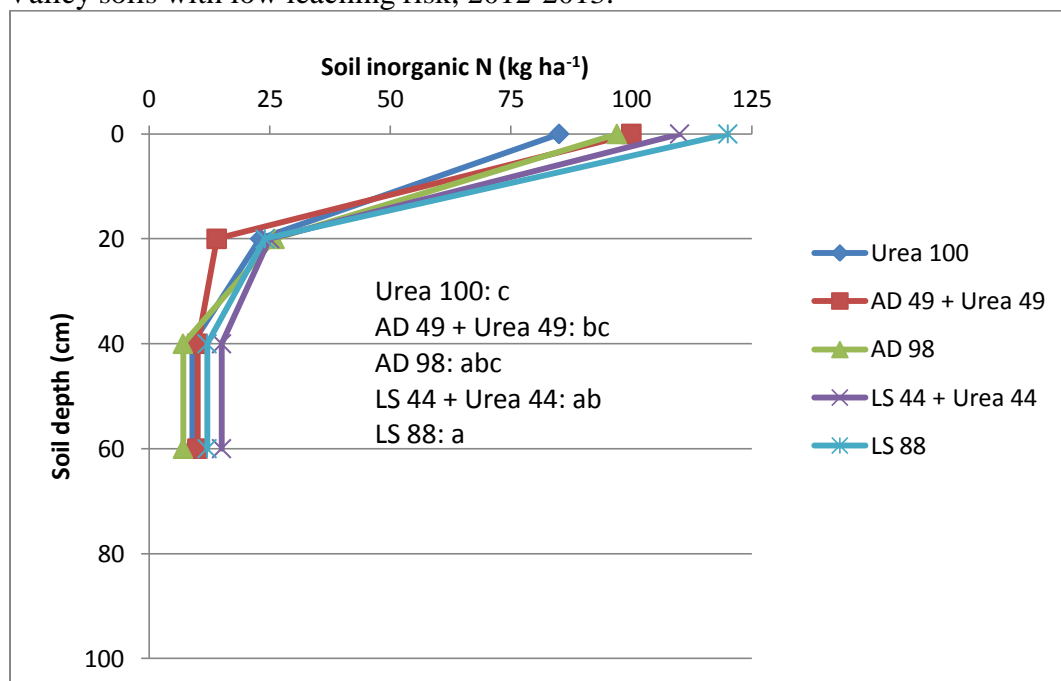
Treatments followed by the same letter resulted in equal soil inorganic N distribution at the 0.05 probability level.

Figure 2.6e: End-of-season mean 2 M KCl extractable soil inorganic N distribution in Coastal Plain soils with high leaching risk, 2013-2014.



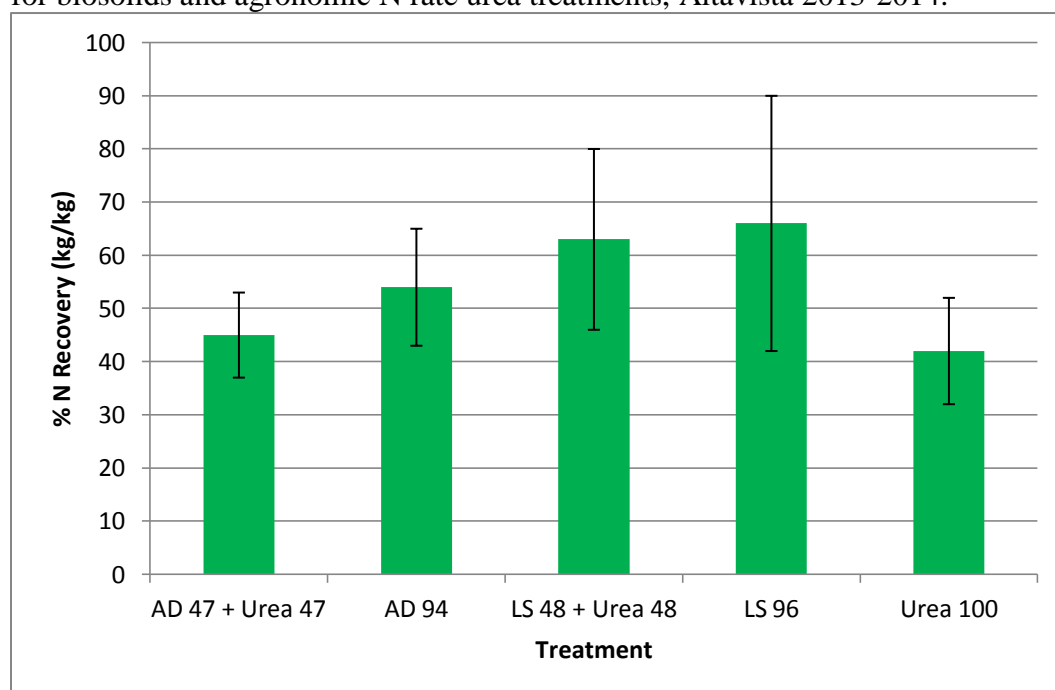
Treatments followed by the same letter resulted in equal soil inorganic N distribution at the 0.05 probability level.

Figure 2.7: End-of-season mean 2 M KCl extractable soil inorganic N distribution in Ridge & Valley soils with low leaching risk, 2012-2013.



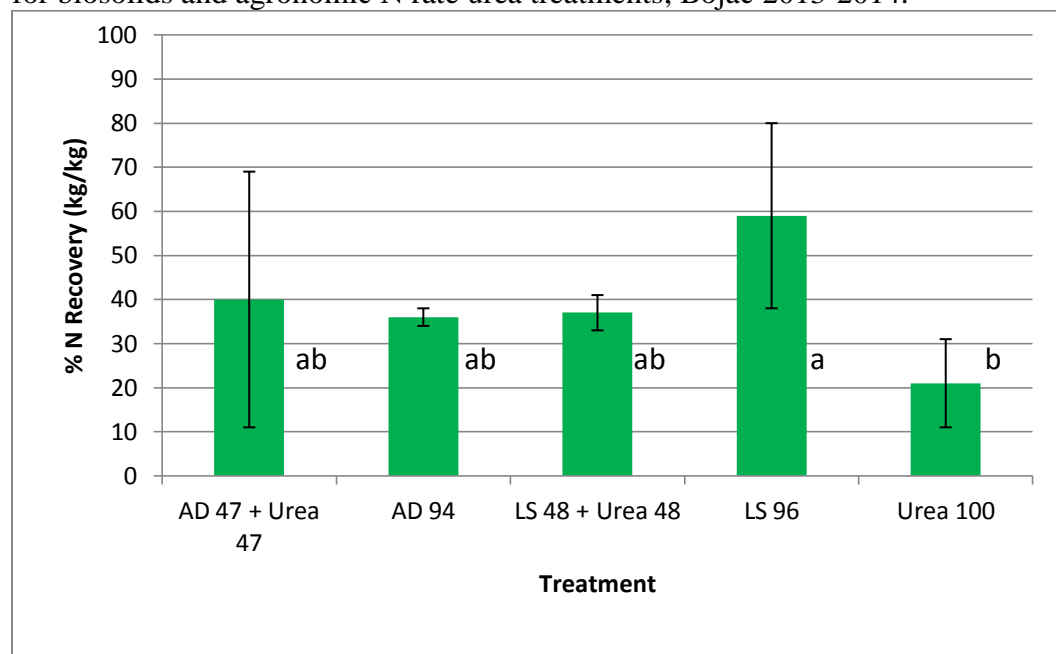
Treatments followed by the same letter resulted in equal soil inorganic N distribution at the 0.05 probability level.

Figure 2.8a: Percent N recovery ( $\{[\text{fert. N uptake} + \text{fert. N resid. in soil}] / [\text{fert. N rate}]\} \times 100\%$ ) for biosolids and agronomic N rate urea treatments, Altavista 2013-2014.



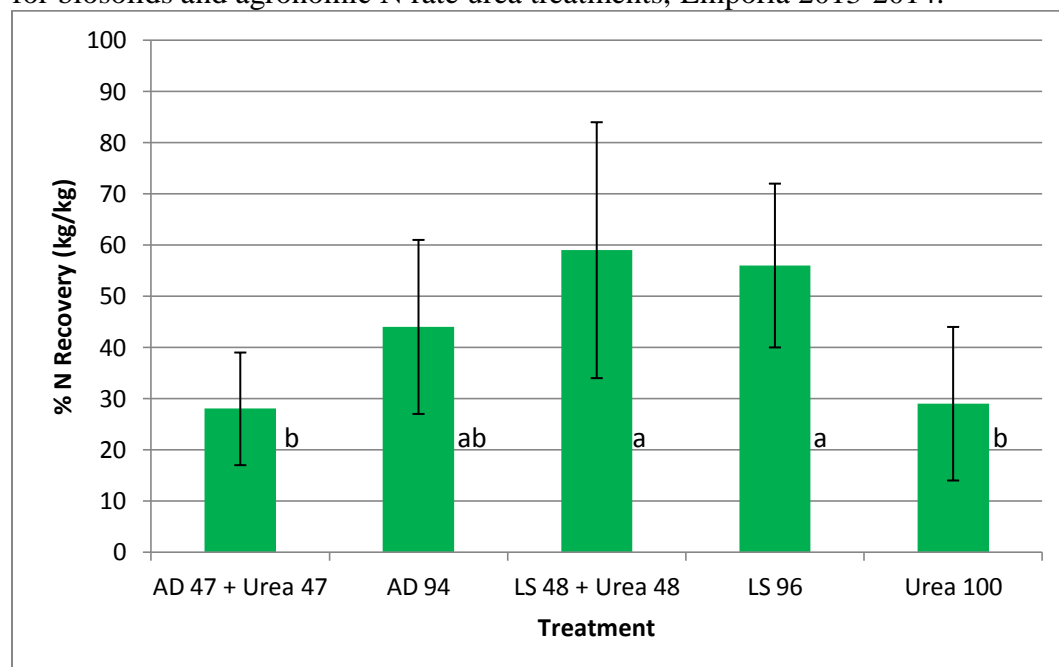
Percent N recoveries followed by the same letter are not statistically different at the 0.05 significance level; error bars represent mean  $\pm$  one standard deviation.

Figure 2.8b: Percent N recovery ( $\{[\text{fert. N uptake} + \text{fert. N resid. in soil}] / [\text{fert. N rate}]\} \times 100\%$ ) for biosolids and agronomic N rate urea treatments, Bojac 2013-2014.



Percent N recoveries followed by the same letter are not statistically different at the 0.05 significance level; error bars represent mean  $\pm$  one standard deviation.

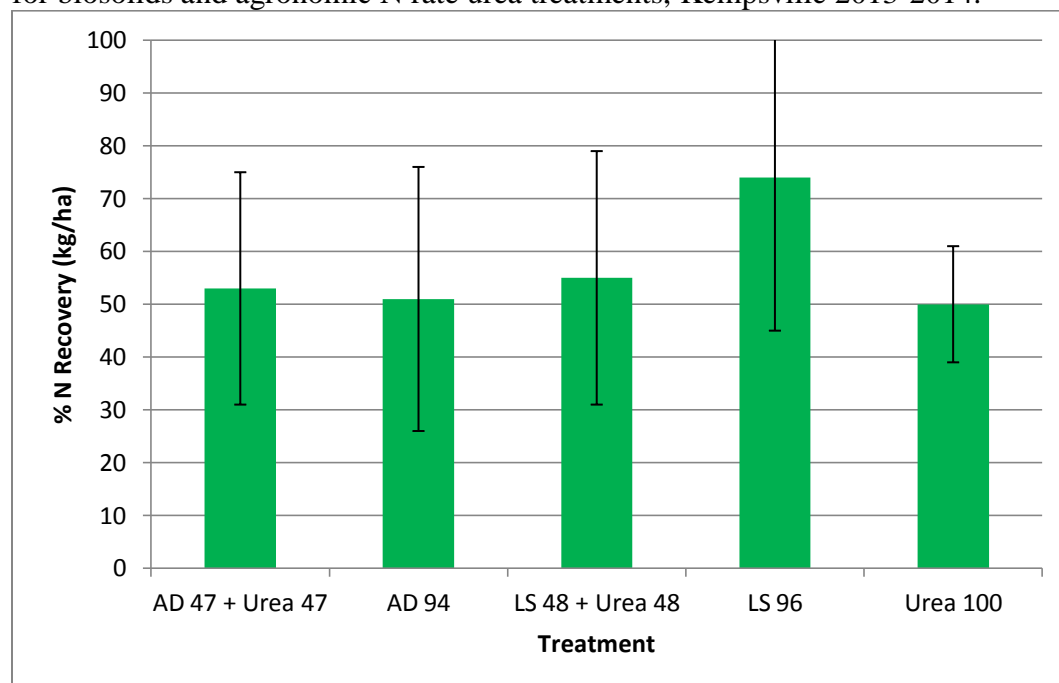
Figure 2.8c: Percent N recovery ( $\{[\text{fert. N uptake} + \text{fert. N resid. in soil}] / [\text{fert. N rate}]\} \times 100\%$ ) for biosolids and agronomic N rate urea treatments, Emporia 2013-2014.



Percent N recoveries followed by the same letter are not statistically different at the 0.05 significance level; error bars represent mean  $\pm$  one standard deviation.

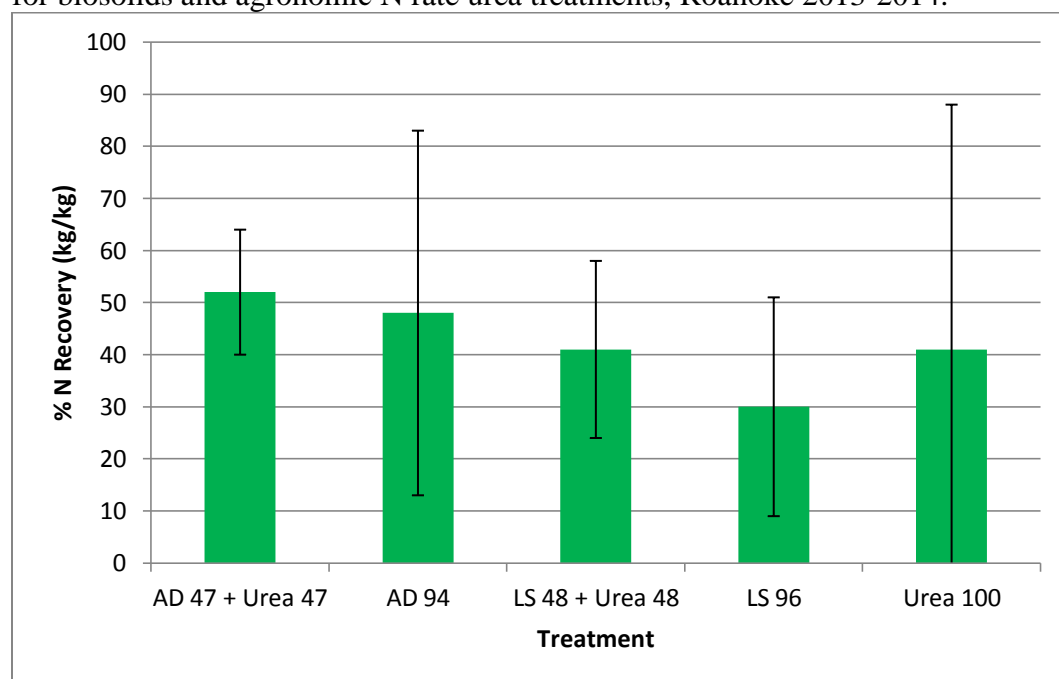


Figure 2.8d: Percent N recovery ( $\{[\text{fert. N uptake} + \text{fert. N resid. in soil}] / [\text{fert. N rate}]\} \times 100\%$ ) for biosolids and agronomic N rate urea treatments, Kempsville 2013-2014.



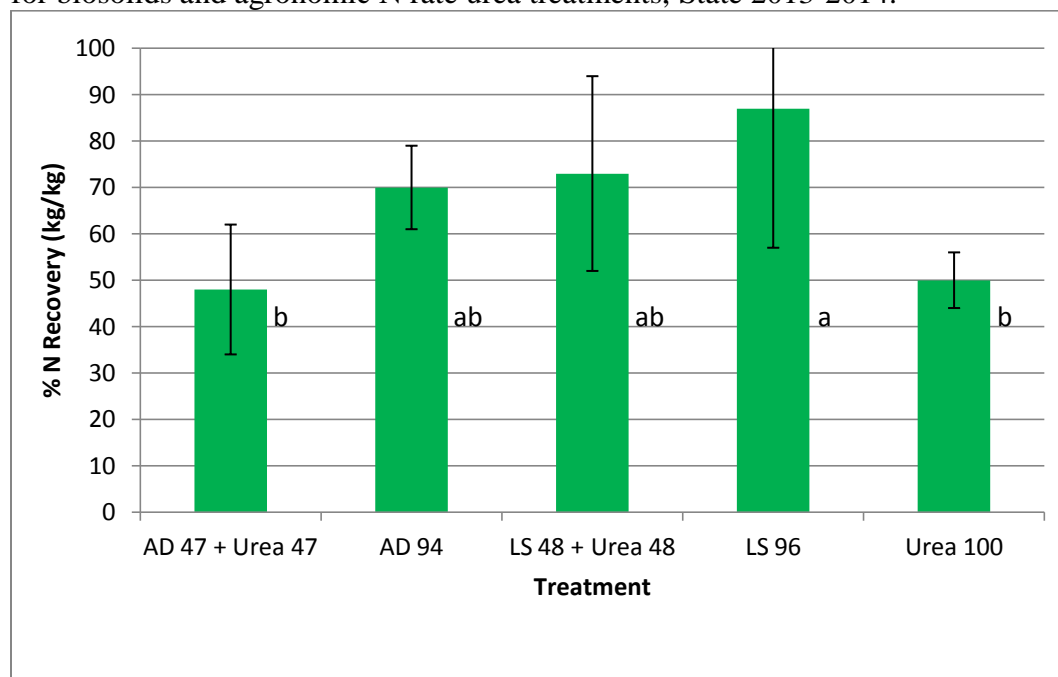
Percent N recoveries followed by the same letter are not statistically different at the 0.05 significance level; error bars represent mean  $\pm$  one standard deviation.

Figure 2.8e: Percent N recovery ( $\{[\text{fert. N uptake} + \text{fert. N resid. in soil}] / [\text{fert. N rate}]\} \times 100\%$ ) for biosolids and agronomic N rate urea treatments, Roanoke 2013-2014.



Percent N recoveries followed by the same letter are not statistically different at the 0.05 significance level; error bars represent mean  $\pm$  one standard deviation

Figure 2.8f: Percent N recovery ( $\{[\text{fert. N uptake} + \text{fert. N resid. in soil}] / [\text{fert. N rate}]\} \times 100\%$ ) for biosolids and agronomic N rate urea treatments, State 2013-2014.



Percent N recoveries followed by the same letter are not statistically different at the 0.05 significance level; error bars represent mean  $\pm$  one standard deviation.

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Table 2.1: Description of research study sites.

Year	County	Soil Series	Soil family	Leaching Risk (VADCR, 2005)
2012	New Kent	Altavista	fine-loamy, mixed, semiactive, thermic Aquic Hapludults	Low
	New Kent	Bojac	coarse-loamy, mixed, semiactive, thermic Typic Hapludults	High
	Augusta	Frederick-Christian	fine, mixed semiactive, mesic Typic Paleudults	Low
	Montgomery	Frederick & Vertrees	fine, mixed semiactive, mesic Typic Paleudults	Low
2013	New Kent	Altavista	fine-loamy, mixed, semiactive, thermic Aquic Hapludults	Low
	New Kent	Bojac	coarse-loamy, mixed, semiactive, thermic Typic Hapludults	High
	King & Queen	Emporia	fine loamy, siliceous, subactive thermic Typic Hapludults	Moderate
	Middlesex	Kempsville	fine-loamy, siliceous, subactive thermic Typic Hapludults	Moderate
	King William	Roanoke	fine, mixed, semiactive, thermic Typic Endoaquults	Low
	King William	State	fine-loamy, mixed, semiactive, thermic Typic Hapludults	Moderate

Table 2.2: Application schedule of each N treatment.

Treatment	0 x Urea-N	0.5 x Urea-N	1.0 x Urea-N	1.5 x Urea-N	0.5 x Biosolids-N†	1.0 x Biosolids-N†
Pre-Plant kg N ha <sup>-1</sup>	0	17	33	50	50	100
Side Dress‡ kg N ha <sup>-1</sup>	0	33	67	100	50	0
Total kg N ha <sup>-1</sup>	0	50	100	150	100	10

† applies to anaerobically digested and lime stabilized, ‡All Side-dress was applied as urea

Table 2.3: Estimated PAN from biosolids applications (VADCR, 2005).

Biosolids type	Estimated Mineralization Rates for Biosolids Organic N in year of land application	Biosolids NH <sub>4</sub> – N Availability, surface application with no incorporation
Anaerobically Digested	0.30 x Biosolids Organic N	0.50 x Biosolids NH <sub>4</sub> – N
Lime Stabilized	0.30 x Biosolids Organic N	0.25 x Biosolids NH <sub>4</sub> – N

Table 2.4: Fall 2012 and 2013 biosolids analyses.

Year	Source	TKN (g kg <sup>-1</sup> )	NH <sub>4</sub> – N (g kg <sup>-1</sup> )	Org. N (g kg <sup>-1</sup> )	Total P (g kg <sup>-1</sup> )	Total K (g kg <sup>-1</sup> )	Solids (g kg <sup>-1</sup> )	pH
2012	Alexandria (AD)	60.3	16.3	44.0	36.3	1.2	310.2	8.61
	Arlington (LS)	30.5	1.8	28.7	14.0	1.4	364.3	12.21
	Blue Plains (LS)	41.4	5.0	36.4	13.6	2.2	287.7	12.20
2013	Alexandria (AD)	55.5	15.2	40.3	35.1	1.4	259.4	8.49
	Blue Plains (LS)	29.0	0.9	28.1	9.8	1.6	382.7	12.24

Table 2.5: Fall 2012 and 2013 actual N – P – K applied with biosolids as kg ha<sup>-1</sup>,

Year	Source	0.5 Agronomic N rate	1.0 Agronomic N rate
2012	Alexandria (AD)	49 – 84 – 2	99 – 168 – 4
	Arlington (LS)	44 – 67 – 7	88 – 134 – 14
	Blue Plains (LS)	46 – 52 – 8	92 – 104 – 16
2013	Alexandria (AD)	47 – 84 – 3	94 – 168 – 6
	Blue Plains (LS)	48 – 54 – 9	95 – 109 – 18

Table 2.6: Wheat Variety planted at each research site.

Year	County	Soil Series	Wheat Variety
2013-2013	New Kent	Altavista	USG 3251
	New Kent	Bojac	USG 3251
	Augusta	Frederick-Christian	SS 5205
	Montgomery	Frederick & Vertrees	SS 5205
2013-2014	New Kent	Altavista	USG 3120
	New Kent	Bojac	USG 3120
	King & Queen	Emporia	USG 3251
	Middlesex	Kempsville	Shirley
	King William	Roanoke	Dynagro 9012
	King William	State	SS 5205

Table 2.7: Fall 2012 pre-plant soil analyses.

Soil	Altavista A	Altavista B	Bojac A	Bojac B	Frederick- Christian A	Frederick- Christian B	Frederick- Vertrees A	Frederick- Vertrees B
Depth (cm)	0 – 15	15 – 30	0 – 15	15 – 30	0 – 15	15 – 30	0 – 15	15 – 30
pH	6.43	6.52	5.86	5.70	5.97	6.92	6.48	6.89
C:N ratio	10.4	-	10.0	-	8.5	-	9.3	-
Total N (mg kg <sup>-1</sup> )	1105	450	892	445	1676	677	2356	1076
NO <sub>3</sub> – N (mg kg <sup>-1</sup> )	6.56	2.47	4.61	2.86	24.62	4.82	48.07	12.16
NH <sub>4</sub> – N (mg kg <sup>-1</sup> )	0.30	0.45	0.30	0.16	0.63	0.37	0.95	0.67
P (mg kg <sup>-1</sup> )	10	4	61	52	53	10	50	17
K (mg kg <sup>-1</sup> )	82	58	40	35	85	30	159	70
Ca (mg kg <sup>-1</sup> )	788	649	604	332	1107	915	1485	1013
Mg (mg kg <sup>-1</sup> )	129	877	90	44	120	110	416	330
P rating	M-	L	VH	H+	H+	M-	H+	M+
K rating	M+	M	M-	L+	M+	L+	VH	M
Ca rating	H-	M+	M+	L+	VH	H	VH	H+
Mg rating	VH	H	H	M-	VH	VH	VH	VH

Table 2.8: Fall 2013 pre-plant soil analyses.

Soil	Altavista		Bojac		Emporia		Kempsville		Roanoke		State	
	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30
Depth (cm)												
pH	5.94	6.10	6.04	6.24	5.67	6.05	6.69	6.84	6.45	6.31	6.07	6.57
C:N	9.7	-	10.1	-	9.6	-	10.6	-	9.6	-	9.6	-
Total N (mg kg <sup>-1</sup> )	939	431	768	381	1138	551	1402	590	1382	562	1193	686
NO <sub>3</sub> – N (mg kg <sup>-1</sup> )	9.11	2.93	5.02	3.28	10.11	4.02	16.44	4.53	8.06	2.91	8.03	3.49
NH <sub>4</sub> – N (mg kg <sup>-1</sup> )	2.36	1.34	1.78	1.08	2.76	1.57	3.37	1.66	2.95	1.09	4.23	1.30
P (mg kg <sup>-1</sup> )	46	11	107	116	44	25	25	5	56	14	93	60
K (mg kg <sup>-1</sup> )	60	53	44	50	81	58	178	135	92	66	62	57
Ca (mg kg <sup>-1</sup> )	549	446	457	433	626	485	1361	737	1103	657	887	784
Mg (mg kg <sup>-1</sup> )	94	101	79	73	25	14	72	51	27	25	74	41
P rating	H+	M	VH	VH	H+	H-	H-	L+	VH	M	VH	VH
K rating	M	M	M-	M-	M+	M	VH	H	H-	M	M	M
Ca rating	M	M-	M-	M-	M+	M	VH	H-	VH	M+	H	H-
Mg rating	H	H+	H-	H-	L+	L	M+	M	L+	L+	H-	M

Table 2.9: Linear responses of mean biomass at GS 25-30 to fall applied urea-N and pre-plant soil inorganic N.

Year	Soil	mean biomass response to urea-N	Pre-plant soil inorganic N (kg ha <sup>-1</sup> )
2012-2013	Bojac	$y = 8.635x + 355.4, R^2 = 0.9555$	17
	Altavista	$y = 7.311x + 1161.2, R^2 = 0.9315$	21
	Frederick-Christian	$y = 2.939x + 706, R^2 = 0.7858$	61
	Frederick & Vertrees	$y = 0.700x + 556, R^2 = 0.2746$	126
2013-2014	Bojac	$y = 0.123x + 399.2, R^2 = 0.0013$	24
	Roanoke	$y = 4.307x + 284.1, R^2 = 0.6815$	30
	Altavista	$y = 0.302x + 347.5, R^2 = 0.7124$	33
	State	$y = 4.673x + 131.9, R^2 = 0.8469$	36
	Emporia	$y = 1.327x + 438.3, R^2 = 0.9838$	37
	Kempsville	$y = 2.160x + 340.3, R^2 = 0.7568$	53



Table 2.10: Midseason 2012-2013 and 2013-2014 mean 2 M KCl extractable soil inorganic N (kg ha<sup>-1</sup>) at GS 25-30, 0 - 30 cm depth.

Soil	ALT		BOJ		EMP	FC	FV	KEM	ROA	STA
Year	2013	2014	2013	2014	2014	2013	2013	2014	2014	2014
Treatment										
AD 0.5	31	9.6 ab	59	5.9 ab	9.4 abc	30	54	11	12 a	11 ab
AD 1.0	35	11 a	61	7.1 a	13 ab	33	57	16	11 a	8.1 b
LS 0.5	34	7.9 b	55	5.9 abc	8.6 b	32	51	14	16 a	11 ab
LS 1.0	32	11 a	62	5.8 ab	14 a	30	58	14	14 a	10 abc
Urea 0	38	8.4 b	63	4.0 c	8.7 bc	36	59	12	5.3 b	5.5 c
Urea 0.5	46	8.0 b	57	4.6 bc	6.9 c	44	54	11	10 ab	11 ab
Urea 1.0	36	8.3 b	64	5.5 abc	8.8 bc	35	60	11	12 ab	12 a
Urea 1.5	39	9.2 ab	61	5.7 ab	11 abc	38	57	8.6	8.1 b	13 a

N concentrations within each column followed by the same letter are not statistically different at the 0.05 probability level

Legend: ALT = Altavista, BOJ = Bojac, EMP = Emporia, FC = Frederick-Christian, FV = Frederick & Vertrees, KEM = Kempsville, ROA = Roanoke, STA = State, AD 0.5 = AD biosolids split applied with urea, AD 1.0 = AD biosolids fall applied at full agronomic N rate, LS 0.5 = LS biosolids split applied with urea, LS 1.0 = LS biosolids fall applied at full agronomic N rate, Urea 0, 0.5, 1.0, and 1.5 = Urea split applied at rates of 0, 50, 100, and 150 kg N ha<sup>-1</sup>, respectively.

Table 2.11: Wheat N use efficiency ([kg fertilizer N assimilated by plant] / [kg N applied]) for urea and biosolids treatments, GS 58 2013-2014.

Soil	Urea 1.0	AD 0.5	AD 1.0	LS 0.5	LS 1.0
Altavista	0.38	0.34	0.43	0.49	0.48
Bojac	0.21 b	0.42 ab	0.35 ab	0.32 ab	0.50 a
Emporia	0.30 b	0.32 b	0.40 b	0.47 ab	0.57 a
Kempsville	0.59	0.54	0.56	0.62	0.67
Roanoke	0.26	0.37	0.44	0.32	0.25
State	0.53	0.49	0.61	0.59	0.63

NUE values within each row followed by the same letter are not statistically different at the 0.05 significance level

Legend: Urea 1.0 = Urea split applied at 100 kg N ha<sup>-1</sup>, AD 0.5 = AD biosolids split applied with urea, AD 1.0 = AD biosolids fall applied at full agronomic N rate, LS 0.5 = LS biosolids split applied with urea, LS 1.0 = LS biosolids fall applied at full agronomic N rate.

Table 2.12: Pre-plant soil inorganic N, grain regression response y-intercept, and soil texture from treatments that received no N.

Year	Soil	Pre-plant soil inorganic N (kg ha <sup>-1</sup> )	y-intercept from regression of mean grain yield response to urea (kg ha <sup>-1</sup> )	Soil texture and past management
2012-2013	BOJ	17	1.95	Coarse-loamy, inorganic N fertilizer
	ALT	21	3.13	Fine-loamy, inorganic N fertilizer
	FC	61	3.97	Fine texture and history of soy milk sludge application
	FV	126	3.02	Fine texture and history of cattle manure application
2013-2014	BOJ	24	1.27	Coarse-loamy, inorganic N fertilizer
	ROA	30	2.09	Fine, inorganic N fertilizer
	ALT	33	2.31	Fine-loamy, inorganic N fertilizer
	STA	36	1.71	Fine-loamy, inorganic N fertilizer
	EMP	37	1.91	Fine-loamy, inorganic N fertilizer
	KEM	53	2.70	Fine-loamy, history of biosolids application

Legend: ALT = Altavista, BOJ = Bojac, EMP = Emporia, FC = Frederick-Christian, FV = Frederick & Vertrees, KEM = Kempsville, ROA = Roanoke, STA = State.

Table 2.13: Comparison of residuals calculated by subtracting predicted grain yield response to urea-N rate from observed grain yield responses to urea- and biosolids-N.

Year	Soil	Urea residuals (Mg ha <sup>-1</sup> )	AD 0.5 residuals (Mg ha <sup>-1</sup> )	AD 1.0 residuals (Mg ha <sup>-1</sup> )	LS 0.5 residuals (Mg ha <sup>-1</sup> )	LS 1.0 residuals (Mg ha <sup>-1</sup> )
2012-2013	ALT	1.11 x 10 <sup>-16</sup> ± 0.55	0.71 ± 0.34	0.19 ± 0.79	0.83 ± 0.51	0.50 ± 0.65
	BOJ	4.16 x 10 <sup>-15</sup> ± 0.53 b	0.64 ± 0.35 a	-0.06 ± 0.69 ab	0.58 ± 0.17 a	-0.15 ± 0.36 b
	FC	5.83 x 10 <sup>-16</sup> ± 0.98 a	-1.50 ± 1.01 b	-0.13 ± 0.69 a	-1.72 ± 0.82 b	-0.73 ± 0.62 ab
	FV	9.52 x 10 <sup>-17</sup> ± 0.75	-0.46 ± 0.85	-0.92 ± 0.92	-0.55 ± 0.30	-0.87 ± 0.54
2013-2014	ALT	1.25 x 10 <sup>-16</sup> ± 0.40 b	0.80 ± 0.24 a	0.60 ± 0.29 a	0.72 ± 0.44 a	0.94 ± 0.29 a
	BOJ	2.70 x 10 <sup>-16</sup> ± 0.40 c	0.75 ± 0.19 b	0.54 ± 0.28 b	1.28 ± 0.44 a	0.99 ± 0.23 ab
	EMP	4.16 x 10 <sup>-16</sup> ± 0.43 d	0.48 ± 0.33 bc	0.22 ± 0.27 cd	1.02 ± 0.17 ab	1.15 ± 0.41 a
	KEM	-2.20 x 10 <sup>-16</sup> ± 0.29 b	0.57 ± 0.26 a	-0.01 ± 0.47 b	0.66 ± 0.21 a	0.68 ± 0.26 a
	ROA	-1.20 x 10 <sup>-16</sup> ± 0.40	0.16 ± 0.56	-0.37 ± 0.52	0.16 ± 0.71	0.16 ± 0.18
	STA	3.05 x 10 <sup>-16</sup> ± 0.29 b	0.69 ± 0.23 a	0.23 ± 0.48 b	0.89 ± 0.31 a	0.88 ± 0.11 a

Distributions within each row followed by the same letter are not statistically different at the 0.05 significance level

Legend: ALT = Altavista, BOJ = Bojac, EMP = Emporia, FC = Frederick-Christian, FV = Frederick & Vertrees, KEM = Kempsville, ROA = Roanoke, STA = State, AD 0.5 = AD biosolids split applied with urea, AD 1.0 = AD biosolids fall applied at full agronomic N rate, LS 0.5 = LS biosolids split applied with urea, LS 1.0 = LS biosolids fall applied at full agronomic N rate.

Table 2.14: Effect of treatments on end-of-season 2012-2013 and 2013-2014 mean 2 M KCl extractable total (NO<sub>3</sub>-N + NH<sub>4</sub>-N) soil inorganic N (kg ha<sup>-1</sup>).

Soil	ALT		BOJ		EMP	FC	FV	KEM	ROA	STA
Year	2013	2014	2013	2014	2014	2013	2013	2014	2014	2014
Depth (cm)	0-80	0-90	0-80	0-90	0-90	0-60	0-60	0-90	0-60	0-30
Treatment										
AD 0.5	47 bcd	67 ab	40 d	59 bc	53	134 bcd	104 abc	62 c	60	32 d
AD 1.0	48 bcd	64 b	66 a	59 b	61	159 bc	97 abc	73 ab	63	45 ab
LS 0.5	66 a	73 ab	41 cd	63 ab	70	184 b	104 a	64 bc	64	49 abc
LS 1.0	45 cd	74 a	53 bc	73 a	57	221 a	81 c	80 a	61	60 a
Urea 0	55 b	58 b	60 ab	57 bc	60	111 e	69 d	68 bc	56	36 bcd
Urea 0.5	43 d	61 b	42 cd	52 c	59	119 de	90 b	68 bc	53	31 cd
Urea 1.0	52 abcd	62 b	58 ab	58 bc	53	138 cd	87 bc	61 c	59	33 cd
Urea 1.5	55 abc	60 b	40 d	63 abc	60	162 bc	91 abc	61 c	57	35 cd

Inorganic N concentrations within each column followed by the same letter are not statistically different at the 0.05 probability level

Legend: ALT = Altavista, BOJ = Bojac, EMP = Emporia, FC = Frederick-Christian, FV = Frederick & Vertrees, KEM = Kempsville, ROA = Roanoke, STA = State, AD 0.5 = AD biosolids split applied with urea, AD 1.0 = AD biosolids fall applied at full agronomic N rate, LS 0.5 = LS biosolids split applied with urea, LS 1.0 = LS biosolids fall applied at full agronomic N rate, Urea 0, 0.5, 1.0, and 1.5 = Urea split applied at rates of 0, 50, 100, and 150 kg N ha<sup>-1</sup>, respectively.

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### **Chapter 3: Rapid estimation of potential soil N mineralization in early spring following fall biosolids applications to winter wheat**

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#### **Abstract**

Land application of biosolids adds organic nitrogen (N) to soils, which increases potential soil N mineralization and supply of plant available N (PAN). The amount of potential soil N mineralization in early spring is critical to winter wheat production because early spring begins the interval of maximum N uptake. We used the Haney-Brinton CO<sub>2</sub>-burst method (Woods End Laboratories, 2002-2013) to quantify the response of potential soil N mineralization in early spring to biosolids fall applied to soft red winter wheat (*Triticum aestivum* L.). Anaerobically digested (AD) and lime stabilized (LS) biosolids were fall applied at 0, 50 and 100 kg PAN ha<sup>-1</sup> at 6 locations in Virginia 2013-2014, all of which were located in the Coastal Plain physiographic province. Soil mineralizable N only responded to fall LS biosolids N rate in coarse textured Coastal Plain soils. Our study shows that the Haney-Brinton CO<sub>2</sub>-burst method does not provide an accurate estimation of spring PAN from fall biosolids applications; however, the Haney-Brinton CO<sub>2</sub>-burst method was able to predict spring PAN for wheat from soils not recently amended with biosolids. Potential N mineralization of soil receiving no recent N amendments rate measured by the Haney-Brinton CO<sub>2</sub>-burst method was the same as estimates of soil organic mineralization calculated a posteriori using pre-plant soil inorganic N, N uptake and residual soil inorganic N to derive the estimate. The sum of pre-plant soil inorganic N and potential N mineralization of the unamended soil was well correlated to grain yield. Therefore,

the Haney-Brinton CO<sub>2</sub>-burst method used in combination with pre-plant soil inorganic N may be useful in mid-season estimation of yield potential for winter small grains.

### **3.1 Introduction**

The utility of biosolids as an N source for winter wheat (*Triticum aestivum* L.) depends on the amount and timing of biosolids organic N mineralization. Maximum winter wheat N uptake in Virginia occurs during the period between stem elongation and the beginning of anthesis (Alley et al., 1993), which typically begins in early to mid-March. Therefore, the amount of PAN and potential N mineralization in the soil in early spring is critical to winter wheat production.

Predicting the amounts and rates of biosolids organic N mineralization has proven difficult. Empirical observations of the amount of biosolids organic N that have mineralized in the year of application are the simplest way to estimate organic N mineralization resulting from future biosolids applications. The Virginia Department of Conservation and Recreation (VADCR) employs coefficients based on empirical research studies (Gilmour et al., 2003) to predict biosolids organic N mineralization for biosolids (VADCR, 2005). Such coefficients are typically imprecise due to the variability of environmental factors that influence N mineralization. Biosolids organic N mineralization rates can vary with different wastewater treatment processes (Wang et al., 2003; Singh and Agrawal, 2008) and may vary between biosolids of the same treatment process from two different sources (Boeira, 2009). The interaction between biosolids N and the characteristics of the soil to which they are applied

(Correa et al., 2012) can also affect N mineralization. Therefore, the amount of N mineralized from biosolids may best be predicted on a case by case basis.

Long term incubations are the most common method of quantifying the release of N from a given biosolids product to specific soils. Such incubations often last for months (He et al., 2000; Hseu and Huang, 2005) and are of limited use if the results are meant to aid in N management decisions that need to be made for a standing crop. Some investigators have developed methods to provide more timely estimates of N mineralization resulting from applications of biosolids to agricultural soils. A method that correlates a short, rapid release of CO<sub>2</sub> following the re-wetting of a dried soil to potential N mineralization has recently been developed by Haney et al. (2008). The release of CO<sub>2</sub> quantifies microbial activity, which is directly related to potential N mineralization (Haney et al., 2008).

Faster methods of N mineralization estimation have great potential applications for winter wheat, given that early spring N availability and management is time sensitive and critical for optimizing grain yields (Alley et al., 1993). Our objective was to test the utility of the Haney-Brinton CO<sub>2</sub>-burst method in quantifying the response of potential soil N mineralization to different types and fall N rates of biosolids.

## **3.2 Materials and Methods**

### **Study sites, soil description, and site management**

Study sites were established at 6 locations 2013-2014 in Virginia, with all sites in the Coastal Plain physiographic province. Four replicates of anaerobically digested (AD) and lime

stabilized (LS) biosolids were surface applied with no incorporation at each site within days of wheat planting. Biosolids were applied at PAN rates of 0, 50, and 100 kg PAN ha<sup>-1</sup>. A description of the sites, including county, soil series and soil family is presented in Table 3.1.

All locations were managed under continuous no-till (NT) in a 2 year, 3 crop rotation of corn-winter wheat-double crop soybean. Soft red winter wheat (*Triticum aestivum* L.) was planted at all locations October 6-20, 2014 following harvest of corn (*Zea mays*,L.). The seeding rate was 69 seedlings per row meter, and rows were spaced 19 cm apart. Herbicide, pesticide and fungicide use decisions were made by each farmer. Grain harvest was performed June 24-27, 2014 at all sites.

### **Weather data collection**

Daily temperature and precipitation data from 2013-2014 were obtained from the National Oceanic and Atmospheric Administration monitoring station at Richmond International Airport in Sandston, Virginia (NOAA, 2014). These data were used to approximate weather conditions in the Coastal Plain physiographic province. Observed mean monthly temperatures and precipitation amounts from each growing season were overlaid with expected mean temperatures and precipitation amounts for comparison (Figures 3.1 and 3.2). Temperature and precipitation data were used to make inferences about winter organic N mineralization.

### **Biosolids analysis**

The source of the dewatered AD biosolids was Alexandria (VA) Renew Enterprises ([www.alexrenew.com](http://www.alexrenew.com)), and the sources of the LS biosolids were Blue Plains (District of Columbia) Advanced Wastewater Treatment Plant ([www.dcwater.com/waste](http://www.dcwater.com/waste)). The LS biosolids from Blue Plains were applied at sites in the Virginia Coastal Plain and LS biosolids from Arlington were applied at sites in the Virginia Valley & Ridge Province. Biosolids were

analyzed for total Kjeldahl N (Clesceri et al., 2012),  $\text{NH}_4\text{-N}$  (Clesceri et al., 2012), organic N (Clesceri et al., 2012), and pH (USEPA, 2009) by A&L Eastern Laboratories. Biosolids results are presented in Table 3.3.

### **Soil and plant sampling and analysis**

Soil samples were randomly collected throughout each site with push probes prior to planting and amendment application. Each sample was taken to a depth of 30 cm. The soil samples were bulk-blended to characterize the sites before amendment application and planting. The soil samples were air dried, ground, and sieved through a No. 10 mesh (2mm equivalent spherical diameter). We analyzed pre-plant soil samples for pH by 1:1 method the Virginia Tech Soil Test Laboratory (Maguire and Heckendorn, 2011) and total C and N were determined by combustion with a Vario MAX CNS macro elemental analyzer (Elementar, Hanau, Germany). Inorganic N was extracted from soils with 2 M potassium chloride (KCl), with soil and KCl combined in a ratio of 1 g of soil to 10 mL of KCl. The combined soil and KCl was shaken mechanically for 30 minutes. The resulting supernatant was filtered through 0.45 micron filter paper to give a filtrate free of suspended solids. The filtrate was then run through a Lachat 8500 Flow Injection Analyzer to determine  $\text{NO}_3\text{-N}$  by QuikChem Method 12-107-04-1-B (Knepel, 2001) and to determine  $\text{NH}_4\text{-N}$  by QuikChem Method 12-107-06-2-A (Hofer, 2001). A description of the pre-plant soil samples, including soil series, surface texture, pH, total C, total N, inorganic N and C:N is presented in Table 3.2.

Soil samples for N mineralization assessment were collected when the wheat was at GS 25-30 (Zadoks et al., 1974), which was March 10-17 at all locations. The plots sampled were four replicates at each location that received 0, 50 or 100 kg PAN  $\text{ha}^{-1}$  as either AD or LS biosolids the previous fall (October 10-20). Four to five soil samples were collected randomly

from the center of each plot with push probes. Each sample was taken to a depth of 30 cm and samples taken within the same plot were bulk-blended to characterize the plot. All samples were promptly air dried, ground, and sieved through a No. 10 mesh (2mm equivalent spherical diameter). Potential soil N mineralization of the soil samples collected at GS 25-30 was estimated by the Haney-Brinton CO<sub>2</sub>-burst method (Woods End Laboratories, 2002-2013). Dried 40 g soil samples were moistened with 20 mL of water and incubated at 23.5°C for 24 hours, which triggered a flush of CO<sub>2</sub>. The burst is measured with the digital color reader (DCR) in ppm. This CO<sub>2</sub>-burst is proportional to microbial biomass and potential N mineralization (Haney et al., 2008). The CO<sub>2</sub> is used to calculate an N-factor, which is then used to calculate potential N mineralization in kg ha<sup>-1</sup> yr<sup>-1</sup> by the following equations:

$$\text{N-factor} = [-0.515 \times \ln(\text{ppm CO}_2)] + 3.2903$$

$$\text{Potential N mineralization in kg ha}^{-1} \text{ yr}^{-1} = \text{N-factor} \times \text{ppm CO}_2$$

Two soil samples were collected near the center of each plot 2-4 days after the wheat was harvested. Samples were taken to a depth of 30 cm with bucket augers and were promptly air dried and ground to pass a No. 10 mesh sieve (2mm equivalent spherical diameter). Inorganic N was extracted from soils with 2 M potassium chloride (KCl), with soil and KCl combined in a ratio of 1 g of soil to 10 mL of KCl. The combined soil and KCl was shaken mechanically for 30 minutes. The resulting supernatant was filtered through 0.45 micron filter paper to give a filtrate free of suspended solids. The filtrate was then run through a Lachat 8500 Flow Injection Analyzer to determine NO<sub>3</sub>-N by QuikChem Method 12-107-04-1-B (Knepel, 2001) and to determine NH<sub>4</sub>-N by QuikChem Method 12-107-06-2-A (Hofer, 2001). One m<sup>2</sup> of plant tissue was sampled at GS 58 (Zadoks et al., 1974). The plant tissue was dried at 60°C, ground to pass a

1mm mesh, and analyzed for N by combustion with a Vario MAX CNS macro elemental analyzer (Elementar, Hanau, Germany). The plant tissue N data were used to estimate N uptake. Pre-plant soil inorganic N ( $N_{i0}$ ), end-of-season soil inorganic N ( $N_{ir}$ ) and N uptake in plots that received no N fertilizer were used to estimate soil organic N mineralization (Est.  $N_{min}$ ) by the following equation:

$$\text{Est. } N_{min} = N \text{ uptake} + N_{ir} - N_{i0}$$

The estimate of growing season soil organic N mineralization relies on the assumption that plots that received no N fertilizer did not lose any N via volatilization or leaching.

Grain samples were collected with a Kincaid 8XP small plot combine and weighed by an onboard computer to calculate grain yield. The grain samples were weighed to calculate yield on a 13.5% moisture basis.

### **Statistical analysis**

All statistical analysis was performed using JMP®Pro 10.0.2 statistical software (SAS Institute Inc., 2012). The response of potential soil N mineralization to fall applied AD and LS biosolids N were quantified with linear regression. Significance of linear fit was set at 0.10. The regressions both biosolids types in each soil were plotted together for comparison of the slopes. The distributions of potential N mineralization estimated by the Haney-Brinton CO<sub>2</sub>-burst method and by mass balance were pooled and tested for normality with the Shapiro-Wilk test. The significance of the Shapiro-Wilk test was set at 0.05. The distributions of estimated N mineralization resulting from the two methods were compared with analysis of variance (ANOVA) when normally distributed and with the Wilcoxon/Kruskal-Wallis rank sums test when not normally distributed. The significance level of distribution comparisons was set at 0.10.



### **3.3 Results and discussion**

#### **Weather prior to soil sampling at GS 25-30**

The activity of mesophilic bacteria that mineralize soil and biosolids organic N is temperature dependent, with an optimum range of 25-37°C. Average winter temperatures in Virginia have a range of 4-15°C (Figures 3.1), and microbial activity increases and decreases with temperatures in this range. Winter temperatures were below average (Figure 3.1), which made the potential for winter soil and biosolids organic N mineralization less than during a typical year (Cassman and Munns, 1980, Wang et al., 2003). Precipitation was consistently above average (Figure 3.2), which presented the potential for leaching of mineralized and nitrified biosolids N.

#### **Soils**

The soils studied varied in texture and pre-plant fertility conditions, which are presented in Table 3.2. All soils studied had a surface texture of silt loam, fine sandy loam or loamy fine sand. The soils with the coarsest surface textures typically had the least total N. All pre-plant soil C measured was assumed to be organic owing to the weakly acid soils of Virginia (Arkley, 1963).

#### **Potential N mineralization**

Potential N mineralization estimated by the Haney-Brinton CO<sub>2</sub>-burst method rarely responded to fall biosolids rate, regardless of biosolids type (Figures 3.3a-f). The Haney-Brinton CO<sub>2</sub>-burst method may not have detected increases in potential soil N mineralization because we applied biosolids to the soil surface in a NT management system. The Haney-Brinton CO<sub>2</sub>-burst method, designed to detect changes of soil-borne potential N mineralization, may not be useful

for assessing NT systems with N sources applied to the soil surface. This is a significant potential disadvantage because NT is an increasingly used soil management system, even where organic by-products are applied.

The few observed increases in potential N mineralization with fall biosolids-N rate were observed in Bojac and Emporia soil series (Figure 3.3b-c), which had the coarsest surface textures of the soils used in our study. Water soluble biosolids C (plus associated N) was more likely to have been transported into the coarser-textured soils. Potential N mineralization responded to LS biosolids N rate in Bojac and to both AD and LS biosolids N rate in Emporia.

#### **Comparison of potential N mineralization with mass balance estimates**

Potential N mineralization estimated with the Haney-Brinton CO<sub>2</sub>-burst method for the 0 kg PAN ha<sup>-1</sup> treatment was equal to that calculated a posteriori via the mass balance method estimates of soil organic N mineralization (Table 3.4). The Haney-Brinton CO<sub>2</sub>-burst method was seemingly able to estimate mineralizable N from a soil sample collected and analyzed with ample time to make supplemental fertilizer N applications.

#### **Relationship of potential N mineralization to grain yield**

The response to added N typically decreases with increases in soil inorganic or potentially mineralizable organic N. The slopes of yield responses to increasing N rate were similar among all soils, so there was no useful factor for predicting the response of grain yield to increasing applied N. Grain yield in zero N fertilization treatments was weakly correlated to potential N mineralization estimated by the Haney-Brinton CO<sub>2</sub>-burst method, pre-plant soil inorganic N, and the sum of the two parameters (Figures 3.4a-c). Correlation between potential N mineralization and zero N grain yield was weakest (Figure 3.4a). Correlation between pre-plant soil inorganic N and zero N grain yield was slightly stronger (Figure 3.4b), which suggests

early season N availability may indicate N availability throughout the season more effectively than the potential N mineralization estimated by the Haney-Brinton CO<sub>2</sub>-burst method.

Correlation of the sum of potential N mineralization and pre-plant soil inorganic N gave a better fit with grain yield than either factor alone (Figures 3.4a-c). Both pre-plant soil inorganic N and potential N mineralization at GS 25-30 are critical to winter wheat production (Alley et al., 1993), and the sum of the pre-plant soil inorganic N and potential N mineralization accounts for both.

The relationships between grain yield in zero N treatments and various N parameters was significant because the soils often yielded differently than was predicted by their VADCR soil productivity group (VADCR, 2005; Table 3.5). For example, Kempsville is in productivity group II (VADCR, 2005) for wheat and was under standard management, but yields resulting from the agronomic N rate were higher than the yield expected for intensive management. This likely occurred because past management (history of biosolids applications) continually increased both pre-plant soil inorganic N and potential N mineralization. Bojac is in the same productivity group as Kempsville (VADCR, 2005) and was managed the same way in our study. However, Bojac treated with the agronomic N rate yielded much lower than what was expected under standard management.

### **3.4 Conclusions**

The value of the Haney-Brinton CO<sub>2</sub>-burst method in predicting spring N mineralization of fall applied biosolids for not-till winter wheat is limited because the method does not appear to account for the expected mineralization from surface-applied organic N sources in no-till systems. The Haney-Brinton CO<sub>2</sub>-burst method provided a quick in-season estimate of

mineralizable N that would have enabled supplemental fertilizer N recommendations for winter wheat in a soil where no organic residual was applied immediately prior to or during the season of production. While the test method may have promise in aiding time sensitive spring N management decisions in winter wheat production, the lack of predictable potential N mineralization where biosolids were applied in the fall under NT reduces the tool's value for sites that receive organic residuals for winter wheat. Estimates of potential N mineralization using the Haney-Brinton CO<sub>2</sub>-burst method summed with pre-plant soil inorganic N may offer a useful tool for such practices due to combining early season readily available N with midseason potentially mineralizable N. This application the Haney-Brinton CO<sub>2</sub>-burst-method may provide more accurate yield estimates than VADCR soil productivity groups. The missing important information is how much N mineralizes between planting and midseason potential N mineralization testing.

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Figure 3.1: Mean monthly temperatures collected by the NOAA monitoring station at Richmond International Airport in Sandston, Virginia.

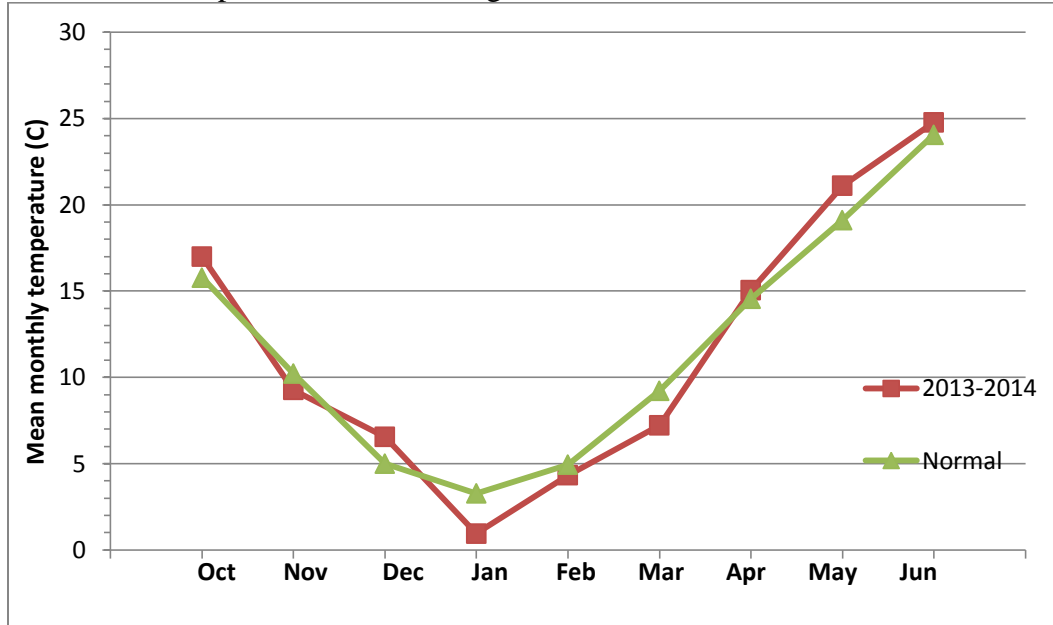


Figure 3.2: Mean monthly precipitation collected by the NOAA monitoring station at Richmond International Airport in Sandston, Virginia.

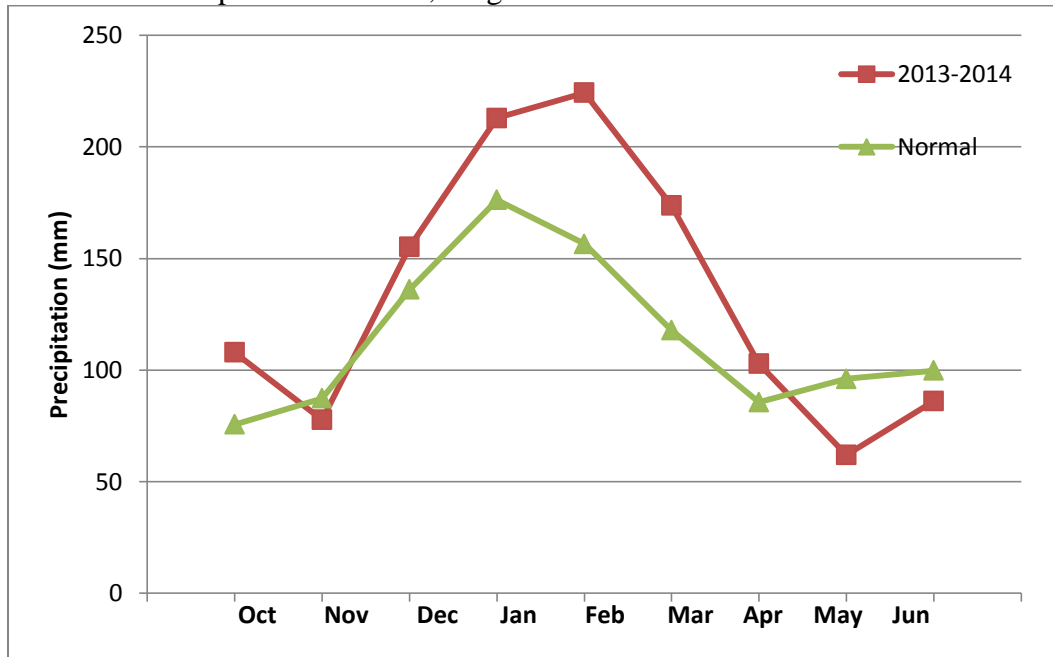


Figure 3.3a: Response of potential N mineralization to biosolids-N rate as predicted by the Haney-Brinton CO<sub>2</sub>-burst method, Altavista soil.

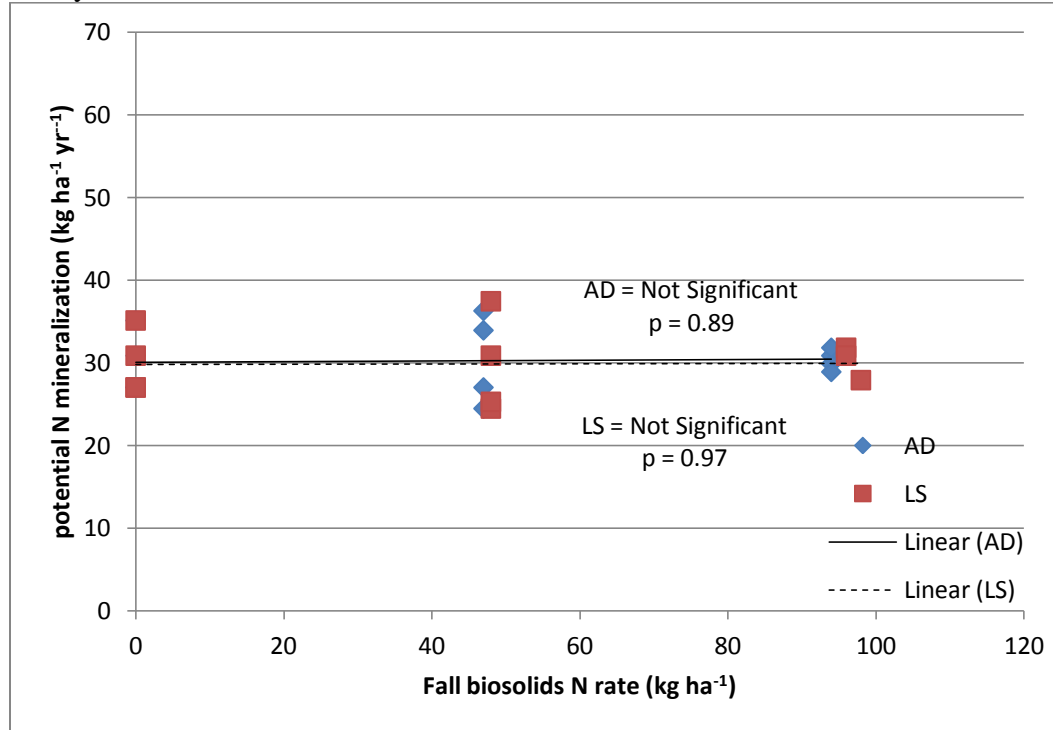


Figure 3.3b: Response of potential N mineralization to biosolids-N rate as predicted by the Haney-Brinton CO<sub>2</sub>-burst method, Bojac soil.

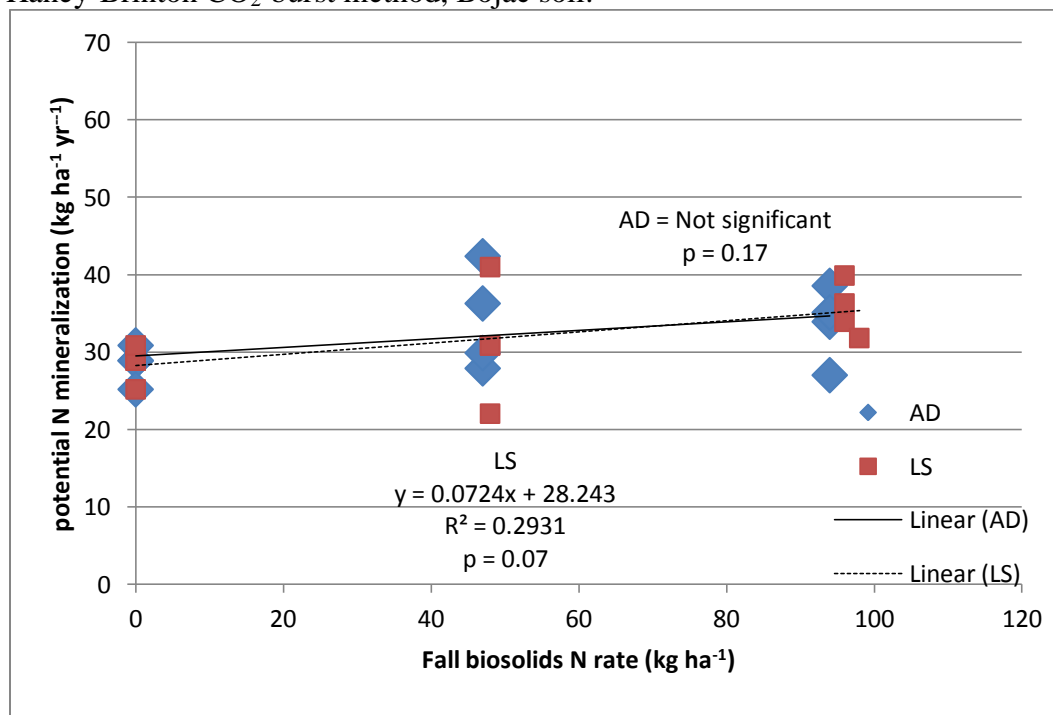




Figure 3.3c: Response of potential N mineralization to biosolids-N rate as predicted by the Haney-Brinton CO<sub>2</sub>-burst method, Emporia soil.

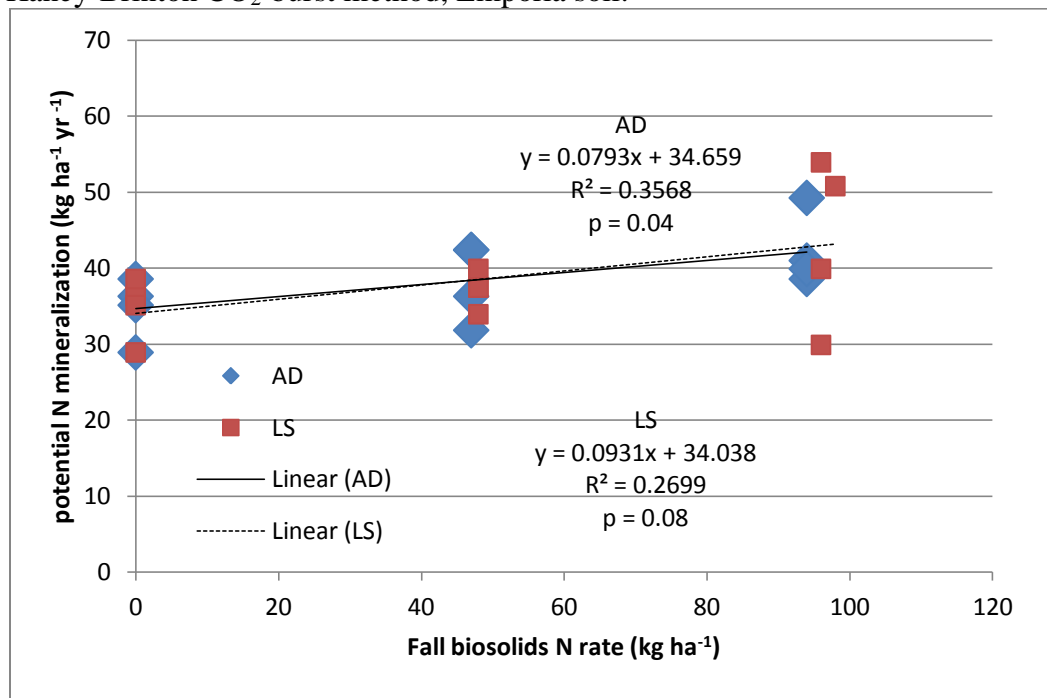


Figure 3.3d: Response of potential N mineralization to biosolids-N rate as predicted by the Haney-Brinton CO<sub>2</sub>-burst method, Kempsville soil.

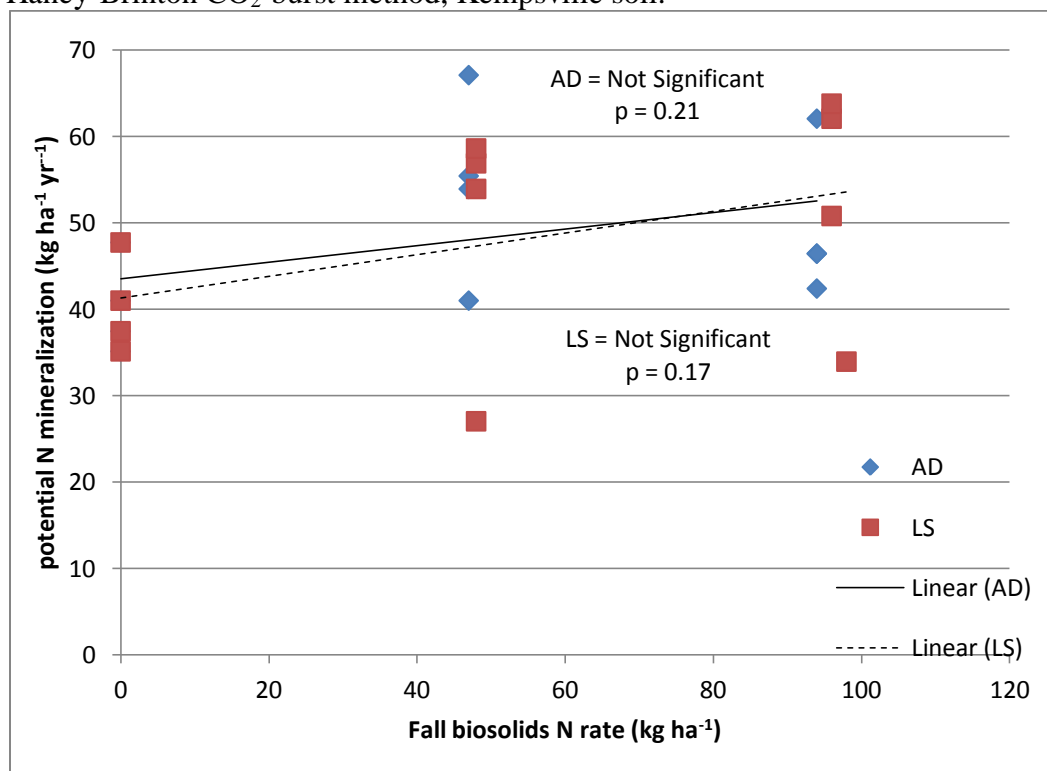


Figure 3.3e: Response of potential N mineralization to biosolids-N rate as predicted by the Haney-Brinton CO<sub>2</sub>-burst method, Roanoke soil.

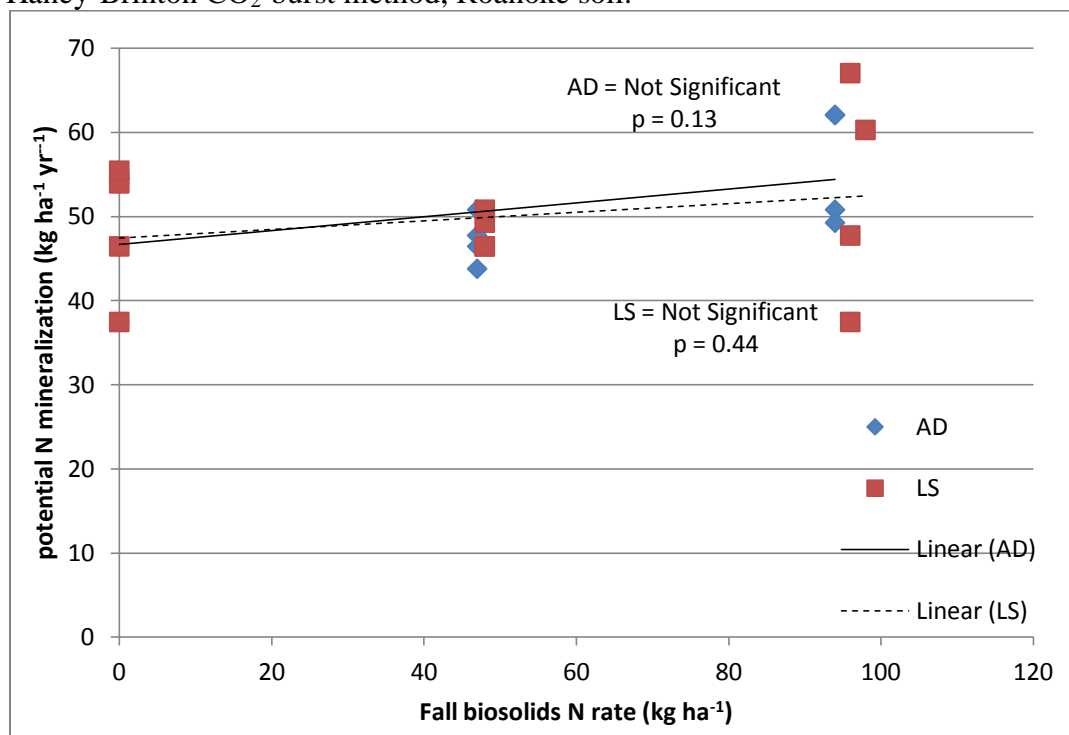


Figure 3.3f: Response of potential N mineralization to biosolids-N rate as predicted by the Haney-Brinton CO<sub>2</sub>-burst method, State soil.

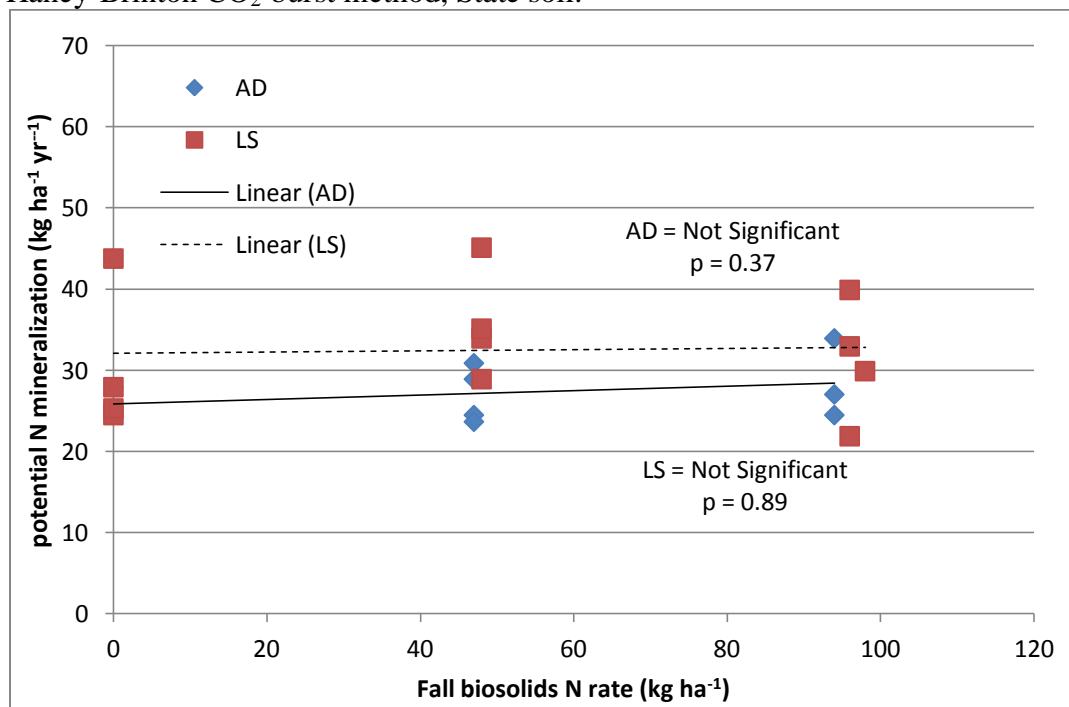


Figure 3.4a: The response of grain yield to potential N mineralization estimated by the Haney-Brinton CO<sub>2</sub>-burst for all soils receiving no N amendments.

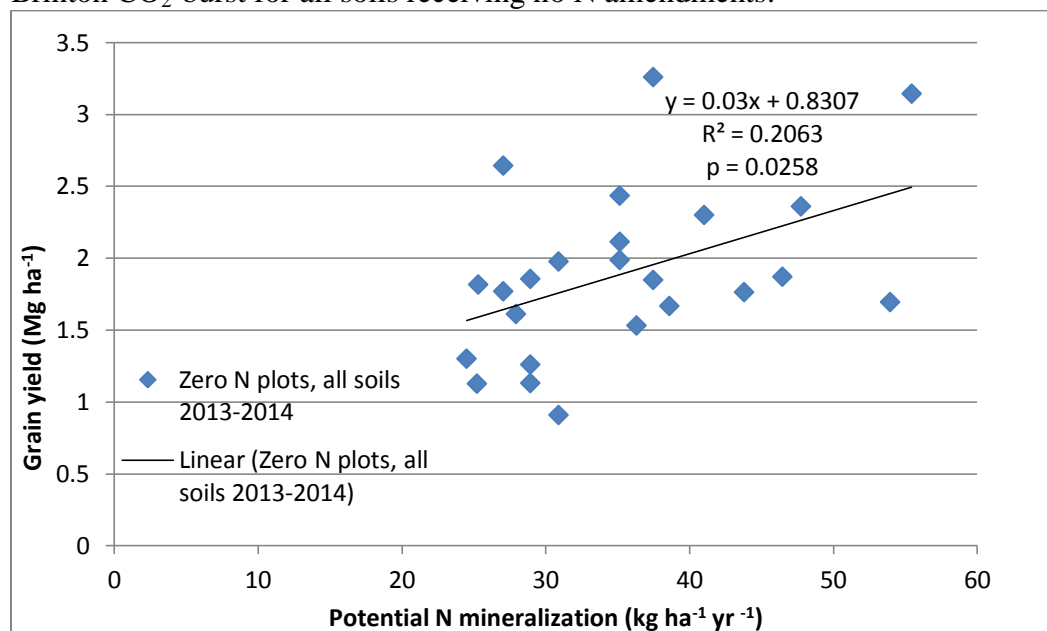


Figure 3.4b: The response of grain yield to pre-plant 2M KCl extractable soil inorganic N for all soils receiving no N amendments.

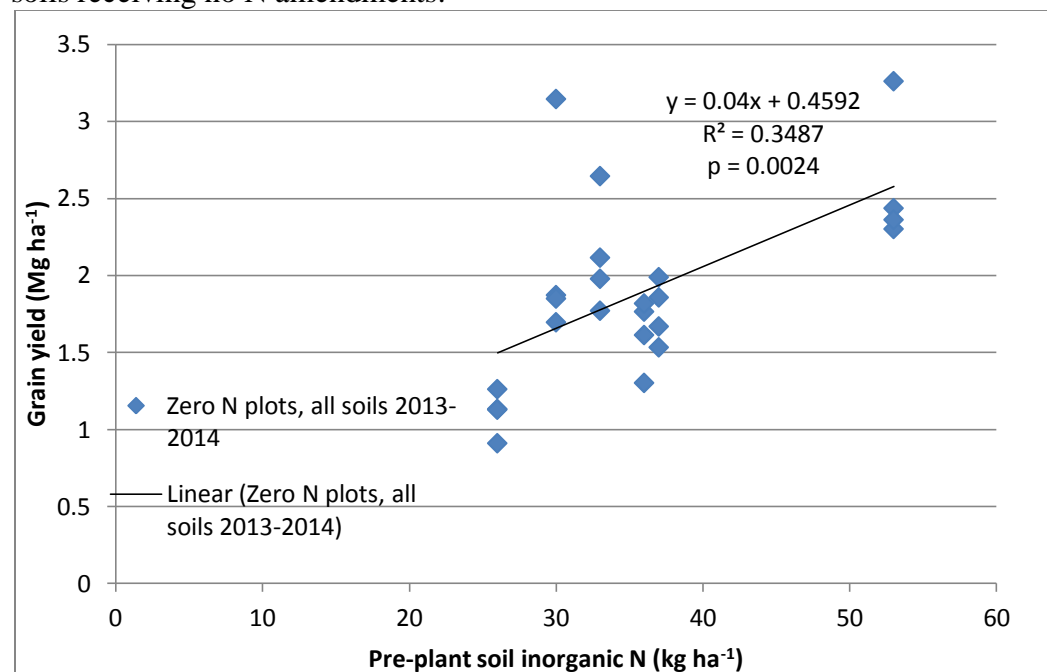
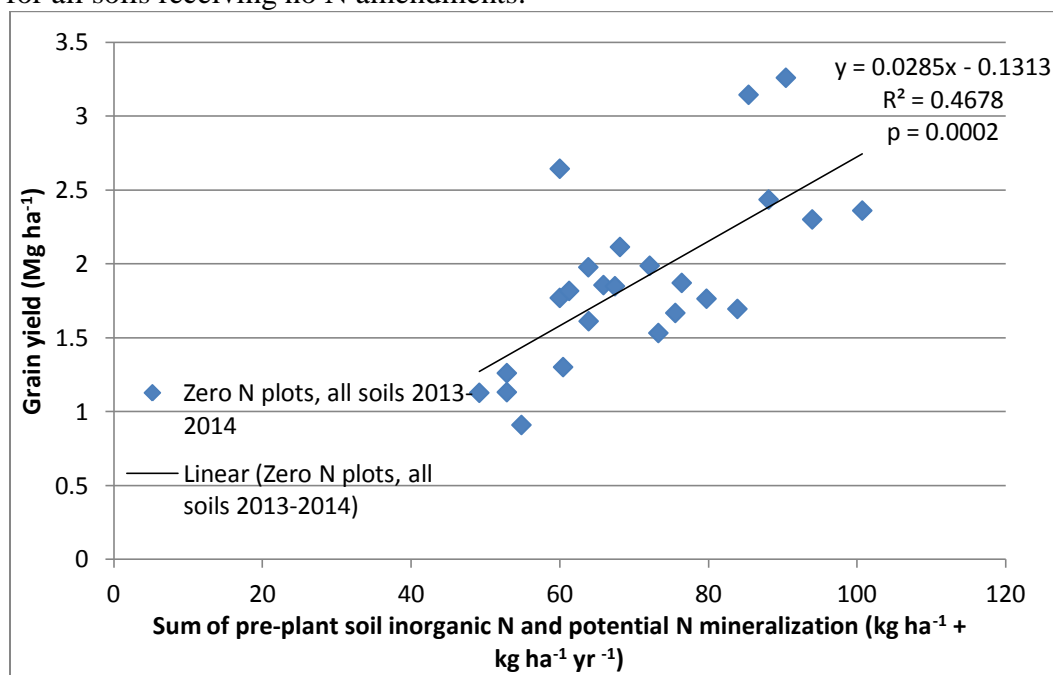


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Table 3.1: Description of soils analyzed for potential N mineralization by the Haney-Brinton CO<sub>2</sub> Burst method.

Soil Series	Soil family
Altavista	fine-loamy, mixed, semiactive, thermic Aquic Hapludults
Bojac	coarse-loamy, mixed, semiactive, thermic Typic Hapludults
Emporia	fine loamy, siliceous, subactive thermic Typic Hapludults
Kempsville	fine-loamy, siliceous, subactive thermic Typic Hapludults
Roanoke	fine, mixed, semiactive, thermic Typic Endoaquults
State	fine-loamy, mixed, semiactive, thermic Typic Hapludults

Table 3.2: Soil texture and pre-planting pH, C and N, 0-30cm depth.

Soil Series	Surface texture	Soil pH	Total C (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	Inorganic N (mg kg <sup>-1</sup> )	C:N
Altavista	Fine sandy loam	6.02	13.3	1.37	16	9.71
Bojac	Loamy fine sand	6.14	11.6	1.15	11	10.1
Emporia	Loamy fine sand	5.86	16.2	1.69	18	9.59
Kempsville	Fine sandy loam	6.77	21.1	1.99	26	10.6
Roanoke	Silt loam	6.38	18.7	1.95	15	9.62
State	Silt loam	6.32	18.0	1.88	17	9.58

Table 3.3: Fall 2013 biosolids analyses results from A&L Eastern Laboratories.

Treatment Process	TKN (g kg <sup>-1</sup> )	NH <sub>4</sub> – N (g kg <sup>-1</sup> )	Org. N (g kg <sup>-1</sup> )	pH
AD	55.5	15.2	40.3	8.49
LS	29.0	0.9	28.1	12.24

Table 3.4: Comparison of potential N mineralization estimated by the Haney-Brinton CO<sub>2</sub>-burst method and soil organic N mineralization by mass balance of pre-plant soil inorganic N, N uptake and residual soil inorganic N, zero N treatment.

Soil	Potential N Mineralization, Haney-Brinton CO <sub>2</sub> -burst method (kg ha <sup>-1</sup> yr <sup>-1</sup> )	Soil organic N mineralization estimated by mass balance (kg ha <sup>-1</sup> )
Altavista	33.5 ± 3.0	30.0 ± 3.9
Bojac	28.5 ± 2.4	25.4 ± 8.7
Emporia	34.7 ± 4.1	28.0 ± 13.0
Kempsville	40.3 ± 5.5	32.2 ± 7.7
Roanoke	48.3 ± 8.2	47.3 ± 10.0
State	30.4 ± 9.1	30.9 ± 7.3

N mineralization estimates followed by the same letter are not statistically different at the 0.10 significance level

Table 3.5: Expected winter wheat yield under standard and intensive management (VADCR, 2005) and mean observed grain yields in resulting from agronomic N rate application.

Soil	Productivity group	Expected grain yield, standard management (Mg ha <sup>-1</sup> )	Expected grain yield, intensive management (Mg ha <sup>-1</sup> )	Mean observed yield with agronomic N rate
Altavista	I	4.30	5.38	4.23
State				3.99
Bojac	II	3.76	4.70	2.82
Emporia				4.26
Kempsville				4.86
Roanoke	III	3.23	4.03	3.88

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## Chapter 4: Overall Conclusions

Winter wheat (*Triticum aestivum* L.) is a good option as a crop to receive biosolids for N fertilization in corn-wheat-soybean crop rotations commonly planted throughout the U.S. mid-Atlantic states due to its crop N needs. Current regulations permit fall applications of biosolids to supply starter N requirements, which typically meet only half the agronomic N need of winter small grains. Fall application of biosolids at the full agronomic N rate is prohibited to reduce the risk of winter N loss; however, the biosolids loading rates that supply 50% of winter small grains N needs are too low to be mechanically or economically feasible for biosolids applicators. Therefore, the underutilization of biosolids for winter small grains fertilization results in the nearly total dependence on inorganic fertilizer as the primary N source in Virginia.

The timing of N application and availability is less important than N rate in maximizing winter wheat grain yield and minimizing estimated N loss in fine and fine-loamy textured soils where N leaching loss risk is low. Therefore, anaerobically digested (AD) and lime stabilized (LS) biosolids can be fall applied at full agronomic N rates to soils with low N leaching loss risk in the Virginia Coastal Plain and Ridge & Valley physiographic provinces. Nitrogen rate, timing of N application, and timing of N availability are critical in increasing grain yield and N recovery in coarser textured soils where risk of leaching loss is moderate or high. The relatively slow rates of LS biosolids PAN supply increased grain yield and slowed the rate of N loss in these soils. Virginia Coastal Plain soils with moderate or high leaching loss risk should only receive fall full agronomic N rate applications of LS biosolids. Splitting the agronomic N rate between fall biosolids and spring urea benefits grain yield in coarse-loamy textured Coastal Plain soils but is not mechanically or economically feasible for biosolids applicators. Fall applications of full agronomic N rate LS biosolids are most beneficial to soft red winter wheat production in

Virginia for increasing N use efficiency, increasing grain yield, and reducing N leaching. Fall applications of full agronomic N rate AD biosolids are at least as efficient as split urea applications.

The utility of biosolids as an N source for winter wheat depends on the amount and timing of biosolids organic N mineralization. Predicting the amounts and rates of biosolids organic N mineralization has proven difficult. Long term incubations are the most common method of quantifying the release of N from a given biosolids product to specific soils. Faster methods of N mineralization estimation have great potential applications for winter wheat, given that early spring N availability and management is time sensitive and critical for optimizing grain yields.

The value of the Haney-Brinton CO<sub>2</sub>-burst method in predicting potential spring N mineralization to fall applied biosolids to winter wheat may be limited to soils that do not receive fall organic amendments, such as biosolids, managed under no-till. The Haney-Brinton CO<sub>2</sub>-burst method provided a quick in-season estimate of mineralizable N that would have enabled supplemental fertilizer N recommendations for winter wheat where no fall organic residuals were applied. Thus, the test method appears to have promise in aiding time sensitive spring N management decisions in winter wheat production with further investigation.

## Appendix A: Unit Conversions

### A.1: Plant Unit Conversions

1. Mid season tissue weights were collected and initially measured in g/m but are reported in kg ha<sup>-1</sup> biomass according to the following conversion:

$$(\text{tissue g/m row}) \times (5.24 \text{ row/m}) \times (1 \text{ kg tissue}/1000 \text{ g tissue}) \times (10000 \text{ m}^2 \text{ ha}^{-1}) = \text{kg tissue ha}^{-1}$$

2. GS 58 biomass weights were collected and initially measured in g/m<sup>2</sup> but are used with Vario MAX CNS macro elemental analyzer (Elementar, Hofnau, Germany) total N percentages to derive total plant N uptake by the following conversion:

$$\left( \frac{\{\text{subsample dry g/m}^2\}}{\{\text{subsample wet g/m}^2\}} \right) \times \text{sample wet g/m}^2 \times (1 \text{ kg tissue}/1000 \text{ g tissue}) \times (10000 \text{ m}^2 \text{ ha}^{-1}) \times (\% \text{ tissue N}/100\%) = \text{kg N ha}^{-1}$$

3. Grain weight (lb) recorded by the Kincaid 8XP small plot combine and grain moisture percentage measured by a Dickey-John GAC2000 grain sampler (DICKEY-john, Auburn, IL) were used to derive grain yield in Mg ha<sup>-1</sup> according to the following conversion:

$$\left( \frac{[43560 \text{ ft}^2/\text{ac}]}{\text{harvested ft}^2} \right) \times (\text{harvested lb}) \times \left( \frac{[100 - \text{grain moisture}]}{86.5} \right) \times \left( \frac{[1.12 \text{ kg ha}^{-1}]}{[\text{lb/ac}]} \right) \times (1 \text{ Mg}/1000 \text{ kg}) = \text{grain yield in Mg ha}^{-1}$$

### A.2: Soil Unit Conversions

1. The Vario MAX CNS macro elemental analyzer (Elementar, Hofnau, Germany) reported total N in percentages; total N is reported in mg/kg according to the following conversion:

$$(\% \text{ N mg}/100 \text{ mg soil}) \times (1000000 \text{ mg soil}/\text{kg soil}) = \text{mg N}/\text{kg soil}$$

2. The Lachat Flow Injection Analyzer reported  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  in mg/L:  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  are reported in  $\text{kg ha}^{-1}$  according to the following conversion:

$$(\text{Liquid N concentration}) \times (\text{extraction dilution factor}) \times (\text{soil bulk density}) \times (1 \text{ kg}/10^6 \text{ mg}) \times (10^6 \text{ dm}^2 \text{ ha}^{-1}) \times (\text{soil sample depth}) = \text{kg N ha}^{-1}$$

Or

$$(\text{mg N}_3 \text{ or } \text{NH}_4\text{-N/L}) \times (0.03 \text{ L KCl}/0.003 \text{ kg soil}) \times (\text{kg soil}/\text{dm}^3 \text{ soil}) \times (1 \text{ kg N}/10^6 \text{ mg N}) \times (10^6 \text{ dm}^2 \text{ ha}^{-1}) \times (\text{dm}) = \text{kg N}_3 \text{ or } \text{NH}_4\text{-N ha}^{-1}$$

## Appendix B: Comparisons of biomass, N uptake, and soil NO<sub>3</sub>-N and NH<sub>4</sub>-N

Table B-1.1: Mean 2M KCl extractable Soil Inorganic N at GS 25-30 to depth of 30 cm, 2012-2013\*

Soil	NO <sub>3</sub> -N (kg ha <sup>-1</sup> )	NH <sub>4</sub> -N (kg ha <sup>-1</sup> )	Inorganic N (kg ha <sup>-1</sup> )
Altavista	4.0	32.4	36.4
Bojac	17.7	42.4	60.1
Frederick-Christian	3.8	31.2	35.0
Frederick & Vertrees	16.5	39.7	56.2

\*no treatment effect in any soil

Table B-1.2: Midseason 2012-2013 and 2013-2014 biomass in kg ha<sup>-1</sup>

Soil	ALT		BOJ		EMP	FC	FV	KEM	ROA	STA
	2013	2014	2013	2014	2014	2013	2013	2014	2014	2014
Treatment										
AD 0.5	1598	514	974 c	644 abc	686	1011	540	498 bc	645 ab	524 a
AD 1.0	1902	660	1651 a	776 a	702	1100	681	671 a	728 a	613 a
LS 0.5	1677	440	1017 c	613 b	608	844	555	451 bcd	603 abc	503 ab
LS 1.0	1991	540	1273 b	954 a	650	1001	566	545 ab	681 ab	639 a
Urea 0	1200	351	393 f	414 cd	435	676	576	320 d	241 d	147 c
Urea 0.5	1226	424	451 ef	330 d	466	802	550	393 cd	383 cd	220 bc
Urea 1.0	1404	388	629 de	498 bc	482	802	555	440 bcd	503 abc	225 bc
Urea 1.5	1546	529	812 cd	367 d	503	838	613	424 bcd	440 bcd	403 ab

Table B-1.3: Midseason 2013-2014 Mean Plant N Uptake in kg N ha<sup>-1</sup>

Soil	Altavista	Bojac	Emporia	Kempsville	Roanoke	State
Treatment						
AD 0.5	12.6 ab	12.1 abcd	16.4 a	14.8 bc	14.9 abc	12.9 abc
AD 1.0	17.6 a	15.8 ab	16.8 a	23.0 a	16.6 ab	17.9 ab
LS 0.5	10.9 bc	11.0 bc	17.8 a	15.1 bc	15.4 abc	15.2 ab
LS 1.0	15.3 ab	24.3 a	18.1 a	18.3 ab	18.7 a	20.6 a
Urea 0	8.2 c	7.7 def	10.3 a	8.8 d	5.0 d	3.7 e
Urea 0.5	10.8 abc	5.6 f	11.6 a	12.0 cd	8.5 cd	5.4 de
Urea 1.0	10.2 bc	8.6 cde	10.3 a	13.0 cd	10.2 bcd	5.9 cde
Urea 1.5	13.7 ab	6.4 ef	11.3 a	11.6 cd	11.3 bcd	10.2 bc

N Uptake amounts followed by the same letter are not statistically different at the 0.05 significance level



Table B-1.4: Mean Grain yield (Mg ha<sup>-1</sup>), End-of-season 2012-2013 and 2013-2014

Soil	ALT		BOJ		EMP	FC	FV	KEM	ROA	STA
Year	2013	2014	2013	2014	2014	2013	2013	2014	2014	2014
Treatment										
AD 0.5 + Urea 0.5	5.06 a	4.37 ab	3.77 a	2.81 bc	4.11 bc	3.26	3.91 abc	5.00 ab	3.98 b	4.07 a
AD 1.0	4.32 a	4.17 ab	3.07 bcd	2.60 bcd	3.84 c	4.63	3.46 c	4.42 ab	3.45 bc	3.61 a
LS 0.5 + Urea 0.5	5.11 a	4.32 ab	3.64 ab	3.37 a	4.68 a	3.01	3.69 abc	5.13 ab	4.02 b	4.30 a
LS 1.0	4.78 a	4.54 a	2.90 cd	3.07 ab	4.81 a	3.99	3.36 c	5.15 ab	4.02 b	4.30 a
Urea 0	2.99 b	2.12 d	1.66 e	1.11 f	1.76 e	4.23	3.02 c	2.59 d	2.14 d	1.62 c
Urea 0.5	3.91 ab	3.22 cd	2.81 d	1.85 e	2.98 d	3.60	3.54 bc	3.75 c	2.93 c	2.64 b
Urea 1.0	4.38 a	3.75 bc	3.50 abc	2.27 de	3.87 c	3.55	4.71 ab	4.62 b	3.95 b	3.67 a
Urea 1.5	4.83 a	4.18 ab	3.44 abcd	2.39 cd	4.50 ab	3.91	4.89 a	5.37 a	4.88 a	4.25 a

Grain yields followed by the same letter are not statistically different at the 0.05 probability level

Table B-1.5: Wheat Lodging in Frederick-Christian Soil, End-of-season 2012-2013

Treatment	Total applied N (kg ha <sup>-1</sup> )	Lodging (% of plot)
AD 0.5 + Urea 0.5	98	50 ab
AD 1.0	98	25 b
LS 0.5 + Urea 0.5	88	55 ab
LS 1.0	88	45 ab
Urea 0	0	0 c
Urea 0.5	50	20 bc
Urea 1.0	100	55 ab
Urea 1.5	150	65 a

Yields or lodging percentages followed by the same letter are not statistically different at the 0.05 probability level

Table B-1.6: 2012-2013 Mean Grain Protein % (w/w)

Soil	Altavista	Bojac	Frederick-Christian	Frederick & Vertrees
Treatment				
AD 0.5 + Urea 0.5	10.4 cd	12.2 bcd	12.8 abc	15.4
AD 1.0	10.4 cd	11.8 d	12.4 abc	15.5
LS 0.5 + Urea 0.5	11.3 ab	12.7 bc	13.4 abc	15.1
LS 1.0	11.5 ab	12.5 bcd	13.5 a	15.6
Urea 0	10.0 d	12.1 bcd	11.8 c	14.8
Urea 0.5	10.2 cd	11.9 cd	12.3 abc	14.8
Urea 1.0	10.9 bc	12.8 ab	12.4 bc	14.8
Urea 1.5	11.9 a	13.6 a	13.6 ab	15.1

Grain protein percentages followed by the same letter are not statistically different at the 0.05 probability level

Table B-1.7: 2012-2013 Mean Grain Fat, Moisture and Starch

Soil	Fat % (w/w)	Moisture% (w/w)	Starch% (w/w)
Altavista	1.5	12.4	53.1
Bojac	1.0	10.1	51.7
Frederick-Christian	0.7	7.4	49.8
Frederick & Vertrees	1.1	8.6	49.3

Table B-1.8: 2013-2014 Mean Total N Uptake in kg ha<sup>-1</sup>

Soil	Altavista	Bojac	Emporia	Kempsville	Roanoke	State
Treatment						
AD 0.5 + Urea 0.5	70 ab	58 ab	62 bc	89 a	72 a	77 ab
AD 1.0	79 a	52 abc	70 bc	91 a	78 a	88 a
LS 0.5 + Urea 0.5	85 a	50 abc	77 ab	98 a	68 a	88 a
LS 1.0	84 a	67 a	87 a	103 a	61 a	91 a
Urea 0	38 c	19 d	32 d	38 b	37 a	31 c
Urea 0.5	52 bc	34 cd	42 d	57 b	52 a	56 b
Urea 1.0	76 a	40 bc	62 bc	97 a	63 a	84 a
Urea 1.5	80 a	42 bc	58 c	92 a	72 a	95 a

N Uptake amounts followed by the same letter are not statistically different at the 0.05 significance level

Table B-2: Midseason 2013-2014 Mean 2M KCl extractable Soil Inorganic N (kg ha<sup>-1</sup>) to depth of 30 cm, Altavista Soil

Treatment	NO <sub>3</sub> – N	NH <sub>4</sub> – N	Inorganic N
AD 0.5	3.8 c	5.7 abc	9.6 ab
AD 1.0	5.2 ab	5.5 a	10.7 a
LS 0.5	4.3 bc	3.7 c	7.9 b
LS 1.0	5.3 a	5.3 ab	10.6 a
Urea 0	3.7 c	4.7 abc	8.4 b
Urea 0.5	4.0 bc	4.0 bc	8.0 b
Urea 1.0	4.0 c	4.3 bc	8.3 b
Urea 1.5	3.7 c	5.5 ab	9.2 ab

N concentrations followed by the same letter are not statistically different at the 0.05 significance level

Table B-3: Midseason 2013-2014 Mean 2M KCl extractable Soil Inorganic N ( $\text{kg ha}^{-1}$ ) to depth of 30 cm, Bojac Soil

	$\text{NO}_3 - \text{N}$	$\text{NH}_4 - \text{N}$	Inorganic N
Treatment			
AD 0.5	1.6 bcd	4.5 ab	5.9 ab
AD 1.0	2.6 a	4.5 a	7.1 a
LS 0.5	2.2 ab	3.8 abc	5.9 abc
LS 1.0	2.0 abc	3.8 ab	5.8 ab
Urea 0	1.4 d	2.6 c	4.0 c
Urea 0.5	1.4 cd	3.2 bc	4.6 bc
Urea 1.0	1.4 d	4.4 a	5.5 abc
Urea 1.5	1.7 abcd	4.0 ab	5.7 ab

N concentrations followed by the same letter are not statistically different at the 0.05 significance level

Table B-4: Midseason 2013-2014 Mean 2M KCl extractable Soil Inorganic N ( $\text{kg ha}^{-1}$ ) to depth of 30 cm, Emporia Soil

	$\text{NO}_3 - \text{N}$	$\text{NH}_4 - \text{N}$	Inorganic N
Treatment			
AD 0.5	2.5 bc	6.9 a	9.4 abc
AD 1.0	3.1 b	9.6 a	12.7 ab
LS 0.5	3.2 b	5.4 a	8.6 b
LS 1.0	6.6 a	7.4 a	14.0 a
Urea 0	1.9 c	6.6 a	8.7 bc
Urea 0.5	2.8 bc	4.1 a	6.9 c
Urea 1.0	2.2 c	6.7 a	8.8 bc
Urea 1.5	3.2 bc	7.4 a	10.6 abc

N concentrations followed by the same letter are not statistically different at the 0.05 significance level

Table B-5: Midseason 2013-2014 Mean 2M KCl extractable Soil Inorganic N ( $\text{kg ha}^{-1}$ ) to depth of 30 cm, Kempsville Soil

	$\text{NO}_3 - \text{N}$	$\text{NH}_4 - \text{N}$	Inorganic N
Treatment			
AD 0.5	4.9 c	5.6 a	10.6 a
AD 1.0	10.3 a	5.7 a	16.0 a
LS 0.5	6.4 abc	7.4 a	13.8 a
LS 1.0	8.9 ab	5.1 a	14.1 a
Urea 0	5.4 bcd	6.4 a	11.8 a
Urea 0.5	5.6 bcd	4.9 a	10.5 a
Urea 1.0	5.2 bcd	5.8 a	10.9 a
Urea 1.5	3.6 d	5.0 a	8.6 a

N concentrations followed by the same letter are not statistically different at the 0.05 significance level

Table B-6: Midseason 2013-2014 Mean 2M KCl extractable Soil Inorganic N ( $\text{kg ha}^{-1}$ ) to depth of 30 cm, Roanoke Soil

	$\text{NO}_3 - \text{N}$	$\text{NH}_4 - \text{N}$	Inorganic N
Treatment			
AD 0.5	6.7 a	5.2 a	11.9 a
AD 1.0	4.9 a	5.7 a	10.6 a
LS 0.5	10.3 a	5.7 a	16.1 a
LS 1.0	8.4 a	5.1 a	13.5 a
Urea 0	2.1 a	3.2 a	5.3 b
Urea 0.5	5.5 a	4.9 a	10.3 ab
Urea 1.0	6.3 a	5.7 a	12.0 ab
Urea 1.5	9.4 a	4.7 a	8.1 b

N concentrations followed by the same letter are not statistically different at the 0.05 significance level

Table B-7: Midseason 2013-2014 Mean 2M KCl extractable Soil Inorganic N ( $\text{kg ha}^{-1}$ ) to depth of 30 cm, State Soil

	$\text{NO}_3 - \text{N}$	$\text{NH}_4 - \text{N}$	Inorganic N
Treatment			
AD 0.5	4.1 a	6.4 a	10.5 ab
AD 1.0	3.5 a	4.5 a	8.1 b
LS 0.5	4.9 a	5.9 a	10.7 ab
LS 1.0	3.0 a	7.4 a	10.4 abc
Urea 0	4.0 a	3.6 a	5.5 c
Urea 0.5	5.2 a	5.7 a	10.9 ab
Urea 1.0	5.9 a	6.0 a	11.8 a
Urea 1.5	5.6 a	6.8 a	12.5 a

N concentrations followed by the same letter are not statistically different at the 0.05 significance level

Table B-8: End-of-season 2012-2013 Mean Residual 2M KCl extractable Inorganic N Content in Altavista soil, 0 – 80 cm

Treatment	$\text{NO}_3\text{-N}$ ( $\text{kg ha}^{-1}$ )	$\text{NH}_4\text{-N}$ ( $\text{kg ha}^{-1}$ )	Inorganic N ( $\text{kg ha}^{-1}$ )
AD 0.5 + Urea 0.5	17.4 a	38.0 a	55.4 abc
AD 1.0	21.0 a	25.4 c	46.5 bcd
LS 0.5 + Urea 0.5	20.8 a	27.3 bc	48.1 bcd
LS 1.0	25.8 a	40.0 a	65.8 a
Urea 0	20.4 a	24.7 c	45.1 cd
Urea 0.5	19.7 a	34.8 ab	54.5 b
Urea 1.0	17.2 a	25.4 c	42.6 d
Urea 1.5	18.4 a	33.4 abc	51.8 abcd

Inorganic N contents followed by the same letter are not statistically different at the 0.05 probability level

Table B-9: End-of-season 2012-2013 Mean Residual 2M KCl extractable NO<sub>3</sub>-N (kg ha<sup>-1</sup>) in Altavista soil with depth

Treatment	0 – 20 cm	20 – 40 cm	40 – 60 cm	60 – 80 cm	total
AD 0.5 + Urea 0.5	9.8 a	3.0 a	2.7 a	1.9 a	17.4 a
AD 1.0	14.6 a	3.3 a	1.7 d	1.5 a	21.0 a
LS 0.5 + Urea 0.5	13.9 a	3.2 a	2.1 bcd	1.7 a	20.8 a
LS 1.0	18.1 a	3.0 a	2.8 ab	1.9 a	25.8 a
Urea 0	13.5 a	2.8 a	2.3 abc	2.1 a	20.4 a
Urea 0.5	13.4 a	2.9 a	1.9 cd	1.6 a	19.7 a
Urea 1.0	11.4 a	2.7 a	2.1 cd	1.1 b	17.2 a
Urea 1.5	11.2 a	2.5 a	2.4 abcd	2.2 a	18.4 a

NO<sub>3</sub>-N concentrations followed by the same letter are not statistically different at the 0.05 probability level

Table B-10: End-of-season 2012-2013 Mean Residual 2M KCl extractable NH<sub>4</sub>-N (kg ha<sup>-1</sup>) in Altavista soil with depth

Treatment	0 – 20 cm	20 – 40 cm	40 – 60 cm	60 – 80 cm	total
AD 0.5 + Urea 0.5	13.0 a	8.7 ab	8.1 ab	8.3 ab	38.0 a
AD 1.0	8.1 b	5.6 c	7.3 c	4.4 d	25.4 c
LS 0.5 + Urea 0.5	9.9 b	6.3 c	5.7 c	5.4 cd	27.3 bc
LS 1.0	14.1 a	9.5 a	8.4 a	8.0 a	40.0 a
Urea 0	8.5 b	6.3 bc	4.8 c	5.1 bcd	24.7 c
Urea 0.5	12.3 a	7.6 bc	8.0 ab	6.9 abcd	34.8 ab
Urea 1.0	8.4 b	6.6 abc	5.5 bc	4.8 cd	25.4 c
Urea 1.5	8.3 b	10.6 abc	5.9 c	8.6 abc	33.4 abc

NH<sub>4</sub>-N concentrations followed by the same letter are not statistically different at the 0.05 probability level

Table B-11: End-of-season 2012-2013 Mean Residual 2M KCl extractable Inorganic N Content in Bojac soil, 0 – 80 cm

Treatment	NO <sub>3</sub> -N (kg ha <sup>-1</sup> )	NH <sub>4</sub> -N (kg ha <sup>-1</sup> )	Inorganic N (kg ha <sup>-1</sup> )
AD 0.5 + Urea 0.5	29.7 d	12.6 bcd	40.0 d
AD 1.0	53.7 a	12.3 a	66.0 a
LS 0.5 + Urea 0.5	32.5 d	8.5 d	41.0 cd
LS 1.0	43.7 ab	8.8 cd	52.5 bc
Urea 0	49.8 abc	10.2 ab	60.0 ab
Urea 0.5	33.1 cd	9.1 bcd	42.1 cd
Urea 1.0	46.7 bc	11.6 a	58.3 ab
Urea 1.5	45.8 ab	10.6 abc	56.4 a

Inorganic N contents followed by the same letter are not statistically different at the 0.05 probability level

Table B-12: End-of-season 2012-2013 Mean Residual 2M KCl extractable NO<sub>3</sub>-N (kg ha<sup>-1</sup>) in Bojac soil with depth

Treatment	0 – 20 cm	20 – 40 cm	40 – 60 cm	60 – 80 cm	total
AD 0.5 + Urea 0.5	22.0 d	4.5 d	2.5 c	2.0 e	29.7 d
AD 1.0	37.3 a	6.7 a	3.9 a	5.8 a	53.7 a
LS 0.5 + Urea 0.5	21.2 d	6.0 bc	2.7 bc	2.8 cde	32.5 d
LS 1.0	29.0 bcd	7.6 ab	3.6 a	3.4 abc	43.7 ab
Urea 0	33.5 abc	7.8 abc	4.8 ab	3.7 ab	49.8 abc
Urea 0.5	22.6 cd	5.5 c	2.6 c	2.4 d	33.1 cd
Urea 1.0	30.0 abc	7.1 ab	5.0 abc	4.5 bcd	46.7 bc
Urea 1.5	32.0 ab	6.9 ab	3.6 abc	3.2 bcde	45.8 ab

NO<sub>3</sub>-N concentrations followed by the same letter are not statistically different at the 0.05 probability level

Table B-13: End-of-season 2012-2013 Mean Residual 2M KCl extractable NH<sub>4</sub>-N (kg ha<sup>-1</sup>) in Bojac soil with depth

Treatment	0 – 20 cm	20 – 40 cm	40 – 60 cm	60 – 80 cm	total
AD 0.5 + Urea 0.5	4.3 bcd	2.8 bc	3.3 a	2.2 abcd	12.6 bcd
AD 1.0	3.8 ab	3.1 a	3.0 a	2.4 a	12.3 a
LS 0.5 + Urea 0.5	3.3 de	1.9 c	1.7 a	1.6 e	8.5 d
LS 1.0	3.1 e	2.3 bc	1.8 a	1.5 de	8.8 cd
Urea 0	3.7 abc	2.1 bc	2.4 a	1.9 bc	10.2 ab
Urea 0.5	3.3 d	2.2 bc	1.7 a	1.9 abc	9.1 bcd
Urea 1.0	4.3 a	2.5 ab	2.5 a	2.5 ab	11.6 a
Urea 1.5	3.6 cd	2.1 bc	2.4 a	2.5 bcde	10.6 abc

NH<sub>4</sub>-N concentrations followed by the same letter are not statistically different at the 0.05 probability level

Table B-14: End-of-season 2012-2013 Mean Residual 2M KCl extractable Inorganic N Content in Frederick-Christian soil, 0 – 60 cm

Treatment	NO <sub>3</sub> -N (kg ha <sup>-1</sup> )	NH <sub>4</sub> -N (kg ha <sup>-1</sup> )	Inorganic N (kg ha <sup>-1</sup> )
AD 0.5 + Urea 0.5	126 bcd	7.9 a	134 bcd
AD 1.0	153 bc	6.2 a	159 bc
LS 0.5 + Urea 0.5	177 b	6.6 a	184 b
LS 1.0	214 a	6.8 a	221 a
Urea 0	105 e	5.9 a	111 e
Urea 0.5	112 de	6.5 a	119 de
Urea 1.0	131 cd	6.0 a	138 cd
Urea 1.5	155 bc	6.3 a	162 bc

Inorganic N contents followed by the same letter are not statistically different at the 0.05 probability level

Table B-15: End-of-season 2012-2013 Mean Residual 2M KCl extractable NO<sub>3</sub>-N (kg ha<sup>-1</sup>) in Frederick-Christian soil with depth

Treatment	0 – 20 cm	20 – 40 cm	40 – 60 cm	Total
AD 0.5 + Urea 0.5	108 bc	11.1 e	7.0 cd	126 bcd
AD 1.0	116 b	30.8 abc	5.5 d	153 bc
LS 0.5 + Urea 0.5	130 bcd	32.1 a	15.1 ab	177 b
LS 1.0	167 a	31.9 a	14.9 a	214 a
Urea 0	88 d	13.3 de	3.7 e	105 e
Urea 0.5	84 cd	18.9 cd	9.5 abc	112 de
Urea 1.0	95 bcd	27.0 ab	9.3 bc	131 cd
Urea 1.5	125 b	19.9 bcd	10.9 bc	155 bc

NO<sub>3</sub>-N concentrations followed by the same letter are not statistically different at the 0.05 probability level

Table B-16: End-of-season 2012-2013 Mean Residual 2M KCl extractable NH<sub>4</sub>-N (kg ha<sup>-1</sup>) in Frederick-Christian soil with depth

Treatment	0 – 20 cm	20 – 40 cm	40 – 60 cm	Total
AD 0.5 + Urea 0.5	4.4 a	1.8 a	1.7 a	7.9 a
AD 1.0	3.3 a	1.5 a	1.4 a	6.2 a
LS 0.5 + Urea 0.5	3.5 a	1.4 a	1.7 a	6.6 a
LS 1.0	3.7 a	1.5 a	1.6 a	6.8 a
Urea 0	2.9 a	1.7 a	1.4 a	5.9 a
Urea 0.5	3.3 a	1.7 a	1.6 a	6.5 a
Urea 1.0	3.1 a	1.5 a	1.5 a	6.0 a
Urea 1.5	3.3 a	1.3 a	1.6 a	6.3 a

NH<sub>4</sub>-N concentrations followed by the same letter are not statistically different at the 0.05 probability level

Table B-17: End-of-season 2012-2013 Mean Residual 2M KCl extractable NO<sub>3</sub>-N (kg ha<sup>-1</sup>) in Frederick-Vertrees soil with depth

Treatment	0 – 20 cm	20 – 40 cm	40 – 60 cm	Total
AD 0.5 + Urea 0.5	82.1 ab	13.0 a	9.2 a	104 abc
AD 1.0	69.0 b	14.9 a	13.0 a	96.9 abc
LS 0.5 + Urea 0.5	81.2 a	13.4 a	9.6 a	104 a
LS 1.0	65.0 b	12.2 a	4.2 a	81.4 c
Urea 0	54.9 c	10.0 b	4.3 a	69.2 d
Urea 0.5	71.9 b	14.2 a	4.0 a	90.1 b
Urea 1.0	67.5 b	15.2 a	4.3 a	87.0 bc
Urea 1.5	63.2 b	22.3 a	5.5 a	91.0 abc

NO<sub>3</sub>-N concentrations followed by the same letter are not statistically different at the 0.05 probability level

Table B-18: End-of-season 2012-2013 Mean Residual 2M KCl extractable  $\text{NH}_4\text{-N}$  ( $\text{kg ha}^{-1}$ ) in Frederick-Vertrees soil with depth

Treatment	0 – 20 cm	20 – 40 cm	40 – 60 cm	Total
AD 0.5 + Urea 0.5	4.4 cd	2.7 a	2.7 a	9.8 c
AD 1.0	5.2 a	2.9 a	2.8 a	11.1 ab
LS 0.5 + Urea 0.5	4.8 abcd	2.8 a	2.8 a	10.3 abc
LS 1.0	4.8 ab	2.6 a	2.7 a	10.1 bc
Urea 0	5.0 abc	3.1 a	3.4 a	11.5 a
Urea 0.5	5.1 a	2.7 a	2.8 a	10.7 b
Urea 1.0	4.5 bcd	2.9 a	2.7 a	10.2 abc
Urea 1.5	4.3 d	2.8 a	2.6 a	9.7 c

$\text{NH}_4\text{-N}$  concentrations followed by the same letter are not statistically different at the 0.05 probability level

Table B-19: End-of-season 2012-2013 Mean Residual 2M KCl extractable Inorganic N Content in Frederick & Vertrees soil, 0 – 60 cm

Treatment	$\text{NO}_3\text{-N}$ ( $\text{kg ha}^{-1}$ )	$\text{NH}_4\text{-N}$ ( $\text{kg ha}^{-1}$ )	Inorganic N ( $\text{kg ha}^{-1}$ )
AD 0.5 + Urea 0.5	104 abc	9.8 c	114 ab
AD 1.0	96.9 abc	11.1 ab	108 ab
LS 0.5 + Urea 0.5	104 a	10.3 abc	114 a
LS 1.0	81.4 c	10.1 bc	91.5 d
Urea 0	69.2 d	11.5 a	80.7 e
Urea 0.5	90.1 b	10.7 b	101 bc
Urea 1.0	87.0 bc	10.2 abc	97.2 bcd
Urea 1.5	91.0 abc	9.7 c	101 abcd

Inorganic N contents followed by the same letter are not statistically different at the 0.05 probability level

Table B-20: End-of-season 2013-2014 Mean 2M KCl extractable soil residual  $\text{NO}_3\text{-N}$  ( $\text{kg ha}^{-1}$ ) with depth, Altavista soil

Depth	0 – 30 cm	30 – 60 cm	60 – 90 cm	total
Treatment				
AD 0.5 + Urea 0.5	25.0 b	3.2 a	2.8 a	31.1 b
AD 1.0	24.3 b	3.7 a	2.3 a	30.3 b
LS 0.5 + Urea 0.5	28.5 ab	3.7 a	2.5 a	34.7 ab
LS 1.0	30.5 a	5.0 a	2.8 a	38.3 a
Urea 0	14.7 d	3.2 a	2.4 a	20.3 d
Urea 0.5	16.3 cd	3.2 a	2.1 a	21.7 cd
Urea 1.0	19.0 c	3.4 a	2.3 a	24.6 c
Urea 1.5	17.5 cd	3.0 a	2.6 a	22.7 cd

Residual  $\text{NO}_3\text{-N}$  concentrations followed by the same letter are not statistically different at the 0.05 significance level



Table B-21: End-of-season 2013-2014 Mean 2M KCl extractable soil residual  $\text{NH}_4\text{-N}$  ( $\text{kg ha}^{-1}$ ) with depth, Altavista soil

Depth	0 – 30 cm	30 – 60 cm	60 – 90 cm	total
Treatment				
AD 0.5 + Urea 0.5	13.7 a	10.8 a	11.4 a	35.3 a
AD 1.0	13.8 a	10.3 a	10.1 a	34.1 a
LS 0.5 + Urea 0.5	14.9 a	11.4 a	12.2 a	38.0 a
LS 1.0	15.1 a	10.5 a	10.1 a	35.7 a
Urea 0	13.4 a	12.7 a	12.0 a	38.1 a
Urea 0.5	12.3 a	13.0 a	13.5 a	38.8 a
Urea 1.0	13.5 a	12.2 a	11.8 a	36.9 a
Urea 1.5	13.2 a	13.4 a	10.6 a	37.2 a

Residual  $\text{NH}_4\text{-N}$  concentrations followed by the same letter are not statistically different at the 0.05 significance level

Table B-22: End-of-season 2013-2014 Mean 2M KCl extractable soil residual inorganic N ( $\text{kg ha}^{-1}$ ) with depth, Altavista soil

Depth	0 – 30 cm	30 – 60 cm	60 – 90 cm	total
Treatment				
AD 0.5 + Urea 0.5	38.8 ab	12.6 a	14.2 a	66.5 ab
AD 1.0	38.1 b	14.0 a	12.3 a	64.4 b
LS 0.5 + Urea 0.5	41.6 abc	15.1 a	14.7 a	73.1 ab
LS 1.0	45.7 a	15.5 a	12.9 a	74.1 a
Urea 0	28.2 cd	15.9 a	14.4 a	58.4 b
Urea 0.5	28.6 d	16.2 a	15.7 a	60.5 b
Urea 1.0	32.4 cd	15.6 a	14.1 a	61.5 b
Urea 1.5	30.7 cd	18.8 a	13.1 a	60.0 b

Residual inorganic N concentrations followed by the same letter are not statistically different at the 0.05 significance level

Table B-23: End-of-season 2013-2014 Mean 2M KCl extractable soil residual  $\text{NO}_3\text{-N}$  ( $\text{kg ha}^{-1}$ ) with depth, Bojac soil

Depth	0 – 30 cm	30 – 60 cm	60 – 90 cm	total
Treatment				
AD 0.5 + Urea 0.5	18.4 b	4.9 a	3.9 a	27.2 ab
AD 1.0	19.6 abc	5.1 a	3.0 a	27.7 ab
LS 0.5 + Urea 0.5	22.7 ab	6.5 a	3.8 a	32.9 a
LS 1.0	26.8 a	7.4 a	4.5 a	38.6 ab
Urea 0	18.3 abc	4.3 a	3.4 a	26.0 bc
Urea 0.5	13.1 c	3.4 a	2.9 a	19.4 c
Urea 1.0	19.5 ab	3.9 a	2.6 a	26.1 bc
Urea 1.5	18.8 ab	4.5 a	4.7 a	28.1 ab

Residual  $\text{NO}_3\text{-N}$  concentrations followed by the same letter are not statistically different at the 0.05 significance level

Table B-24: End-of-season 2013-2014 Mean 2M KCl extractable soil residual  $\text{NH}_4\text{-N}$  ( $\text{kg ha}^{-1}$ ) with depth, Bojac soil

Depth	0 – 30 cm	30 – 60 cm	60 – 90 cm	total
Treatment				
AD 0.5 + Urea 0.5	10.9 a	9.2 a	11.3 a	31.4 a
AD 1.0	12.0 a	8.3 a	11.4 a	31.7 a
LS 0.5 + Urea 0.5	12.5 a	8.3 a	9.3 a	30.1 a
LS 1.0	13.3 a	10.5 a	10.3 a	34.1 a
Urea 0	12.4 a	8.9 a	9.7 a	31.0 a
Urea 0.5	14.3 a	8.8 a	9.5 a	32.6 a
Urea 1.0	11.8 a	9.4 a	10.3 a	31.6 a
Urea 1.5	14.9 a	11.2 a	9.1 a	35.2 a

Residual  $\text{NH}_4\text{-N}$  concentrations followed by the same letter are not statistically different at the 0.05 significance level

Table B-25: End-of-season 2013-2014 Mean 2M KCl extractable soil residual inorganic N ( $\text{kg ha}^{-1}$ ) with depth, Bojac soil

Depth	0 – 30 cm	30 – 60 cm	60 – 90 cm	total
Treatment				
AD 0.5 + Urea 0.5	29.2 bc	14.0 a	15.3 a	58.6 bc
AD 1.0	31.6 bc	13.4 a	14.4 a	59.4 b
LS 0.5 + Urea 0.5	35.2 ab	14.8 a	13.0 a	63.0 ab
LS 1.0	40.1 a	17.8 a	14.8 a	72.8 a
Urea 0	30.6 bc	13.3 a	13.1 a	57.0 bc
Urea 0.5	27.3 c	12.2 a	12.4 a	52.0 c
Urea 1.0	31.3 bc	13.4 a	12.9 a	57.6 bc
Urea 1.5	33.8 b	15.7 a	13.8 a	63.3 abc

Residual inorganic N concentrations followed by the same letter are not statistically different at the 0.05 significance level

Table B-26: End-of-season 2013-2014 Mean 2M KCl extractable soil residual  $\text{NO}_3\text{-N}$  ( $\text{kg ha}^{-1}$ ) with depth, Emporia soil

Depth	0 – 30 cm	30 – 60 cm	60 – 90 cm	total
Treatment				
AD 0.5 + Urea 0.5	12.6 b	1.9 b	4.3 a	18.9 b
AD 1.0	21.6 a	1.9 b	3.4 a	26.9 a
LS 0.5 + Urea 0.5	20.1 a	2.7 a	2.1 a	24.5 a
LS 1.0	17.7 a	1.8 c	2.4 a	21.9 a
Urea 0	18.7 ab	2.6 ab	4.0 a	25.3 ab
Urea 0.5	19.0 ab	2.0 b	2.5 a	23.6 ab
Urea 1.0	14.1 b	1.8 b	2.0 a	17.9 b
Urea 1.5	17.3 ab	1.9 b	4.7 a	24.0 ab

Residual  $\text{NO}_3\text{-N}$  concentrations followed by the same letter are not statistically different at the 0.05 significance level

Table B-27: End-of-season 2013-2014 Mean 2M KCl extractable soil residual  $\text{NH}_4\text{-N}$  ( $\text{kg ha}^{-1}$ ) with depth, Emporia soil

Depth	0 – 30 cm	30 – 60 cm	60 – 90 cm	total
Treatment				
AD 0.5 + Urea 0.5	15.9 a	9.2 a	8.5 a	33.6 a
AD 1.0	14.9 a	10.2 a	8.4 a	33.5 a
LS 0.5 + Urea 0.5	25.2 a	11.2 a	9.7 a	45.1 a
LS 1.0	14.4 a	10.2 a	10.4 a	35.0 a
Urea 0	14.2 a	11.5 a	8.5 a	34.3 a
Urea 0.5	16.3 a	9.9 a	8.7 a	34.9 a
Urea 1.0	17.8 a	9.3 a	7.9 a	35.1 a
Urea 1.5	15.6 a	11.4 a	8.5 a	35.5 a

Residual  $\text{NH}_4\text{-N}$  concentrations followed by the same letter are not statistically different at the 0.05 significance level

Table B-28: End-of-season 2013-2014 Mean 2M KCl extractable soil residual inorganic N ( $\text{kg ha}^{-1}$ ) with depth, Emporia soil

Depth	0 – 30 cm	30 – 60 cm	60 – 90 cm	total
Treatment				
AD 0.5 + Urea 0.5	28.6 a	11.1 a	12.8 a	52.5 a
AD 1.0	35.5 a	12.1 a	11.8 a	60.5 a
LS 0.5 + Urea 0.5	45.3 a	13.9 a	11.8 a	69.6 a
LS 1.0	32.1 a	12.0 a	12.9 a	57.0 a
Urea 0	23.9 a	14.2 a	12.5 a	59.5 a
Urea 0.5	35.3 a	11.9 a	11.2 a	58.5 a
Urea 1.0	31.9 a	11.2 a	9.9 a	53.0 a
Urea 1.5	33.0 a	13.3 a	13.2 a	59.5 a

Residual inorganic N concentrations followed by the same letter are not statistically different at the 0.05 significance level

Table B-29: End-of-season 2013-2014 Mean 2M KCl extractable soil residual  $\text{NO}_3\text{-N}$  ( $\text{kg ha}^{-1}$ ) with depth, Kempsville soil

Depth	0 – 30 cm	30 – 60 cm	60 – 90 cm	total
Treatment				
AD 0.5 + Urea 0.5	30.0 ab	2.4 a	1.8 a	27.2 bcd
AD 1.0	23.8 bcd	2.5 a	1.8 a	28.1 abcd
LS 0.5 + Urea 0.5	20.8 d	2.0 a	1.9 a	24.7 d
LS 1.0	37.1 ab	2.4 a	1.7 a	41.7 ab
Urea 0	30.5 a	2.1 a	1.7 a	34.4 a
Urea 0.5	26.8 ab	2.2 a	2.4 a	31.3 ab
Urea 1.0	21.5 cd	2.2 a	1.6 a	25.3 cd
Urea 1.5	23.9 bc	2.6 a	1.8 a	28.3 bc

Residual  $\text{NO}_3\text{-N}$  concentrations followed by the same letter are not statistically different at the 0.05 significance level

Table B-30: End-of-season 2013-2014 Mean 2M KCl extractable soil residual  $\text{NH}_4\text{-N}$  ( $\text{kg ha}^{-1}$ ) with depth, Kempsville soil

Depth	0 – 30 cm	30 – 60 cm	60 – 90 cm	total
Treatment				
AD 0.5 + Urea 0.5	15.6 a	10.4 a	8.5 c	34.4 b
AD 1.0	19.0 a	14.5 a	10.9 ab	44.4 a
LS 0.5 + Urea 0.5	18.9 a	10.1 a	9.9 abc	38.9 ab
LS 1.0	16.0 a	11.6 a	11.0 ab	38.6 ab
Urea 0	16.9 a	8.6 a	8.6 c	34.0 b
Urea 0.5	15.6 a	9.9 a	11.8 a	36.9 b
Urea 1.0	16.6 a	10.2 a	9.1 bc	35.9 b
Urea 1.5	14.2 a	9.8 a	9.2 bc	33.1 b

Residual  $\text{NH}_4\text{-N}$  concentrations followed by the same letter are not statistically different at the 0.05 significance level

Table B-31: End-of-season 2013-2014 Mean 2M KCl extractable soil residual inorganic N ( $\text{kg ha}^{-1}$ ) with depth, Kempsville soil

Depth	0 – 30 cm	30 – 60 cm	60 – 90 cm	total
Treatment				
AD 0.5 + Urea 0.5	45.6 abc	12.8 a	10.4 a	62.2 c
AD 1.0	42.7 abc	17.0 a	12.7 a	72.5 ab
LS 0.5 + Urea 0.5	39.7 c	12.1 a	11.8 a	63.6 bc
LS 1.0	53.1 ab	14.0 a	12.7 a	79.8 a
Urea 0	47.4 a	10.7 a	10.3 a	68.4 bc
Urea 0.5	42.4 bc	12.1 a	14.0 a	68.2 bc
Urea 1.0	38.1 bc	12.4 a	10.8 a	61.2 c
Urea 1.5	38.0 c	12.4 a	10.9 a	61.4 c

Residual inorganic N concentrations followed by the same letter are not statistically different at the 0.05 significance level

Table B-32: End-of-season 2013-2014 Mean 2M KCl extractable soil residual  $\text{NO}_3\text{-N}$  ( $\text{kg ha}^{-1}$ ) with depth, Roanoke soil

Depth	0 – 30 cm	30 – 60 cm	total
Treatment			
AD 0.5 + Urea 0.5	27.3 a	2.4 a	29.1 ab
AD 1.0	23.9 ab	2.5 a	26.4 abc
LS 0.5 + Urea 0.5	27.7 a	2.0 a	29.8 a
LS 1.0	28.2 a	2.4 a	30.6 a
Urea 0	19.6 bc	2.1 a	21.7 c
Urea 0.5	20.6 bc	2.2 a	22.7 bc
Urea 1.0	22.7 abc	2.2 a	24.9 abc
Urea 1.5	17.8 c	2.6 a	20.5 c

Residual  $\text{NO}_3\text{-N}$  concentrations followed by the same letter are not statistically different at the 0.05 significance level

Table B-33: End-of-season 2013-2014 Mean 2M KCl extractable soil residual  $\text{NH}_4\text{-N}$  ( $\text{kg ha}^{-1}$ ) with depth, Roanoke soil

Depth	0 – 30 cm	30 – 60 cm	total
Treatment			
AD 0.5 + Urea 0.5	17.7 a	13.7 a	31.3 b
AD 1.0	20.1 a	16.6 a	36.7 ab
LS 0.5 + Urea 0.5	21.2 a	12.9 a	34.1 abc
LS 1.0	17.2 a	13.0 a	30.2 c
Urea 0	20.3 a	13.9 a	34.2 abc
Urea 0.5	17.7 a	12.8 a	30.5 c
Urea 1.0	21.1 a	14.6 a	36.1 a
Urea 1.5	20.1 a	16.8 a	37.0 abc

Residual  $\text{NH}_4\text{-N}$  concentrations followed by the same letter are not statistically different at the 0.05 significance level

Table B-34: End-of-season 2013-2014 Mean 2M KCl extractable soil residual inorganic N ( $\text{kg ha}^{-1}$ ) with depth, Roanoke soil

Depth	0 – 30 cm	30 – 60 cm	total
Treatment			
AD 0.5 + Urea 0.5	44.8 a	16.0 bc	60.0 a
AD 1.0	44.0 ab	19.1 ab	63.1 a
LS 0.5 + Urea 0.5	48.9 a	14.9 c	63.8 a
LS 1.0	45.4 ab	15.4 bc	60.8 a
Urea 0	39.9 b	16.0 bc	55.9 a
Urea 0.5	38.2 b	15.0 c	53.2 a
Urea 1.0	42.3 ab	16.8 abc	59.4 a
Urea 1.5	38.0 b	19.4 a	57.4 a

Residual inorganic N concentrations followed by the same letter are not statistically different at the 0.05 significance level

Table B-35: End-of-season 2013-2014 Mean 2M KCl extractable soil residual inorganic N ( $\text{kg ha}^{-1}$ ) 0 – 30 cm, State soil

	$\text{NO}_3 - \text{N}$	$\text{NH}_4 - \text{N}$	Inorganic N
Treatment			
AD 0.5 + Urea 0.5	19.8 cd	13.3 a	32.1 d
AD 1.0	28.5 ab	16.3 a	44.8 ab
LS 0.5 + Urea 0.5	30.5 ab	18.7 a	49.1 abc
LS 1.0	39.8 a	20.2 a	60.1 a
Urea 0	22.8 bc	13.3 a	36.1 bcd
Urea 0.5	16.3 d	15.1 a	31.4 cd
Urea 1.0	16.8 cd	16.3 a	33.1 cd
Urea 1.5	19.8 cd	16.1 a	35.0 cd

Residual inorganic N concentrations followed by the same letter are not statistically different at the 0.05 significance level