ABSTRACT

DODD, DANNY RYAN. Laboratory Analyses to Estimate Plant-Available Nitrogen in Land-Applied Biosolids. (Under the direction of Dr. Jeffrey G. White).

Nitrogen availability coefficients (NAC) are used to determine appropriate rates for application of municipal biosolids to crop land by estimating the percentage of total N that will become available to plants the first year. The basis and origin of the current North Carolina Department of Agriculture and Consumer Services (NCDA&CS) NAC are not well known. They may have been derived for sewage sludge rather than for biosolids conforming to USEPA water-quality rules promulgated in 1993. In addition, the coefficients only account for differences in some wastewater treatment methods and not for varying soil types nor application rates. This may lead to improper fertilization with adverse agronomic, economic, and environmental consequences. The objectives of this study were to: 1) evaluate three laboratory methods to estimate plant-available N (PAN) and NAC from three contrasting regional biosolids (BS) compared to NH_4NO_3 (AN): Cary pellet (CP), Raleigh plus (R+), and Orange Water and Sewer Authority (OWASA) cake (OWC); 2) determine whether PAN and NAC depend on soil type and application rate by applying these N sources at five rates to representative North Carolina soils, two from both the Piedmont and Coastal Plain; and 3) compare the tests among themselves and to the results of a field trial. The laboratory tests included a short-term (7 d) anaerobic incubation, the Amino-Sugar N Test (ASNT), and a long-term (112 d) aerobic incubation. The field test compared CP and AN applied to tall fescue (*Festuca arundinacea*). Biosolids were applied at 0, 0.5, 1.0, 1.5, and 2.0 times the realistic yield expectation rate for fescue on a Wedowee sandy loam soil. All tests used the appropriate NCDA&CS NAC for a broadcast application: 30, 17, and 28% for

CP, OWC, and R+, respectively; AN was assumed 100% plant available. Cary pellet contained more total N (65 g kg^{-1}) than OWC (49 g kg^{-1}) and R+ (7 g kg^{-1}). In the anaerobic incubation, CP and OWC yielded similar concentrations of N across all four soils with greater concentrations than AN in the Noboco and Wedowee soils. Anaerobic N from R+ was consistently lower than the other N sources. Average anaerobic incubation total inorganic N recoveries for AN, CP, OWC, and R+ were 73, 37, 25, and 15%, respectively, but varied among soils and rates. Average anaerobic PAN recoveries for AN, CP, OWC, and R+ were 73, 125, 130, and 50%, respectively. According to this incubation, NAC for CP and OWC underestimated PAN and overestimated for R+. The magnitudes of the differences were judged agronomically important. The OWC ASNT-N was substantially higher than the other N sources and the same for CP and AN across all soils. Raleigh+ ASNT-N was lower than all other sources. Aerobic incubation N from AN across most soils was similar to CP, substantially greater than R+, and slightly lower than OWC. The field trial showed no N source X PAN rate interaction, but there were statistically significant main effects of those factors. Fescue yield, N content and uptake, and apparent N recovery increased with PAN rate. Fescue yield from CP was 800 kg ha^{-1} more than AN, and N concentration was 0.25 percentage points greater. Nitrogen uptake from CP was 27 kg ha^{-1} higher than AN. Apparent N recovery from CP was greater than AN by 16 percentage points. Field test results suggested that the NAC for CP underestimated PAN. From the results of all the laboratory tests and the field trial, we conclude that soil type and biosolids rate can affect biosolids N mineralization such that existing NAC could not consistently estimate PAN. The magnitudes of some of the differences observed were judged agronomically important.

Laboratory Analysis to Estimate Plant-Available Nitrogen in Land-Applied Biosolids

by
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BIOGRAPHY

Danny ‘Ryan’ Dodd was born in raised in mountains of Asheville, NC. He obtained his B.S. in Environmental/Earth Science from UNC Asheville in 2008. He began a M.S. program in Soil Science and minor in GIS at NC State in January 2010. Throughout his time as an undergraduate and before moving to Raleigh, Ryan worked as a forestry technician at the Bent Creek Experimental Forest, a research support staff member at the Mountain Horticultural Crops Research and Extension Center in Fletcher, NC, and as white water raft guide on the French Broad River in Hot Springs, NC. Ryan’s professional interests include: soil fertility, waste management, conservation, forestry, and related applications of GIS for each. In his increasingly smaller amount of personal time, he enjoys long distance backpacking, white water boating, playing and recording drums and bass, gardening, computers, animals, and the Green Bay Packers (National Football League team).

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CHAPTER 1: RESEARCH INTRODUCTION AND LITERATURE REVIEW

1.0 Research Focus

Biosolids are nutrient rich, solid, semisolid, or liquid remains of treated municipal sewage sludge. Land applying biosolids at an agronomically correct rate in order to meet the nitrogen (N) requirement of a crop and to minimize water pollution by leaching of nitrate (NO_3) is essential to proper biosolids management. Information regarding the actual amount of N mineralized from biosolids is not well documented. The North Carolina Department of Agriculture and Consumer Services (NCDA&CS) currently provides different N availability coefficients to estimate the percentage of total N that will become available to plants during the first year following land application of biosolids that have been produced using different treatment (composted, aerobic, anaerobic, lime stabilized, etc.) and application (broadcast, incorporate, injected, irrigated) methods. The coefficients used in this research ranged from 0.17 for anaerobically treated municipal biosolids, to 0.30 for "other" municipal biosolids. Broadcast was chosen for the application method for all biosolids (McGinnis et al., 2011). However, the derivation of these coefficients is not well understood and they only account for some sludge treatment methods and not differences between soil types. These distinctions may have substantial impacts on N mineralization of land-applied biosolids due to the diversity of North Carolina receiving soils. In addition, the origins of the coefficients are not documented and appear to predate current institutional memory of the NCDA&CS.

As a result of the lack of documentation supporting the currently used coefficients, further evaluation of N mineralization of three different regional biosolids on four different representative soils at five rates was compared to NH_4NO_3 using three different laboratory

tests commonly used to estimate plant-available N (PAN). Additionally, a growth response field trial was conducted. The research was intended to either provide supporting evidence for existing N availability coefficients, or suggest potential modification of them if needed. In addition, the research evaluated the practicality of using one or all of the N tests studied for rapid routine estimation of plant available N in biosolids. Results from the three laboratory tests were compared with a view toward determining which among them was best at estimating PAN of land-applied municipal biosolids.

1.1 Hypotheses

1. Different biosolids will mineralize differently on different soils and when applied at different rates, which will be reflected in laboratory tests
2. The different laboratory tests correlate with each other and can be used to estimate biosolids PAN in the field

1.2 Overall Objectives

1. Test two biological incubations and one laboratory procedure to predict N mineralization from different biosolids applied to different soils at different rates
2. Determine if laboratory assays provide better estimates of biosolids PAN than established availability coefficients
3. Determine the practicality of these tests for routine estimation of biosolids PAN
4. Test crop response to mineralization of biosolids in the field

LITERATURE REVIEW

1.3 Background

Human waste management is an issue that has spanned all societies throughout civilization. Community waste management was originally very simple due to low population densities, and surrounding land or waterways were capable of dealing with the low volume of material that was being applied. However, increasing populations led to larger volumes of waste being produced, and it became unreasonable and inconvenient to simply apply the material to agricultural land. The fundamental methods of disposal included burying, burning, and placement in water bodies (Fahm, 1980). The majority of waste produced was dumped into streams and rivers in an attempt to remove it from the community. Some communities, such as the Indians, Chinese, Japanese, and early Greeks and Romans developed sophisticated methods of using their waste as a valuable soil amendment to increase agricultural production several millennia ago. However, in modern society, disposal of waste into local water bodies, landfills, and by incineration continued in thousands of cities across the United States until the early 1970's when the Federal Water Pollution Control Amendments of 1972 (Clean Water Act) was passed. This amendment significantly reduced the amounts of toxic substances being released into water as sanitary sewer systems were developed and implemented. As knowledge continues to grow, methods to collect, treat, and properly dispose of human waste are being developed to protect water quality and ultimately human health (Fahm, 1980), and greater emphasis is being placed upon land application of the byproducts of waste water treatment. As a result, a thorough

understanding of the consequences of land application of municipal waste water products is needed.

1.4 Biosolids Use

The by-product of treatment of raw sewage is known as sewage sludge or "sludge." From this sludge a product called "biosolids" can be produced through various wastewater treatment methods. Biosolids are nutrient rich, solid, semisolid, or liquid remains of treated municipal sewage sludge. They are differentiated from sewage sludge in that they have been treated to meet land application standards in the Part 503 United States Environmental Protection Agency (USEPA) regulations (United States Environmental Protection Agency, 1994) promulgated in 1993. The term biosolids was created in 1991 by the Name Change Task Force of the Water Environment Federation (WEF), formerly known as the Federation of Sewage Works Associations. This was done to differentiate between raw, untreated sludge from treated and tested sewage sludge that was capable of being used as a beneficial soil amendment. The term "sludge" generally had a negative connotation to the general public and the name change was an attempt to rebrand the product. Over 250 names were suggested, but biosolids won the vote (Sludge News, 2012). Although the name was adopted by the waste water treatment industry and does not appear in the Part 503 regulations, it is now commonly used by the USEPA (USEPA, 2009) and other Government regulatory agencies. The North Carolina Department of Environment and Natural Resources (NCDENR) Division of Water Quality (DWQ) and the USEPA set standards for managing biosolids and defined three classes of biosolids quality. Class B biosolids can be applied to

land only on sites that have received permits from the State. Class A biosolids have very low levels of metals and pathogens, can be applied to land without a permit or used to help grow crops for human consumption and by the general public in gardens, landscaping, etc. Exceptional Quality (EQ) biosolids are of the highest quality. They are class A biosolids that meet more stringent limits on metals. They are a useful soil amendment. Approximately 5.3 to 7.7 million metric tons (dry weight) of biosolids are produced per year by publicly owned treatment plants in the United States, and significant increases are expected in the next several decades (National Research Council, 1996), (Parr, J.F and Hormick, S.B., 1993), (Chenxi et al., 2008). Approximately 60% of the biosolids produced are being land applied (Environmental Protection Agency: Municipal and Industrial Solid Waste Division, 1999). Finding and developing mutually beneficial uses for this material for both the wastewater treatment plants and the public is a top priority for the waste management industry.

Biosolids are a source of macro- and micronutrients essential to crop growth. Many products contain high amounts of nitrogen (N), phosphorus (P), and potassium (K) and can be used as a substitute for commercial fertilizers. Although biosolids vary widely in chemical, biological, and physical properties depending on the source of the material, treatment, and handling methods, there is likely to be value in most products produced (Oberle and Keeney, 1994). Also, additions of biosolids into the soil increases soil organic matter content, thus improving soil structure, pH, buffering capacity, and inherent fertility (Stevenson and Cole, 1999). It is possible that crop land that has been taken out of production due to high erodibility and planted in perennial grasses and trees would greatly benefit from application of biosolids by enhancing their productivity and protecting them

from further degradation. After a period of remediation, some of these lands may be brought back into an acceptable level of crop production (Parr, J.F and Hormick, S.B., 1993). In addition to the agronomic value of land applying biosolids, there is also economic value as biosolids are often offered at little cost to growers and can be used as an alternative to costly commercial fertilizers (Faust and Oberst, 1996).

1.5 Health and environmental concerns

The most important health and environmental concerns associated with land application of municipal biosolids include pathogens, potentially toxic metals, and plant nutrients, which can pollute soils and ground and surface waters . Most of the N contained within typical biosolids is in the organic fraction (> 90%, Table 1). Therefore, it must be converted from the organic form to inorganic ammonium (NH_4) via a natural microbial process called mineralization in order for the N to be utilized by plants. Once NH_4 is present it can be converted to NO_3 via nitrification. Nitrate is very soluble in water and not strongly adsorbed to soil because of the lack of soil anion exchange sites. Thus, NO_3 becomes very mobile when water movement is substantial and is subject to leaching (Havlin et al., 2005). Nitrate is a potential environmental pollutant because it can enter surface water bodies which are often N limited and stimulate rapid growth of algae and aquatic weeds. When the vegetation dies, decomposition by microorganisms consumes dissolved oxygen (O_2) and can lead to anoxic conditions. This process is known as eutrophication, which degrades water quality for many uses and can lead to fish kills (Brady and Weil, 2002). Ammonium is not considered an environmental pollutant as it is either converted to organic N through plant

uptake processes or held on the negatively charged cation-exchange sites of soil particles, greatly hindering movement to ground and surface waters. Although NO_3 is also converted to organic N through plant uptake processes, it is generally considered to be much more mobile in the environment that NH_4.

Nitrate in drinking water is a potential threat to human and animal health. In excess, it can cause methemoglobinemia in livestock and infants, which decreases the oxygen-carrying capacity of blood. It has also been implicated in stomach and other cancers (Stevenson and Cole, 1999). The USEPA has a maximum contaminant level (MCL) for nitrate in drinking water of 10 ppm (10 mg L^{-1}) N as NO_3 (equivalent to 45 mg L^{-1} as NO_3); the World Health Organization (WHO) has a guideline of 11 mg N L^{-1} as NO_3-N (equivalent to 50 mg NO_3 L^{-1}) (WHO, 2004). For the past 50 years and counting, the actual human health effect of nitrate in drinking water has been a subject of much debate. Powlson et al., (2008) offer a thorough comparison of interpretations of available data on the issue and no consensus was reached. Arguments' stating that nitrate is not a significant human health concern claim that reported cases of infant methaemoglobinaemia (blue-baby syndrome) are associated with shallow wells and are a result of bacteria in human or animal excrement, not exposure to nitrate *per se*. Indications of a link between nitrate intake and stomach cancer are also said to been over-ridden by more recent research. Several positive effects of nitrate on human health have also been found such as controlling gastroenteritis, cardiovascular health, decreased coronary disease, and reduced blood pressure (Powlson et al., 2008).

Despite the arguments against nitrate being a human health concern, others remain confident that caution is still needed. It was postulated that although normal physiological

concentrations of nitric oxide within the body have beneficial effects, chronic exposure to elevated concentrations caused by chronic inflammation may be associated with cancer. Health benefits attributed by others to nitrate intake from vegetables may instead be attributable to intake of antioxidants from the vegetables. Certain subgroups within a population are also thought to be more susceptible than others to adverse impacts connected with nitrate. Previous studies are often thought of as inadequately designed and could be a reason why there is a lack of evidence for a link between nitrate intake and various cancers from population surveys. Overall, it was stated that there is an urgent need for a comprehensive, independent study to determine whether the current nitrate limit for drinking water is scientifically justified or whether it could be safely raised (Powlson et al., 2008).

Concern has also been expressed that N_2O released into the atmosphere through denitrification can reduce the amount of ozone (O_3) in the atmosphere. This would decrease the atmosphere's ability to screen out ultraviolet radiation (Foth and Ellis, 1997). Greater exposure to ultraviolet radiation can lead to a prevalence of skin cancers and the associated implications of such (Powlson et al., 2008).

Heavy or toxic metals in biosolids are another concern, largely due to industrial discharge of heavy metals and toxic organics into the sanitary sewer system. However, pretreatment of industrial discharge prior to entry into municipal waste streams has been shown to reduce the concentration of heavy metals such as cadmium (Cd) (Lue-Hing et al., 1980), another result of water quality legislation. The extent of this risk of heavy metal concentrations in biosolids has been shown to be minimal, especially in Class A and B biosolids (National Research Council, 1996). Crop tissue trace-metal concentration has been

shown to increase with repeated land application of biosolids (Berti and Jacobs, 1996). The pH and organic matter content of the soil strongly affect heavy metal availability. At a pH < 6 trace-elements are more soluble (with the exception of molybdenum and selenium) (Page et al., 1987). However, many biosolids are lime-stabilized for pathogen control, which increases the pH of the biosolids and reduces the solubility of most metals.

Biosolids may contain infectious disease organisms such as pathogenic bacteria, viruses, protozoa, and parasites, and public concern has been expressed as a result. Specific examples include bacteria such as *Salmonella* and *Shigella*; viruses such as hepatitis, Rota, and Norwalk; and parasites associated with giardiasis, cryptosporidiosis, taeniasis, and ascariasis. The primary method of exposure is ingestion, and the use of contaminated biosolids on crops that are eaten raw provides a risk to the consumer. There are many stages of wastewater and sludge treatment, many aimed at mitigating different health concerns (see below). Restrictions such as not allowing crops to be harvested for at least two weeks after land application and limiting access to applied areas further achieves safety. Although more research is needed about potential risks of infectious diseases in land applied biosolids, existing studies have shown the risks to be miniscule under proper management strategies compared to everyday exposure from other sources (National Research Council, 1996; Ramulu, 2001).

There is also concern about pharmaceuticals, personal care products (PPCPs), fire retardants, and other chemicals in biosolids (commonly referred to as organic wastewater

contaminants. However, more research is needed to make any definitive statements (Chenxi et al., 2008), and related topics are not covered in this research.

1.6 Biosolids Treatment Processes

Incoming waste streams, wastewater treatment, and biosolids production can differ greatly from facility to facility. As a result, biosolids have varying levels of nutrients, pathogens, heavy metals, and organic contaminants. The Clean Water Act Amendments of 1987 required the USEPA to develop regulations to protect human health and the environment from any reasonably anticipated adverse effects of certain pollutants that might be present in sewage sludge biosolids. This regulation, Standards for the Use or Disposal of Sewage Sludge (Title 40 of the Code of Federal Regulations [CFR], Part 503), was published in the Federal Register (58 FR 9248 to 9404) on February 19, 1993, and became effective on March 22, 1993. It is generally referred to as "the Part 503 rule" and also as "Part 503" (United States Environmental Protection Agency, 1994). The Part 503 rule established requirements for the final use or disposal of biosolids when they are: (i) applied to land to condition the soil or fertilize crops or other vegetation grown in the soil, (ii) placed on a surface disposal site for final disposal, or (iii) fired in a biosolids incinerator. The rule also provides provisions on placement of biosolids in a municipal solid waste landfill. Part 503 defines sewage sludge as a solid, semi-solid, or liquid residue generated during the treatment of domestic sewage in a treatment works. Sewage sludge includes scum or solids removed in primary, secondary, or advanced wastewater treatment processes and any material derived from sewage sludge (e.g., a blended sewage sludge/fertilizer product) but does not include

girt and screenings or ash generated by the firing of sewage sludge in an incinerator (United States Environmental Protection Agency, 1994).

Part 503 defines land application of biosolids as the application of biosolids to land to either condition the soil or to fertilize crops or other vegetation grown in the soil. Approximately 60% of the biosolids produced are land applied or further treated for use as compost (Environmental Protection Agency: Municipal and Industrial Solid Waste Division, 1999). Of the biosolids that are land applied, 74% are applied to agricultural land, 22% for exceptional quality treatment, 3% for land reclamation, and 1% applied to forestland (Beecher et al., 2007). All biosolids applied to land must meet the ceiling concentrations for pollutants. The ceiling concentrations are the maximum concentration limits for 10 heavy metal pollutants in biosolids, specifically, arsenic, cadmium, chromium, copper, lead, mercury, molybdenum, nickel, selenium, and zinc. Molybdenum, however, is being evaluated by the EPA using additional data developed since 2000, including results from the Targeted National Sewage Sludge Survey released in 2009. Regulatory decisions will be made thereafter for a revised numeric standard in land applied biosolids. Other chemicals in biosolids identified in 2003 for further assessment include: barium, beryllium, chloroaniline, 4-, fluoranthene, manganese, pyrene, silver, nitrate, and nitrite (USEPA, 2009). The EPA states that "Biosolids must also meet either pollutant concentration limits, cumulative pollutant loading rate limits, or annual pollutant loading rate limits for these same heavy metals. Either Class A or Class B pathogen requirements and site restrictions must be met before biosolids can be land applied (United States Environmental Protection Agency, 1994)." The two classes differ on the level of pathogen reduction that has been obtained.

Class A biosolids have pathogen levels below detectable limits. Biosolids are designated Class B if pathogens are detectable but have been reduced to levels that do not pose a threat to public health and the environment as long as actions are taken to prevent exposure to them after their use or disposal. Also, specified vector (any organism that carries and transmits an infectious pathogen into another living organism) attraction reduction must be met (United States Environmental Protection Agency, 1994).

1.7 Raleigh plus biosolids treatment methods

The "Raleigh plus" (R+) biosolids product used in this research came from the Neuse River Wastewater Treatment Plant (NRWWTP) in Raleigh, NC. It is an advanced or tertiary aerobic wastewater treatment facility with a capacity of 60 million gallons a day, and is treating an average of 44 million gallons a day. The aerobic treatment process is C enhanced to aid in N removal. Waste water treatment is continuous flow and the biosolids treatment is a batch process. Advanced or tertiary treatment means that wastewater undergoes three stages of treatment: primary, secondary, and advanced treatment. Primary treatment is a physical process that removes debris, sand, heavy organic solids, and grease and oils. Secondary treatment is a biological process referred to as "activated sludge" in which microorganisms convert NH_4-N to N gas through nitrification/denitrification. Secondary clarification separates the microorganisms from the treated water and returns them to the biological process. Advanced treatment is the process of filtering the clarified water in sand filters and disinfecting the water by ultraviolet (UV) light before the water is metered and returned to the Neuse River (City of Raleigh, 2012).

The specific treatment methods of the remaining solids include twice dewatering the sludge with belt presses to make a cake that is approximately 20 to 23% solids. The cake is then mixed with lime kiln dust at a mixture ratio of approximately 5:1 (5 sludge: 1 lime kiln dust). Next, there are a series of temperature checks and laboratory analyses completed to verify the mix has met 40 CFR Part 503 regulations for Class A. Specific treatment methods include vector attraction method #6 (alkaline stabilization) and alternative #5g (pasteurization) for pathogen reduction. The requirements for alkaline stabilization involve raising the pH to at least 12, measured at 25ºC, and without the addition of more alkaline material, maintain a pH of a least 12 for 2 hours in addition to maintaining a pH of at least 11.5 without addition of more alkaline material for an additional 22 hours. The conditions of this option ensure that the biosolids can be stored for at least several days, transported, and then used or disposed without the pH falling to a point where vector attraction occurs. Pasteurization involves maintaining the temperature of the biosolids at 70ºC or higher for 30 minutes or longer, further reducing pathogen levels of the biosolids from alkaline stabilization alone (United States Environmental Protection Agency, 1994). Raleigh plus is distributed as a soil amendment to agricultural and institutional operations in the region. It is also available as a liming agent with supplemental nutrients. The NRWWTP also utilizes the services of a private firm that receives dewatered primary sludge and produces a Class A biosolids product in the form of compost. Once the regulatory requirements are met, the compost is distributed to the public as a soil amendment and growing media (City of Raleigh, 2012) (T.J. Lynch, personal communication).

1.8 OWASA cake biosolids treatment methods

The Orange County Water and Sewer Authority (OWASA) biosolids product used in this research came from the Mason Farm Wastewater Treatment Plant in Carrboro, N.C. The waste water treatment plant WWTP treats approximately 8 million gallons per day of domestic wastewater (sewage) from the Carrboro-Chapel Hill communities. The WWTP also accepts and treats about 1 million gallons per year of septage pumped from septic tanks serving the surround rural area. Approximately four dry tons of biosolids are produced each day from the WWTP. Some of the biosolids are applied in liquid form to agricultural land and the majority are dewatered and transported to a private composting facility in Chatham County to make a soil additive for landscaping. The OWASA plant has liquid land application permits for a total of 1,156 acres of farm land in Orange, Chatham, and Alamance counties. Nearly 90% (1,013 acres) is privately owned. The remaining 143 acres are owned by OWASA as part of a 700-acre tract in Orange County (Orange Water and Sewer Authority, 2012).

The specific treatment methods of the OWC biosolids involve processing of the primary sludge via a gravity belt thickener to ensure a consistent moisture content of approximately 80%. The cold, thickened sludge is then sent to a series of anaerobic digesters via a batch process to achieve pathogen reduction and vector attractant reduction. Anaerobic digestion involves treatment of sludge in the absence of air for a specific mean cell residence time at a specific temperature. Values are between 15 days at 35ºC to 55ºC and 60 days at 20ºC (United States Environmental Protection Agency, 1994). Anaerobic digestion at the OWASA WWTP involves a series of four tanks with varying temperature and storage time

(average total digestion time of 36.6 days). The first stage of anaerobic digestion is at a thermophilic temperature (58-60ºC). Second stage is once again at a thermophilic temperature. The batch is held for 22 hours and Part 503 time and temperature requirements are met at this stage. Third stage is once again at a thermophilic temperature followed by the fourth stage which is at a mesophilic temperature (35-38ºC). The temperature of the digesting sludge, the operation of the recirculation pumps, and digester mixers are monitored continuously. The process pH and alkalinity are checked once per week, in addition to gas production. After completion of anaerobic digestion, biosolids are then moved to in-house storage tanks until the liquid biosolids are applied to land or the biosolids are dewatered via a rotary press and sent to a commercial composting facility. All OWASA biosolids meet the trace metal requirements necessary to qualify for the EQ designation of the USEPA and NCDWQ because of their low concentrations of trace metals. Their ability to consistently meet these low levels of trace metals reflects the lack of industrial dischargers into the community sewer system (Orange Water and Sewer Authority,); (personal communication).

1.9 Cary pellet biosolids treatment methods

The Cary pellet biosolids product used in this research comes from the South Cary Water Reclamation Facility (SCWRF) in Apex, N.C. It has a capacity of 12.8 million gallons per day and is a tertiary biological nutrient removal (BNR) treatment plant that receives wastewater from the collection system on the south side of Cary. Biological nutrient removal removes N and P from the wastewater through the use of microorganisms under different environmental conditions in the treatment process (Town of Cary, 2012). The

Town began operating a biosolids dryer in December, 2005 to provide a cost effective and flexible biosolids reuse program. The facility takes liquid biosolids, a byproduct of the main wastewater treatment processes, from the North and South Cary Water Reclamation facilities and converts them, using heat, to a dry BB-sized pellet. The pellets meet strict state and federal guidelines required to achieve a Class A EQ rating and provides the Town with the most options for safe reuse or cost effective disposal. From 2006—2008, the facility treated an average of 28 million gallons of biosolids per year and produced an average of 3,100 tons of pellets per year for the agricultural market. The waste generated by a typical family in a year is about 100 pounds of fertilizer. The Town markets its round fertilizer pellets as "Cary Enviro Gems," and attempts have been made to make these available for purchase to the general public as a soil amendment (Town of Cary, 2012).

1.10 Plant Available Nitrogen, Mineralization, and Nitrification Process

For organic N to be of use to plants, it must be converted to NH_4 through mineralization and to NO_3 via nitrification. Plant available N (PAN) consists primarily of $NH_4 + NO_3$. More than 95 % of N in many municipal biosolids is organic (Table 1). The N exists in the form of proteins, amino acids, amino sugars, and other complex N compounds. Mineralization occurs through aminization and ammonification by heterotrophic bacteria and fungi (Haynes, 1986). Aminization converts the proteins in residues to amino acids, amines, and urea. A diverse population of aerobic and anaerobic bacteria, fungi, and actinimycetes then convert these products to ammonia (NH_3) through ammonification. Ammonia reacts

with soil water to produce NH_4 and is either nitrified, absorbed by plants, used as a substrate for heterotrophic bacteria, fixed, or volatilized (Havlin et al., 2005).

In the majority of agricultural soils, NH_4 is rapidly converted to NO_3 through nitrification. Ammonium is oxidized to nitrite (NO_2) by species such as *Nitrosomonas* bacteria and then oxidized further to NO_3 by species such as *Nitrobacter* bacteria through a two-step process by the following equations:

$$2NH_4 + 3O_2 \rightarrow 2NOH_2 + 2H_2O + 4H$$

$$2NO_2 + O_2 \rightarrow 2NO_3$$

Net Reaction:

$$NH_4 + 2O_2 \rightarrow NO_3 + H_2O + 2H$$

Nitrosomonas and nitrobacter are autotrophic bacteria that obtain their energy from the oxidation of N and carbon (C) from CO_2 (Havlin et al., 2005).

Table 1. Analysis of the three biosolids products being used in this research, displaying total and inorganic Nitrogen and percent dry matter.

Biosolids	Total N	N-inorg	N-org	% Inorg	% Org	% Dry Matter
	---------- mg kg^{-1} ----------					
R+	6939	294	6645	4	96	52
CP	65500	3296	62207	5	95	95
OWC	48801	1673	47128	3	97	20

Nitrification, like all microbial driven processes, is affected by environmental conditions. Nitrification rates are generally highest under the following conditions (Havlin et al., 2005; Haynes, 1986; Schepers and Raun, 2008):

- moisture contents are at field capacity (70 to 80% of total spore space)
- aerobic conditions
- pH ~ 7 to 9
- proper substrate (presence of NH_4)
- temperature of 25 to 35° C

1.11 Estimating Plant Available Nitrogen

Maximizing N use efficiency (NUE) and minimizing environmental loss of both dissolved and gaseous N from land-applied biosolids requires accurate predictions of PAN. Estimates of bioavailability of N vary greatly depending on what time frame is being considered (a month, a growing season, a year, etc.), recent crop management and amendment history, biotic and abiotic soil characteristics, and environmental factors such as temperature and soil water content. The first-year N availability coefficient (NAC) is the proportion of total N that becomes plant-available in one growing season. In North Carolina, they are used to estimate PAN by equations [1] and [2] provided by the NCDA&CS (McGinnis et al., 2011):

[1] Nutrient available (PAN) (lb/ton) = [nutrient concentration (mg/kg) ÷ 1,000,000] × NAC × NM × 2000 × (DM% ÷ 100)

[2] Nutrient available (lb/1000 gal) = [nutrient concentration (mg/L) ÷ 1,000,000] × NAC × NM × 8340

Where:

- NAC = nutrient availability coefficient
- NM = nutrient multiplier
- DM% = dry matter percent

The NM is only relevant for P and K to allow expression of P as phosphate (P_2O_5) and K as potash (K_2O). The NM for P and K are 2.29 and 1.20, respectively. For all other nutrients and elements, the NM is 1. Available N calculations are based on an NAC and total N for solid waste or Total Kjeldahl Nitrogen (TKN) for liquid waste, unless an inorganic nitrogen (IN-N) test was conducted by the NCDA&CS. If the inorganic N test was conducted, available N is determined as the sum of available organic N (OR-N) and available IN-N (equation [3]) (McGinnis et al., 2011):

[3] Available N = available IN-N + available OR-N

In North Carolina, once PAN has been estimated, application rates are determined based on realistic yield expectations (RYE) and soil- and crop-specific N application factors (NC Nutrient Management Workgroup, 2003). These are intended to meet crop N requirements and minimize excessive N application to protect the environment.

1.12 Nitrogen Availability Indices

Estimation of soil N availability has been a goal of many research projects since the early 1900's (Bundy and Meisinger, 1994). Numerous methods of estimating soil N availability have been developed and tested. Common methods used include chemical extraction, biological incubations, crop response trials, and soil NO_3-N tests. Chemical indices are generally more convenient than biological indices, but do not simulate the action of microorganisms in release of plant-available forms of soil N. Chemical indices are, however, rapid, precise, and more convenient than biological incubations, and are used frequently as a result (Haynes, 1986). If a rapid and relative assessment of N availability among soils differing in past management is desired, techniques such as digestion with 2 M KCl, steam distillation, and UV absorbance are useful. Other field methods include the residual mineral N assessment, the pre-plant NO_3-N test, and the pre-sidedress NO_3-N test. However, if N recommendations for crop production based on soil N availability are required, other methods will likely be most beneficial due to low correlation with field measured N availability (Hong et al., 1990). Other methods generally include biological soil incubations under aerobic of anaerobic conditions that promote N mineralization from organic sources followed by measurement of the inorganic N produced (Bundy and Meisinger, 1994). Short term C mineralization is often measured simultaneously since soil organic matter decomposition links C and N cycles in the soil (Schepers and Raun, 2008). In situ incubations are useful in quantification of N mineralization under field conditions and the relatively new amino sugar N test has been shown to detect responsiveness of soils planted in corn to N fertilization (Khan et al., 2001). The concentration of soil organic

matter N has also been used as an index for N availability, although the relationship has not been significant enough to be predictive (Schepers and Raun, 2008). The USEPA states that N mineralization or biosolids in the first year is variable and can range from 10-50%+. Due to this variability in N mineralization, it is recommended that mineralization studies be conducted on specific biosolids (USEPA, 1995).

1.13 Anaerobic Incubation

Utilizing anaerobic conditions (the absence of oxygen) for biosolids incubation is one biological index to provide a relative assessment of N availability. Generally, the soil is saturated to promote anaerobic conditions and incubated for approximately 7 d. Anaerobic microbes mineralize organic N to NH_4-N and soil is extracted with 2 M KCl. An aliquot of the extract is then analyzed for inorganic N (NH_4- + NO_3-N). Originally proposed by Waring and Bremner (1964), and recommended by Keeney (1982) and Bundy and Meisinger (1994), this method has several advantages. It is simple to establish due to minimal apparatus or reagent requirements and has a short incubation period (7 d). Moisture adjustment concerns are eliminated and a higher temperature (which stimulates more rapid mineralization than aerobic incubations) can be used since optimum temperature for nitrification is not an issue. This allows for routine use and rapid turnover as a predictive tool of biosolids N mineralization. Aerobic incubation is also commonly used to estimate potentially mineralizable N in soils (Stanford and Smith, 1972). It generally involves incubating soil for an extended period of time (30 weeks) and sampling and analyzing the soil for inorganic N throughout the incubation. Short-term incubations do not necessarily reflect the long term N-

supplying capacity of a soil (Haynes, 1986). Aerobic incubations provide an estimate of the temporal N mineralization under ideal field conditions, and as a result, may represent field conditions better than other incubations. Waring and Bremner (1964) originally found a strong positive correlation ($r = 0.96$, $p < 0.001$) between the results of two week anaerobic and aerobic incubations at 30ºC, with a total of 39 samples. Only NH_4-N was measured since no oxidation of ammonium occurred under the incubation conditions. Osborne and Storrier (1976) found that the anaerobic incubation accounted for 72% of the average yield variation of N and 60% of the variation in average N uptake across five rates of N fertilization from urea, ammonium sulphate, and sodium nitrate. The incubation was related to plant yield response across all fertilizers used, especially urea (R^2=80), and statistically significant only with urea in terms of nitrogen uptake (R^2=68). Keeney and Bremner (1966) evaluated several incubation and chemical methods of obtaining an index of soil N availability in major Iowa soil types planted in common ryegrass (*Lolium multiflorum*). They used one aerobic and two anaerobic incubations. The aerobic incubation was at 30ºC for 14 days and the two anaerobic incubations were at 30ºC for 14 days and 40ºC for 7 days. Methods and duration of soil storage before analysis were also examined (field-moist, air-dried, and air-dried and stored from 0 to 48 weeks). Air-drying of the 25 soils used resulted in a large increase in the mineralizable N value obtained by the aerobic or the anaerobic 30ºC incubation, but led to a small decrease in the mineralizable N value obtained by the anaerobic 40ºC method. Mineralizable N increased in all air dried soil stored for up to 24-weeks at which point both anaerobic methods began to decrease and the aerobic method increased to 48-weeks. The effects of air-drying and air-dry storage were statistically significant and

were different for different soils. Generally, the results indicate that soil samples should be air-dried and stored for 8 to 24 weeks for routine use in soil testing laboratories. It was also determined that the aerobic and anaerobic incubation at 40ºC provide a good index of soil nitrogen availability for the second plus third cuttings of ryegrass, when native inorganic N is not distorting results (average correlation coefficients = 0.72 and 0.76, respectively, and 0.50 and 0.56 for the first cutting). It was postulated that the relationship between mineralizable N and plant uptake was confounded in the first cutting due to much of the N being derived from the inorganic N present in the soils at the initiation of the greenhouse study. Evidence supporting this assumption is represented by the fact that correlations of inorganic N in the soil vs. N uptake in the first and second cutting were 0.93 and 0.69, respectively. The anaerobic incubation at 30ºC was not highly related to N uptake (average correlation coefficient = 0.54). Erratic results of both anaerobic incubations were attributed to un-decomposed organic material floating to the surface of the water-logged soils. Therefore, the conclusion can be drawn that an anaerobic incubation similar to what was used by Kenney and Bremner (1966) can be a valid estimate of the availability of soil N that has been amended with biosolids (Debosz et al., 2002) (Stark and Clapp, 1980).

Ryan et al., (1971) completed a study on biological methods for obtaining an index of soil N availability to ‘X-1605’ grain sorghum (*Sorghum vulgares*) under different levels of N fertilization on fifteen Kentucky soils. The ability for a routine test for estimating the capacity of soil to supply N to plants for the southern United States is difficult due to generally low levels of native N in those soils, high losses of applied N, and variability of soil and climate (such as precipitation exceeding evapotranspiration, a warmer climate that

promotes microbial activity year-round, and different seasonal mineralization rates). A water-logged (anaerobic) incubation was carried out for 7 or 14 days at 30 or 40ºC on both amended and un-amended soils from Kentucky. Contrary to the results from Keeney and Bremner (1966), Ryan et al., (1971) found the values from aerobic and anaerobic incubation methods were closely related to the amount of N taken up by the first sorghum harvest. Although the reasoning for this contradictory evidence was not completely understood in the study, it was generally attributed to soil pre-treatment methods, low amounts of native N, differences in test crop, and greenhouse temperature. They found that the amount of extractable NH_4-N present after anaerobic incubation of unamended soils was related to the amount of N taken up by sorghum (Average $R^2 = 0.53$), with the exception of the 7-day 40ºC incubation ($R^2 = 0.12$). A greater coefficient of determination occurred when the incubation was carried out for 14 days compared to 7 days, although it was suggested that the difference between 3 to 12 days was not substantial, and larger N mineralization values were found for 30ºC than 40ºC at both lengths of incubation. Fourteen day incubation indices from unamended aerobic and anaerobic incubation at 30ºC were equally precise at measuring N uptake. Other anaerobic incubations were not as precise as the aerobic incubation.

Muruganandam et al. (2008) (Muruganandam et al., 2009) used the anaerobic incubation methods of Bundy and Meisinger, (1994) to measure potential N mineralization of soil aggregates to evaluate the activities of nitrogen-mineralization enzymes associated with three tillage systems. It was assumed that the mineralization potential measured by this method presumably represents the population size of N mineralizers, as the optimum moisture and temperature conditions required for microbial growth and activity were

provided. Tillage, aggregate size, and the interactions between tillage system and aggregate size were different. The potential N mineralization rate of all the aggregate size fractions of the no-till and chisel systems were 1.5 to two times greater than for the moldboard system (Muruganandam et al., 2009). This type of application for the anaerobic incubation shows the usefulness of the test as a part of larger evaluations.

Selmer-Olsen (1974) described the ability to perform the anaerobic incubation in 2 N KCl solutions as opposed to H_2O due to the fact that chloride inhibits NO_3 loss by approximately 70% during incubation, but does not significantly affect mineralization. Also, 2 N KCl is commonly used in determination of ammonium and nitrate in soil extracts after incubation which eliminates additional steps of addition of chemicals or dilution steps making it very reasonable for routine analysis. The correlation coefficient of nitrogen uptake by ryegrass against the sum of ammonium and nitrate N found after anaerobic incubation of 35 dry stored soil samples in 2 N KCl was 0.89 and only 0.41 if the correlation is only considering ammonium. As a result, it appears as if incubation in 2 N KCl gives as good results as when performed in water.

1.14 Evidence against the validity of anaerobic incubations

Although the above studies, among others, show a strong correlation between anaerobically produced NH_4-N and field measurements of N availability, others have found poor correlations. Fox and Piekielek, (1983) conducted 67 experiments throughout Pennsylvania over a 6-year period. It was found that a number of N-availability tests (Boiling 0.01 M $CaCl_2$ and 0.01 M $NaHCO_3$-extractable N, autoclave extractable NH_4-N,

total soil N, Walkley-Black soil organic matter, soil NO_3^-, H_2SO_4-extractable, and KCL-extractable N) were not well correlated with the soil N available to a field grown in corn (Fox and Piekielek, 1983). In an attempt to develop a method of predicting soil N availability, a 7 d anaerobic incubation at 40ºC was used in a subsequent study (Fox and Piekielek, 1983). They found the NH_4-N ranged from 25.1 to 86.9 mg kg^{-1} with a mean of 55.5 mg kg^{-1}. The amount mineralized was poorly correlated with field measured N availability via N fertilizer response trials in corn (r=0.31) when using all experiment sites, and was similar to the poor correlation of field measured N availability and chemical indexes listed above. A different index of field-measured N availability (relative N uptake) also resulted in a poor correlation with field measured N availability (r=0.35). Anaerobically mineralized NH_4-N was well correlated with total soil N and modified Keeney and Bremner boiling 0.01 M $CaCl_2$ extractable N (r=0.79 and 0.74, respectively), which is another determination of inorganic N similar to the well documented KCl extraction. Certain soils that were outliers were removed in hopes of improving the correlation but the correlation was not improved to a practically useful level.

McCracken et al. (1989) established a study to examine the ability of selected soil indices to detect management-induced differences in soil N availability on corn at a single site in a Maury silt loam (fine-silty, mixed, mesic Typic Paleudalf), specifically: anaerobic incubation and KCl-extractable NO_3-N. Results indicated that the anaerobic incubation index failed to correlate significantly with any crop parameters studied (N uptake, ear leaf N concentration, dry matter production, and grain yield). Differences were found between management practices but were not reflected in crop response. The index falsely predicted

greater N mineralization on the control plots than one that had received N fertilization, and predicted similar soil N availability for vetch and no-cover crop plots, with less for the rye plots. KCL-extractable NH_4-N and NO_3-N alone (no incubation) proved to be the best index of predictive N availability from a soil sampled at 2 weeks after planting. However, extractable NH_4-N had no effect on the correlation. As a result, it was concluded that the soil NO_3-N test has more power than other indices tested to estimate long-term management effect on soil N availability. Shallow profile sampling for NO_3-N after corn planting has been shown to have similar value in a humid climate (Magdoff et al., 1984).

Despite the contradictory evidence, Boone (1990) suggested that the differences in N test values are merely a result of differences in technique. The suggestion is that in the field, N dynamics are a result of the net sum of N immobilization and N mineralization, typically under aerobic conditions. Therefore, it is likely that anaerobic incubation measures N released primarily from the microbial biomass (Myrold, 1987) and (Azam et al., 1988). Also, anaerobic conditions stimulate mineralization (Haynes, 1986) and lower net immobilization (Bartholomew, 1965). As a result, anaerobic incubations give a measure of an active N pool but do not represent N dynamics between mineralization and immobilization found in situ (Boone, 1990). Such incubations do, however, provide a relative measure of the soil's ability to release N for plant growth (Keeney, 1982, Bundy and Meisinger, 1994).

1.15 Aerobic Incubation

In response to rapid changes in N fertilizer use and greater awareness of related environmental consequences, methods for assessing the N supplying capacities of soil as an aid in predicting N fertilizer needs are required. Early studies of soil N mineralization were relatively short-term (7 to 14 d) and were driven by the need for rapid and reliable methods for routine assessment of soil N availability. Long term studies did not meet those practical requirements but may provide vital information. Stanford and Smith (1972) originally proposed a 30-week incubation period at 35ºC with sampling dates on 2, 4, 8, 12, 16, 22, and 30 weeks on 39 soils. Mineral N was removed by leaching with $CaCl_2$ followed by a nutrient solution devoid of N before incubation. Excess water was removed under vacuum and optimal soil water conditions were maintained by applying suction at 60 cm of mercury. Nitrogen mineralization rates were found to be relatively constant throughout the 30-week period for 23 soils and 15 of the remaining soils experienced slight declines with continued incubation. Differences between rates were attributed to pretreatment effects (drying, freezing, or fumigation), lag in microbial activity and/or assimilation of N by organisms, or slight changes in pH. The quantity of soil N mineralized in a given time is dependent upon temperature, available water, rate of oxygen replenishment, pH, amount and nature of plant residues, and level of other nutrients, but the main factors considered in this study were soil pH and plant residues. The pH of the soils was generally little affected by incubation and the microbial population was assumed to not be a limiting factor, except, perhaps, during the initial period of incubation in certain soils. It was suggested that N mineralization rates are proportional to the amount of potentially mineralizable N in the soil, that the pattern of N

mineralization follows first-order kinetics, and that an N mineralization potential value (N_o) can be estimated from $\log(N_o - N_t) = \log N_o - kt/2.303$. Here, k = an estimate of the rate constant, and t = time. It was also suggested that there was a similarity among soils with respect to the principal sources of mineralizable N due to the fact that the mineralization rate constants associated with the determined values of N_o were similar for a broad range of soils. Stanford (1972) concluded that N_o is a definable soil characteristic which may be of value in estimating N supplying capacities of soils under specified environmental conditions. It also provides a common basis for evaluating various chemical and biological availability indices under a broad range of soil conditions and for making quantitative estimates of N mineralization in the field.

1.16 Research on aerobic incubation with biosolids

Sommers et al. (1981) conducted a study to characterize organic N mineralization in various sewage sludges. The study was conducted before common use of the term "biosolids." The study was also completed before the promulgation of the Part 503 regulations in 1993, and was the study cited for the estimated N mineralization rates in the USEPA Process Design Manual: Land Application of Sewage Sludge and Domestic Septage (USEPA, 1995). Previous studies were cited that found organic N mineralization rates of 4 to 48% in a 16 week incubation period (Ryan et al., 1973) and 36 to 41% in another study (Sabey et al., 1975) using an anaerobically digested sludge. Substantially lower organic N mineralization rates (2.3 to 4.2%) were found in another study using both anaerobically digested and activated sludge (Premi and Cornfield, 1971). Based on plant uptake of N, it

has been estimated that 4 to 29% of the organic N in sludge can be mineralized (Sabey, 1977). Other studies have also evaluated organic N mineralization for biosolids produced in different ways. Magdoff and Chromec (1977) found that 14 to 25% of the organic N mineralized for anaerobically treated sludge and from 36 to 41% for aerobically digested sludges during a 13 week laboratory incubation. The amount of organic N mineralized in raw-, digested-, and activated sludges was found to be 7, 21, and 60%, respectively, for a 42 day incubation (Stephenson, 1955). This study also found that composting decreased the amount of N mineralization as much as 30%. Soil characteristics may influence the rate of organic N mineralization also (Tester et al., 1977). The influence of the soil factor was considered in the design of this research thesis.

Field experiments have also been conducted to estimate organic N mineralization. Kelling et al. (1977) concluded that approximately 50% of the organic N applied was mineralized within 3 weeks after sludge application. Years two and three showed rates of 25 and 15% respectively (Kelling et al., 1977). Magdoff and Amadon (1980) found approximately 50% of the organic N mineralized in a field experiment, and similar results were found during laboratory incubations for 17 weeks (Magdoff and Amadon, 1980).

The objectives of Sommers et al. (1981) were to determine N mineralization for a variety of sewage sludges obtained from different regions of the U.S., compare two different incubation methods of doing such, and evaluate several extraction procedures for estimating N mineralization from sewage sludges. The sludges came from MI, WA, WI, IL, OH, IN, MD, CO, AZ, and CA. The incubation methods included a leaching incubation (Stanford and Smith, 1972) and closed system static incubation. The sludges used included primary,

primary plus waste activated, raw-CaO treated, raw Zimpro treated, waste activated, anaerobically digested, aerobically digested, raw-CaO treated and composted, and anaerobically digested and composted, with anaerobically digested being the majority. It is also very important to note that all sludges were air-dried and ground to less than 60 mesh prior to use. This could have had an impact on N mineralization due to the fact that the grinding increased the available surface area and air drying could have affected the N dynamics of the products. The soil used was a Fincastle silt loam (fine-silty, mixed mesic Aeric Ochraqualf) collected in West Lafayette, IN and was air-dried and ground to less than 20 mesh. The original soil pH was 5.9. The anaerobically digested sludges were variable in organic N content.

For all incubations, the control treatment (no N added) was subtracted from the subsequent sampled amounts. A curvilinear relationship between N mineralized and time was found for nearly all sludges. Mineralization occurred rapidly during the initial 3-4 weeks and then the rate decreased substantially. The curvilinear relationships meant that N mineralization was able to be estimated by first order kinetics. The amounts of N mineralized in the static procedure generally exceeded those obtained for the leaching methods, likely due to removal of potentially decomposable soluble organic N during leaching with 0.01 M $CaCl_2$. Therefore, it was concluded that the leaching procedure will generally underestimate organic N mineralization. The average percent of N mineralized for the leaching and the static incubation was 17.5% and 15.9%, respectively. The ranges of N mineralization from anaerobic sludges were 2.1 to 26.7%, and 4.5 to 12% for composted sludges. Composting was found to reduce mineralization from 27.9 to 9.4% for raw sludge

that had been treated with CaO before and after composting and 13.7 to 12% in anaerobically digested sludge. In general, the greatest amounts of mineralizable N were in primary or waste activated sludges and further treatment was shown to reduce N mineralization (Sommers et al., 1981).

Overall, it was stated that the amount of mineralization of organic N in sewage sludge was proportional to the total organic N content. Moreover, the potential for N immobilization was the greatest when the overall C/N ratio of the sludge exceeds 20/1. The rate constants calculated were comparable to those presented in previously cited studies. Sommers et al. (1981) stated that current guidelines used to calculate the amounts of sewage sludge applied to land assume that 20 to 25% of the organic N is mineralized during the first year after application, although no official Government document or research is cited to support this statement. They also stated, as a result of their research, that different N mineralization percentages be used for various sludge types. Ultimately, they recommended using the following N mineralization percentages: 25% for raw and primary sludges, 40% for waste activated sludges, 15% for anaerobically digested sludges, and 8% for composted sludges. Sludges treated with wet air oxidation need individual study, they recommended. The authors did not mention the effect of application method on these percentages. However, it was mentioned earlier in the study that NH_3-N loss via volatilization was dependent upon the method of sludge application, initial soil moisture content, and sludge pH. They did not find a chemical extractant that accurately predicted the amount of potentially mineralizable N in sewage sludge (Sommers et al., 1981).

Soil and agronomic research on land application of municipal wastewater treatment residuals in North Carolina essentially ceased with the retirement from the Department of Soil Science at North Carolina State University of Dr. Larry King in 2001. All of King's research involved wastewater treatment residuals that pre-dated the implementation in March 1993 of the USEPA Title 40 Part 503 rule governing disposal of sewage sludge biosolids in order to protect human health and the environment. Municipal waste water treatment has undergone substantial process changes since implementation of Part 503, including treatments specifically designed to process sludge into biosolids meeting 503 standards for land application. King (1984) established an experiment to determine the availability of the organic N in a variety of municipal, industrial, and animal wastes by aerobically incubating them with soil for 16-weeks. The results of this laboratory work were used to develop multiple regression models to predict N availability. Of the sludges used, total N content was generally greater in municipal sludges aerobically digested than in those anaerobically digested. Nitrogen in aerobic sludges was generally more plant-available than that in anaerobic sludges. Most waste additions reduced soil pH due to nitrification with the exception of products treated with CaOH. This is contrary to Stanford (1972) where pH was only slightly affected by incubation. The differences in effect on pH might be attributed to the removal of mineral N initially present by leaching with $CaCl_2$ and treatment with a nutrient solution containing additional Ca in Stanford (1972). When actual PAN was predicted using multiple regressions of actual PAN as the independent variable and predicted PAN as the dependent, an R^2 value of 0.87 was yielded. Using the model only for municipal sludges, the prediction was within ±30% of actual values found in the incubation studies.

Applying the model to other data (Parker and Sommers, 1983b) was largely unsuccessful. King concluded that models can be developed to estimate PAN fairly accurately for a given set of wastes and soils, but use of such models for other wastes and soils was not yet feasible. This conclusion speaks to the potentially important effect of different soils in N mineralization of biosolids. Independent variables of the model developed to predict PAN were characteristic of the wastes: organic N, inorganic N, and total N content; organic C, C/N ratio, and inorganic to organic N ratio. Total N was measured via the Kjeldahl procedure, inorganic N extracted with 2 M KCl and steam distillation, and C content estimated by a chemical oxygen demand procedure developed for use with compost. Carbon to N ratios of the wastes was a significant term in the model developed, which is an important factor to consider when deciding to use only the solids from liquid wastes. The model suggests availability of organic N in liquid wastes would be different if inorganic N from the liquid fraction were present during incubation.

Gilmour et al., (1996) developed a short-term method that could be used to assess long-term decomposition characteristics for biosolids. Twenty-four biosolids/soil combinations were incubated at 25ºC for a period of about 60 d. Soil water content was adjusted to 40% of the soil water holding capacity. Two models were developed: simultaneous decomposition and sequential decomposition. The simultaneous decomposition model assumes that rapidly and slowly decomposable biosolids C fractions decompose simultaneously and independently of each other. The Sequential Decomposition Model assumes that the rapidly decomposable biosolids C fraction decomposes completely before decomposition of the slowly decomposable fraction. Evidence exists of long-term

decomposition being used to estimate PAN (Gilmour and Skinner, 1999). The 24 biosolids/soil combinations used to compute the rapid decomposition fraction percentages and rate constants had been treated via anaerobic digestion of the biosolids/soil mixtures and stored for different periods of time before incubation. The rapid fraction ranged from 11 to 52% of the total biosolids C for the sequential model and from 12 to 43% for the simultaneous decomposition model. Mean values were 25 and 28%, respectively, and were not different. The simultaneous model to describe biosolids decomposition is limited to situations where long-term decomposition data are available. Long-term decomposition data is required for this model due to lack of correlation with the rapid fraction rate constant. The sequential model should be used where the percent decomposition at 7 d is known for a given biosolids-soil combination. Correlation of the rate constant vs. the percentage of biosolids C in the rapid fraction resulted in an $r^2 = 0.85$. Ultimately, it was proposed that a single measurement, percent decomposition at 7 d, could be used to asses long-term decomposition for similar biosolids. The relationship between predicted decomposition using the sequential decomposition model and observed decomposition resulted in an $r^2 = 0.76$. The equation used should not be applied to biosolids with small rapid fractions (<10%) such as those stored for extended periods, composted, or biosolids not originating from municipalities. Under certain circumstances, long term decomposition is a good estimator of PAN (Gilmour et al., 2003b).

Gilmour (1999) conducted a study to quantify biosolids PAN under field conditions and to propose methods including computer simulation to estimate biosolids PAN in a land application program. Six biosolids were evaluated over a 2-yr period and aerobic incubations

were used to obtain decomposition kinetics compared with field studies. Estimates of PAN using the research methods revealed all factors analyzed to be independent and statistically significant in the following order: C/N ratio, Organic N, and biosolids total N content (r^2 = 0.96, 0.90, and 0.84, respectively). Approximately 45% of the biosolids organic N and 40% of the biosolids total N was in plant available forms during the field growing season. It was suggested that annual mineralization rate percentages recommended by the EPA need revision due to the fact that the seasonal percentages found in this work were dramatically larger than annual mineralization rates. The Decomposition model (Gilmour and Clark, 1988) described in this research employs first-order kinetics to estimate rates of C and N transfer among biosolids (rapid and slow fractions), microbial biomass (indigenous, new), and soil organic matter (newer and decomposable, recalcitrant) pools. Nitrogen mineralized from biosolids is equal to the C decomposed divided by the biosolids C/N ratio. The Sequential model described previously was used and operates under the assumption that the rapidly decomposable portion of biosolids precedes slow biosolids fraction decomposition. New microbial biomass forms as biosolids decompose using a microbial efficiency of 0.4. The soil organic matter pool is assigned a C/N ratio of 10, while biomass has a C/N ratio of 8. Model inputs included average monthly air temperature, precipitation, and pan evaporation. Soil temperature was assumed to equal air temperature and soil water potential was obtained by a water balance plus the water release curve for a typical silt loam soil. The relationship between PAN from the equation used by Gilmour and PAN estimated using the Decomposition model provided an estimate of growing season PAN that is statistically significant, but a lower correlation than those based on analytical measures described above

($r^2 = 0.67$). It was postulated that the ranges of land application situations have yet to be characterized because of the effects of biosolids' properties, soils, weather, cropping systems, and application methods on biosolids PAN. Computer simulation is proposed as one approach to account for these variables that affect decomposition rates and mineralization of organic N. Computer simulation can also be used to extend estimates of growing season PAN to an annual basis. Use of the computer model and weather data makes the approach site-specific, while analytical data for a specific biosolids make the approach biosolids specific. Gilmour (1988) concluded that the Decomposition model accounts for variability that the constant factor approach commonly used (20% of the organic N assumed mineralized during the first year and yearly) does not consider. Predictions of PAN were consistently higher from the model than from the constant factor approach, indicating that the latter would lead to over application of waste-water sludge and result in related environmental consequences.

In an attempt to determine if first year overall PAN values predicted by the Decomposition model were related to analytical data, several analytical sludge properties were evaluated for two sludge products. Two relationships were obtained and one sludge's predicted PAN percentage was strongly correlated with % NH_4-N ($r = 0.88$) and the other was highly correlated with % organic-N ($r = 0.98$). Both sludges were anaerobically digested. It was postulated that for certain sludges, PAN can be estimated from analytical data (Gilmour and Clark, 1988).

In one of the most comprehensive studies examining biosolids PAN to date, Gilmour et al. (2003a) combined laboratory and field studies with computer simulation to characterize

the amount of PAN released when municipal biosolids were land applied to agronomic crops. In addition to aerobic laboratory incubations, field studies were conducted in Arkansas, Michigan, Virginia, and Washington from 1998 to 1999. The incubations were used to characterize biosolids decomposition kinetics and used along with biosolids analytical data in estimation of biosolids PAN. One-hundred grams of dry weight soil were placed in a 946-mL bottle and biosolids were either mixed or placed on the soil as appropriate to simulate common application methods of incorporating or broadcasting, respectively. Soil water content was adjusted to near field capacity and samples were incubated at 25ºC up to approximately 74 or 200 days. Agronomic crops used in the field trials included sorghum sudan grass, tall fescue, and corn. The computer simulation model used was Decomposition ((Gilmour and Skinner, 1999), and is as described above. Biosolids production methods of the 25 biosolids studied included anaerobic digestion, aerobic digestion, lime stabilization, oxidation ditch, and stabilization via lagoon. The mean organic N content was 41,700 mg kg^{-1} and mean C:N ratio was 7.5, and were similar to other studies (Gilmour et al., 1996). The inorganic fraction of the biosolids was predominately NH_4-N with small amounts of NO_3-N. Laboratory decomposition (measured by collecting evolved carbon dioxide in 1 M NaOH base traps) results followed typical patterns up to 75 days in 1998 and 240 days in 1999 at the Washington location soil. The decomposition data can then be combined with other factors to estimate PAN. The 14 biosolids in the Washington study included anaerobically and aerobically digested, oxidation ditched, and long term storage lagoon products. Mean decomposition across both years and all biosolids studied across all locations ranged from 3 to 54% with an approximate overall mean of 23.5% (from laboratory results). Biosolids that

had been stabilized in a lagoon had much lower decomposition, which ranged from 3 to 10% with a mean of 7%. In 1998, net N mineralization was widely variable ranging from 0 to 59% with a mean of 30%, which was similar to results of Gilmour (1996) where results of 20 to more than 50% and a mean of 35% were found. Based on the results of regression analysis between percent net N mineralization (y) and percent decomposition (x), Gilmour (2003), stated that percent net N mineralization is similar to percent decomposition on average, but that the relationship may not hold for individual biosolids. The majority of the biosolids that had not been stabilized in a lagoon were found to have two decomposition phases: rapid and slow fractions.

The mean value of total biosolids added was 452 kg N ha^{-1} with mean total N content of 48,500 mg N kg^{-1}. The range of PAN observed during the growing season for all field locations and both years was 200 to 47,200 mg kg^{-1} with a mean of 18,900 mg N kg^{-1}. Observed PAN ranged from 9 to 74% of the total N in the biosolids with a mean value of 37%. Similar studies found mean ranges from 20 to 24% (King, 1984); (Parker and Sommers, 1983a). Linear regressions were run for observed PAN versus biosolids organic N, total N, and C/N ratio in an attempt to estimate observed PAN from analytical data. The best relationship was between observed PAN and biosolids total N ($r^2 = 0.67$, $p<0.001$) across all biosolids, locations, and years, and was similar to that observed by Gilmour and Skinner (1999). However, it was stated that use of this relationship should be limited to biosolids and weather scenarios similar to those in Gilmour (2003). Computer simulations used environmental factors of temperature, precipitation (plus irrigation), and, in some cases, potential evapotranspiration to estimate growing season PAN under actual conditions. The

model predicted observed growing-season PAN relatively well ($r^2 = 0.72$, $p<0.0001$). These results, along with those of Gilmour and Skinner (1999) support the use of the computer simulation model Decomposition in estimating PAN in biosolids. It was also suggested that decomposition using mean decomposition kinetics can be used where rate constants for the biosolids being used are not available.

There was also linear relationship between first-year N mineralization and growing season N mineralization ($r^2 = 0.73$, $p<0.0001$) (Gilmour et al., 2003a) in the laboratory results. The mean values for the growing season and the full year were 27 and 37% of the organic N, respectively. It was postulated that the difference between first-year and growing season N mineralization represents the percentage of biosolids organic N that is potentially mobile as environmental pollutant, in addition to other N loss factors (leaching, volatilization, etc.). When project data was compared (Decomposition simulation parameters, first year N mineralization % (y) vs. Growing season N Mineralization % (x), and biosolids treatment processes), it was suggested that biosolids treatment processes should not be used to categorize N mineralization factors for biosolids unless extensive stabilization has occurred. This is due to the variation from the N mineralization percentages provided by the EPA (USEPA, 1995) for specific biosolids production categories (primary, anaerobically or aerobically digested, and composted) found by Gilmour, (2003). Differences in availability coefficients provided by the NCDA&CS are distinguished by *some* sewage-sludge treatment methods and method of land-application. Mean first-year N mineralization percentage for the data used to draw a correlation between first year and growing season mineralization was 40% for non-stabilized biosolids and 14% for biosolids that have

undergone extensive stabilization (lagoon or composted). Mean first-year mineralization percentages for Arkansas, Michigan, Virginia, and Washington were 42, 37, 41, and 34%, respectively, with an overall mean of 38.5%. It was recommended that areas with different weather than the study sites used require estimation of those means using the Decomposition model and actual or mean first-order rate constants. The Decomposition model accounts for soil texture. It was ultimately recommended that biosolids be grouped into not stabilized or stabilized by lagoon storage or composted in regard to organic N mineralization. Generally, Gilmour (2003) describes biosolids mineralization as highly variable and dependent on many factors. The computer simulation model Decomposition provides somewhat reliable estimates of PAN for field situations. North Carolina, and the Piedmont specifically, need further and updated research on estimates made of biosolids PAN in a single growing season. The methods used in this research were aimed at providing this information for biosolids used in these areas.

1.17 Amino Sugar Nitrogen Test

Soil testing for NO_3 is considered by some to be the best option for identifying sites where N fertilization will not produce a yield response by corn (Bundy and Meisinger, 1994). Two soil NO_3 tests are most commonly used: the preplant NO_3 test (PPNT) and the presidedress NO_3 test (PSNT). For the PPNT, profile samples are collected in the early spring to a depth of 60 or 90 cm to account for carryover of mineral N from previous cropping (Schmitt and Randall, 1994). The PSNT is sampled at a depth of 30 cm in late spring so that soil N mineralization can be taken into account and supplemented, if necessary,

by sidedressing (Bundy and Andraski, 1993). The PSNT has been recommended more widely than the PPNT in the eastern USA, but usage has been limited by the need to collect soil samples during the growing season and delays in N fertilization until testing can be completed. Its effectiveness can also be nullified if adverse weather conditions delay sidedressing. Spatial and temporal variability of soil NO_3 concentrations, which depend on mineralization, immobilization, nitrification, denitrification, leaching, and plant uptake, limit the usefulness of these tests. This resulted to the fact that a one-time test for soil NO_3 is likely going to be of little value for predicting crop N availability throughout the growing season, especially in humid regions where N-cycle processes are continuously extensive (Khan et al., 2001).

Khan states that a soil test for N that estimates a labile organic fraction that supplies the plant through mineralization would be ideal, as exterior environmental impacts would be minimized and subsequently, reduce variability in soil test levels. This would make time of soil sampling less critical and soil N availability could potentially be predicted on the basis of a one-time test prior to the growing season. Khan (2001) developed a simple technique involving the use of amino sugar N to detect sites that will not be responsive to N fertilization. Results indicated that the lowest test value for any nonresponsive soil was 34% higher than the highest value for any responsive soil, and on average, the difference in amino sugar N was more than 200%, suggesting that high test values result in no response to N fertilization of corn. Actual values for nonresponsive soils were 237 to 435 mg N kg^{-1} and responsive soils were from 72 to 223 mg N kg^{-1}. It was determined that 2 M NaOH and a 5-h diffusion period at 48 to 50ºC was the best compromise in terms of speed, convenience,

sensitivity, and resolution in determination of the amino sugar N fraction. Strong correlation was obtained between Amino Sugar N and Soil Test-N ($r^2 = 0.82$). Soil test N was determined by permanganate reduced iron modification of a semimicro-Kjeldahl procedure (Bremner and Mulvaney, 1982). Soil samples were collected from previous work done in 1990 to 1992 in Illinois that had been stored in Mason jars and frozen. Along with this information and additional work, it was determined that the test successfully recovers Amino Sugar N and NH_4-N while NO_3- and NO_2-N were undetectable. Using these fractions of recoverable N, 25 Illinois soils (0-30 cm) were classified correctly as being responsive or non-responsive to N fertilization with a critical test value range of 225 to 235 mg N kg^{-1}. This test has obvious value for improving N fertilizer efficiency, increasing the profitability of corn production, and reducing the adverse environmental effects of excessive N fertilization (Khan et al., 2001).

Gilmour and Skinner, (1999) and Mulvaney et al., (2001) also found strong evidence that the amino sugar N fractions of soil organic N can be used to predict responsiveness of corn to N fertilization. Eighteen varying soil hydrolysate samples were prepared and analyzed for total hydrolyzable N, NH_4-N, (NH_4 + amino sugar)-N, and amino acid N from soils taken from N response trials conducted for corn at 18 sites in 1990, at 29 sites in 1991, and 28 sites in 1992 throughout Illinois. Five of the 18 soils were selected for use in an incubation study to evaluate potential mineralization and detect changes in hydrolyzable N fractions throughout a time period of three months. Large ranges were found in content and distribution of hydrolyzable N. A fivefold range was found in total hydrolyzable N, a 13-fold range in amino acid N, a threefold range in hydrolyzable NH_4-N, and an 11 fold range in

amino sugar N. Mulvaney et al. (2001) postulated that amino acids and amino sugars may differ in the extent to which they occur as stable humic forms, and hence in their tendency to undergo mineralization. If a particular form of soil organic N is highly labile, then the concentration of this form should be inversely related to crop responsiveness to N fertilization, and a distinct difference should exist between responsive and nonresponsive soils. Their results indicated that total hydrolyzable N, amino acid N, and hydrolyzable NH_4-N could not be used to estimate soil N availability due to large overlap between data for responsive and nonresponsive soils. However, amino sugar N was statistically significant at the 0.001 probability level. The lowest value for any nonresponsive soil exceeded the highest value for any responsive soil by more than 30%, and on average, the difference was nearly threefold. Similar results were found by Khan et al. (2001). Further evidence of a close relationship between soil amino sugar content and non-responsiveness to N fertilization was shown by correlations of soil chemical properties with check plot yield and N fertilizer response. Amino sugar N content had r-values of 0.79 and -0.82 when correlated with check-plot yield and N-fertilizer response, respectively, and both were statistically significant at the 0.001 probability level. These coefficients of correlation were higher than those for all other variables correlated with check-plot yield and N fertilizer response, such as organic C (r-values of 0.55 and -0.60, respectively), total N (0.52 and -0.55), total hydrolyzable N (0.59 and -0.61), amino acid N (0.55 and -0.67), and hydrolyzable NH_4-N (0.34 and -0.48).

Of the seven responsive soils, considerable variation in magnitude of response existed. There was some indication that the variation was related to their content of amino sugar N and suggested the possibility of a quantitative soil test in addition to a means of

detecting nonresponsive sites (Mulvaney et al., 2001). The most responsive soil had the lowest concentration of amino sugar N, whereas two of the three least responsive soils had the highest concentrations of amino sugar N. In laboratory aerobic incubations performed, mineral N was greater in nonresponsive soils than responsive, and as mineral N decreased, so did concentrations of amino sugar N.

These results indicate that soil amino sugar N fraction is a key factor in identifying the responsiveness of corn to N fertilization. The clear distinction in different forms of N that was observed between soils from seven responsive and 11 nonresponsive sites in the N response study, high correlations between amino sugar N and check-plot yield or fertilizer-N response, and greater production of mineral N by nonresponsive than responsive soils during laboratory incubation (which was always accompanied by a decrease in amino sugar N), support use of this organic N fraction to identify sites where corn can be grown profitably without the use of N fertilizer (Mulvaney et al., 2001). As a result, the test might provide a relative index of N mineralization in soils amended with biosolids.

Williams et al. (2007a) conducted a study to evaluate three different soil N tests for practicality, precision, and ability to correlate with corn economic optimum N rate (EONR) and fertilizer response on southeastern U.S. soils. The soil N tests used were the Illinois soil N test (ISNT), as described by Khan et al. (2001); the gas pressure test (GPT) as described by Picone et al. (2002); and the incubation and residual N test (IRNT), as described by Bundy and Meisinger (1994) and Crozier et al., (2003). Soil samples from Williams (2007a) were collected from the sites of 16 N-response trials from 2001 to 2003 where different mineralizable and residual N levels were expected. The ISNT was determined to be the most

practical test because it was the easiest to perform, most precise, and could be completed in 1 d. The R^2 for a correlation of the ISNT, GPT, and humic matter percent vs Delta Yield (maximum yield minus check yield) were 0.49, 0.60, and 0.35, respectively. Additionally, the coefficient of determination for a correlation of the ISNT, GPT, IRNT, and NO_3-N vs EONR were 0.90, 0.62, 0.33, and 0.41, respectively. These results indicate the potential of the ISNT and GPT to account for mineralizable and residual soil N levels and thus improve current corn N recommendations in the humid southeastern USA (Williams et al., 2007a).

Williams et al., (2007b) also determined EONR and ISNT levels in representative southeastern soils in 35 N-response trials in the Piedmont, Middle, and Lower Coastal Plains of North Carolina from 2001 to 2004. They found the ISNT to be strongly correlated with EONR for well or poorly drained sites (r^2 = 0.88 and 0.78, respectively), but had insufficient data to establish correlations for very poorly drained or severely drought-stressed sites. Expression of the ISNT on a mass per unit volume basis vs. EONR only slightly improved correlation (r^2 = 0.88 and 0.79 for well and poorly drained sites, respectively), and these improvements were deemed not justifiable given the effort required for soil bulk density determinations. Regressions of ISNT vs. minimum, average, and maximum EONR based on different N-fertilizer-cost: corn/price ratios (11.4:1, 7.6:1, and 5:1, respectively) showed strong correlations with EONR for well-drained sites (r^2 = 0.77, 0.87, and 0.87, respectively) and poorly drained sites (r^2 = 0.84, 0.78, and 0.70, respectively). The ISNT-EONR correlations were different among the cost/price ratios for well-drained sites, but not different for poorly drained sites. Their results indicated that the well and poorly drained soil ISNT concentration models for predicting EONR were relatively robust and showed promise as a

tool for N management. It was stated, however, that further research is needed to calibrate and validate the average EONR vs. ISNT concentration relationships under grower conditions. They postulated that the ISNT could be used to modify current yield-based N fertilizer recommendations or to develop new recommendations based on ISNT. A possible strategy for using the ISNT to predict corn N need could be to take a preplant soil sample for ISNT analysis, apply a small amount of starter N at planting, then apply sidedress N between V4 and V10 with total N rates based on the ISNT.

It is important to note that Williams et al. (2007a, b) and Spargo and Alley (2008) used an incubator for the ISNT test rather than the hot plate used by Khan et al., (2001) and Mulvaney and Khan (2001). Spargo (2007) demonstrated that the use of an incubator set to 50ºC reduced both the quantity of recovered N and the sensitivity of the assay when compared with the standard ISNT. However, improved measurement precision was achieved as were several ancillary benefits include eliminating the need to rotate jars and reduced labor demand. It was postulated that one person can titrate approximately 100 samples a day using an autotitrator, plus the required time for the additional steps. It was concluded that greater sample throughput may be achieved with a smaller laboratory footprint using the incubator method. This could further allow the ISNT to be used for routine use in commercial and institutional soil testing laboratories for estimation of PAN is a growing season (Spargo and Alley, 2008).

Despite the successful results of using the ISNT to detect responsiveness to N fertilization of corn, others have found contradictory results. Laboski et al. (2008) used data from 96 corn N rate response trails across Iowa, Illinois, Michigan, Minnesota, Nebraska,

and Wisconsin to evaluate the usefulness of the ISNT in identifying nonresponsive fields, predicting EONR, and estimating mineralizable N. Corn was grown following several crops. Contrary to the findings of Williams et al. (2007a,b), Laboski et al. (2008) found that the ISNT could not accurately predict nonresponsive sites, nor could it reliably estimate EONR, even when subsetting the data based on soil drainage class and previous crop. Laboski et al. (2008) found the ISNT to be strongly correlated to organic matter ($r = 0.96$) at the sites studied. Klapwyk and Ketterings (2006) also found the ISNT to be strongly correlated with OM ($r = 0.95$) for soils in New York. Laboski et al. (2008) found the ISNT to be strongly correlated with total N ($r = 0.90$) and postulated that it appeared to be measuring a relatively constant fraction of total N. Further elaboration on this was not provided. Khan et al. (2001) and Klapwyk and Ketterings (2005) also found strong correlations between the ISNT and total N, although they did not explore that relationship. Laboski et al. (2008) concluded that the ISNT appears to measure a constant fraction of total N for a wide range of soils rather than readily mineralizable fractions of soil organic N as would be required to assess the soil's contribution to the available N supply. Ultimately, the ISNT was not suggested for use in adjusting N rate recommendations for corn in the North Central Region (Corn Belt) of the United States (Laboski et al., 2008). Other studies have had similar results of the failure of the ISNT to function as described by Khan et al., (2001) in Iowa (Barker et al., 2006) and Wisconsin (Osterhaus et al., 2008).

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CHAPTER 2: MATERIALS AND METHODS

2.0 Nitrogen Sources and Application Rates

Three different biosolids were evaluated via several established laboratory procedures: 1) “Raleigh plus” from the Neuse River Wastewater Treatment Plant (NRWWTP) in Raleigh, NC (Wake Co.), 2) “OWASA cake” from Mason Farm Wastewater Treatment Plant in Carrboro, NC (Orange Co.), 3) “Cary Pellet” from the South Cary Water Reclamation Facility (SCWRF) in Apex, N.C (Wake Co.). These biosolids were compared to ammonium nitrate (NH_4NO_3; AN). Raleigh plus is a twice-dewatered lime-stabilized and pasteurized cake originating from an aerobic treatment process with C enhanced N removal. The OWC is the result of anaerobic digestion via a batch process to achieve pathogen reduction and is processed via a gravity belt thickener. The CP biosolids are processed via biological nutrient removal, and they are heat treated to form a BB-sized pellet. The fresh biosolids were collected directly from the production facilities in December 2010. The OWC and R+ biosolids were stored in a refrigerator at 4ºC in polyethylene bags to maintain their moisture content and CP was stored in a mesh sack at room temperature. Ammonium nitrate was from Fisher Scientific, 100% reagent grade, and granular. A subsample of each source was analyzed for a suite of total nutrient and heavy metal contents (N: NO_3, NH_4, urea, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Bo, N, Cd, Pb, Na); pH; soluble salts; C; dry matter; lime/$CaCO_3$ equivalent) by the Plant/Waste/Solution/Media Analysis Section of the Agronomic Division of the NCDA&CS (Tucker et al., 2007).

The relative response of four N sources added at four rates to four soils was evaluated in the anaerobic incubation and the ASNT. . The design was a 4 X 4 X 4 factorial: N Source

X Rate X Soil, augmented with a zero N source-zero rate control. This design has been denoted an “augmented factorial” or “factorial plus" (Piepho et al., 2006); (Marini, 2003). These experiments were completely randomized designs (CRD) with four replications within each soil. The tests were carried out by soil type (one soil per day for each of the four soils and repeated 4 times) and not by replication (the entire experiment being replicated four times). That resulted in the soil factor essentially being a block. Therefore, no definitive statements about differences among soils could be made due to soil being confounded with block. Data were still analyzed as if the experiment was run by replication, but discussion of differences between soils cannot be statistically proven with the available data. In addition, data were also analyzed by soil to interpret the differences within each soil. The results of that analysis were proven statistically with the available data

The target N application rates were based on the North Carolina Realistic Yield Expectation (RYE; NC Nutrient Management Workgroup, 2003) for tall fescue (*Festuca arundinacea*), a common biosolids-receiving crop, on a Wedowee sandy loam in Nash Co., NC, the site of the field trial. The RYE N rate so calculated was 144.5 kg N ha^{-1}; NH_4NO_3 was applied at this rate. Given that much of the total N in most biosolids does not become available the first year, we used the existing NCDA&CS “first-year-nutrient availability coefficients” (McGinnis et al., 2011) for N to estimate the amount of biosolids needed to release 127 kg of plant available N ha^{-1} in all laboratory tests (Table 2.1). The difference between the NH_4NO_3 and biosolids rates was due to a calculation error. Nutrient availability of the biosolids was calculated by equations [1] and [2], as specified by the NCDA&CS (McGinnis et al., 2011) and as described previously in section 1.11:

[1] Nutrient available (PAN) (lb/ton) = [nutrient concentration (mg/kg) ÷ 1,000,000] × NAC × NM × 2000 × (DM% ÷ 100)

[2] Nutrient available (lb/1000 gal) = [nutrient concentration (mg/L) ÷ 1,000,000] × NAC × NM × 8340

Where:

- NAC = nutrient availability coefficient
- NM = nutrient multiplier
- DM% = dry matter percent

The NCDA&CS N coefficients differ somewhat based on the biosolids production process and were chosen accordingly. The application rates were 0.5, 1, 1.5 and 2 X the RYE, augmented by a zero N control. Adjusted PAN and total N added for each RYE interval and N source are shown in Table 2.2. Bulk samples for the anaerobic incubation and the ASNT were made using 100 g of soil for the three biosolids and 150 g of soil for AN in slider-sealable 0.045 mm-thick plastic bags with dimensions of 17.7 x 20.3 cm. Amendments were added to the bulk soil at the calculated rates described above and mixed by hand to yield a homogeneous mixture. Method specific subsamples were taken from each bulk sample, as described below. The aerobic incubation methods varied slightly, and were as described in section 2.5.

2.1 Soil Collection and Chemical-Physical Characterization

Soil series that are typically permitted to receive regional biosolids were identified by querying several local biosolids land applicators. Based on their guidance, representative and diverse soil series were selected from two of North Carolina's three physiographic regions, two from the Piedmont: Vance sandy clay loam (fine, mixed, semiactive, thermic Typic Hapludults) and Wedowee sandy loam (fine, kaolinitic, thermic Typic Kanhapludults); and two from the Coastal Plain: Norfolk loamy sand (fine-loamy, kaolinitic, thermic, Typic Kandiudults) and Noboco loamy sand (fine-loamy, siliceous, subactive, thermic Oxyaquic Paleudults). Using NCDA-NRCS soil maps (USDA-NRCS, 2011) and aerial photographs, suitable sites that had no record of previous application of municipal biosolids were identified for collecting samples of these soils. The Vance sandy loam, ~6% slope (Kleiss et al., 1993), was collected under non-managed sod at the Upper Piedmont Research Station, Reidsville, NC (Rockingham Co.). The Wedowee sandy loam, 2-6% slope (USDA-NRCS, 2011) was collected under fescue sod bordering biosolids field experiment near Spring Hope, NC (Nash Co.; described in subsequent sections). The Norfolk loamy sand, ~2-6% slope (Kleiss, 1981), was collected under non-managed sod at the Central Crops Research Station, Clayton, NC (Johnston Co.). The Noboco loamy fine sand, ~0-2% slope (USDA-NRCS, 2011), was collected under non-managed sod bordering a research field at the Williamsdale Biofuels Field Laboratory, Wallace, NC (Duplin Co.). Approximately 130 L of soil were collected from one location (4 m^2) at each site through a depth of ~20 cm. As a result, inferences can only be made about the specific locations in which the soil was collected. Broad inferences about the soil series itself cannot be made. The soil consisted primarily of

the surficial Ap horizon (“plow layer”), but for the piedmont soils, included some of the clayey Bt horizon. A moist subsample was sent to NCDA&CS Agronomic Division Soil Test Section for routine fertility and chemical analysis (Mehlich-3 P, K, Ca, Mg, S, Cu, Mn, Zn, Na, cation exchange capacity and base saturation, pH/acidity/lime requirement; humic matter; soil class, and weight-to-volume ratio). Soil was sieved to pass a 2-mm sieve and stored under a covered shed in ~19 L plastic buckets, sealed with a lid, and covered with a tarp until use in laboratory analyses.

2.2 Soil Water Container Capacity

Based on the method of Cassel and Nielsen (1986), container water holding capacity was determined on four replications of each soil using 600 mL glass Buchner funnels with fritted disc fitted with a hollow aluminum column filled with settled air-dried soil. The mass of the air-dried soil in the core was recorded before placing it in the Buchner Funnel. Moisture was added such that the soil was saturated from the bottom up, thus minimizing air trapped in the soil core. The apparatus was sealed and 30 kPa pressure was applied for 24 hours using a Meriam Instrument pressure device to allow for equilibration and drainage of excess water. The soil core was removed from the funnel and the mass was recorded. The weight of the water in each soil was divided by the dry soil weight and multiplied by 100 to obtain an estimate of percent container capacity. Eighty percent of the calculated container capacity was used for the aerobic incubation experiment. The biosolids products used had moisture contents ranging from 5 to 80%. Therefore, moisture content and application rates

of each biosolid were accounted for in the moisture adjustment calculation prior to adjustment.

2.3 Amino-Sugar Nitrogen Test (ASNT)

Laboratory procedures were adopted from Khan et al. (2001) and as described above. A subsample of 1.0±0.01 g of the soil/N-source mixture was removed from each bag and placed into a 0.47-L (1 pint) Mason jar. The samples were treated with 10 mL of 2 M NaOH. A 60-mm petri dish was filled with 5 mL of H_3BO_3 indicator solution (bromocresol green and methyl red) and attached to the jar lid so as to be suspended above the soil solution. The jar lid was immediately attached to the jar (air tight) and the entire assembly was heated in an incubator at 49ºC (±1º) for 5 hours, a modification suggested by Spargo et al., (2007) and also used by Williams (2007a,b). After the 5 h incubation, samples were allowed to cool to room temperature, Petri dishes were removed from the jars, and the indicator solution was diluted with 5 mL of deionized water. The diluted indicator solution was titrated using a standardized H_2SO_4 solution (approximately 0.01 M) to an endpoint established on the basis of color (University of Illinois, 2004). Soil test concentrations (mg kg^{-1}) were calculated as S x T, where S is milliliters of H_2SO_4 used in titrating, T is the titer (µg N mL^{-1}; T = 280 µg N mL^{-1} for 0.01 M H_2SO_4) of H_2SO_4 (Khan et al., 2001; University of Illinois, 2004). This test was also repeated on the N sources alone (with no soil) in a separate analysis. One equivalent gram of dry N source (on a wet-weight basis) was used for each N source.

2.4 Anaerobic Incubation

Laboratory procedures were adopted from Bundy and Meisinger (1994) and as described above. From the bulk mixture, 15 ± 0.01 g of soil-N-source mixture was placed into a 120 mL extraction receptacle (cup). Fifty milliliters of deionized water was added to the receptacle and swirled gently to minimize adhesion of the mixture to the walls of the container. The samples were placed in an incubator for 7 days at 40±1ºC. The samples were removed from the incubator and extracted with 50 mL of 2 M KC at a 1:3.3 soil/solution ratio, and poured through a No. 42 Whatman filter paper, as described by Bremner and Keeney (1966). Samples were decanted into 20-mL scintillation vials, sealed, and placed in a freezer until analysis of inorganic-N (NH_4 + NO_3) of the filtrate on the Lachat flow-injection auto analyzer. The percentage of PAN recovered was calculated as the total amount of inorganic N (NH_4 + NO_3) that was recovered from the NAC estimates of PAN added (Table 2.2). The percentage of total N recovered was calculated as a percentage of inorganic N recovered from the total N added (Table 2.2).

2.5 Aerobic Incubation

Laboratory procedures were adopted from Bendy and Meisinger (1994), Montalvo (2008), and Moore (2001). The experimental design was a 4 X 4 X 4 X 2 X 7 factorial: N Source X Rate X Soil X Moisture X Sampling times (0, 3, 7, 14, 28, 56, and 112 d), augmented with a zero N source-zero rate control, all with four replications. The application rates were as described above and were augmented by a zero N control (Tables 2.1 and 2.2). To ensure sufficient sample quantity, N application rates were calculated and mixed on the

basis of 600 grams of soil. Each 600-g amended bag was mixed by hand in replication 1 and blended in a standard kitchen blender for replications 2-4 to ensure greater homogeneity and destruction of any remaining aggregates. Each 600 g bulk sample was split into two 300 g subsamples and placed into slider-sealable 0.045 mm-thick plastic bags with dimensions of 17.7 x 20.3 cm. Each 300 g bag was adjusted to an estimate of 80% of container capacity (as described above) and was weighed. Moistened soils were periodically weighed to readjust water content whenever moisture loss was greater than 5% on a weight basis, although little change was noticed throughout all sampling days and replications. Samples were incubated in a constant temperature room at 20±2ºC and were sampled for KCl-extractable NH_4- and NO_3-N (Bremner and Keeney 1966) at each of the seven sampling dates previously described by taking individually two 10 cm^3 (approximately 10-15 g) volumetric scoops. One of these subsamples was extracted with 50 mL of 1 M KCl, an approximate ratio 1:3.3-5 soil mixture: solution ratio, shaken for 60 min on a mechanical shaker, and filtered through a No. 42 Whatman filter paper. Samples were decanted into 20-mL Scintillation vials and placed in a freezer until analysis of inorganic-N (NH_4 + NO_3) of the filtrate on the Lachat flow-injection auto analyzer. The other 10 cm^3 subsample was weighed, oven dried at 105ºC, and weighed once more in order to calculate the subsample dry mass, calculate dilutions factors, and express the results on a dry weight basis. It was assumed that the subsamples were of equal weight.

2.6 N Mineralization Field Study

In an attempt to further understand the N mineralization dynamics of land applied municipal biosolids, a field trial was initiated in fall 2010 on private land with no previous history of biosolids application in Spring Hope (Nash County), NC. The experiment consisted of a 2 X 4 factorial, N source X N rate, augmented with a zero-rate control and implemented in a randomized complete block design with four replications. The N sources were only CP and AN in this experiment due to logistic issues, and were applied at the same rates as described above and in Table 2.2. The entire field site was 32.9 by 20.7 m. Individual plots were 3.7 by 3.76 m. There were three 2.03-m wide alleys between each block. The two N sources were applied on 15 November 2010 to an existing stand of tall fescue. On 17 May 2011, 76.20 cm swaths were cut from the center of each plot using a sickle bar mower, raked, and weighed. Grab samples were collected, dried at 65ºC for 48 hr., and reweighed to calculate tissue moisture and dry matter yield on a per hectare basis. Only one cutting was taken due to a dry summer. The dry plant material was analyzed for total nutrient and heavy metal content at the NCDA&CS Plant Analysis section (McGinnis et al., 2011). Subsequent to the work described here, plots will be split and N sources will be reapplied to split plots in order to evaluate residual and cumulative effects of N applications. Nitrogen uptake was calculated as the N concentration in the forage multiplied by the yield (N uptake = N concentration * yield). Apparent N recovery (ANR) was calculated as the amount of N uptake from a fertilized plot minus the N uptake of the appropriate control plot, divided by the amount of PAN added to the appropriate plot, and multiplied by 100, as

describe by Good et al. (2004); (ANR = [(N uptake amended – N uptake control) / PAN applied] * 100).

2.7 Statistical Analysis

For each laboratory study and the field trial, an analysis of variance was performed using the PROC MIXED of SAS Version 9.2 (SAS Institute, Cary, NC, 2012). Regression analyses were performed using PROC REG and PROC MIXED procedures in SAS. Mean separation was performed using the PDMIX800 macro (Piepho, 2012).

Table 2.1. Nitrogen availability coefficients (NAC) used for biosolids used in this research.

Biosolids	Waste Type	Waste Source	Application Method	Total-N	Inorganic-N	Organic-N
OWASA Cake	Municipal	Anaerobic	Broadcast	0.17	0.80	0.17
Raleigh Plus	Municipal	Lime Stabilized	Broadcast	0.28	0.80	0.28
Cary Pellet	Municipal	Other	Broadcast	0.30	0.25	0.30

Table 2.2. Plant-Available N rate and Total N applied for each N source used in this research.

N Source	RYE Interval	PAN Rate	Total N Applied
		---------- kg ha^{-1} ----------	
AN	0.5	73	73
	1.0	145	145
	1.5	218	218
	2.0	290	290
Cary Pellet	0.5	64	214
	1.0	127	427
	1.5	191	641
	2.0	254	854
OWASA Cake	0.5	64	332
	1.0	127	663
	1.5	191	995
	2.0	254	1326
Raleigh plus	0.5	64	210
	1.0	127	419
	1.5	191	629
	2.0	254	838

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CHAPTER 3: SOILS AND BIOSOLIDS CHARACTERIZATION

3.0 Biosolids Characterization

Chemical and physical properties of all biosolids used in this research are illustrated in Figure 3.1 and Table 3.2. Analysis of N content specifically revealed that CP contained the highest amount of total N with 65,500 mg kg^{-1}, followed by OWC with 48,801mg kg^{-1}, and R+ with 6,939 mg kg^{-1} present (Table 3.2). This was not surprising given the quality of the three biosolids (Class A EQ), and is typical of WWTP with efficient denitrification processes. As a result, all biosolids are at least 95% organic material. The dry matter proportions of the biosolids varied as could be predicted from their natures: the dried CP had the highest dry matter (95%), the double-dewatered R+ intermediate (52%), and the OWC dewatered cake the lowest (20%).

Other chemical characteristics of note are the high phosphorus (P) contents of CP (34,900 mg kg^{-1}) and OWC (25,635 mg kg^{-1}). Raleigh plus had a relatively small amount with only 1,765 mg kg^{-1}. Raleigh plus is most commonly used as a liming agent and its liming ability is represented in the chemical data (Table 3.2). It has a calcium (Ca) content of 247,362 mg kg^{-1}, a calcium carbonate equivalency (CCE %) of 74.3, an agricultural lime equivalent (ALE) of 2.2, and a pH of 11.2. This is a result of mixing the sludge with a lime kiln dust and alkaline pasteurization, as discussed in the Biosolids Treatment Processes section. Cary pellet and OWC have little value as a liming agent. The iron (Fe) content of the CP (42,700 mg kg^{-1}) is over four times greater than the next highest product (R+; 9,928 mg kg^{-1}). Iron chloride ($FeCl_2$ or $FeCl_3$) is frequently used in wastewater treatment as a flocculent and to remove P and may be the source of the high Fe level in CP. Heavy metal

contents of all biosolids are generally low, and CP has a SO_4 content (24,300 mg kg^{-1}) that is 2.5 times greater than the next highest product (OWC; 307,708 mg kg^{-1}). Total organic carbon (TOC) varied between each biosolids with CP (417,000 mg kg^{-1}) > OWC (307,708 mg kg^{-1}), > R+ (128,538 mg kg^{-1}), the same order as total N content. The C:N ratio of each product is R+ (18.5) > CP (6.4) > OWC (6.3).

3.1 Soil Characterization

Chemical and physical properties of all four soils used throughout this research are shown in Table 3.2. The soils used consisted of a Norfolk loamy sand, a Noboco loamy sand, a Vance sandy clay loam, and a Wedowee sandy loam. The Norfolk and Noboco soils had the highest HM content with 0.56 and 0.76%, respectively. The Vance and Wedowee soils both had a HM content of 0.36%. The soils range in bulk density from 1.04 to 1.39 g cm^{-3}. The base saturation of the Wedowee soil was the highest at 94%, followed by the Noboco soil with 87%, the Vance soil with 78%, and the Norfolk soil with 47%. The pH of the four soils was typical of NC soils and ranged from 5.1 to 6.8. The Noboco soil had relatively high amounts of P (662.4 kg ha^{-1}) compared to the other soils. Data on the inorganic N fractions were not available.

Table 3.1. Chemical composition of the three biosolids used throughout this research. Biosolids were selected from three regional waste water treatment plants in the Piedmont of NC. All results are on a dry weight basis unless otherwise indicated. Cells marked with (-) indicate data not available.

Biosolids Property	Cary Pellet (CP)	OWASA Cake (OWC)	Raleigh plus (R+)
Dry Matter (%) ‡	95	20	52
pH‡	5.8	6.4	11.2
C:N ratio	6.4	6.3	18.5
CCE% G]	-	0.3	74.3
ALE (tons) ₮	-	1717	2.2
	---------- mg kg^{-1} ----------		
TKN	65,500	48,801	6,939
Organic-N	62,210	47,128	6,645
Inorganic-N	3,293	1,673	294
NH_4-N	3,290	1,665	260
NO_3-N	3	8	34
P	34,900	25,635	1,765
K	6,810	2,357	2,914
Ca	16,100	20,559	247,362
Mg	5,150	4,534	3,747
Na	1,100	1,105	508
Fe	42,700	7,642	9,928
Al	6,900	-	-
Mn	779	425	105
Cu	286	341	64
Zn	702	678	149
Cd	2	1	-
Cr	42	-	-
Ni	22	9	-
Pb	11	10	-
As	5	-	-
Se	2	-	-
Mo	11	-	-
Cl	831	-	1,021
B	59	68	68
Soluble salts	182	182	358
SO_4	24,300	9,569	418
Carbon (TOC) ‡	417,000	307,708	128,538

‡ Wet weight basis

Table 3.1 Continued.

Ǥ Acid neutralizing capacity of waste as a percentage of pure $CaCO_3$
Ŧ Amount of waste required to equal neutralizing value of one ton of ag lime

Table 3.2. Selected properties of the four soils used in this research. Cells marked with (-) indicate data not available. Analyses performed according to Tucker et al., (1997).

	---- N.C. Coastal Plain Soil ----		----- N.C. Piedmont Soil -----	
Soil Property	Norfolk	Noboco	Vance	Wedowee
Surface texture	loamy sand	loamy sand	sandy clay loam	sandy loam
Percent humic matter	0.56	0.76	0.36	0.36
Weight / Volume, (g cm^{-3})	1.39	1.25	1.04	1.22
CEC ‡, (cmol kg^{-1})	3.4	6.7	4.9	8.4
Percent Base Saturation	47	87	78	94
Exchangeable Acidity, (cmol kg^{-1})	1.8	0.9	1.1	0.5
pH	5.1	6.2	5.7	6.8
P, (kg $ha^{-1)}$	160.8	662.4	247.2	237.6
K, (kg ha^{-1})	105.6	176.0	222.9	160.3
Ca % Ŧ	32	76	54	77
Mg % Ŧ	12	8	19	14
Mn, (kg ha^{-1})	8.0	22.1	36.5	76.8
Zn, (kg ha^{-1})	4.1	17.9	14.6	16.8
Cu, (kg ha^{-1})	1.1	2.3	9.4	4.4
S, (kg ha^{-1})	-	21.1	25.9	18.2
Na, (kg ha^{-1})	0	0	0	0

‡ Cation exchange capacity
Ŧ Percentage of CEC occupied
¶ Mn availability for first crop
Ǥ Mn availability for second crop

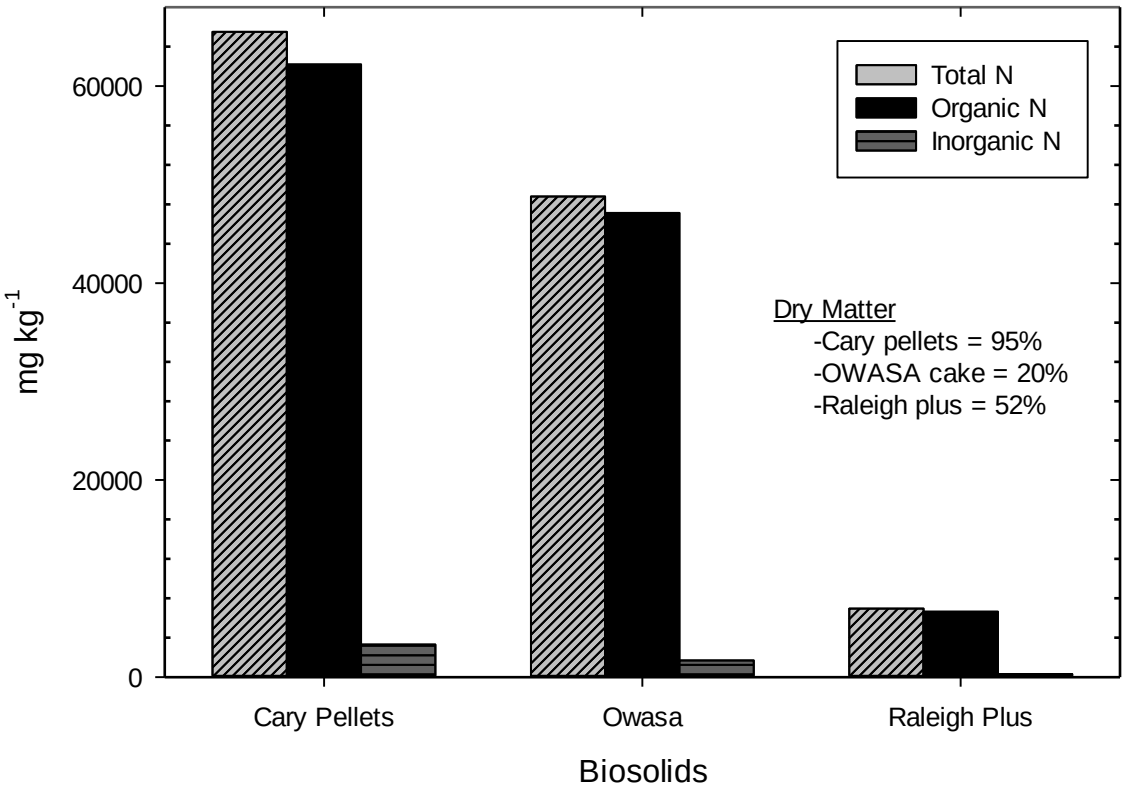

Figure 3.1. Total nitrogen (N), organic N, inorganic N, and percent dry matter for the three biosolids studied: Raleigh plus: City of Raleigh Neuse River Waste Water Treatment Plant, Raleigh, NC; Cary Pellets: Town of Cary, NC; OWASA cake: Orange County Water and Sewer Authority, Carrboro, NC. CP, Cary pellets, OWC, OWASA cake, R+, Raleigh Plus.

CHAPTER 4: ANAEROBIC INCUBATION

4.0 Anaerobic Incubation Total Inorganic N

The NCDA&CS provided an analysis of the percent humic matter (HM) of each soil tested. Humic matter as determined by the NCDA&CS method is strongly correlated with soil organic matter (Gonese and Weber, 1998) . The organic matter present in each soil was assumed to be the main source of mineralizable N in the unamended control soils. The Noboco loamy sand had the highest % HM with 0.76, followed by Norfolk with 0.56, and Vance and Wedowee both with 0.36 (Table 3.2). The two loamy sand surface textured soils had greater % HM than the less sandy soils. As a result, Noboco and Norfolk soils were expected to yield the highest anaerobic N in the unamended controls due to the greater amounts of organic N. However, the Noboco, Norfolk, Vance, and Wedowee soils released approximately 22, 20, 13, and 42 mg kg^{-1} of anaerobic N, respectively. Wedowee inherently released more N via anaerobic incubation than the other three soils despite having a relatively low % HM. Therefore, the differences in HM likely do not fully explain the differences found in anaerobic N. Potential explanations include inherent differences in these soils' ability to mineralize organic matter, likely due to differing soil chemical, physical, and/or microbiological properties, which could have important implications for mineralization of biosolids added to these soils.

4.0.0 Analysis of Variance

The average anaerobic incubation total inorganic N of the unamended-soil controls was subtracted from the amended samples so that the effect of the amendments alone could

be identified. The analysis of variance of all treatment factors tested is illustrated in Table 4.1. The three-way interaction of RYE rate*N source*Soil was statistically significant, as were the two way interactions of N source*Soil and RYE rate*N source. The two-way interaction of RYE rate*soil was on the brink of statistical significance at the 0.05 level, but was considered important enough to investigate. All main effects were also significant. As a result, the ANOVA was broken down by soil type to simplify the analysis (Table 4.2). Throughout all soil types, the RYE*N source interaction was statistically significant. As a result, the simple effects of rate were investigated for each N source in each soil.

4.0.1 Regression Analysis: Simple Effects, Noboco loamy sand

Results of a regression analysis of the response of anaerobic incubation total inorganic N to rates of three biosolids (CP, OWC, and R+) and NH_4NO_3 is illustrated in Figure 4.1. Each regression line represents the simple effect of RYE rate for each N source (The x-axis represents RYE intervals of the Realistic Yield Expectation recommended rate, which were estimates of PAN from the biosolids based on NAC described previously and in Table 2.1). Regression equations, model significance, and R2 values are illustrated in Table 4.3. The best fit model for all regressions was linear. In the Noboco loamy sand soil (Fig. 4.1A), the mean of the anaerobic N of the controls was 22.1 mg kg-1, an amount intermediate among the four soils tested. OWASA cake and CP yielded the greatest amount of anaerobic incubation total inorganic N across all rates. Separation of the observed means showed no difference between the two models (Table 4.4). Both CP and OWC's linear increase was statistically significant between each RYE interval. However, NH_4NO_3 and R+ responded to

RYE rates differently than CP and OWC, and was evidence of the RYE rate*N source interaction—that is—the response to RYE rate depended on the N source. Ammonium nitrate was generally higher than R+ across all rates, but mean separation showed the only difference was at the 2 X RYE interval (Table 4.4). Interestingly, CP and OWC yielded much greater amounts of anaerobic N than did NH_4NO_3, and R+ yielded less. First year N availability coefficients (NAC) were used to calculate biosolids rates in an attempt to standardize the amount of inorganic N released from each N source. Inorganic NH_4NO_3 was assumed to be 100% plant-available upon dissolution of the prills. Therefore, if the anaerobic incubation accurately predicted N mineralized from the N sources, and the first year NAC were correct, all regression lines should have been coincident. Evaluation of the anaerobic incubation and the precision of the NAC are further discussed in subsequent sections.

4.0.2 Regression Analysis: Simple Effects, Norfolk loamy sand

Results of a regression analysis of the simple effects of RYE rate for each N source for the Norfolk loamy sand soil are illustrated in Figure 4.1B (anaerobic N vs. RYE rate). The mean anaerobic N of the control samples was 19.1 mg kg^{-1}, an amount intermediate among the four soils tested. Cary pellet appeared to have yielded a greater amount of anaerobic N than OWC, but mean separation showed no differences between the observed means (Table 4.5) of both biosolids. However, AN anaerobic N was greater in this soil than in the Noboco loamy sand (Figure 4.1). Mean separation showed differences between AN and CP in the 0.5 X and 1.0 X RYE intervals only, and differences in the 0.5 X RYE

intervals between AN and OWC. Raleigh plus mineralized less anaerobic N than all other N sources, with the exception of the 0.5 X RYE interval of AN. The response of R+ to RYE rate was different from the other N sources and was evidence of the RYE rate*N source interaction shown by the ANOVA (Table 4.2). In the Norfolk soil, the NAC appeared to have estimated PAN from CP and OWC correctly at most rates, but did not do so in the Noboco soil. The discrepancy is due to the amount of AN recovery between the two soils, as all other N sources were similar in the Noboco and Norfolk soil. As described in subsequent sections, the difference in recovery in Anaerobic N is due to the variation in recovery of anaerobic NO_3-N.

4.0.3 Regression Analysis: Simple Effects, Vance loamy sand

The results of a regression analysis for the Vance sandy clay loam soil are illustrated in Figure 4.1C. The mean of the anaerobic N of the controls samples was 12.9 mg kg^{-1}, the lowest of the four soils tested. Cary pellet, OWC, and AN reacted similarly across all rates. Mean separation (Table 4.6) showed no differences among the observed means for those three N sources across all RYE rates. Such results would be expected if both the anaerobic incubation was precise and the NAC used were correct. Raleigh plus produced unexpected results. The lowest rate of 0.5 X RYE had the most anaerobic N of all four N sources, which *decreased* as the application rate increased. Originally, this anomaly was attributed to a labeling error. However, the samples were rerun and similar results were found. Examination of mean separation (Table 4.6) showed no differences among RYE rate intervals of R+, and the regression equation was not statistically significant (Table 4.3).

Therefore, the results were similar to that of the Norfolk soil in terms of differences among RYE rates, but different in terms of magnitude of anaerobic N. There was also a difference among RYE rates of R+ in the Norfolk and Vance soils. This difference in anaerobic N response between soils was evidence of the RYE rate*N source*Soil interaction (Table 4.1). Moreover, CP, OWC, and AN had statistically significant linear models (Table 4.3). Since the model for R+ was not statistically significant, evidence was provided of the RYE rate*N source interaction (Table 4.2) because of the difference in response to RYE rate between the R+ and other N sources. Once again, soil type affected the anaerobic N mineralized from certain N sources.

4.0.4 Regression Analysis: Simple Effects, Wedowee sandy loam

The results of a regression analysis for the Wedowee sandy loam is illustrated in Figure 4.1D. The mean of the control samples was 41.7 mg kg^{-1}, the highest of the four soils tested. Once again, CP and OWC mineralized similarly across all rates, with OWC yielding the greatest amount of anaerobic N. However, mean separation (Table 4.7) showed no differences in the observed means between CP and OWC across all RYE rates, as was the case for all four soils. Ammonium nitrate yielded less anaerobic N than both CP and OWC, as was the case in the Noboco loamy sand. As was the case for the Noboco soil, the difference in AN anaerobic N was due to the anaerobic NO_3-N mineralized from the Wedowee soil after correction for the control treatments. It appeared that not all N added via AN was recovered by the incubation (Fig. 4.4). Ammonium nitrate was different than CP and OWC at each RYE interval with the exception of 0.5 X RYE interval of CP. Raleigh

plus again also differed from the other N sources across all rates. The RYE intervals of 0.5 X and 1.0 X had negative means (-12.1 and -0.8 mg kg^{-1}, respectively) when the control samples were subtracted (Table 4.7). This suggested that soil microbes assimilated the available N into their biomass, thereby immobilizing the PAN. Anaerobic N mineralized from R+ increased among the 0.5 X, 1.5 X, and 2.0 X RYE intervals (Table 4.7). The difference in response between N sources provided evidence of the RYE rate*N source interaction (Table 4.2). Overall, the magnitude of anaerobic N was lower in the Wedowee soil than all other soils, and suggested that different soils mineralize biosolids differently.

4.0.5 Summary of Anaerobic Incubation Total Inorganic N

As previously discussed, the % HM of each soil did not fully explain the differences in anaerobic N found in the unamended controls, and suggested that properties other than HM affected N mineralization in the soils tested. Based on the waste analysis reports of the biosolids studied, CP had the highest amount of total N, followed by OWASA cake, and R+ had the least (Fig. 3.1). The results of this anaerobic incubation showed that CP and OWC yielded the highest amount of anaerobic N across all soils and RYE rates, but there was no statistically significant difference between the observed means of each. Raleigh plus yielded the least anaerobic N of the four N sources among all soils. Also, certain N sources reacted differently across different soils. The fact that AN appeared to have mineralized differently among different soils (Fig. 4.1) is evidence that soil type affects N mineralization, and was illustrated by the statistically significant three-way interaction of RYE rate*N source*Soil in the overall ANOVA (Table 4.1). For example, anaerobic incubation N for AN was

different from CP and OWC in the Wedowee soil, but was not different from them in the Vance soil. Additionally, R+ yielded substantial amounts of anaerobic N in the Noboco, Norfolk, and Vance soils, but yielded very little in the Wedowee soil. Cary pellet and OWC mineralized similarly throughout all soils. The only noticeable difference between those two biosolids was that the magnitude of anaerobic N in the Wedowee soil was slightly lower than then other three soils. Raleigh plus was consistently the most different from the other N sources across all four soils tested.

A main objective of this research was to investigate whether generalized NAC based on biosolids treatment type and application method should be used for land application of municipal biosolids, or if the NAC should also be based on soil type and/or rate. Mineralization of the biosolids did not always equal that of AN. For example, CP and OWC were substantially greater than AN in the Noboco and Wedowee soils, and R+ was substantially lower than AN throughout most of the soils and rates. This information suggests that either the anaerobic test did not accurately predict N release for all N sources or that the first year NAC provided by the NCDA&CS are not correct. As mentioned in the disclaimer in section 3.0, incubations were grouped by soil. The order of incubations, from first to last, was Norfolk, Wedowee,Vance, and Noboco. There was no consistent trend of anaerobic N from AN in that order, so the differences are not likely attributed to an artifact of the experiment method. Although N data on the unamended soils was not available, it is possible that there was some N immobilization in the Noboco and Wedowee soils that were amended with AN due to high C/N ratios. Therefore, information on inherent soil N is important to have when conducting an anaerobic incubation. It is also possible that some of

the N was bound by the clays of the Noboco and Wedowee soils, but the 1 M KCl used to extract the inorganic N should have removed the majority of the N the was present. Moreover, the biosolids contain many other nutrients other than N (Table 3.1), which may have aided in microbial N mineralization of the biosolids in certain soils. Ammonium nitrate contains only N, and could explain the differences in anaerobic N among the four soils.

Application rate also affected the anaerobic N response in all soils. The slopes of the regression equations varied among N sources (Table 4.3), and among soils. This meant that the response to application rate depended on N source, and was evidence of the RYE rate*N source interaction found throughout all soils (Table 4.2). These hypotheses are further investigated in subsequent sections.

4.1 Anaerobic Incubation Ammonium and Nitrate

The mean values of the controls for NH_4-N for the Noboco, Norfolk, Vance, and Wedowee soils were 21.5, 17.9, 1.6, and 41.7 mg kg^{-1}, respectively (Fig. 4.2), and are in the same order of magnitude as anaerobic incubation total inorganic N. The mean values of the controls for NO_3-N for the Noboco, Norfolk, Vance, and Wedowee soils were 0.66, 1.82, 12.13, and 0 mg kg^{-1}, respectively (Fig. 4.3).

4.1.0 Analysis of Variance

The average anaerobic incubation NH_4- and NO_3-N of the appropriate controls were subtracted from the amended samples so that the effect of the amendments alone could be identified. The analysis of variance of all parameters tested is illustrated in Table 4.8 for

anaerobic NH_4-N, and Table 4.15 for anaerobic NO_3-N. All main effects and all interactions were significant except RYE rate*Soil for NH_4-N. The anaerobic NH_4-N was similar to the anaerobic total inorganic N, and anaerobic NO_3-N differed. All main effects were significant. As a result, the ANOVA's were broken down by soil type to simplify the analysis (Tables 4.9 and 4.16). For all soils, all main effects and interactions were significant except for RYE rate and RYE rate*N source for NO_3-N. Based on significant interactions, and to be consistent with the previous analyses, simple effects of RYE rate of each N source were investigated for each soil type.

4.1.1 Regression Analysis: Simple Effects

Results of regression analyses of the response of anaerobic incubation NH_4-N are illustrated in Fig. 4.2 and in Fig. 4.3 for NO_3-N. Regression equations, model significance, and R^2 values for anaerobic NH_4-N and NO_3-N are illustrated in Tables 4.10 and 4.17, respectively. The best fit model for all regressions was linear. All models were statistically significant for anaerobic NH_4-N and most were not significant for anaerobic NO_3-N. The anaerobic NH_4-N regressions essentially paralleled and dominated anaerobic total inorganic N release except for AN on the Norfolk (Fig. 4.2B) and Vance (Fig. 4.2C) soils which released varying amounts of NO_3-N (Fig. 4.3B and 4.3C, respectively).

Biosolids that were added to the Noboco loamy sand showed no anaerobic NO_3-N release. However, there was a detectable amount of NO_3 from AN (Fig. 4.3A), and mean separation (Table 4.18) showed a statistically significant increase in the observed means from the low to high RYE rates. Ammonium nitrate was also different from the biosolids at the

high rates (1.5 X and 2.0 X RYE). Norfolk loamy sand amended with AN (Fig. 4.3B) produced a greater amount of anaerobic NO_3-N than the Noboco loamy sand (Fig. 4.3A), and NO_3-N increased with rate to a maximum of 59 mg kg^{-1} (Table 4.19). All three biosolids did not release any statistically significant amounts of NO_3-N, as illustrated by the lack of significance of the models for each (Table 4.17) and means comparisons in Table 4.19. For AN, the Vance sandy clay loam produced the greatest amounts of NO_3-N of the four soils tested (Fig. 4.3C). Some NO_3-N was recovered by AN in all soils except for the Wedowee. According to the statistically significant regression equation in the Vance soil (Table 4.17), NO_3-N also mineralized from R+, but mean separation was unable to declare any differences among rates (Table 4.20). Also, all observed means of R+ were different from AN in the Vance soil, (Table 4.20). The Wedowee sandy loam produced no anaerobic NO_3-N (Fig. 4.3D). The production of NO_3-N was not expected due to the anaerobic conditions which should have fostered the reduction of NO_3 to NH_4. Possible explanations for the occurrence of NO_3 in some of the soils are discussed in subsequent sections.

4.2 Total Inorganic Nitrogen Recovery

4.2.0 Analysis of Variance

The analysis of variance of all treatment factors tested is illustrated in Table 4.22. The three-way interaction of RYE rate*N source*Soil was statistically significant, as were the two-way interaction of N source*Soil and all main effects. As a result the ANOVA was broken down by soil type to simplify the analysis (Table 4.23). The two-way interaction of

RYE rate*N source was statistically significant for the Norfolk and Vance soils, but not for the Noboco and Wedowee soils. However, the main effect of N source was statistically significant in all soils, while the main effect of RYE rate was significant only for the Vance soil. For the soils where there was no RYE rate*N source, interaction (Noboco and Wedowee), these main effects can be relied upon. However, for the other soils with the interaction, simple effects needed to be examined. However, in order to maintain consistency with previous analyses, simple effects of RYE rate for each N source were investigated for each soil.

4.2.1 Regression Analysis: Simple Effects, Noboco loamy sand

Results of regression analyses of the response of total inorganic N recovery to N rate for each soil are illustrated in Figure 4.4. Regression equations, model significance, and R2 values are illustrated in Table 4.24. Noboco loamy sand regressions are shown in Figure 4.4A. The best fit model for all N sources was linear. The anaerobic incubation recovered the greatest proportion of inorganic N from AN, as was expected. The AN model was not statistically significant (Table 4.24) and there were no differences among RYE rates (Table 4.25), as illustrated by the lack of significance of the RYE rate*N source interaction coupled with the non-significant main effect of RYE Rate (Table 4.23). Surprisingly, an average of only 51% of the added AN-N was recovered by the anaerobic incubation in this soil (Table 4.29). It was hypothesized that the incubation would recover close to 100% of the added AN-N from all soils. Cary pellets had the second highest total inorganic N recovery. The model was statistically significant (Table 4.24) and there was a difference between the 0.5 X RYE rate and all other rates (Table 4.25). The average recovery of CP at the 0.5 X RYE rate

was 41.2% and 33.9% across the other rates, with an overall average of 35.7%. The first-year NAC provided by the NCDA&CS for biosolids generated like CP and broadcast applied was 30% and 40% for incorporated (McGinnis et al., 2011). There was no specific coefficient for the CP biosolids (heat treated and pelleted), so the "other" designation was used. As a result, this anaerobic incubation suggested that the NAC recommended by the NCDA&CS slightly underestimated the amount of N mineralization of CP for broadcast application methods, and overestimated for incorporated application methods for the Noboco loamy sand soil, if the incubation was precise. A broadcast application method was used to calculate the biosolids application rates in this incubation, but the biosolids were hand-mixed with the soil before incubation. The difference between the two coefficients would lead to application rates of 128 and 170 kg of PAN ha^{-1}, respectively, a difference of 43 kg PAN ha^{-1}. This difference may explain why the recovery of CP was less than 40% (35.7%), and suggested that the NAC used for CP in this soil is close to being correct, according to this test.

After AN and CP, OWC had the next highest total inorganic N recovery (Fig. 4.4A). The model was on the brink of statistical significance and there were no differences between RYE rates. Averaged across all application rates, the incubation recovered 25.1% of the total added N. The NAC recommended by the NCDA&CS for this type of biosolids that have been broadcast is 17% and 20% for incorporated. The results of this incubation for OWC show that the current NAC underestimated the amount of mineralizable N. Raleigh plus had the lowest inorganic N recovery in this soil. The model for R+ was statistically significant (Table 4.24), but there were no difference in the observed means at each RYE interval (Table

4.25). The average recovery across all rates was 12.8%, substantially lower than the 28% recommended by the NCDA&CS for broadcast application, and even lower for incorporated (40%). The results from R+ were not surprising due to the advanced N removal techniques that were used to produce the R+ biosolids. Although there is a category of NAC's for lime-stabilized biosolids, it does not accurately reflect the entire sewage sludge treatment process used to produce those biosolids, as additional steps are taken to reduce the N content of the biosolids. Such techniques are not commonly used in lime-stabilized sewage sludge.

4.2.2 Regression Analysis: Simple Effects, Norfolk loamy sand

Results of the regression analysis for the Norfolk loamy sand soil are illustrated in Figure 4.4B. The incubation recovered the most inorganic N from AN, as was the case in the Noboco soil. The linear model, however, was not significant (Table 4.24), and there were no differences between RYE intervals (Table 4.26). The average recovery of AN was 92%; much higher than the 51.2% in the Norfolk soil (Table 4.29). This strongly suggested that soil type affected PAN recovery in this incubation, and is evidence of the RYE rate*N source*Soil interaction (Table 4.22). Also, the recovery of AN was close to 100%, as was hypothesized. The next highest recovery was once again from the CP. A quadratic model was highly statistically significant and there were differences among RYE intervals (Table 4.26), evidence of rate as an important factor in estimating N release. The average recovery across all rates for CP was 44.2%, higher than the NAC's recommended by the NCDA&CS (30% for broadcast and 40% for incorporated). Following CP in magnitude of inorganic N recovery was OWC. The model was statistically significant but there were no differences

between observed RYE interval means. The average recovery across all RYE rates was 25.1%. The NAC's recommended by the NCDA&CS were 17 and 20% for broadcast and incorporated, respectively. Raleigh plus had the lowest inorganic N recovery. The quadratic model was significant and there were some differences across RYE rate. The 0.5 X RYE rate recovered an average of 19% of the total inorganic N, and only 8% at the 2.0 X RYE interval. As a result, the differences among RYE rates would be agronomically important. The average recovery was 11.8% (Table 4.29). The NAC's recommended by the NCDA&CS were 28 and 40% for broadcast and surface incorporated, respectively. As mentioned previously, the unexpected results from R+ can likely be explained by the biosolids treatment method.

4.2.3 Regression Analysis: Simple Effects, Vance sandy clay loam

Results of the regression analysis for the Vance sandy clay loam are illustrated in Figure 4.4C. Ammonium nitrate again had the highest recovery and was close to 100% across all RYE rates, with an average recovery of 95.9% (Table 4.27). Raleigh plus produced unexpected results. Its model was quadratic and there were differences in the observed means between the 0.5 X RYE rate and all others that would be agronomically important. This anomaly is not well understood, as explained previously. The average recovery of R+ was 39% across all rates, but the large recovery at the 0.5 X RYE interval skews the average (22.9% when the 0.5 X RYE interval is excluded). Cary pellet had the next highest inorganic N recovery. The linear model for CP was significant, and there were differences across the observed means of the RYE intervals, but the differences would not

likely be agronomically important. The average recovery was 38.1%. OWASA cake had the lowest recovery in the Vance soil. The model was not significant and there were no differences across RYE intervals. The average recovery across all RYE intervals was 24.6% (Table 4.29).

4.2.4 Regression Analysis: Simple Effects, Wedowee sandy loam

Results of the regression analysis for the Wedowee sandy loam is illustrated in Figure 4.4D. As in the Noboco soil, AN had a much lower inorganic N recovery than expected. The linear model was not significant (Table 4.25) and there were no differences between the observed RYE interval means (Table 4.24). The average recovery from AN was 52.2% (Table 4.29). Cary pellet recovered the second most inorganic N in this soil. The linear model was not significant and there were no differences in the observed means of RYE intervals. The average recovery was 31.1%. OWASA cake had the next highest recovery of inorganic N. The model was highly statistically significant and there were differences in the observed means of the 0.5 X RYE interval and the 1.5 X and 2.0 X RYE intervals that would be agronomically important (e.g., a difference of 76 kg PAN ha^{-1}). The average recovery of OWC was 24.3%. Raleigh plus once again had the lowest inorganic N recovery. The quadratic model was highly statistically significant (Table 4.24) and there were differences between the 0.5 X RYE interval and all others (e.g., the differences resulted in immobilization of inorganic N to some inorganic N being plant-available, respectively) (Table 4.28). Averaged across all rates, the recovery was -2%, In light of the low total N

content of Raleigh Plus (Fig. 3.1), this may have been due to microbial immobilization of N in R+ amended samples in this soil.

4.2.5 Summary of Anaerobic Incubation Total Inorganic N Recovery

The purpose of this analysis was to evaluate the total inorganic N recoveries of four N sources applied to four soils and at five different rates via anaerobic incubation, and compare and contrast those recoveries to the first year nutrient availability coefficients recommended by the NCDA&CS. As described previously, there was substantial variability in recovery between soil types. Only in the Norfolk and Vance soils did the AN-N recovery approach 100%. The Noboco and Wedowee soils recovered substantially less AN (Table 4.29). The lack of ~100% recovery of AN across all soils means that soil type affected the N recovery of the anaerobic incubation. It also suggested some type of N loss, either by microbial N immobilization due to low C/N ratios of those soils or N being bound by the clays. There was further evidence of variability among soils with the recovery of R+. The recovery varied across the four soils tested. Cary pellet and OWC behaved similarly to each other across all soils in terms of rate response and magnitude of anaerobic N yielded, but the Wedowee soil yielded less anaerobic N for all four N sources than the other soils. This information suggested that soil type should be considered when estimating the percentage of inorganic N availability from land-application of biosolids. There was also some variation in inorganic N recovery at different RYE rates of some N sources. When a difference existed, recovery generally decreased as RYE rate increased, and was often an agronomically important difference. This evidence suggested that application rate should also be considered when

estimating the percentage of inorganic N available from land-applied biosolids. Evaluation of the effectiveness of the anaerobic incubation itself is discussed in subsequent sections.

The relative magnitude of inorganic N recovery from the N sources was consistent among soils, with the exception of R+ in the Vance soil. From largest to smallest, the general inorganic N recoveries followed the order of: AN > CP > OWC > R+. Although averages can be misleading, especially when there are statistically significant interactions among RYE rate*N Source*Soil, they were examined in order to make generalizations. The average N recoveries of each N source within each soil and the overall averages are shown in Table 4.29. The average recoveries for AN, CP, OWC, and R+ were 72.8, 37.3, 24.8, and 15.4%, respectively. There were no substantial differences in the averages when the soils were grouped by region i.e., Piedmont vs. Coastal plain. The 73% recovery of AN-N was concerning and suggested a problem with the anaerobic incubation. However, the low recovery was only found in two of the four soils tested, and was likely a result of NO_3-N recovery and/or microbial N immobilization. The recovery of 37% for CP suggested that the first year NAC of 30% recommended by the NCDA&CS for broadcast applied biosolids under estimated the amount of N that will become available from this product. The NAC for CP that was surface incorporated was 40% and slightly overestimated the N that will become plant-available from this product. The 25% recovery of the OWC suggested that the NAC coefficient of 17% recommended by the NCDA&CS for broadcast applied is also an under estimation, as is the NAC for surface incorporated (20%). The recovery of 15% for R+ was lower than the 28% recommended by the NCDA&CS for broadcast applied and the 40% for surface incorporated, and suggested that the currently used NAC's overestimated the amount

of first year N availability in such a biosolid. The fact that R+ recovered less inorganic N than estimated by the NCDA&CS is not surprising given the advanced treatment and stabilization methods that are employed to reduce the N content of the product.

4.3 Plant-Available Nitrogen Recovery

4.3.0 Analysis of Variance

The analysis of variance of all treatment factors tested is illustrated in Table 4.30. The three-way interaction of RYE rate*N source*Soil was statistically significant, as were all two-way interactions, and main effects. As a result, the ANOVA was broken down by soil type to simplify the analysis (Table 4.31). The two-way interaction of RYE rate*N source was statistically significant for all soils except the Noboco loamy sand, for which the main effects of both N source and RYE rate were significant. To maintain consistency with previous analyses, simple effects of RYE rate for each N source were investigated for each soil.

4.3.1 Regression Analysis: Simple Effects

Results of regression analyses of the response to RYE rate of PAN recovery, and of NH_4-N and NO_3-N recovery as a proportion of PAN are illustrated in Figures 4.5, 4.6, and 4.7, respectively. Regression equations, model significance, and R^2 values are illustrated in Table 4.32. Most of the best fit models were linear, but there were some quadratic models, as well. In the Noboco sandy loam soil (Fig 4.5A), OWC and CP were not different from each other (Table 4.33) and the observed means were statistically significantly higher than AN. As discussed previously, AN only recovered 51.2% of the PAN applied (Table 4.29).

OWASA cake and CP recovered an average of 131 and 120% of the estimated PAN applied. Only 42.1% of the Raleigh plus PAN was recovered. Ammonium nitrate and R+ were coincident throughout all RYE intervals except for 2.0 X RYE where PAN recovery of AN exceeded that of Raleigh Plus. The CP linear model was statistically significant and the observed mean of the 0.5 X RYE intervals was greater than the other rates. This suggested that rate is an important factor to considering when evaluating PAN recovery in an anaerobic incubation in this soil.

The Norfolk loamy sand soil was similar to the Noboco soil in that CP and OWC recovered more PAN than all other N sources (Fig. 4.5B), and CP and OWC were not different from each other (Table 4.34). Plant-available N recovery of both CP and OWC decreased with increasing RYE rate at an agronomically important amount. At all rates except 0.5 X RYE, the OWC PAN recovery was not different than that of AN. The linear model of AN was not statistically significant and there were no differences across all RYE intervals. The average PAN recovery across all rates was 95.7% for AN, and as discussed previously, was close to the expected recovery of 100%. However, the average PAN recoveries of CP and OWC were 148 and 133.2%, respectively, which implied that the NAC's used for those biosolids underestimated the amount of PAN. The R+ quadratic model was statistically significant and there were differences between the lowest and highest RYE rates. Plant-available N recovery of R+ was different from all other N sources except for the 0.5 X RYE interval, where Raleigh Plus and AN had similar recoveries, The average PAN recovery from R+ across all rates was 36.3% (Table 4.37).

In the Vance sandy clay loam (Fig. 4.5C), the anaerobic test once again recovered the most PAN from the CP and OWC, and the lines were coincident according to separation of the observed means (Table 4.35). The average PAN recoveries of the two biosolids were 128.2 and 128.3%, respectively (Table 4.37). The PAN recovery from both biosolids was not different from that of AN across all rates. The average PAN recovery for AN across all rates was 95.9%, and the model was not statistically significant. In this soil, it appeared as if the NAC coefficients recommended by the NCDA&CS for CP and OWC were close to being correct, and the relative recoveries were different than in the others soils. The Raleigh plus recovery in this soil was an anomaly not easily explained, as previously described.

In the Wedowee sandy loam soil, PAN recovery of OWC and CP were coincident (Table 4.36), averaging PAN recoveries of 126.7 and 104.4% across all RYE intervals (Table 4.37). Recovery of PAN from OWC and CP was different from that of AN across all rates except the 0.5 X RYE interval of CP (Table 4.36). The AN model was not significant, and there were no differences in the observed means across all RYE intervals. The average PAN recovery of AN in this soil was only 52.2% and was much lower than expected. The PAN recovery of R+ was different from AN across all RYE intervals, averaging -6.7%, likely a result of microbial immobilization of N in samples amended with R+.

4.3.2 Anaerobic Incubation Evaluation

In an attempt to evaluate the effectiveness of the anaerobic incubation in recovering the added N, AN was used for quality control. It was hypothesized that the anaerobic incubation would recover 100% of the total inorganic N added via AN. Total PAN, NH_4-N,

and NO_3-N were evaluated. Regressions of the NH_4-N recovery from NH_4NO_3 are illustrated in Figure 4.6. If the anaerobic test functioned as expected, NH_4-N recovery should have been approximately 200% of the added NH_4 because AN is 50% NH_4 and 50% NO_3, and due to reduction of the added NO_3 to NH_4. The average NH_4-N recoveries for the Noboco, Norfolk, Vance, and Wedowee soils were 85.3, 99.5, 103.5, and 104.2%, respectively. The averages of anaerobic incubation NO_3-N recoveries are illustrated in Figure 4.7. The average NO_3-N recoveries of the Noboco, Norfolk, Vance, and Wedowee soils were 17, 84.5, 85.8, and 0.1%, respectively. The substantial amount of NO_3-N recovery was unexpected as full NO_3 reduction via dissimilatory reduction to NH_4 (DNRA) was expected. Microbial NO_3 assimilation is a reductive process in which NO_3 is reduced to NH_4, and NH_4 is rapidly assimilated into amino acids by specific enzymes: NO_3- → NO2- → NH_4+ → R-NH2 (Coyne, 1999). The fact that substantial amounts of NO_3-N were recovered in three of the four soils tested and only ~100% of the added NH_4-N was recovered required exploration. One hypothesis was that the soil-biosolids mixture was not completely anaerobic or had not been anaerobic long enough for full NO_3 reduction. This hypothesis seems unlikely given that little to no NO_3 was recovered from the Wedowee (Fig. 4.7D) and the Noboco soils (Fig 4.7A). Undecomposed organic material that floated to the surface during the incubations could have also led to erratic results (Keeney and Bremner, 1966).

Another possibility was that there was not enough C available for the microbial population to reduce the NO_3. The three biosolids products produced little to no NO_3 across all soils (Fig. 4.3), perhaps due to the large amount of C in the biosolids. Ammonium nitrate is 100% inorganic and contains no C source for the microbes. The % HM of the soils was referenced

as a potential explanation, but no consistent trend was found. For example the Norfolk loamy sand had relatively high HM content relative to the Wedowee sandy loam, yet the Norfolk soil still recovered an average of 84.5% of the added NO_3 from AN across all RYE rates. The Noboco loamy sand had the highest % HM but recovered an average of only 17% NO_3 across all RYE rates.

Another hypothesis is that some of the soils did not have an adequate population of anaerobic microbes to reduce the large amount of NO_3 added via AN. All soils were air dried before incubation which could have negatively affected the anaerobic microbe population. No consistent trend was identified related to the surface textures of the soils and its effect on NO_3 reduction. Particle size analysis of all soils test would likely be beneficial in interpretation of an anaerobic incubation.

To the author's knowledge, previous studies have assumed no NO_3 would be recovered from an anaerobic incubation and did not measure it (WARING and BREMNER, 1964), (Bundy and Meisinger, 1994), (Keeney, 1982), (Stanford, 1982), (Muruganandam et. al, 2008). Also, the effectiveness of the anaerobic incubations were often correlated with field studies and NH_4 recovery was not commonly investigated (WARING and BREMNER, 1964). The data presented in this study suggested that the PAN recovery of an inorganic N source should be evaluated for quality control and/or that there was a problem with this specific experiment that was not identified.

4.3.3 Summary of Plant-Available Nitrogen Recovery

The purpose of this analysis was to evaluate the PAN recoveries from anaerobic incubation of four N sources applied to four soils at five different rates , and compare and contrast these those predicted by first year NAC recommended by the NCDA&CS. As described previously, there was variability in recovery between soil types, and recovery of AN was not 100% for each soil. Both CP- and OWC-treated soils consistently lead to higher recovery than AN. Averaged across all soils, the PAN recovery of CP and OWC was 130 and 125%, respectively (Table 4.37). This provided evidence that the NAC recommended by the NCDA&CS underestimated the amount of PAN released from those biosolids. The R+ biosolids PAN recovery averaged across all four soils tested was 50%. That meant that the NAC coefficients used overestimated the N release from this product by a factor of two. There were also statistically significant differences in PAN recovery across some RYE rates for some N sources that were agronomically important, which suggested that application rate is also an important factor to consider when estimating PAN from biosolids. There were no differences in PAN recovery across RYE intervals for AN, but there were for some of the biosolids. That suggested that some characteristic (s) of the biosolids affect N mineralization differently when applied at different rates.

It was also determined that the anaerobic incubation did not recover expected amounts of either NH_4 or NO_3 from AN across all soils. As a result, it was hypothesized that PAN recovery from an inorganic fertilizer such as AN needs to be evaluated when conducting an anaerobic incubation, which is not commonly done. Such information can be

useful in evaluating the precision of the test and standardizing PAN from organic sources relative to an inorganic one.

4.4 Conclusions

This study used an anaerobic incubation to study the N release dynamics (mineralization) of three NC biosolids applied to four NC soils, at five different rates. The relative differences in N release and N recovery were evaluated and compared to estimates of PAN release using NAC currently recommended by the NCDA&CS. Generally, it was found that different biosolids mineralized N differently when applied to different soils and at different rates, as hypothesized. Current estimates provided by the NCDA&CS only account for differences in some biosolids treatment and application methods. Therefore, these results showed that soil type and application rate need to be considering when estimating PAN release. Also, the specific NAC were evaluated for precision. According to the anaerobic incubation, current NAC underestimated PAN release of CP and OWC, and overestimated PAN release for R+, and the differences were often agronomically important. For example, in terms of PAN recovery and using overall averages (Table 4.37), the differences in PAN application rates from the RYE goal and recovery from the anaerobic incubation for AN, CP, OWC, and R+ was 39, -32, -38, and 65 kg PAN ha^{-1}. That is, AN recovered 39 kg PAN ha^{-1} less than expected, CP recovered 32 kg PAN ha^{-1} more than expected, OWC recovered 38 kg PAN ha^{-1} more than expected, and R+ recovered 63 kg PAN ha^{-1} less than expected. The differences in PAN recovery were on the brink of agronomic importance (greater than 20 kg N ha^{-1}) for CP and OWC, but the difference in R+ recovery was important. Also, the

averages used in calculation of the differences do not take into account the larger differences that existed between individual RYE rates and soils. As a result, according to this study, the currently recommended coefficients likely need modification, but further correlation with field research is needed.

Table 4.1. Analysis of variance for anaerobic incubation total inorganic N from anaerobic incubation of four N sources (3 biosolids, NH_4NO_3) applied at four rates on four soils. Average anaerobic N content of the control samples was subtracted from the amended samples.

Effect	Numerator DF	Denominator DF	F Value	Pr > F
RYE rate	3	192	274.65	<0.0001
N source	3	192	283.34	<0.0001
Soil	3	192	67.51	<0.0001
RYE rate*N source	9	192	24.04	<0.0001
RYE rate*Soil	9	192	1.71	0.0892
N source*Soil	9	192	18.09	<0.0001
RYE rate*N source*Soil	27	192	2.17	0.0014

Table 4.2. Analysis of variance for anaerobic incubation total inorganic N from anaerobic incubation of four N sources (3 biosolids, NH_4NO_3) applied at four rates; grouped by soil. Average anaerobic N content of the control samples was subtracted from the amended samples.

Effect	Numerator DF	Denominator DF	F Value	Pr > F
		Noboco loamy sand		
RYE rate	3	48	128.69	<0.0001
N source	3	48	161.28	<0.0001
RYE rate*N source	9	48	9.51	<0.0001
		Norfolk loamy sand		
RYE rate	3	48	120.86	<0.0001
N source	3	48	147.43	<0.0001
RYE rate*N source	9	48	9.69	<0.0001
		Wedowee sandy loam		
RYE rate	3	48	37.05	<0.0001
N source	3	48	5.52	0.0025
RYE rate*N source	9	48	7.49	<0.0001
		Vance sandy clay loam		
RYE rate	3	48	62.74	<0.0001
N source	3	48	178.44	<0.0001
RYE rate*N source	9	48	4.44	0.0003

Table 4.3. Regression equations, model significance, and R^2 values from a 7-day anaerobic incubation. Independent variable was RYE rate and dependent variable was anaerobic incubation total inorganic N.

Parameter	n	Equation†	Model P	R^2
		Noboco loamy sand		
OWASA cake	16	y = 9.27 + 64.67x	< 0.0001	0.94
Cary pellet	16	y = 11.60 + 56.10x	< 0.0001	0.97
NH_4NO_3	16	y = -2.36 + 35.33x	< 0.0001	0.72
Raleigh plus	16	y = 10.19 + 13.28x	< 0.0001	0.71
		Norfolk loamy sand		
OWASA cake	16	y = 17.61 + 56.71x	< 0.0001	0.88
Cary pellet	16	y = 19.69 + 63.78x	< 0.0001	0.98
NH_4NO_3	16	y = -4.78 + 64.89x	< 0.0001	0.87
Raleigh plus	16	y = 11.19 + 8.36x	0.0284	0.30
		Wedowee sandy loam		
OWASA cake	16	y = 24.15 + 47.18x	0.0004	0.85
Cary pellet	16	y = 8.61 + 50.73x	< 0.0001	0.86
NH_4NO_3	16	y = 12.07 + 21.23x	0.0004	0.61
Raleigh plus	16	y = -18.71 + 15.93x	0.0001	0.66
		Vance sandy clay loam		
OWASA cake	16	y = 11.00 + 61.19x	< 0.0001	0.75
Cary pellet	16	y = 8.91 + 63.21x	< 0.0001	0.96
NH_4NO_3	16	y = -1.19 + 64.17x	< 0.0001	0.80
Raleigh plus	16	y = 82.92 - 14.41x	0.1077	0.17

† x = Realistic Yield Expectation Interval; y = Anaerobic Incubation Total Inorganic N (mg kg^{-1})

Table 4.4. Anaerobic incubation total inorganic N from a Noboco loamy sand amended with four N sources: simple effect of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

RYE rate	Nitrogen Source								
	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- mg kg^{-1} ----------								LSD
0.5	16.0 B†	c§	39.3 A	d	41.4 A	d	16.9 B	c	13.5
1.0	31.7 B	bc	66.6 A	c	74.6 A	c	22.3 B	bc	14.1
1.5	51.1 B	ab	98.9 A	b	105.7 A	b	32.1 B	ab	25.5
2.0	68.4 B	a	122.0 A	a	138.8 A	a	35.8 C	a	22.2
LSD	28.6		11.8		21.3		10.7		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)
§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 4.5. Anaerobic incubation total inorganic N from a Norfolk loamy sand amended with four N sources: simple effect of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

RYE rate	Nitrogen Source								
	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- mg kg^{-1} ----------								LSD
0.5	24.9 B†	c§	50.6 A	d	48.2 A	c	17.6 B	a	16.4
1.0	62.5 B	b	84.5 A	c	71.3 AB	c	18.7 C	a	21.5
1.5	96.0 A	a	116.3 A	b	102.0 A	b	18.8 B	a	21.0
2.0	121.9 A	a	146.3 A	a	132.5 A	a	31.5 B	a	30.9
LSD	32.3		11.2		27.0		14.9		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)
§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 4.6. Anaerobic incubation total inorganic N from a Vance sandy clay loam amended with four N sources: simple effect of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

	Nitrogen Source								
RYE rate	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- mg kg^{-1} ----------								LSD
0.5	30.2 B†	c§	41.2 B	d	41.9 B	c	81.7 A	a	28.6
1.0	60.8 A	bc	71.9A	c	72.4 A	bc	56.6 A	a	44.6
1.5	96.6 A	ab	102.1 A	b	101.7 A	ab	67.1 B	a	27.6
2.0	123.5 A	a	136.5 A	a	134.1 A	a	54.2 B	a	44.7
LSD	41.1		17.4		46.7		37.4		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 4.7. Anaerobic incubation total inorganic N from a Wedowee sandy clay loam amended with four N sources: simple effect of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

	Nitrogen Source								
RYE rate	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- mg kg^{-1} ----------								LSD
0.5	21.7 B†	b§	31.2 AB	c	45.6 A	c	-12.1 C	b	21.9
1.0	35.5 B	ab	63.7 A	b	72.1 A	b	-0.8 C	ab	18.2
1.5	42.5 B	ab	84.4 A	ab	99.9 A	a	5.4 C	a	19.9
2.0	54.7 B	a	108.8 A	a	114.9 A	a	12.4 C	a	28.2
LSD	21.9		26.3		24.7		14.8		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 4.8. Analysis of variance for anaerobic incubation NH_4-N from anaerobic incubation of four N sources (3 biosolids, NH_4NO_3) applied at four rates on four soils. Average anaerobic N content of the control samples was subtracted from the amended samples.

Effect	Numerator DF	Denominator DF	F Value	Pr > F
RYE rate	3	192	293.82	<0.0001
N source	3	192	579.31	<0.0001
RYE rate*N source	9	192	39.97	<0.0001
Soil	3	192	71.26	<0.0001
RYE rate*Soil	9	192	1.29	0.2419
N source*Soil	9	192	19.83	<0.0001
RYE rate*N source*Soil	27	192	2.30	0.0006

Table 4.9. Analysis of variance for anaerobic incubation NH_4-N from anaerobic incubation of four N sources (3 biosolids, NH_4NO_3) applied at four rates; grouped by soil. Average anaerobic N content of the control samples was subtracted from the amended samples.

Effect	Numerator DF	Denominator DF	F Value	Pr > F
		Noboco loamy sand		
RYE rate	3	48	165.05	<0.0001
N source	3	48	281.27	<0.0001
RYE rate*N source	9	48	17.03	<0.0001
		Norfolk loamy sand		
RYE rate	3	48	128.23	<0.0001
N source	3	48	292.4	<0.0001
RYE rate*N source	9	48	13.41	<0.0001
		Wedowee sandy loam		
RYE rate	3	48	62.76	<0.0001
N source	3	48	184.85	<0.0001
RYE rate*N source	9	48	4.59	0.0002
		Vance sandy clay loam		
RYE rate	3	48	33.96	<0.0001
N source	3	48	59.22	<0.0001
RYE rate*N source	9	48	13.52	<0.0001

Table 4.10. Regression equations, model significance, and R^2 values from a 7-day anaerobic incubation. Independent variable was RYE rate and dependent variable was anaerobic incubation NH_4-N.

Parameter	n	Equation†	Model P	R^2
		Noboco loamy sand		
OWASA cake	16	y = 9.05 + 65.00x	< 0.0001	0.94
Cary pellet	16	y = 12.02 + 56.06x	< 0.0001	0.97
NH_4NO_3	16	y = 3.64 + 23.90x	< 0.0001	0.75
Raleigh plus	16	y = 9.80 + 12.34x	0.0001	0.66
		Norfolk loamy sand		
OWASA cake	16	y = 19.95 + 56.50x	< 0.0001	0.88
Cary pellet	16	y = 22.01 + 63.61x	< 0.0001	0.98
NH_4NO_3	16	y = -0.12 + 32.71x	< 0.0001	0.86
Raleigh plus	16	y = 10.84 + 7.42x	0.0597	0.23
		Wedowee sandy loam		
OWASA cake	16	y = 24.15 + 47.18x	< 0.0001	0.85
Cary pellet	16	y = 8.61 + 50.73x	< 0.0001	0.86
NH_4NO_3	16	y = 12.16 + 21.10x	0.0004	0.61
Raleigh plus	16	y = -19.10 + 15.39x	0.0001	0.66
		Vance sandy clay loam		
OWASA cake	16	y = 22.89 + 60.94x	< 0.0001	0.80
Cary pellet	16	y = 20.82 + 62.98x	< 0.0001	0.98
NH_4NO_3	16	y = 0.76 + 32.64x	< 0.0001	0.81
Raleigh plus	16	y = 95.88 - 24.97x	0.0153	0.35

† x = Realistic Yield Expectation Interval; y = Anaerobic Incubation NH_4-N (mg kg^{-1})

Table 4.11. Anaerobic incubation NH_4-N from a Noboco loamy sand amended with four N sources: simple effects of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

	Nitrogen Source								
RYE rate	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- mg kg^{-1} ----------								LSD
0.5	14.7 B†	c§	40.0 A	d	41.2 A	d	16.4 B	c	13.6
1.0	28.5 B	bc	66.8 A	c	750 A	c	20.6 B	bc	12.8
1.5	40.2 B	ab	98.9 A	b	105.8 A	b	30.0 B	ab	19.8
2.0	50.7 B	a	122.7 A	a	139.2 A	a	33.9 B	a	16.9
LSD	17.9		12.2		20.7		11.3		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 4.12. Anaerobic incubation NH_4-N from a Norfolk loamy sand amended with four N sources: simple effects of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

	Nitrogen Source								
RYE rate	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- mg kg^{-1} ----------								LSD
0.5	13.8 B†	c§	52.8 A	d	50.5 A	c	17.2 B	a	16.9
1.0	35.0 B	b	86.7 A	c	73.4A	c	16.8 C	a	15.2
1.5	51.3 C	ab	118.3 A	b	103.9 A	b	17.0 C	a	14.5
2.0	62.9 B	a	148.3 A	a	134.5 A	a	29.5 C	a	25.3
LSD	16.4		11.0		27.1		15.7		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$) subtracted from the amended soil concentration. CP, Cary pellets, OWC, OWASA cake, R+, Raleigh Plus.

Table 4.13. Anaerobic incubation NH_4-N from a Vance sandy clay loam amended with four N sources: simple effects of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

	Nitrogen Source								
RYE rate	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- mg kg^{-1} ----------								LSD
0.5	16.9 C†	c§	53.0 B	d	53.6 B	c	92.7 A	ab	21.0
1.0	33.4 B	bc	83.5 A	c	84.1 A	bc	55.0 AB	ab	35.0
1.5	50.4 B	ab	113.6 A	b	113.2 A	ab	62.3 B	ab	28.6
2.0	65.6 B	a	148.0 A	a	145.5 A	a	48.7 B	b	32.5
LSD	20.8		10.5		39.2		38.3		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 4.14. Anaerobic incubation NH_4-N from a Wedowee sandy loam amended with four N sources: simple effects of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

	Nitrogen Source								
RYE rate	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- mg kg^{-1} ----------								LSD
0.5	21.7 B†	b§	31.2 AB	c	45.6 A	c	-12.8 C	b	21.8
1.0	35.5 B	ab	63.7 A	b	72.1 A	b	-1.7 C	ab	17.9
1.5	42.4 B	ab	84.4 A	ab	99.9 A	a	4.1 C	a	19.9
2.0	54.6 B	a	108.8 A	a	114.9 A	a	10.9 C	a	28.1
LSD	21.9		26.3		24.7		14.1		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 4.15. Analysis of variance for anaerobic incubation NO_3-N from anaerobic incubation of four N sources (3 biosolids, NH_4NO_3) applied at four rates on four soils. Average anaerobic N content of the control samples was subtracted from the amended samples.

Effect	Numerator DF	Denominator DF	F Value	Pr > F
RYE rate	3	192	25.09	<0.0001
N source	3	192	251.92	<0.0001
RYE rate*N source	9	192	16.89	<0.0001
Soil	3	192	24.35	<0.0001
RYE rate*Soil	9	192	4.72	<0.0001
N source*Soil	9	192	60.37	<0.0001
RYE rate*N source*Soil	27	192	3.22	<0.0001

Table 4.16. Analysis of variance for anaerobic incubation NO_3-N from anaerobic incubation of four N sources (3 biosolids, NH_4NO_3) applied at four rates; grouped by soil. Average anaerobic N content of the control samples was subtracted from the amended samples.

Effect	Numerator DF	Denominator DF	F Value	Pr > F
		Noboco loamy sand		
RYE rate	3	48	6.92	0.0006
N source	3	48	29.44	<0.0001
RYE rate*N source	9	48	6.24	<0.0001
		Norfolk loamy sand		
RYE rate	3	48	26.15	<0.0001
N source	3	48	301.85	<0.0001
RYE rate*N source	9	48	24.13	<0.0001
		Wedowee sandy loam		
RYE rate	3	48	1.25	0.3007
N source	3	48	28.76	<0.0001
RYE rate*N source	9	48	0.75	0.6634
		Vance sandy clay loam		
RYE rate	3	48	7.21	0.0004
N source	3	48	81.41	<0.0001
RYE rate*N source	9	48	3.61	0.0017

Table 4.17. Regression equations, model significance, and R^2 values from a 7-day anaerobic incubation. Independent variable was RYE rate and dependent variable was anaerobic incubation NO_3-N.

Parameter	n	Equation†	Model P	R^2
		Noboco loamy sand		
OWASA cake	16	y = 0.219 - 0.333x	0.0461	0.046
Cary pellet	16	y = -0.423 + 0.035x	0.9353	0.001
NH_4NO_3	16	y = 11.429 - 6.002x	0.0004	0.604
Raleigh plus	16	y = 0.401 + 0.934x	0.0730	0.212
		Norfolk loamy sand		
OWASA cake	16	y = -1.832 + 0.013x	0.9715	0.000
Cary pellet	16	y = -1.835 + 0.027x	0.945	0.000
NH_4NO_3	16	y = -4.660 + 32.177x	< 0.0001	0.864
Raleigh plus	16	y = 0.345 + 0.940x	0.0977	0.184
		Wedowee sandy loam		
OWASA cake	16	y = 0.00003 - 0.00002x	0.6702	0.013
Cary pellet	16	y = 0.00003 - 0.00002x	0.1887	0.120
NH_4NO_3	16	y = -0.088 + 0.125x	0.1592	0.136
Raleigh plus	16	y = 0.387 + 0.547x	0.1034	0.148
		Vance sandy clay loam		
OWASA cake	16	y = -12.144 + 0.018x	0.9957	0.000
Cary pellet	16	y = -12.134 + 0.006x	0.9985	0.000
NH_4NO_3	16	y = -2.726 + 30.530x	0.0002	0.646
Raleigh plus	16	y = -13.621 + 10.484x	0.0125	0.369

† x = Realistic Yield Expectation Interval; y = Anaerobic Incubation NO_3-N (mg kg^{-1})

Table 4.18. Anaerobic incubation NO_3-N from a Noboco loamy sand amended with four N sources: simple effects of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

	Nitrogen Source								
RYE rate	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- mg kg^{-1} ----------								LSD
0.5	1.23 A†	b§	-0.66 A	a	0.22 A	a	0.51 A	a	2.8
1.0	3.24 A	b	-0.19 A	a	-0.41 A	a	1.72 A	a	3.9
1.5	10.99 A	ab	-0.02 B	a	-0.17 B	a	2.11 B	a	8.0
2.0	17.69A	a	-0.66 B	a	-0.42 B	a	1.93 B	a	7.8
LSD	11.6		1.9		1.9		2.2		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 4.19. Anaerobic incubation NO_3-N from a Norfolk loamy sand amended with four N sources: simple effects of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

	Nitrogen Source								
RYE rate	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- mg kg^{-1} ----------								LSD
0.5	11.10 A†	b§	-1.82 B	a	-1.82 B	a	0.42 B	a	3.1
1.0	27.48 A	b	-1.82 B	a	-1.82 B	a	1.84 B	a	9.3
1.5	44.66 A	a	-1.77 B	a	-1.82 B	a	1.85 B	a	8.3
2.0	59.00 A	a	-1.80 B	a	-1.80 B	a	1.98 B	a	11.1
LSD	16.6		1.9		1.8		2.4		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 4.20. Anaerobic incubation NO_3-N from a Vance sandy clay loam amended with four N sources: simple effects of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

	Nitrogen Source								
RYE rate	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- mg kg^{-1} ----------								LSD
0.5	12.54 A†	c§	-12.13 B	a	-12.13 B	a	-11.66 B	a	20.8
1.0	26.62 A	bc	-12.13 B	a	-12.13 B	a	0.82 B	a	20.3
1.5	45.42 A	ab	-12.12 C	a	-12.13 C	a	4.02 B	a	14.8
2.0	57.15 A	a	-12.12 B	a	-12.10 B	a	4.75B	a	23.9
LSD	29.3		15.9		15.9		16.4		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 4.21. Anaerobic incubation NO_3-N from a Wedowee sandy loam amended with four N sources: simple effects of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

	Nitrogen Source								
RYE rate	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- mg kg^{-1} ----------								LSD
0.5	0.00 A†	a§	0.00 A	a	0.00 A	a	0.68 A	a	0.8
1.0	0.00 B	a	0.00 B	a	0.00 B	a	0.88 A	a	0.8
1.5	0.10 B	a	0.00 B	a	0.00 B	a	1.26 A	a	0.6
2.0	0.18 B	a	0.00 B	a	0.00 B	a	1.46 A	a	0.9
LSD	0.4		0		0		1.5		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 4.22. Analysis of variance for anaerobic incubation total inorganic N recovery (%) from anaerobic incubation of four N sources (3 biosolids, NH_4NO_3) applied at four rates on four soils.

Effect	Numerator DF	Denominator DF	F Value	Pr > F
RYE rate	3	192	6.58	0.0003
N source	3	192	271.6	<0.0001
Soil	3	192	1.49	<0.0001
RYE rate*N source	9	192	48.17	0.1543
RYE rate*Soil	9	192	1.51	0.1456
N source*Soil	9	192	16.93	<0.0001
RYE rate*N source*Soil	27	192	2.9	<0.0001

Table 4.23. Analysis of variance for anaerobic incubation total inorganic N recovery (%) from anaerobic incubation of four N sources (3 biosolids, NH_4NO_3) applied at four rates; grouped by soil.

Effect	Numerator DF	Denominator DF	F Value	Pr > F
		Noboco loamy sand		
RYE rate	3	48	0.9	0.4466
N source	3	48	55.39	<0.0001
RYE rate*N source	9	48	0.35	0.9507
		Norfolk loamy sand		
RYE rate	3	48	0.56	0.6425
N source	3	48	186.3	<0.0001
RYE rate*N source	9	48	2.22	0.0364
		Wedowee sandy loam		
RYE rate	3	48	0.71	0.5534
N source	3	48	42.69	<0.0001
RYE rate*N source	9	48	1.31	0.2569
		Vance sandy clay loam		
RYE rate	3	48	6.17	0.0012
N source	3	48	70.63	<0.0001
RYE rate*N source	9	48	4.45	0.0003

Table 4.24. Regression equations, model significance, and R^2 values from a 7-day anaerobic incubation. Independent variable was RYE rate and dependent variable was anaerobic incubation total inorganic N recovery (%).

Parameter	n	Equation†	Model P	R^2
		Noboco loamy sand		
OWASA cake	16	$y = 28.84 - 2.99x$	0.0862	0.20
Cary pellet	16	$y = 42.72 - 5.6x$	0.0005	0.59
NH_4NO_3	16	$y = 47.60 + 2.83x$	0.6822	0.01
Raleigh plus	16	$y = 19.30 - 5.22x$	0.0143	0.36
		Norfolk loamy sand		
OWASA cake	16	$y = 33.44 - 6.34x$	0.0144	0.36
Cary pellet	16	$y = 64.07 - 25.58x + 6.41x^2$	<0.0001	0.82
NH_4NO_3	16	$y = 78.47 + 10.79x$	0.2251	0.10
Raleigh plus	16	$y = 32.79 - 33.27x + 10.55x^2$	0.0054	0.55
		Wedowee sandy loam		
OWASA cake	16	$y = 33.26 - 7.19x$	0.0009	0.56
Cary pellet	16	$y = 35.15 - 3.28x$	0.2752	0.08
NH_4NO_3	16	$y = 73.55 - 17.12x$	0.1355	0.15
Raleigh plus	16	$y = -28.69 + 37.98x - 11.10x^2$	<0.0001	0.76
		Vance sandy clay loam		
OWASA cake	16	$y = 29.18 - 3.69x$	0.3462	0.06
Cary pellet	16	$y = 44.21 - 4.87x$	0.0066	0.42
NH_4NO_3	16	$y = 92.81 + 2.42x$	0.8274	0.00
Raleigh plus	16	$y = 154.87 - 164.20x + 47.67x^2$	<0.0001	0.86

† x = Realistic Yield Expectation Interval; y = Anaerobic Incubation total inorganic N recovery (%)

Table 4.25. Percent recovery of total N added from anaerobic incubation of a Noboco loamy sand amended with four N sources: simple effects of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

	Nitrogen Source								
RYE rate	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- % ----------								LSD
0.5	49.5 A†	a§	41.2 A	a	28.0 AB	a	18.1 B	a	21.6
1.0	49.2 A	a	35.0 B	b	25.2 B	a	11.9 C	a	11.0
1.5	52.9 A	a	34.6 AB	b	23.8 BC	a	11.4 C	a	21.6
2.0	53.0A	a	32.0 B	b	23.5 BC	a	9.6 C	a	14.1
LSD		33.0		5.3		7.8		8.6	

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)
§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 4.26. Percent recovery of total N added from anaerobic incubation of a Norfolk loamy sand amended with four N sources: simple effects of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

	Nitrogen Source								
RYE rate	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- % ----------								LSD
0.5	77.2 A†	a§	53.1 B	a	32.6C	a	18.8 C	a	18.7
1.0	96.9 A	a	44.4 B	b	24.1BC	ab	10.0 C	ab	28.1
1.5	99.2 A	a	40.7 B	bc	24.1 BC	ab	10.0 C	b	19.1
2.0	94.5 A	a	38.4 B	c	22.4 C	b	8.4 C	b	15.8
LSD		39.0		6.0		9.9		9.82	

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)
§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 4.27. Percent recovery of total N added from anaerobic incubation of a Vance sandy clay loam amended with four N sources: simple effects of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

	Nitrogen Source								
RYE rate	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- % ----------								LSD
0.5	93.6 A†	a§	43.3 BC	a	28.3C	a	87.4 AB	a	44.3
1.0	94.2 A	a	37.7 B	ab	24.5 B	a	30.3 B	b	29.3
1.5	99.9 A	a	35.7 B	b	22.9 C	a	23.9 C	b	9.6
2.0	95.7 A	a	35.8 B	b	22.7 B	a	14.5 B	b	28.4
LSD	53.1		6.7		18.4		22.7		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)
§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 4.28. Percent recovery of total N added from anaerobic incubation of a Wedowee sandy loam amended with four N sources: simple effects of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

	Nitrogen Source								
RYE rate	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- % ----------								LSD
0.5	67.3 A†	a§	32.7 AB	a	30.8 AB	a	-12.9 B	b	51.3
1.0	55.0 A	a	33.4 B	a	24.4 B	ab	-0.4 C	a	14.5
1.5	43.9 A	a	29.5 B	a	22.5 B	b	1.9 C	a	12.7
2.0	42.4A	a	28.6 B	a	19.4 C	b	3.3 D	a	7.9
LSD	52.4		13.9		8.0		7.9		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)
§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 4.29. Averages of total inorganic N recoveries (%) across all RYE intervals by soil type and averaged across all four soils.

N Source	Average Total Inorganic N Recovery (%)
Noboco loamy sand	
NH_4NO_3	51.2
Cary pellet	35.7
OWASA cake	25.1
Raleigh plus	12.8
Norfolk loamy sand	
NH_4NO_3	92.0
Cary pellet	44.2
OWASA cake	25.1
Raleigh plus	11.8
Vance sandy clay loam	
NH_4NO_3	95.9
Cary pellet	38.1
OWASA cake	24.6
Raleigh plus	39.0
Wedowee sandy loam	
NH_4NO_3	52.2
Cary pellet	31.1
OWASA cake	24.3
Raleigh plus	-2.0
Overall Average	
NH_4NO_3	72.8
Cary pellet	37.3
OWASA cake	24.8
Raleigh plus	15.4

Table 4.30. Analysis of variance for anaerobic incubation PAN recovery (%) from anaerobic incubation of four N sources (3 biosolids, NH_4NO_3) applied at four rates on four soils.

Effect	Numerator DF	Denominator DF	F Value	Pr > F
RYE rate	3	192	26.3	<0.0001
N source	3	192	185.07	<0.0001
Soil	3	192	57.51	<0.0001
RYE rate*N source	9	192	4.36	<0.0001
RYE rate*Soil	9	192	4.98	<0.0001
N source*Soil	9	192	22.37	<0.0001
RYE rate*N source*Soil	27	192	6.84	<0.0001

Table 4.31. Analysis of variance for anaerobic incubation PAN recovery (%) from anaerobic incubation of four N sources (3 biosolids, NH_4NO_3) applied at four rates; grouped by soils.

Effect	Numerator DF	Denominator DF	F Value	Pr > F
		Noboco loamy sand		
RYE rate	3	48	5.03	0.0041
N source	3	48	143.25	<0.0001
RYE rate*N source	9	48	0.82	0.5971
		Norfolk loamy sand		
RYE rate	3	48	0.0001	0.0001
N source	3	48	116.39	<0.0001
RYE rate*N source	9	48	2.96	0.0070
		Wedowee sandy loam		
RYE rate	3	48	1.03	0.3882
N source	3	48	125.99	<0.0001
RYE rate*N source	9	48	3.9	0.0009
		Vance sandy clay loam		
RYE rate	3	48	15.75	<0.0001
N source	3	48	3.82	0.0155
RYE rate*N source	9	48	9.32	<0.0001

Table 4.32. Regression equations, model significance, and R^2 values from a 7-day anaerobic incubation. Independent variable was RYE rate and dependent variable was anaerobic incubation PAN recovery (%).

Parameter	n	Equation†	Model P	R^2
		Noboco loamy sand		
OWASA cake	16	$y = 150.52 - 15.59x$	0.0862	0.20
Cary pellet	16	$y = -18.86 + 143.59x$	0.0005	0.59
NH_4NO_3	16	$y = 47.60 + 2.83x$	0.9822	0.01
Raleigh plus	16	$y = 63.64 - 17.22x$	0.0143	0.36
		Norfolk loamy sand		
OWASA cake	16	$y = 174.56 -33.12x$	0.0144	0.36
Cary pellet	16	$y = 215.36 -85.98x + 21.55x^2$	<0.0001	0.82
NH_4NO_3	16	$y = 78.47 + 10.79x$	0.2251	0.10
Raleigh plus	16	$y = 108.15 -109.72x + 34.79x^2$	0.0054	0.55
		Wedowee sandy loam		
OWASA cake	16	$y = 173.61 -37.55x$	0.0009	0.56
Cary pellet	16	$y = 118.16 - 11.02x$	0.2752	0.08
NH_4NO_3	16	$y = 73.56 - 17.12x$	0.1355	0.15
Raleigh plus	16	$y = 94.63 + 125.24x - 36.61x^2$	< 0.0001	0.76
		Vance sandy clay loam		
OWASA cake	16	$y = 152.32 - 19.24x$	0.3462	0.06
Cary pellet	16	$y = 148.60 - 16.35x$	0.0066	0.42
NH_4NO_3	16	$y = 92.81 + 2.42x$	0.8274	0.00
Raleigh plus	16	$y = 510.77 - 541.51x + 157.23x^2$	< 0.0001	0.86

† x = Realistic Yield Expectation Interval; y = Anaerobic Incubation PAN recovery (%)

Table 4.33. Percent recovery of total plant-available nitrogen from anaerobic incubation of a Noboco loamy sand amended with four N sources: simple effects of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

	Nitrogen Source								
RYE rate	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- % ----------								LSD
0.5	49.5 B†	a§	138.7 A	a	145.9 A	a	59.7 B	a	46.6
1.0	49.2 B	a	117.5 A	b	131.6 A	a	39.4 B	a	24.4
1.5	52.9 B	a	116.3 A	b	124.2 A	a	37.7 B	a	27.6
2.0	53.0 B	a	107.6 A	b	122.4 A	a	31.6 C	a	18.1
LSD	33.0		17.8		40.6		28.3		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)
§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 4.34. Percent recovery of total plant-available nitrogen from anaerobic incubation of a Norfolk loamy sand amended with four N sources: simple effects of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

	Nitrogen Source								
RYE rate	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- % ----------								LSD
0.5	77.3 B†	a§	178.4 A	a	170.1 A	a	62.1 B	a	57.3
1.0	96.9 B	a	149.1 A	b	125.8 AB	ab	32.9 C	ab	34.7
1.5	99.2 B	a	136.7 A	bc	119.9 AB	ab	22.2 C	b	22.5
2.0	94.5 B	a	129.0 A	c	116.9 AB	b	27.8 C	b	26.0
LSD	39.0		20.1		51.9		32.4		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)
§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 4.35. Percent recovery of total plant-available nitrogen from anaerobic incubation of a Vance sandy clay loam amended with four N sources: simple effects of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

	Nitrogen Source								
RYE rate	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- % ----------								LSD
0.5	93.6 B†	a§	145.4 B	a	147.6 B	a	288.2 A	a	98.7
1.0	94.2 A	a	126.8 A	ab	127.6 A	a	99.9 A	b	77.7
1.5	99.9 AB	a	120.1 A	b	119.6 A	a	78.8 B	b	32.4
2.0	95.7 A	a	120.4 A	b	118.2 A	a	47.8 B	b	36.4
LSD	53.1		22.5		95.9		75.0		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)
§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 4.36. Percent recovery of total plant-available nitrogen from anaerobic incubation of a Wedowee sandy loam amended with four N sources: simple effects of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

	Nitrogen Source								
RYE rate	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- % ----------								LSD
0.5	67.3 B†	a§	110.0 AB	a	160.7 A	a	-42.7 C	b	72.4
1.0	55.0 B	a	112.4 A	a	127.1 A	ab	-1.4 C	a	31.4
1.5	43.9 B	a	99.2 A	a	117.5 A	b	6.3 C	a	22.6
2.0	42.4 B	a	96.0 A	a	101.3 A	b	10.9 C	a	24.7
LSD	52.4		46.9		41.6		26.1		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)
§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 4.37. Averages of PAN recoveries (%) across all RYE intervals by soil type and averaged across all four soils.

N Source	Average Recovery (%)
Noboco loamy sand	
NH_4NO_3	51.2
Cary pellet	120.0
OWASA cake	131.0
Raleigh plus	421.0
Norfolk loamy sand	
NH_4NO_3	92.0
Cary pellet	148.3
OWASA cake	133.2
Raleigh plus	36.3
Vance sandy clay loam	
NH_4NO_3	95.9
Cary pellet	128.2
OWASA cake	128.3
Raleigh plus	128.7
Wedowee sandy loam	
NH_4NO_3	52.2
Cary pellet	104.4
OWASA cake	126.7
Raleigh plus	-6.7
Overall Average	
NH_4NO_3	72.8
Cary pellet	125.2
OWASA cake	129.8
Raleigh plus	50.1

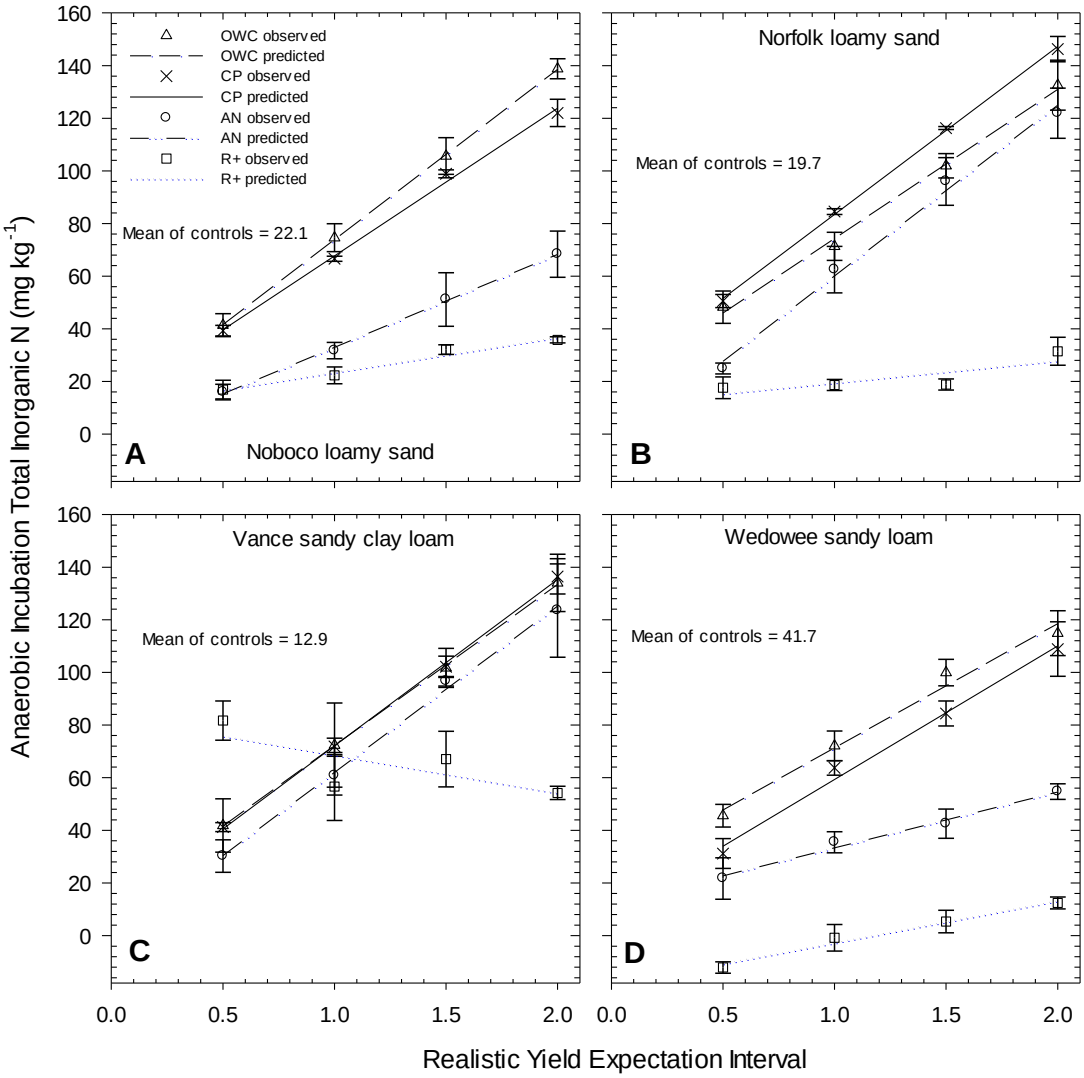

Figure 4.1. Anaerobic Incubation Total Inorganic N vs. Realistic Yield Expectation Interval. Regression analysis of the simple effects of RYE rate for each N source in each soil type studied during a 7-day anaerobic incubation. Nitrogen sources included three biosolids and NH_4NO_3, each mixed at five rates with two representative coastal plain soils: Noboco loamy sand (A) and Norfolk loamy sand (B), and two representative piedmont soils: Vance sandy clay loam (C) and Wedowee sandy loam (D). Nitrogen rates were determined as 0, 0.5 X, 1.0 X, 1.5 X, and 2.0 X the North Carolina Realistic Yield Expectation Database (North Carolina Nutrient Management Workgroup) N rate for Fescue on a Wedowee coarse sandy loam soil. The 1.0 RYE rates were 144.5 kg ha^{-1} for NH_4NO_3 and 127 kg ha^{-1} for the three biosolids; the differences were due to a calculation error, but were grouped by RYE interval in the above figure. Anaerobic Incubation Total Inorganic N = NH_4 + NO_3. Error bars represent the standard error of the individual means. Average N content of the control samples was subtracted from the amended samples.

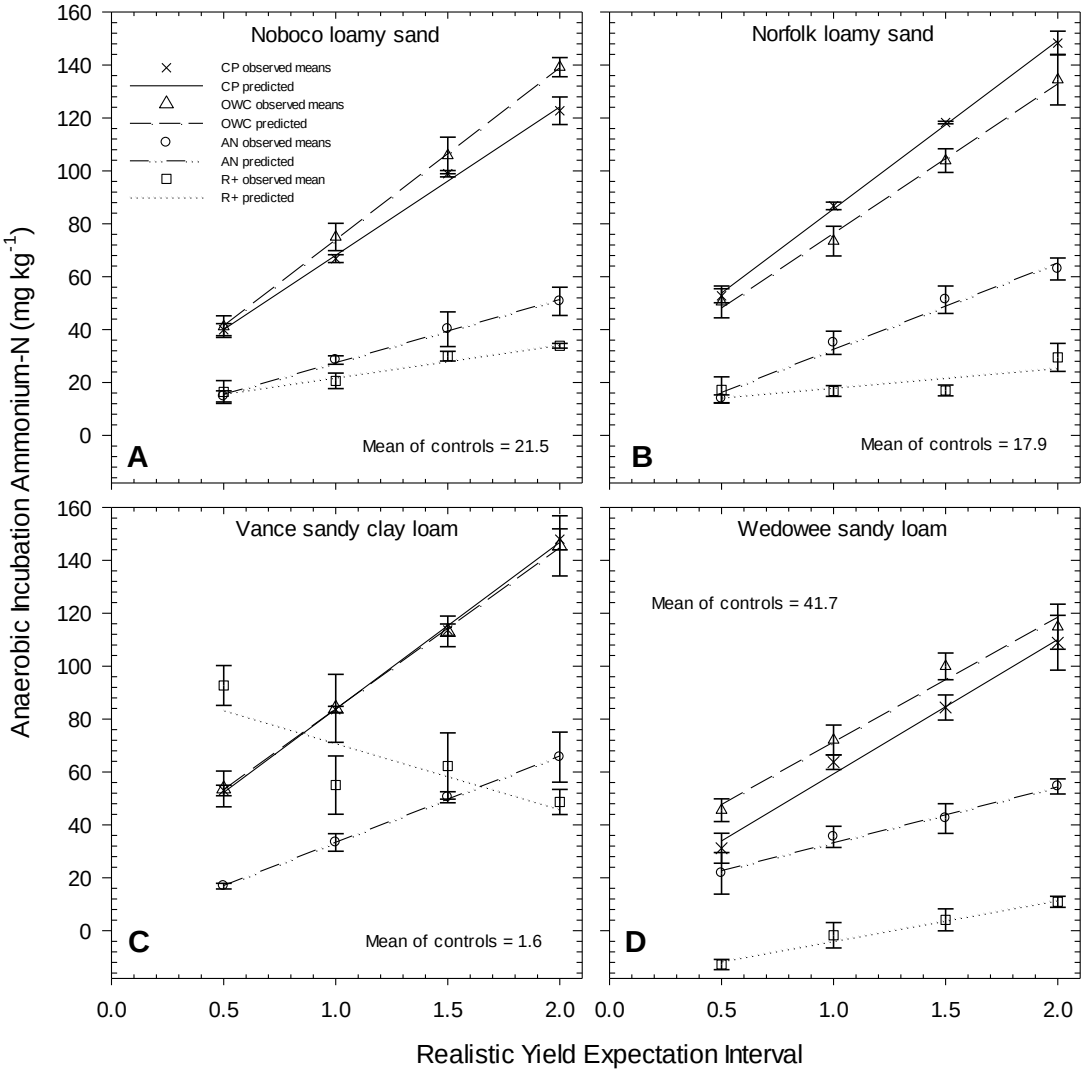

Figure 4.2. Anaerobic incubation NH_4-N vs. Realistic Yield Expectation Interval. Regression analysis of the simple effects of RYE rate for each N source in each soil type studied during a 7-day anaerobic incubation. N sources included three biosolids and NH_4NO_3, each mixed at five rates with two representative coastal plain soils: Noboco loamy sand (A) and Norfolk loamy sand (B), and two representative piedmont soils: Vance sandy clay loam (C) and Wedowee sandy loam (D). Nitrogen rates were determined as 0, 0.5 X, 1.0 X, 1.5 X, and 2.0 X the North Carolina Realistic Yield Expectation Database (North Carolina Nutrient Management Workgroup) N rate for Fescue on a Wedowee coarse sandy loam soil. The 1.0 RYE rates were 144.5 kg ha^{-1} for NH_4NO_3 and 127 kg ha^{-1} for the three biosolids; the differences due to a calculation error, but are grouped by RYE interval in the above figure. Anaerobic Incubation Total Inorganic N = NH_4 + NO_3. Error bars represent the standard error of the individual means. Average N content of the control samples were subtracted from the amended samples.

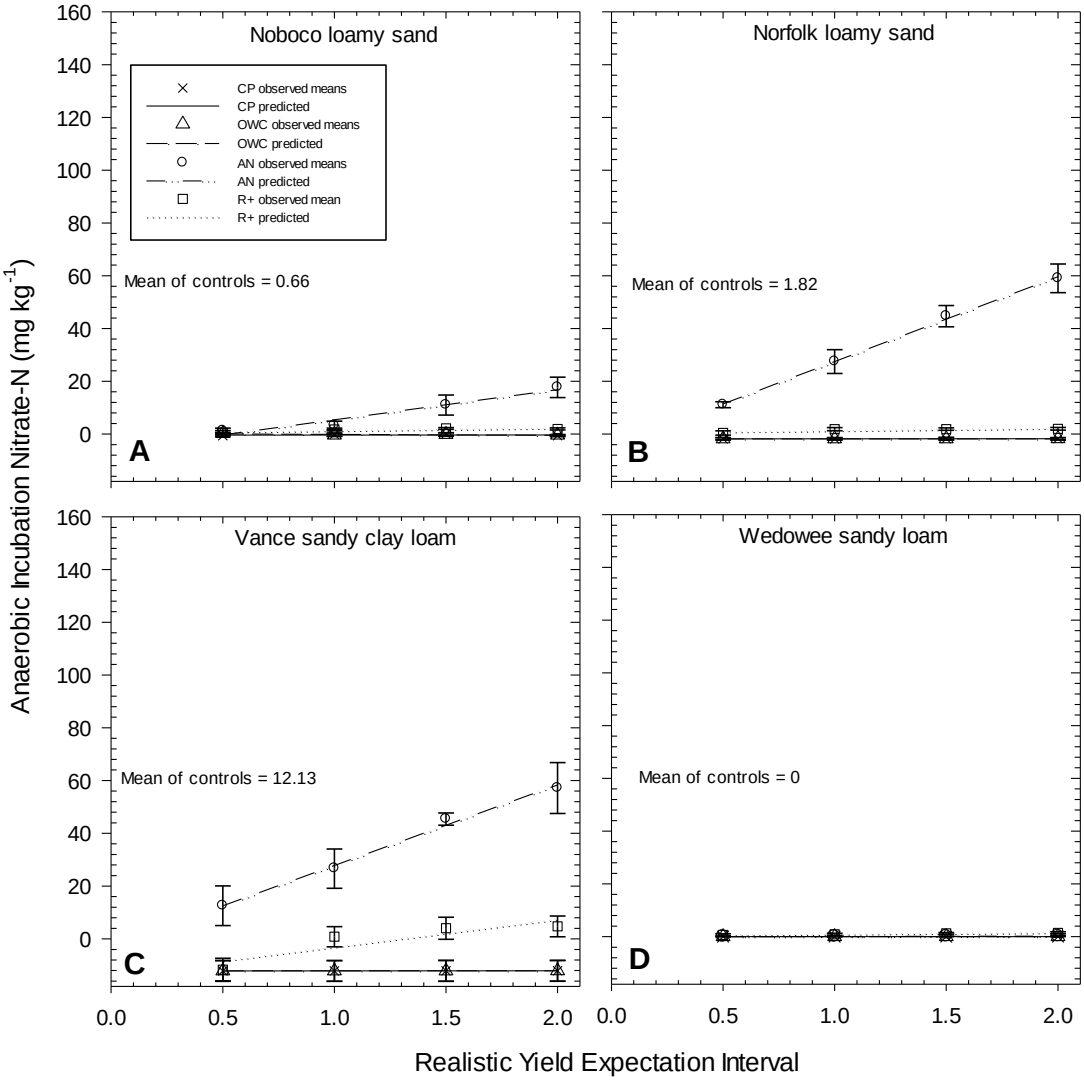

Figure 4.3. Anaerobic incubation NO_3-N vs. Realistic Yield Expectation Interval. Regression analysis of the simple effects of RYE rate for each N source in each soil type studied during a 7-day anaerobic incubation. N sources included three biosolids and NH_4NO_3, each mixed at five rates with two representative coastal plain soils: Noboco loamy sand (A) and Norfolk loamy sand (B) and two representative piedmont soils: Vance sandy clay loam (C) and Wedowee sandy loam (D). Nitrogen rates were determined as 0, 0.5 X, 1.0 X, 1.5 X, and 2.0 X the North Carolina Realistic Yield Expectation Database (North Carolina Nutrient Management Workgroup) N rate for Fescue on a Wedowee coarse sandy loam soil. The 1.0 RYE rates were 144.5 kg ha^{-1} for NH_4NO_3 and 127 kg ha^{-1} for the three biosolids due to a calculation error, but are grouped in the above figure. Anaerobic Incubation Total Inorganic N = NH_4 + NO_3. Error bars represent the standard error of the individual means. Average N content of the control samples were subtracted from the amended samples.

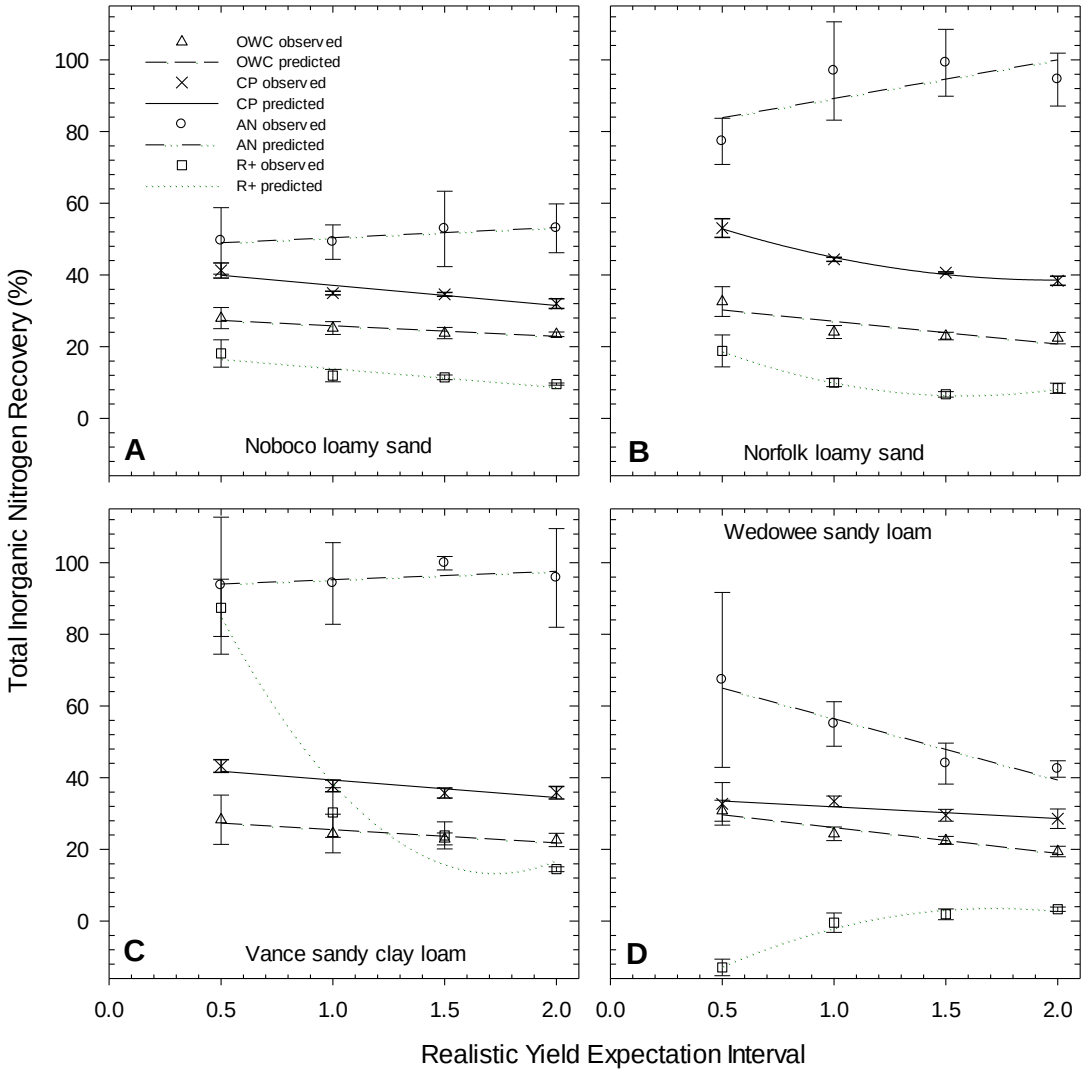

Figure 4.4. Anaerobic incubation total inorganic N recovery (%) vs. Realistic Yield Expectation Interval. Regression analysis of the simple effects of RYE rate for each N source in each soil type studied during a 7-day anaerobic incubation. N sources included three biosolids and NH_4NO_3, each mixed at five rates with two representative coastal plain soils: Noboco loamy sand (A) and Norfolk loamy sand (B) and two representative piedmont soils: Vance sandy clay loam (C) and Wedowee sandy loam (D). Nitrogen rates were determined as 0, 0.5 X, 1.0 X, 1.5 X, and 2.0 X the North Carolina Realistic Yield Expectation Database (North Carolina Nutrient Management Workgroup) N rate for Fescue on a Wedowee coarse sandy loam soil. The 1.0 RYE rates were 144.5 kg ha^{-1} for NH_4NO_3 and 127 kg ha^{-1} for the three biosolids due to a calculation error, but are grouped in the above figure. Anaerobic Incubation Total Inorganic N = NH_4 + NO_3. Error bars represent the standard error of the individual means.

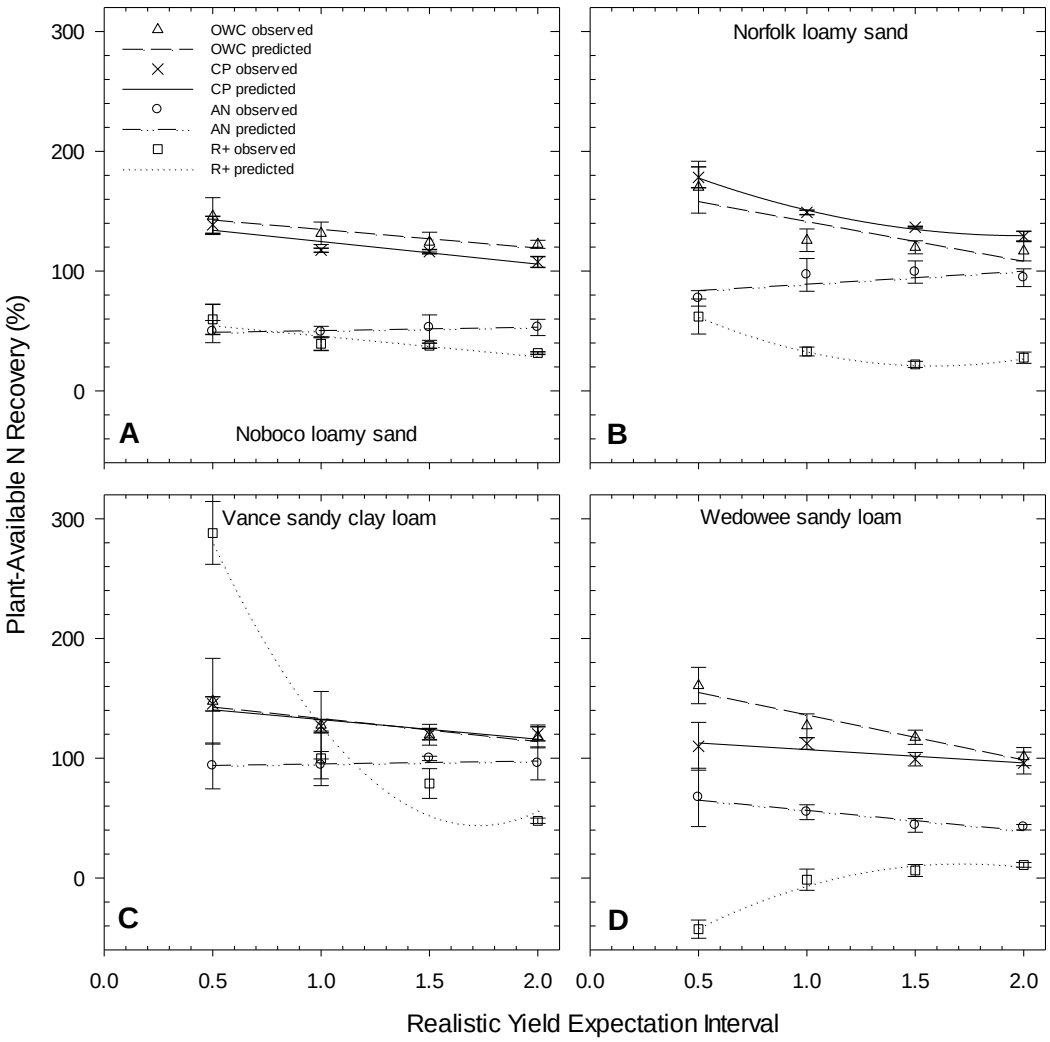

Figure 4.5. Anaerobic incubation plant-available N recovery (%) vs. Realistic Yield Expectation Interval. Regression analysis of the simple effects of RYE rate for each N source in each soil type studied during a 7-day anaerobic incubation. N sources included three biosolids and NH_4NO_3, each mixed at five rates with two representative coastal plain soils: Noboco loamy sand (A) and Norfolk loamy sand (B) and two representative piedmont soils: Vance sandy clay loam (C) and Wedowee sandy loam (D). Nitrogen rates were determined as 0, 0.5 X, 1.0 X, 1.5 X, and 2.0 X the North Carolina Realistic Yield Expectation Database (North Carolina Nutrient Management Workgroup) N rate for Fescue on a Wedowee coarse sandy loam soil. The 1.0 RYE rates were 144.5 kg ha^{-1} for NH_4NO_3 and 127 kg ha^{-1} for the three biosolids due to a calculation error, but are grouped in the above figure. Anaerobic Incubation Total Inorganic N = NH_4 + NO_3. Error bars represent the standard error of the individual means.

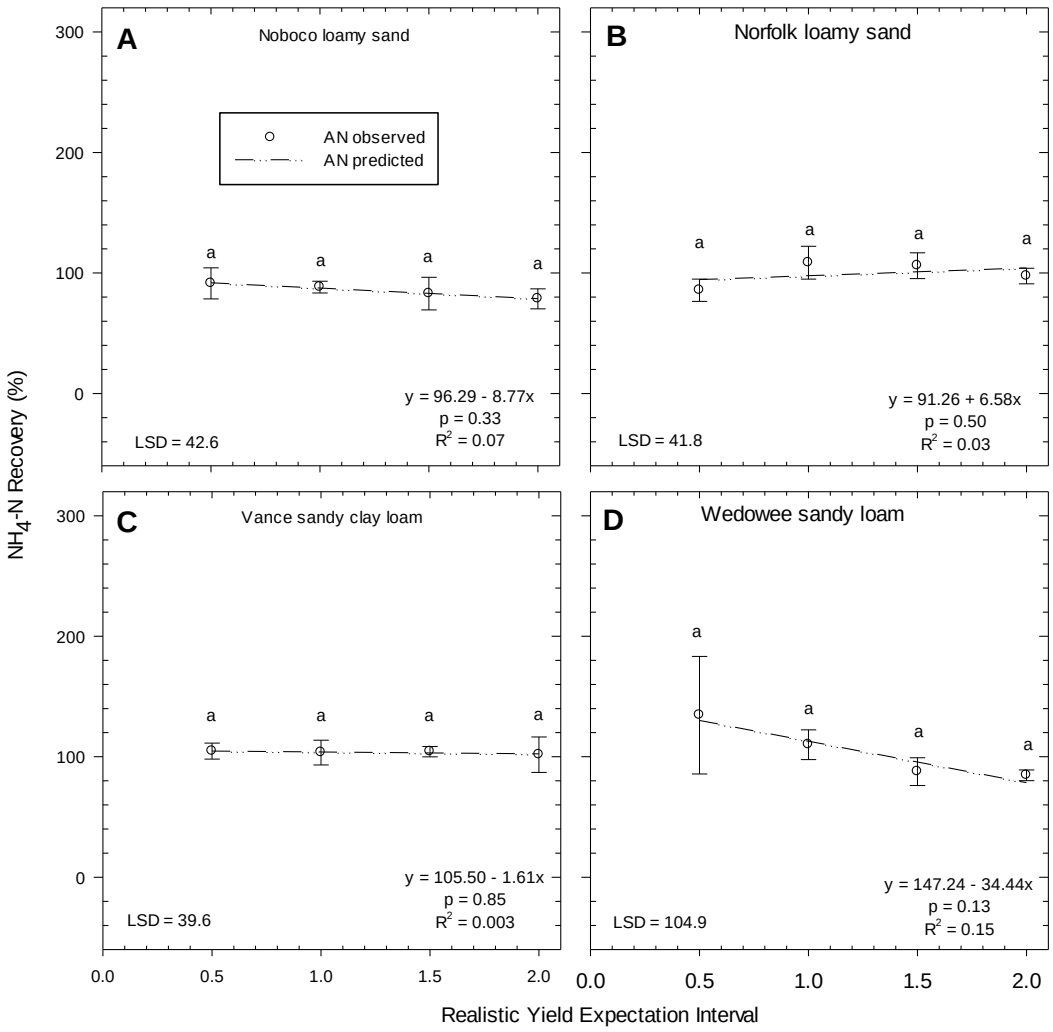

Figure 4.6. Anaerobic incubation NH_4-N recovery (%) vs. Realistic Yield Expectation interval. Regression analysis of the simple effects of RYE rate for each N source in each soil type studied during a 7-day anaerobic incubation. N sources included three biosolids and NH_4NO_3, each mixed at five rates with two representative coastal plain soils: Noboco loamy sand (A) and Norfolk loamy sand (B) and two representative piedmont soils: Vance sandy clay loam (C) and Wedowee sandy loam (D). Nitrogen rates were determined as 0, 0.5 X, 1.0 X, 1.5 X, and 2.0 X the North Carolina Realistic Yield Expectation Database (North Carolina Nutrient Management Workgroup) N rate for Fescue on a Wedowee coarse sandy loam soil. The 1.0 RYE rates were 144.5 kg ha^{-1} for NH_4NO_3 and 127 kg ha^{-1} for the three biosolids due to a calculation error, but are grouped in the above figure. Anaerobic Incubation Total Inorganic N = NH_4 + NO_3. Error bars represent the standard error of the individual means.

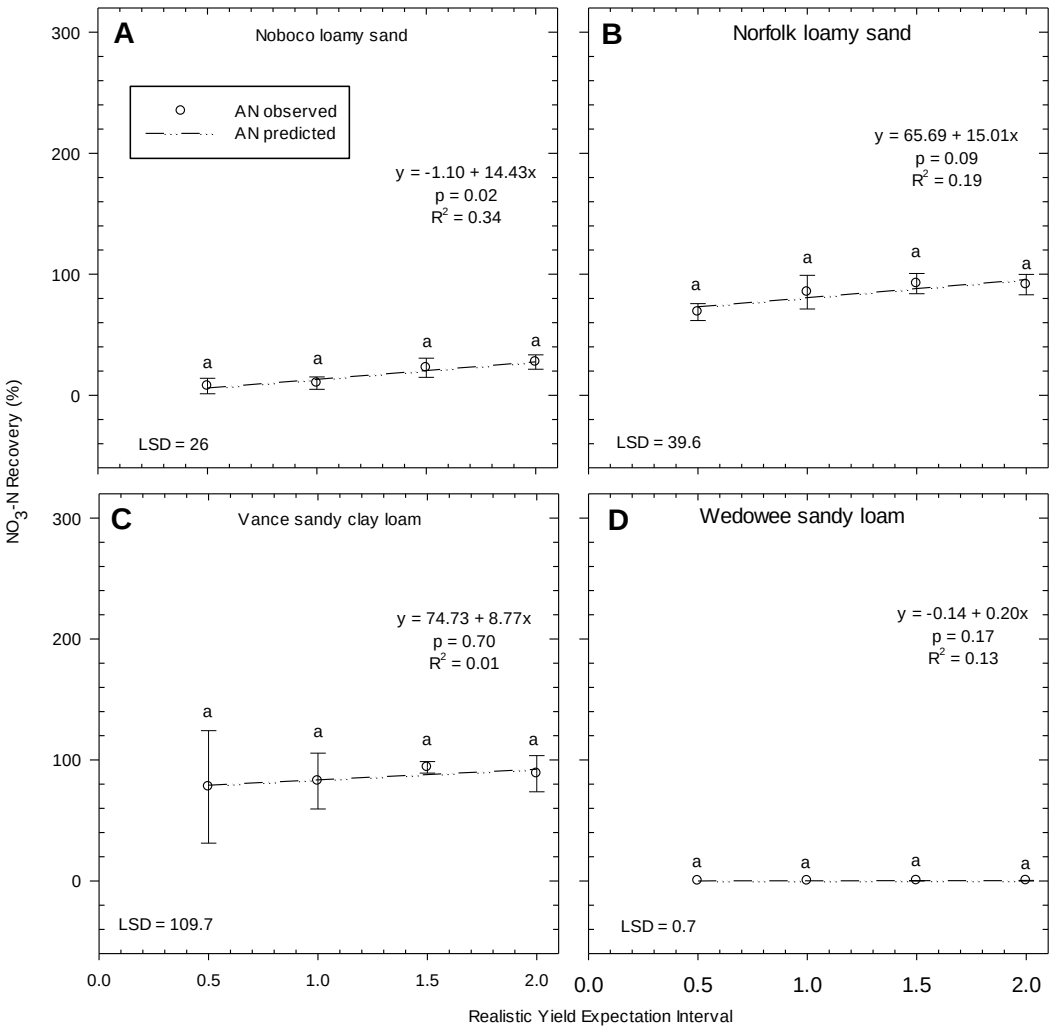

Figure 4.7 Anaerobic incubation NO_3-N recovery (%) vs. Realistic Yield Expectation Interval. Regression analysis of the simple effects of RYE rate for each N source in each soil type studied during a 7-day anaerobic incubation. N sources included three biosolids and NH_4NO_3, each mixed at five rates with two representative coastal plain soils: Noboco loamy sand (A) and Norfolk loamy sand (B) and two representative piedmont soils: Vance sandy clay loam (C) and Wedowee sandy loam (D). Nitrogen rates were determined as 0, 0.5 X, 1.0 X, 1.5 X, and 2.0 X the North Carolina Realistic Yield Expectation Database (North Carolina Nutrient Management Workgroup) N rate for Fescue on a Wedowee coarse sandy loam soil. The 1.0 RYE rates were 144.5 kg ha^{-1} for NH_4NO_3 and 127 kg ha^{-1} for the three biosolids due to a calculation error, but are grouped in the above figure. Anaerobic Incubation Total Inorganic N = NH_4 + NO_3. Error bars represent the standard error of the individual means.

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CHAPTER 5: AMINO SUGAR NITROGEN TEST

5.0 Amino-Sugar N Content

5.0.0 Analysis of Variance

The average ASNT contents of the appropriate controls were subtracted from the amended samples so that the effect of the amendments alone could be identified. The analysis of variance of all parameters tested is illustrated in Table 5.1. The three-way interaction of RYE rate*N source*Soil was not statistically significant, nor was the two-way interaction of RYE rate*Soil. The two-way interaction of RYE rate*N source was statistically significant. The two-way interaction of N source*Soil was not statistically significance at the 0.05 level, but was assumed important enough to evaluate. Due to the statistically significant interaction involving soil type, the results were reanalyzed by soil type (Table 5.2). Throughout all soil types, the RYE rate*N source interaction was statistically significant. As a result, the simple effects of RYE rate were investigated for each N source in each soil.

5.0.1 Regression Analysis: Simple Effect of RYE Rate, Noboco loamy sand

Results of regression analyses of the response of ASNT-N to application of three biosolids (CP, OWC, and R+) and NH_4NO_3 is illustrated in Figure 5.1. Each regression line represents the simple effect of RYE rate for each N source. Regression equations, model significance, and R^2 values are illustrated in Table 5.3. All but one best fit model was linear. In the Noboco loamy sand soil (Fig. 5.1A), the mean of the ASNT-N of the controls was 78.4

mg kg^{-1} (175.6 kg ha^{-1}), and was an amount intermediate among the four soils tested. OWASA cake yielded the greatest amount of ASNT-N across all application rates. Separation of the observed means showed differences between the OWC and all other N sources at all rates except the 0.5 RYE interval, in which OWC was not different from AN or CP, and the 1.5 RYE interval where it was not different than AN (Table 5.4). The linear model for OWC was statistically significant and ASNT-N increased with increasing application rates. The ASNT-N response from OWC was different than the responses of AN, CP, and R+. The models for CP and R+ were not statistically significant and ASNT-N did not change across RYE interval means for any of the N sources other than OWC. Undoubtedly, the lack of differences was at least partially due to the large errors of the N sources. The Noboco soil was the first soil tested, and the large errors may have been attributable to the learning curve of the experimental procedure. The order of the other soils tested was Norfolk, Wedowee, and Vance. The lack of statistically significant differences among RYE rates among all N sources except OWC was evidence of the RYE rate*N source interaction—that is—the ASNT-N response to RYE rate depended on the N source. However, the regression model for AN was significant, albeit with a low R^2, indicating that when all four rates were considered together, ASNT-N did tend to increase with AN rate. This result was expected because the ASNT recovers both NH_4-N initially present in addition to NH_4 from the amino-sugar organic fraction. The ASNT-N from the AN regression appeared to be greater than CP and R+ across most rates, but separation of the observed means showed that there was no difference among any of those N sources. This would be expected to be the case if the ASNT accurately predicted N released from the N sources and

the first year NAC provided by the NCDA&CS were correct. Also, the ASNT-N averaged across all rates for R+ was 3 mg kg^{-1}, and suggested that there was very little mineralizable N from R+ when added to the Noboco soil.

5.0.2 Regression Analysis: Simple Effect of RYE Rate, Norfolk loamy sand

Results of the regression analyses of the response of ASNT-N to application of four different N sources (as described above) are illustrated in Figure 5.1B. The mean of the ASNT-N of the controls was 65.9 mg kg^{-1} (147 kg ha^{-1}), which was the lowest ASNT-N among the four soils tested. OWASA cake again yielded the most ASNT-N across all RYE rates. Interestingly, the best fit model for OWC in the Norfolk soil was quadratic, as opposed to a best fit linear model in all the other soils, and was highly statistically significant. This provided evidence of the RYE rate*soil interaction (Table 5.1) and shows that the ASNT-N response depended on the soil type. Separation of the observed means showed that OWC ASNT-N was greater than in all the other N sources across all RYE rates (Table 5.5). Additionally, ASNT-N increased with increasing application rate. Ammonium nitrate had the second most ASNT-N of the four N products added. However, the observed means were only different from CP at the 1.5 RYE intervals. The observed means of AN were different from R+ at RYE intervals of 0.5, 1.5, and 2.0. Raleigh plus had the lowest ASNT-N across all rates, but was only different from CP at an RYE interval of 1.5, and there was no difference among RYE rates. The R+ ASNT-N averaged across all RYE rates was -0.3 mg kg^{-1} and was evidence of a loss of NH_4-N in the Norfolk soil amended with R+. Perhaps some N transformations (i.e. nitrification) occurred as the samples were prepared for

treatment. As in the Noboco soil, the general magnitude of ASNT-N response was similar among all N sources and RYE intervals, but there were more differences among the N sources and RYE rate in the Norfolk soil, likely a result of the smaller errors (Table 5.5).

5.0.3 Regression Analysis: Simple Effect of RYE Rate, Vance sandy clay loam

Results of a regression analysis of the response of ASNT-N to application of four different N sources (as described above) are illustrated in Figure 5.1C. The control mean ASNT-N was 92.8 mg kg^{-1} (207 kg ha^{-1}), which, along with the Wedowee soil, was greater than the other soils. OWASA cake again yielded the most ASNT-N across all RYE rates, the best fit model was linear, and the model was highly statistically significant (Table 5.3). OWASA cake ASNT-N was greater than all other N sources with the exception of CP and R+ at the 0.5 RYE interval, (Table 5.6). Ammonium nitrate and CP were the next highest ASNT-N contents and the observed means of the two were only statistically significantly different at the 1.5 RYE rate where AN was greater than CP. Ammonium nitrate ASNT-N increased with increasing RYE rates between the 0.5 and 1.0, and the 1.0 and 2.0 intervals. The ASNT-N from the 2.0 RYE rate of CP was higher than all other rates of CP. Raleigh plus once again yielded the least ASNT-N across all RYE rates. The linear regression appears to be decreasing with increasing application rate, but the model was not statistically significant, and there was no difference in any observed means at varying RYE rates. The mean values of the 1.0, 1.5, and 2.0 RYE rates were negative (-6.7, -8.8, and -4.6, respectively), and an overall average of -4 mg kg^{-1} across all rates was evidence of NH_4-N loss and/or transformations, as described previously. The observed means of R+ were lower

than those of all other N sources at the 1.5 and 2.0 RYE intervals. As in the other soils, there was evidence of varying amounts of ASNT-N between certain N sources. Presuming that the ASNT is a precise method of estimating N mineralization, then all models should have been coincident given that they were all applied at the same inherent or NAC estimated PAN rate. Since the magnitude of ASNT-N was different for different N sources, the currently used NAC coefficients might be incorrect.

5.0.4 Regression Analysis: Simple Effect of RYE Rate, Wedowee sandy loam

Results of a regression analysis of the response of ASNT-N to application of four different N sources (as described above) are illustrated in Figure 5.1D. The mean ASNT-N was 92.3 mg kg^{-1} (206.8 kg ha^{-1}), equivalent to the other soil with the highest ASNT-N, the Vance sandy clay loam. As was the case in all other soils, OWC yielded the most ASNT-N across all RYE rates. The best fit model was linear and it was statistically significant. The ASNT-N increased with increasing application rate (Table 5.7). The observed means of OWC were greater than the observed means of all other N sources across all RYE intervals. The N sources that yielded the next most ASNT-N were AN and CP. Both models were linear and on the brink of statistical significance at $\alpha = 0.05$ (Table 5.3). There were no differences in the observed means between AN and CP at any RYE rates, and neither N source increased in ASNT-N as RYE rate increased. Raleigh plus yielded the least ASNT-N of the four N sources tested. A linear model best fit the data but it was not statistically significant. There were no differences in the observed means between different RYE rates. However, after subtracting the control values from R+ amended samples, all observed means

were negative and had an overall average of -17.9 mg kg^{-1} (Table 5.7). These were the lowest values of the four soils tested and suggested NH_4-N loss and/or transformation. Ultimately, the observed means were not different from the observed means of AN and CP. Additionally, the overall magnitude of all N sources ASNT-N appeared less than that of the Noboco and Norfolk soils. For example, the maximum ASNT-N value from OWC at the 2.0 RYE interval in the Wedowee soil was 110 mg kg^{-1}. That same RYE interval and N source in the Noboco and Norfolk soils was approximately 140- (Fig. 5.1A) and 162 mg kg^{-1} (Fig. 5.1B), respectively.

5.0.5 Summary of Amino-Sugar N Test

The ASNT used in this research involved applying four different N sources to four different soils at five different rates in order to evaluate the relative differences in ASNT-N, and compare and contrast the results with the currently used first year NCDA&CS NAC. The two piedmont soils—Vance sandy clay loam and Wedowee sandy loam—yielded the most residual ASNT-N (unamended controls). Interestingly, those two soils had the lowest percent HM, at 0.36%. Humic matter as determined by the NCDA&CS method is strongly correlated with soil organic matter (Gonese and Weber, 1998). It was hypothesized that soils with the greatest percentage of HM would produce the most ASNT-N, but that was not the case. However, the Vance and the Wedowee soils had equivalent HM and ASNT-N. The Noboco loamy sand and Norfolk loamy sand had HM percentages of 0.76 and 0.56%, respectively, and those control soils had lower ASNT-N than the Piedmont soils. The residual ASNT-N from the Noboco soil was higher than that of the Norfolk soil. As a result,

it appeared as if unamended soils with relatively low HM content yielded greater amounts of ASNT-N. However, the lower HM content soils also generally yielded a lower magnitude of ASNT-N when N sources were added. As a result, it was not possible to definitely establish a direct relationship between HM content and ASNT-N. A possible explanation could that the relative ages of the organic matter in the soils were highly variable. For example, even though the Noboco soil had the highest HM percentage, a majority of that organic material could have been older, more highly decomposed and recalcitrant than that organic matter of the other soils. Other explanations include inherent differences in these soils' ability to mineralize organic matter, likely due to differing soil chemical, physical, and/or microbiological properties, which could have important implications for mineralization of biosolids-N added to these soils. No data were available to definitively postulate on this hypothesis.

Throughout the ASNT, the OWC yielded the most ASNT-N. The ASNT-N responses of CP and AN were similar across all soils and rates. Raleigh plus generally yielded less ASNT-N than the other three N sources, although the observed means were frequently the same as those of CP and AN. Assuming that the ASNT accurately and precisely predicts N mineralization/release, this data suggested that the NAC recommended by the NCDA&CS are not entirely correct. Ammonium nitrate is assumed to be 100% plant-available upon dissolution of the prills. Estimated NAC's were used in an attempt to add the same amount of PAN as was added via AN. If the NAC were correct, all regression models should have been coincident. However, it appeared as if the NAC for OWC underestimated the N mineralization for that N source. The NAC used for R+ appeared to have overestimated the

amount of N mineralization from that product, although at certain RYE intervals, R+ was no different than AN, which was the goal of using different NAC for different biosolids (Table 2.1). The ASNT-N response from AN and CP was coincident throughout most soils and rates. Therefore, it appeared as if the NAC used for CP was satisfactory. Moreover, the relative magnitudes of ASNT-N appeared to vary between the coastal plain and piedmont soils, although we have not tested this statistically. The coastal plain soils appeared to yield a greater magnitude of ASNT-N than the piedmont soils across all N sources and RYE rates. This difference suggested that soil type affected the N mineralization of the biosolids and, as a result, should be considered when estimating first year N mineralization.

5.1 ASNT on Biosolids Only

The ASNT test was conducted on 1.0 g (dry weight) of each of the four biosolids alone; that is, not added to soil (Table 5.7; Fig. 5.2). Additionally, CP was pulverized via mortar and pestle to achieve a powdered consistency in order to detect any differences between the pelleted and powder forms of the biosolids. As expected, AN had the largest ASNT-N value of the N sources with 68,878 mg kg^{-1}. OWASA cake had the second highest ASNT-N value with 9,790 mg kg^{-1}. Both the pelleted and powdered CP biosolids returned similar ASNT-N values, with 4,302 and 4,600 mg kg^{-1}, respectively, which suggested that the particle size of the products had little to no influence on ASNT-N. Raleigh plus yielded the lowest ASNT-N value with 263 mg kg^{-1}, which was expected given the advanced N removal techniques used to produce the biosolids (as previously discussed). The fact that OWC had a test value over twice as high as CP was surprising because CP had 65,000 mg kg^{-1} of Total

Kjeldahl N (TKN) vs. 48,801 mg kg^{-1} TKN for the OWC (Fig. 3.1). Cary pellet also had approximately twice the amount of inorganic N with 3,293 mg kg^{-1} vs. 1,673 mg kg^{-1} from the OWC (Table 3.1). The NH_4 and NO_3 concentrations of CP were 3,290 and 3 mg kg^{-1}, respectively, and for OWC the concentrations were 1,665 and 8 mg kg^{-1}, respectively. The higher test value of the OWC suggested either that its organic matter contained a greater proportion of AS-N or that its AS-N was more easily digested, volatilized, and captured by the method than the CP. The results could also help explain the ASNT-N results of the biosolids-soil mixture (Figure 5.1), as OWC was consistently higher than the other N sources. Perhaps the results of the ASNT did not accurately reflect the mineralizable N in the soil-biosolids mixture due to a lack of precision of the experimental method. This information suggested that when using the ASNT as done in this research, testing the N sources alone may provide valuable information.

To evaluate the effectiveness of the ASNT-N recovery, inorganic AN was used as a control. The ASNT recovers NH_4 from the NaOH- digested amino sugar organic N fraction. Therefore, it was hypothesized that the ASNT would recover 100% of the added NH_4 from AN. However, the recovery of NH_4 was only 40.5%. This result suggested that the ASNT did not capture all of the NH_4 volatilized as NH_3. As the jar lids were removed, a strong odor of gaseous NH_3 was detected and suggested loss of a substantial amount of NH_3 since it was not trapped by the boric acid. It is possible that the boric acid trap had become saturated with NH_3 and was no longer able to trap any additional NH_3 due to the large amount of NH_4 added via AN (173.4 mg). Stoichiometric calculations supported this hypothesis. This was only an issue with ammonium nitrate as the NH_3 concentrations of the biosolids were not high

enough to saturate the boric acid. However, as biosolids vary widely in NH_3 concentrations, the trapping capacity of the boric acid should be evaluated prior to running this test with biosolids. Khan (2001) found 97 to 102% recoveries using ^{15}N-labeled $(NH_4)_2SO_4$ or glucosamine added to soil, but did not exceed 6.5% with labeled glycine and was undetectable with labeled NO_3 or NO_2. If saturation of the boric acid indicator solution did occur, a higher volume or concentration of boric acid might be used or a smaller amount of N source tested.

5.2 Correlation of ASNT to Anaerobic Incubation

A linear regression of the treatment means of both the ASNT vs. anaerobic incubation total inorganic N is illustrated in Figure 5.3. The regression equation is $y = 46.79 + 0.69x$, with an $R^2 = 0.46$, and the model is statistically significant at $\alpha = 0.05$. As displayed by this information, the ASNT-N values explained 46% of the variability in the anaerobic N values, and the remaining 54% was unexplained. The anaerobic incubation is generally considered to be a satisfactory laboratory test to evaluate the relative differences in potentially mineralizable N (Bundy and Meisinger, 1994). To the author's knowledge, the ASNT has not been used to predict N mineralization of biosolids. The results of this correlation suggested that other factors are contributing to the variability between the two tests other than the ASNT values alone. This result was not surprising given the substantial differences between the experimental methods of each test. The anaerobic incubation is a biological incubation measuring total PAN and the ASNT is a chemical procedure measuring inherent NH_4 and NH_4 from the amino sugar N organic N fraction. Based on these results, using the

ASNT to predict anaerobic N test values would not be recommended due to the unexplained variability that existed between the two variables.

5.3 Conclusions

Across all soils and RYE rates, OWC yielded the greatest amount of ASNT-N of the four N sources tested. Ammonium nitrate and CP were generally coincident across all soils, and R+ ASNT-N was generally lower than all other biosolids. There were differences in ASNT-N magnitude between the coastal plain (Noboco and Norfolk) and the piedmont (Vance and Wedowee) soils. The ASNT-N response to RYE rate was generally similar across all soils, but there were some variability which suggested that soil type affected ASNT-N values. Presuming the ASNT is a precise test of N mineralization from biosolids, it appeared as if the NAC used for OWC underestimated PAN from those biosolids. The NAC for CP appeared satisfactory, and the NAC for R+ slightly underestimated PAN from those biosolids. Performing the ASNT on the N sources only (no soil) also resulted in ASNT-N values from OWC that were greater than the other biosolids, despite the lower total and inorganic N of OWC vs. that of CP. Less than half of the AN added was recovered which suggested that the experimental method used needed to be improved before testing the N sources only. When correlating the ASNT and the anaerobic incubation, the ASNT explained 45% of the variability in the anaerobic test values.

Table 5.1. Analysis of variance from an amino sugar nitrogen test of four N sources (3 biosolids, NH_4NO_3) applied at four rates on four soils. Average ASNT-N value of the control samples was subtracted from the amended samples.

Effect	Numerator DF	Denominator DF	F Value	Pr > F
RYE rate	3	192	58.85	<0.0001
N source	3	192	281.81	<0.0001
Soil	3	192	19.04	<0.0001
RYE rate*N source	9	192	18.49	<0.0001
RYE rate*Soil	9	192	1.32	0.2271
N source*Soil	9	192	1.83	0.0644
RYE rate*N source*Soil	27	192	0.75	0.8045

Table 5.2. Analysis of variance from an amino sugar nitrogen test of four N sources (3 biosolids, NH_4NO_3) applied at four rates; grouped by soil. Average ASNT-N value of the control samples was subtracted from the amended samples.

Effect	Numerator DF	Denominator DF	F Value	Pr > F
		Noboco loamy sand		
RYE rate	3	48	8.65	0.0001
N source	3	48	30.30	<0.0001
RYE rate*N source	9	48	2.18	0.0402
		Norfolk loamy sand		
RYE rate	3	48	55.83	<0.0001
N source	3	48	237.27	<0.0001
RYE rate*N source	9	48	16.54	<0.0001
		Wedowee sandy loam		
RYE rate	3	48	12.44	<0.0001
N source	3	48	104.31	<0.0001
RYE rate*N source	9	48	6.77	<0.0001
		Vance sandy clay loam		
RYE rate	3	48	28.42	<0.0001
N source	3	48	115.53	<0.0001
RYE rate*N source	9	48	10.46	<0.0001

Table 5.3. Regression equations, model significance, and R^2 values from an Amino-Sugar Nitrogen Test. Independent variable was RYE rate and dependent variable was Amino-Sugar Nitrogen Content (mg kg^{-1}).

Parameter	n	Equation†	Model P	R^2
		Noboco loamy sand		
OWASA cake	16	$y = 3.08 + 66.53x$	0.0001	0.67
Cary pellet	16	$y = 1.04 + 11.51x$	0.2360	0.10
NH_4NO_3	16	$y = -21.58 + 39.12x$	0.0226	0.32
Raleigh plus	16	$y = -7.80 + 6.84x$	0.4420	0.04
		Norfolk loamy sand		
OWASA cake	16	$y = 53.24 - 30.37x + 41.70x^2$	< 0.0001	0.91
Cary pellet	16	$y = -3.40 + 16.91x$	< 0.0001	0.74
NH_4NO_3	16	$y = -4.19 + 26.68x$	0.0008	0.56
Raleigh plus	16	$y = -5.37 + 4.28x$	0.1328	0.15
		Vance sandy clay loam		
OWASA cake	16	$y = -2.79 + 53.97x$	< 0.0001	0.72
Cary pellet	16	$y = -1.10 + 14.28x$	0.0001	0.66
NH_4NO_3	16	$y = -7.70 + 22.09x$	< 0.0001	0.74
Raleigh plus	16	$y = 3.05 - 5.62x$	0.1699	0.13
		Wedowee sandy loam		
OWASA cake	16	$y = -7.60 + 61.67x$	< 0.0001	0.79
Cary pellet	16	$y = -11.55 + 10.92x$	0.0798	0.20
NH_4NO_3	16	$y = -12.73 + 12.52x$	0.0364	0.28
Raleigh plus	16	$y = -11.02 - 5.50x$	0.3874	0.05

† x = Realistic Yield Expectation Interval; y = ASNT-N (mg kg^{-1})

Table 5.4. ASNT nitrogen from a Noboco loamy sand amended with four N sources: simple effects of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

	Nitrogen Source								
RYE rate	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- mg kg^{-1} ----------								LSD
0.5	3.7 AB†	a§	5.9 AB	a	34.1 A	c	-5.4B	a	37.9
1.0	1.1 B	a	17.9 B	a	76.0 A	bc	-0.2B	a	42.4
1.5	53.0 AB	a	10.4 B	a	96.6A	ab	3.9 B	a	72.7
2.0	51.5 B	a	27.6 B	a	138.2 A	a	4.7 B	a	60.4
LSD	69.1		43.9		60.7		42.2		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 5.5. ASNT nitrogen from a Norfolk loamy sand amended with four N sources: simple effects of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

	Nitrogen Source								
RYE rate	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- mg kg^{-1} ----------								LSD
0.5	10.7 B†	b§	3.6 BC	b	45.9 A	c	-3.4C	a	9.3
1.0	16.0 B	b	13.6 B	b	72.4 A	bc	-3.2 B	a	26.7
1.5	44.1 B	a	26.2 C	a	93.7 A	b	5.6D	a	17.8
2.0	45.9 B	a	27.6 BC	a	161.9 A	a	0.9 C	a	26.8
LSD	28.0		11.5		28.1		11.4		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the least squares means test ($p \leq 0.05$) subtracted from the amended soil concentration.

Table 5.6. ASNT nitrogen from a Vance sandy clay loam amended with four N sources: simple effects of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

	Nitrogen Source								
RYE rate	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- mg kg^{-1} ----------								LSD
0.5	0.6 B†	c§	7.3AB	b	21.3 A	c	4.1 AB	a	18.2
1.0	17.2 B	b	14.8 B	b	63.7 A	b	-6.7 B	a	24.7
1.5	28.2 B	ab	13.4 C	b	61.6 A	b	-8.8 D	a	14.4
2.0	33.7 B	a	31.5 B	a	112.0A	a	-4.6 C	a	27.3
LSD	15.8		9.4		35.7		16.8		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the least squares means test ($p \leq 0.05$)

Table 5.7. ASNT nitrogen from a Wedowee sandy loam amended with four N sources: simple effects of N source by RYE rate and RYE rate by N source. Control N concentrations were subtracted from the amended soil concentration.

	Nitrogen Source								
RYE rate	NH_4NO_3		Cary pellet		OWASA cake		Raleigh Plus		
	---------- mg kg^{-1} ----------								LSD
0.5	-8.1 B†	a§	-8.5 B	a	14.3 A	c	-11.0B	a	21.5
1.0	1.3 B	a	0.9 B	a	65.6A	b	-20.5 B	a	34.6
1.5	8.1 B	a	9.1 B	a	88.6 A	ab	-19.6 B	a	31.0
2.0	10.5 B	a	7.0 B	a	109.5 A	a	-20.5 B	a	32.2
LSD	26.1		27.4		36.7		29.6		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 5.7. Amino Sugar nitrogen test values from 1.0 g (dry matter) of four N sources alone; not mixed with soil. Cary pellet was tested in the pelleted form and pulverized to a powder to investigate any differences attributable to the particle size of the biosolids. Error bars represent the standard error of the individual means.

N Source	ASNT-N (mg kg^{-1})	Standard Error
NH_4NO_3	68,878	402
OWASA cake	9,790	151
Cary pellet (powder)	4,600	31
Cary pellet	4,302	38
Raleigh plus	263	8

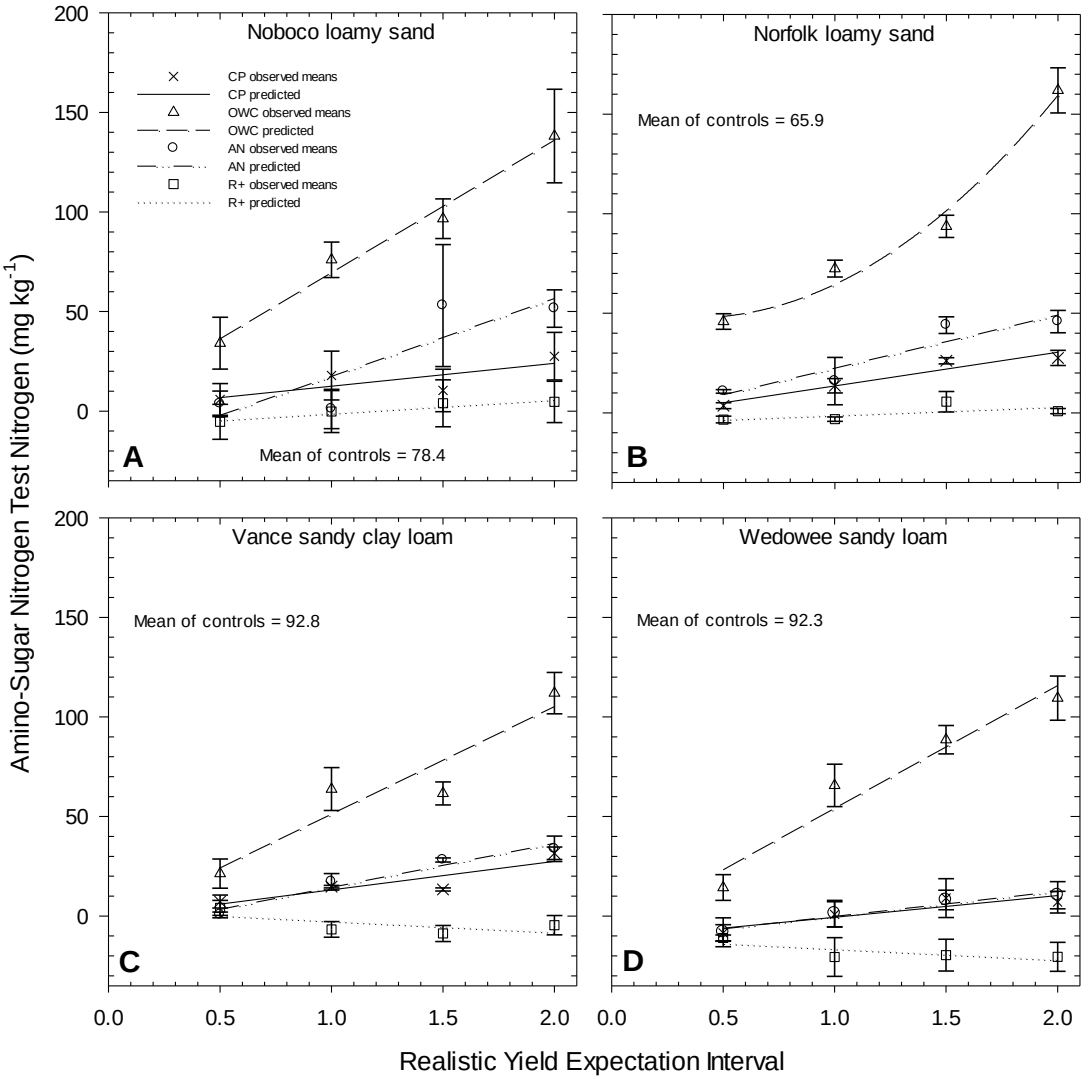

Figure 5.1. Amino-Sugar Nitrogen Content vs. Realistic Yield Expectation Interval. Regression analysis of the simple effects of RYE rate for each N source in each soil type studied during an ASNT. Nitrogen sources included three biosolids and NH_4NO_3, each mixed at five rates with two representative coastal plain soils: Noboco loamy sand (A) and Norfolk loamy sand (B), and two representative piedmont soils: Vance sandy clay loam (C) and Wedowee sandy loam (D). Nitrogen rates were determined as 0, 0.5 X, 1.0 X, 1.5 X, and 2.0 X the North Carolina Realistic Yield Expectation Database (North Carolina Nutrient Management Workgroup) N rate for Fescue on a Wedowee coarse sandy loam soil. The 1.0 RYE rates were 144.5 kg ha^{-1} for NH_4NO_3 and 127 kg ha^{-1} for the three biosolids; the differences were due to a calculation error, but were grouped by RYE interval in the above figure. Error bars represent the standard error of the individual means. Average ASNT-N content of the control samples was subtracted from the amended samples.

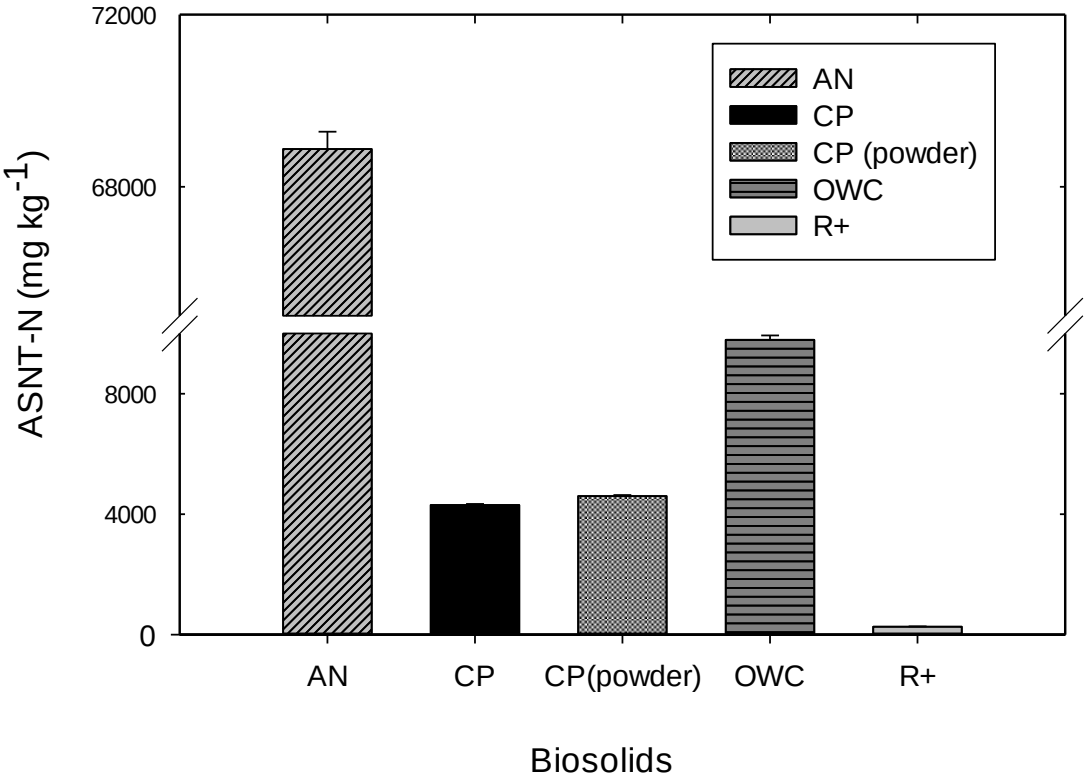

Figure 5.2. Amino sugar nitrogen content of four N sources alone; not mixed with soil. Cary pellet was tested in the pelleted form and pulverized to a powder to investigate any differences attributable to the particle size of the biosolids. Error bars represent the standard error of the individual means. CP, Cary pellets, OWC, OWASA cake, R+, Raleigh Plus, AN, NH4NO3.

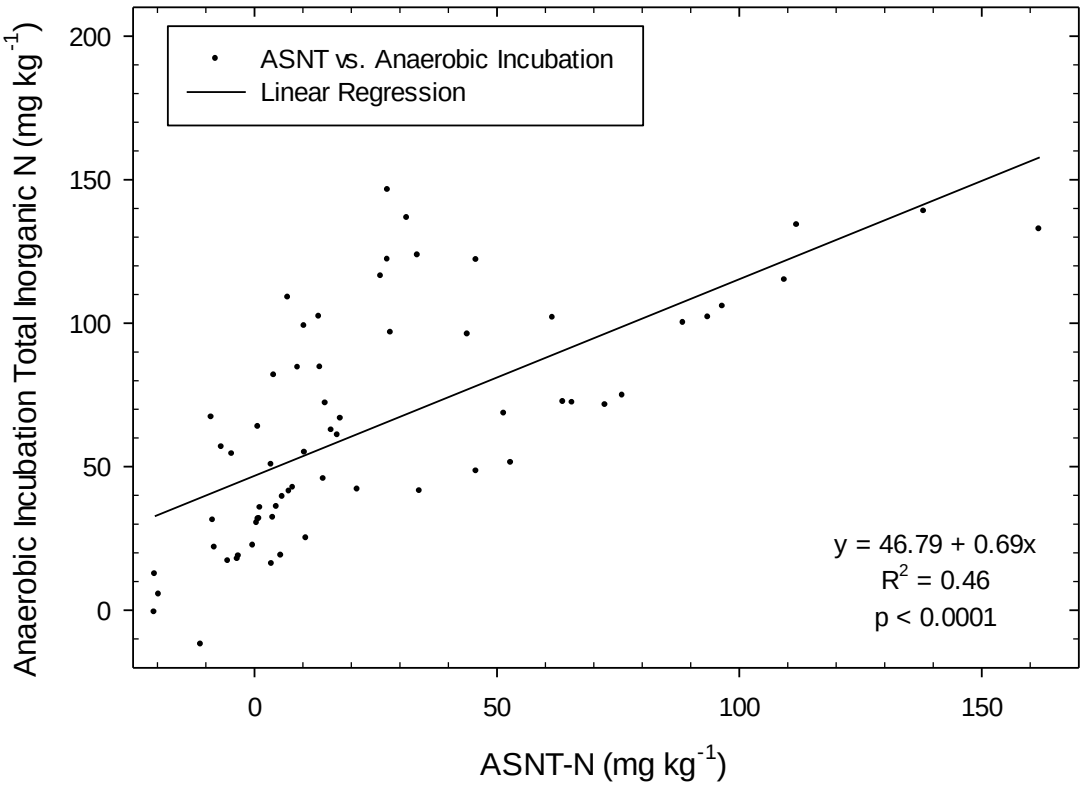

Figure 5.3. Linear regression of the test values from the ASNT vs. the anaerobic incubation total inorganic N.

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CHAPTER 6: GROWTH RESPONSE FIELD TRIAL

6.0 Response of Tall Fescue Growth to Biosolids Application

6.0.0 Analysis of Variance

Within each block, the yield of the four control plots was subtracted from that of the amended plots so that the effect of the amendments alone could be identified. The analysis of variance of all treatment factors tested is illustrated in Table 6.1. The interaction of N source and PAN application rate was not statistically significant. Therefore, main effects of both N source and PAN rate were examined. The main effect of PAN rate was highly statistically significant. The main effect of N source was not significant at the 0.05 level and had a p-value of 0.0604. However, for the purposes of this research, it was considered to be statistically significant (Snedecor and Cochran, 1989).

6.0.1 Regression Analysis: Main Effect of PAN Rate

Results of a regression analysis of the response of tall fescue yield to application of Cary Pellet and NH_4NO_3 are illustrated in Figure 6.1. The regression line represents the main effect of PAN rate; that is, the response to PAN rate did not depend on the N source. The average yield of the control plots was 2,300 kg ha^{-1}. The best-fit model was linear and the equation across all application rates was Yield response = 1137.73 + 18.50 PAN. The model was statistically significant and had an $R^2 = 0.58$. The yield response at the application rates of 72, 146, 217, and 289 kg PAN ha^{-1} were 2072, 4303, 5369, and 6171 kg ha^{-1}, respectively. Mean separation showed that the yield response from an application rate of 72 kg PAN ha^{-1} was different than all other rates. Also, an application rate of 144.5 kg

PAN ha^{-1} was not different from 217 kg PAN ha^{-1}, but was different from a rate of 289 kg PAN ha^{-1}. The rate of 217 was not different from rates of 144.5 or 289 kg PAN ha^{-1}. Interestingly, a peak yield was not reached with the rates applied in this trial when plotted as the main effect of PAN rate. It is generally expected to reach a point where there is no longer a response to N fertilization. It was expected to reach that critical point somewhere close to the highest rate used in this research, but such did not end up being the case. Generally, forage dry matter responses to N fertilizer fall within the range 10 to 50 kg dry matter per kg of N. Moreover, in temperate regions, fescue yields will continue to increase up to N rates of 370 to 448 kg ha^{-1} annually (Barnes et al., 2003). Given that range, it was not surprising to see the linear response of the main effect of PAN rate since the highest application rate was only 289 kg N ha^{-1}. Also, the quadratic response of the simple effect of CP (Fig. 6.2) suggested that more PAN was applied via CP than the NAC estimated. However, some nutrient(s) other than N may have limited yield response to AN, and CP may have supplied such nutrient(s).

6.0.2 Main Effect of N Source

The main effect of N source is also shown in Fig. 6.1. Averaged across all rates, CP and AN yielded 4,900 and 4,100 kg ha^{-1}, respectively. Mean separation showed that the two main effect values are different, using an LSD value of 814 kg ha^{-1}. The fact that CP produced a higher yield than AN was interesting, because it suggested that the pellets provided better nutritional value to the tall fescue than AN. This may have been due to the fact that the soil was deficient in one or several nutrients that were a part of the biosolids.

This deficiency may have inhibited N uptake and utilization in plots that were amended with AN. The CP product contained substantial amounts of P (34,900 mg kg^{-1} ; 78,176 kg ha^{-1}), K (6,810 mg kg^{-1} ; 15,254 kg ha^{-1}), Ca (16,100 mg kg^{-1} ; 36,064 kg ha^{-1}), SO_4 (24,300 mg kg^{-1} ; 54,432 kg ha^{-1}), and C (417,000 mg kg^{-1} ; 934,090 kg ha^{-1}) (Table 3.1). The soil test recommendations by the NCDA&CS for the field location recommended the addition of 134 to 224 kg N ha^{-1}, 56 to 78 kg K_2O ha^{-1}, and 17 to 22 kg S ha^{-1}, and those additions were not made for this research. Therefore, addition of these nutrients into the soil may have resulted in greater N use by the forage.

Another hypothesis could have been an inaccuracy in the first-year N availability coefficient of 0.3 provided by the NCDA&CS (McGinnis et al., 2011) for broadcast application of the biosolids. If the increase in yield response of the CP over AN was due to a greater supply of N, than this data suggested that the current coefficient underestimated the amount of N mineralized from the CP, since AN was assumed to be 100% plant available and both amendments were applied at the same estimated PAN rate. Part of the problem may have been attributed to the fact that the coefficient that was used for CP was classified as "Other." That is, there was no specific category for the heat-treated and pelleted product. Since the "Other" waste source classification is meant to be a catch-all for municipal waste products not otherwise listed, it was not surprising that the coefficient did not accurately predict the percentage of N mineralization in the first year. It is likely that a specific classification for the CP would be beneficial.

One additional hypothesis for the difference between the two N sources is that mineralization of CP might have been better synchronized with forage N uptake. Ninety five

percent of the total N in CP was organic (Fig. 3.1). As a result, the organic N had to be mineralized by soil microbes to inorganic N over time in order to become plant available. Ammonium nitrate, on the other hand, is 100% inorganic and the N was available to plants upon dissolution of the prills. Therefore, AN was more subject to N loss via leaching, volatilization, and possibly denitrification before the fescue could utilize the N. Distinguishing the feasibility of these hypotheses is discussed further as other data from the field trial were analyzed.

6.0.3 Simple Effects

Although not warranted by the analysis of variance, the simple effects of both CP and AN are illustrated in Figure 6.2. The LSD means separation is shown in Table 6.2. A quadratic model was the best fit for the simple effect of the CP: Yield = -1605.14 + (62.41 PAN) + (-0.12 PAN^2). The model was statistically significant and had an $R^2 = 0.76$. Mean separation for the simple effect of rate for the CP showed that the application rate of 72 kg PAN ha^{-1} was different from all other rates, and that all other rates were not different from each other at the 0.05 alpha levels (Table 6.2). The yield responses for the 72, 145, 217, and 289 kg ha^{-1} application rates were 1640, 2065, 1737, and 2550 kg PAN ha^{-1}, respectively.

A linear model was the best fit for AN (Fig. 6.2). The regression equation was Yield response = 693.79 + 18.69 PAN. The model was statistically significant, and had an $R^2 = 0.57$. Mean separation shows that there were no differences in yield response between application rates (Table 6.2). The mean yield responses for the 72, 145, 217, and 289 kg

PAN ha^{-1} application rates were 1927, 3595, 4701, and 6060 kg ha^{-1}, respectively. As described by the lack of significance of the N source*PAN rate interaction, there were no differences between CP and AN at any single rate (Table 6.2). To further illustrate the inability to declare the CP and AN regression lines different, plots of each regression line with the 95% confidence interval were shown (Figs. 6.3a,b). The confidence intervals overlap throughout the range of application rates. This information, along with the mean separation and the ANOVA, justify interpretation of the main effects of PAN and N source (Fig 6.1).

6.1 Percent N of Forage Biomass

6.1.0 Analysis of Variance

The average percent N of the four control plots was subtracted from the amended plots so that the effect of the amendments alone could be identified. The analysis of variance of all treatment factors tested is illustrated in Table 6.1. The interaction of N source and PAN application rate was not statistically significant. Therefore, main effects of both N source and PAN rate were examined. The main effect of PAN was highly statistically significant. The main effect of N source was also statistically significant.

6.1.1 Regression Analysis: Main Effect of PAN Rate

Results of a regression analysis of the response of tall fescue N concentration to application of CP and AN are illustrated in Figure 6.4. The regression line represents the main effect of PAN rate; that is, the response to PAN rate did not depend on the N source.

The average N concentration of the control plots was 1.15%, and was within the typical range of 1-4% (Barnes et al., 2003). The best-fit model was linear and the equation across all application rates was Percent N response = -0.536 + 0.003 PAN. The model was statistically significant and had an $R^2 = 0.28$. The mean N concentration response at application rates of 72, 145, 217, and 289 kg PAN ha^{-1} resulted in mean N concentration response values of -0.07, 0.04, and 0.39%, respectively. Mean separation showed that the percent N response from an application rate of 72 kg ha^{-1} was different from PAN rates of 217 and 289 kg PAN ha^{-1}, respectively. A PAN rate of 145 kg PAN ha^{-1} was different from a rate of 289 kg PAN ha^{-1} only. The highest rate of 289 kg PAN ha^{-1} was different from all others.

The mean N concentration of the control plots was greater than means of the 72 and 145 kg PAN ha^{-1} rates. This meant that the N concentration initially decreased as CP and AN were added to the forage. According to Greenwood et al. (1991), the concentration of N declines as plants grow, even when there is a sufficient supply of N and other nutrients (Greenwood et al., 1991). This is due to an increase in the proportion of structural and storage tissues which contain small amounts of N. Greenwood et al. (1991) also stated that the growth rate declines with decline in percent N. The increase in structural and storage tissues could explain why the N concentration decreased in the lower rates applied in this research. However, contrary to Greenwood et al. (1991), the decrease in % N did not continue in this research. The 289 kg PAN ha^{-1} application rate resulted in an N concentration higher than the control samples, and growth rate did not decrease throughout the rates applied (Fig. 6.1). It is possible that the forage could have approached luxury

consumption due to the N concentration increasing, but there is no definitive evidence of that in this data because the yield continued to increase across all PAN rates.

The N concentrations of the forage harvested in this research were lower those than found by Balasko (1977) in the middle-latitude regions of the USA. The forage in that research was harvested in December and January, and had approximately equal yields as those found in this research from N application rates of only 60 kg ha^{-1} via $NaNO_3$ or AN in spring and after each of three summer harvests. The N concentrations of the control plots were 1.34 and 1.21%, similar to the 1.15% in this research. Plots that received 60 kg ha^{-1} of PAN via NH_4NO_3 yielded N concentrations of 1.49 and 1.38% for December and January, respectively. Those concentrations are similar to the concentrations found in this research at application rates of 289 kg PAN ha^{-1}, almost 5 times higher than the rates used in Balasko (1977).

6.1.2 Main Effect of N Source

The main effect of N source is also shown in Fig. 6.4. Averaged across all rates, CP had a percent N yield response of 0.14 percentage points and AN had a yield response of -0.11 percentage points. Mean separation shows that the two main effect values were different. As hypothesized with the yield data, it is possible that: CP supplied more N to the forage, N release was better synchronized with forage uptake, and/or nutrients other than N in the CP facilitated better N assimilation.

6.1.3 Simple Effects

Although not warranted by the analysis of variance, the simple effects of both CP and AN are illustrated in Figure 6.5. The LSD mean separation is shown in Table 6.3. A linear model was the best for the both N sources. The regression equation for the best fit line for CP was % N response = -0.429 + 0.0003 PAN. The model was statistically significant and it had an $R^2 = 0.29$. Mean separation for the simple effect of rate for CP show that the only difference was between the lowest and highest rates (72 and 289 kg ha^{-1}) at the $p = 0.05$ level (Table 6.3). The LSD values for the 72, 145, 217, and 289 kg PAN ha^{-1} application rates were 0.41, 0.49, 0.47, and 0.69 percentage points, respectively.

A linear model was also the best fit for AN (Fig. 6.5). The regression equation was % N response = -0.643 + 0.003x. The model was statistically significant and had an $R^2 = 0.32$. The LSD mean separation showed that the 72 kg PAN ha^{-1} rate was different from the 217 and 289 kg PAN ha^{-1} rates (Table 6.3). The highest rate of 289 kg PAN ha^{-1} was different from all other rates. As described by the lack of significance of the N source*PAN rate interaction (Table 6.1), there were no differences between CP and AN at any single rate. As with the simple effect yield plots shown in Fig 6.3a,b, the confidence intervals overlapped throughout the predicted range, further justifying the interpretation of the main effect of PAN and N source.

6.2 N Uptake of Forage Biomass

6.2.0 Analysis of Variance

Within each block, the average N uptake of the four control plots was subtracted from that of the amended plots so that the effect of the amendments alone could be identified. The control mean average N uptake was 28.4 kg ha^{-1}. The analysis of variance for all treatment factors tested is illustrated in Table 6.1. The interaction of N source and PAN application rate was not statistically significant. Therefore, main effects of both N source and PAN rate were examined. The main effect of PAN rate was highly statistically significant. The main effect of N source was also statistically significant.

6.2.1 Regression Analysis: Main Effect of PAN Rate

Results of a regression analysis of the response of tall fescue N uptake to application of CP and AN are illustrated in Figure 6.6. The regression line represents the main effect of PAN rate; that is, the response to PAN rate did not depend on the N source. The best fit model was linear and the equation across all application rates was N uptake response = -20.07 + 0.41 PAN. The model was statistically significant and had an $R^2 = 0.55$. The N uptake responses at the 72, 145, 217, and 289 kg PAN ha^{-1} rates were 8.5, 44.3, 62.3, and 101.4 kg N ha^{-1}, respectively. Mean separation shows that the N uptake response from a PAN rate of 72 kg PAN ha^{-1} was different from a PAN rate of 217 and 289 kg PAN ha^{-1}. A rate of 145 kg PAN ha^{-1} was different from a rate of 289 kg PAN ha^{-1}. The highest rate of 289 kg PAN ha^{-1} was different from all others.

6.2.2 Main Effect of N Source

The main effect of N source is also shown in Fig. 6.6. Averaged across all PAN rates, CP had an N uptake response of 67.8 kg ha^{-1} and AN had an N uptake response of 40.5 kg N ha^{-1}. Mean separation showed that the two main effect values were different. As with both forage biomass yield and N concentration responses, plots treated with CP had greater N uptake response than AN. As hypothesized with the yield and percent N data, it is possible that CP supplied more N to the forage; N release (mineralization) was better synchronized with N uptake, or nutrients other than N in the CP facilitated N uptake. If CP supplied more N to the forage than AN, than the NAC used for CP could be underestimating the amount of CP PAN.

Cogger et al. (1999) found values of N uptake in their control plots to be 60 to 112 kg ha^{-1} in 1993 and 1994 in western Washington in a study to evaluate N recovery from Class A biosolids applied to two cool-season perennial grasses and to make practical recommendations for biosolids application rates for those grasses. Their values were much higher than the 28.4 kg ha^{-1} found in our control plots, which suggested substantial differences in soil and/or forage properties. They also applied heat-dried and dewatered biosolids at rates ranging from 100 to 300 kg total N ha^{-1} twice a year, slightly lower than the total N rates used for CP in this research (213 to 854 kg total N ha^{-1}). The N uptake values from those products ranged from 39 to 335 kg ha^{-1} for the heat-dried biosolids and from 27 to 325 kg ha^{-1} for the dewatered biosolids when the average value of the controls for both years were subtracted from the N uptake values, as done in this research. Differences could be attributed to differing harvest schedules (Cogger et al. (1997) harvested between three to six

times at the early boot growth stage), differing soil characteristics, climate, and biosolids treatment processes. Cogger et al. (1997) used both Tall fescue and perennial ryegrass in their field trials, but all comparisons here were related to tall fescue.

6.2.3 Simple Effects

Although not warranted by the analysis of variance, the simple effects of both CP and AN are illustrated in Figure 6.7. The LSD mean separation is shown in Table 6.4. A linear model was the best for both N sources. The regression equation for the best fit line for CP was N uptake response = -13.43 + 0.45 PAN. The model was statistically significant and it had an $R^2 = 0.58$. Mean separation for the simple effect of rate for CP show that the only difference was between the lowest and highest rates (72 and 289 kg ha^{-1}) at the 0.05 level (Table 6.4).

A linear model was also the best fit for AN (Fig. 6.7). The regression equation was N uptake response = -26.71 + 0.37 PAN. The model was statistically significant and had an $R^2 = 0.68$. The LSD mean separation showed that the 72 kg PAN ha^{-1} rate was different from the 217 and 289 kg PAN ha^{-1} rates (Table 6.4). The highest rate of 289 kg PAN ha^{-1} was different from all other rates. As described by the lack of a significant N source*PAN rate interaction (Table 6.1), there were no differences between CP and AN at any single rate. As with the simple effect yield plots shown in Fig 6.3a, b, the confidence intervals overlapped throughout the predicted range, further justifying the interpretation of the main effect of PAN and N source.

6.3 Apparent N Recovery of Forage Biomass

6.3.0 Analysis of Variance

The analysis of variance for the ANR is shown in Table 6.1. The interaction of N source and PAN application rate was not statistically significant. Therefore, main effects of both N source and PAN rate were examined. The main effect of PAN rate was highly statistically significant. The main effect of N source was also statistically significant.

6.3.1 Regression Analysis: Main Effect of PAN Rate

Results of a regression analysis of tall fescue apparent N recovery versus PAN rate averaged over N source are illustrated in Figure 6.8. The regression line represents the main effect of PAN rate; that is, the response to PAN rate did not depend on N source The best fit model was linear and the equation across all application rates was ANR = 9.53 + 0.09 PAN. The model was statistically significant and had an $R^2 = 0.15$. The mean ANR's for the 72, 145, 217, and 289 kg PAN ha^{-1} rates were 11.7, 30.7, 28.8, and 35.1%. Mean separation showed that the only difference in ANR was between the lowest PAN rate of 72 kg PAN ha^{-1} and 289 kg PAN ha^{-1}. It was hypothesized that ANR would decrease as higher rates of fertilizer were added, but these results contradicted that hypothesis. This may have been due to the development of root system which allowed for better uptake of the available N. Other possibilities include soil moisture variations among the plots or slight N contamination from hairy vetch found in some plots that was not accounted for by the control plots. Whitehead (1995) stated that cool-season forage grasses fertilized with inorganic N will typically have an ANR range from 50 to 80% (Whitehead, 1995). Cogger et al. (2001) postulated that ANR

from organic N sources such as biosolids applied on a total N basis is less than ANR from synthetic fertilizers, because only a portion of the N from the organic material becomes available for plant uptake during the year of application. However, in our work, first year PAN availability coefficients were used in an attempt to apply the same amount of PAN in each N source. In their results, ANR stayed the same across several biosolids application rates and in a few instances, decreased slightly. In the results presented in this study, ANR was the same across three of the four PAN rates, but was slightly higher at the highest rate. Given that yield and N uptake increased linearly with application rate used, the results are not surprising.

6.3.2 Main Effect of N Source

The main effect of N source is also illustrated in Fig. 6.8. Averaged across all PAN rates, CP had an ANR of 34.7% and AN had an ANR of 18.4%. Mean separation showed that the two main effect values were different. As with forage yield, percent N concentration, and N uptake, plots treated with CP had greater values than AN when the main effect of N source was investigated. This information further supported the hypotheses that CP supplied more N to the forage; N release (mineralization) was better synchronized with N uptake, or nutrients other than N in the CP facilitated ANR.

6.3.3 Simple Effects

Although not warranted by the analysis of variance, the simple effects of both CP and AN are shown in Fig. 6.9. The LSD mean separation is shown in Table 6.5. A linear model

was the best for both N sources. The regression equation for the best fit line for CP was ANR = 20.50 + 0.08 PAN. However, the model was not statistically significant and had an $R^2 = 0.13$. The LSD mean separation showed that there were no differences in PAN rates at the 0.05 level (Table 6.5). The LSD values for the 72, 145, 217, and 289 kg ha^{-1} application rates were 32.9, 27.1, 16.3, and 25.6%, respectively.

A linear model was also the best fit for AN (Fig. 6.9). The regression equation was ANR = -1.44 + 0.11 PAN. The model was barely statistically significant at the 0.05 level and had an $R^2 = 0.23$. The LSD mean separation showed that there were no differences between any PAN rates. As described by the lack of significance of the N source*PAN rate interaction (Table 6.1), there were no differences between CP and AN at any single rate. As with the simple effect plots shown in Fig 6.3a, b, the confidence intervals overlapped throughout the predicted range. These circumstances provide further justification for the interpretation of the main effect of PAN and N source.

6.4 Relationship of Field Trial to Laboratory Tests

The results of this field trial were similar to that of the anaerobic incubation previously described (Chapter 4) in terms of the relative difference in magnitudes between AN and CP. For example, in the anaerobic incubation, the anaerobic N content from CP was higher than the anaerobic N content from AN in two of the four soils, and similar in the other two soils. This information suggested that the NAC used for CP underestimated the amount of anaerobic N from CP. As was the case in the results of this field trial, application of CP to a Wedowee soil lead to greater fescue yield, higher N concentration, greater N uptake, and

greater N recovery. Those results also suggested that the NAC for CP underestimated the amount of PAN from those biosolids, although other nutrients were also added as a part of the CP biosolids and could have mitigated other limiting nutrients. However, the results from this field trial are not similar to those of the ASNT. The ASNT-N content for CP and AN was coincident throughout most RYE rates and soils. That information suggested that the NAC for CP was satisfactory, according to the ASNT.

6.5 Conclusions

One year of a field trial testing growth response of tall fescue to application of biosolids compared to AN was completed. Application rates of the biosolids were determined based on N content and a first year N availability coefficient of 0.3 for a broadcast application method, as recommended by the NCDA&CS. Parameters tested included yield, N concentration, N uptake, and apparent N recovery. Compared to AN, plots treated with the biosolids produced greater amounts of each parameter. Three potential explanations were described for the differences between the biosolids and AN, none of which can be definitively proven given the available data. Other nutrients in the biosolids may have led to increased crop growth and/or better N assimilation. The biosolids used contained substantial amounts of K, Ca, P, and S. These other nutrients could have satisfied a deficiency in the soil which allowed for better utilization of soil N by the tall fescue. Moreover, the biosolids contain substantial amounts of C which may have improved structure and overall soil quality.

Most importantly to this work, these results suggested that the N availability coefficient of 0.3 underestimated the actual amount of PAN from the biosolids. If the coefficient was accurate, there likely would have been little difference in the parameters tested. The statistically significant difference in the main effect of N source across all treatment effects tested would likely be agronomically important to growers. For example, plots that received CP grew 800 kg ha^{-1} more fescue than those that received AN (Fig. 6.1). Moreover, plots that received CP had an N concentration of 0.25 more percentage points (Fig. 6.4), 27 kg ha^{-1} more N uptake (Fig. 6.6), and an ANR of 16 more percentage points (Fig. 6.8) than those that received AN, thereby improving the nutritional quality of the forage.

Another possible explanation is that the N release from the biosolids was better synchronized with the tall fescue N requirements. Due to the fact that most of the N in the biosolids was organic, mineralization had to occur over time in order for the N to become plant-available. Ammonium nitrate was considered to be 100% plant available upon dissolution, and was therefore subject to greater N losses. The time-released N from the biosolids may be the reason the tested parameters were consistently greater for the biosolids. Overall, more field research is needed to investigate the discrepancies found between the biosolids and NH_4NO_3.

Table 6.1. Analysis of variance of the four treatment factors analyzed from a field trial that tested growth response of tall fescue to application of NH_4NO_3 (AN) and Cary pellet (CP) biosolids applied at four rates. Within each block, the average value of all four parameters of the four control blocks was subtracted from that of the amended plots so that the effect of the amendments alone could be identified.

Effect	Numerator df	Denominator df	F Value	Pr > F
		Yield		
N source	1	21	3.94	0.0604
PAN rate	3	21	18.69	<0.0001
N source*PAN rate	3	21	0.62	0.6090
		Percent N		
N source	1	21	7.94	0.0103
PAN rate	3	21	10.45	0.0002
N source*PAN rate	3	21	0.03	0.9911
		N uptake		
N source	1	21	7.23	0.0137
PAN rate	3	21	14.48	<0.0001
N source*PAN rate	3	21	0.30	0.8263
		Apparent N Recovery		
N source	1	21	8.19	0.0094
PAN rate	3	21	3.24	0.0427
N source*PAN	3	21	0.23	0.8759

Table 6.2. Yield response of tall fescue to PAN rate and N source (Cary pellets and NH_4NO_3): simple effects of N source by PAN rate and PAN rate by N source. Control yields were subtracted from those with PAN treatments.

	Nitrogen Source				
PAN rate	NH_4NO_3		Cary pellet		
kg N ha^{-1}	---------- mg kg^{-1} ----------				L.S.D
72	1927 A†	a§	2217 A	b	1640
145	3495 A	a	5010 A	a	2065
217	4701 A	a	6038 A	a	1737
289	6060 A	a	6281 A	a	2550
L.S.D	3000		2117		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 6.3. Nitrogen concentration response of tall fescue PAN rate and N source (Cary pellets and NH_4NO_3): simple effects of N source by PAN rate and PAN rate by N source. Control yields were subtracted from those with PAN treatments.

	Nitrogen Source				
PAN rate	NH_4NO_3		Cary pellet		
kg N ha^{-1}	---------- mg kg^{-1} ----------				L.S.D
72	-0.41 A†	c§	-0.21A	b	0.41
145	-0.20 A	bc	0.07 A	ab	0.49
217	-0.10 A	b	0.18 A	ab	0.47
289	0.26 A	a	0.51 A	a	0.69
L.S.D	0.29		0.70		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 6.4. Nitrogen uptake response of tall fescue to PAN rate and N source (Cary pellets and NH_4NO_3): simple effects of N source by PAN rate and PAN rate by N source.

	Nitrogen Source				
PAN rate	NH_4NO_3		Cary pellet		
kg N ha^{-1}	---------- mg kg^{-1} ----------				L.S.D
72	2.9 A†	c§	14.0 A	b	23.8
145	27.4 A	bc	61.2 A	ab	39.1
217	44.8 A	b	79.9 A	ab	35.4
289	86.7 A	a	116.1 A	a	74.0
L.S.D	39.3		71.0		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)
§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

Table 6.5. Apparent N recovery (ANR) of tall fescue to PAN rate and N source (Cary pellets and NH_4NO_3): simple effects of N source by PAN rate and PAN rate by N source.

	Nitrogen Source				
PAN rate	NH_4NO_3		Cary pellet		
kg N ha^{-1}	---------- mg kg^{-1} ----------				L.S.D
72	4.1 A†	a§	19.4 A	a	32.9
145	19.0 A	a	36.9 A	a	27.1
217	20.7 A	a	40.2 A	a	16.3
289	30.0 A	a	42.3 A	a	25.6
L.S.D	33.8		36.4		

† Within rows, means followed by the same capital letter are not significantly different as determined by the LSD test ($p \leq 0.05$)
§ Within columns, means followed by the same lowercase letter are not significantly different as determined by the LSD test ($p \leq 0.05$)

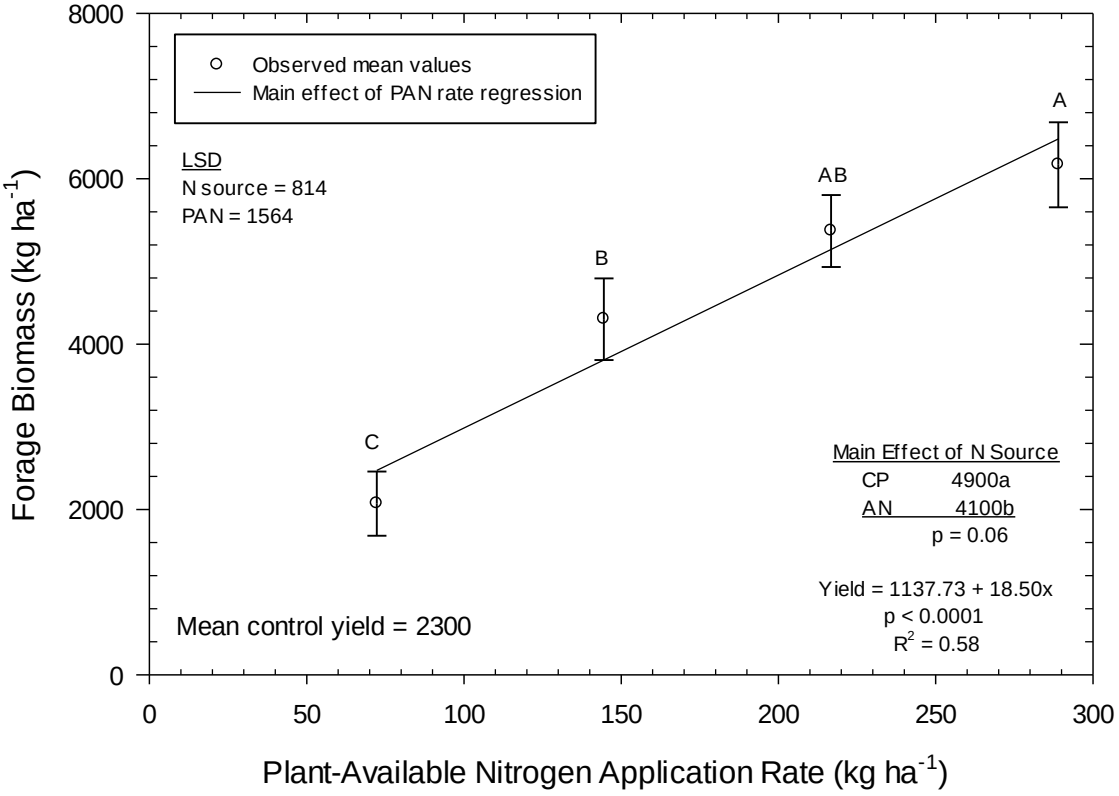

Figure 6.1. Linear regression of the main effect of PAN application rate and the main effect of N source vs. yield response. Amendments applied in the fall of 2010 and harvested in the summer of 2011. Average yield of the control plots were subtracted from the amended plots. Error bars represent the standard error of the individual means. Main effect means followed by the same capital letter (N rate) or lowercase letter (N source) are not significantly different by Fisher's Protected LSD, $\alpha = 0.05$.

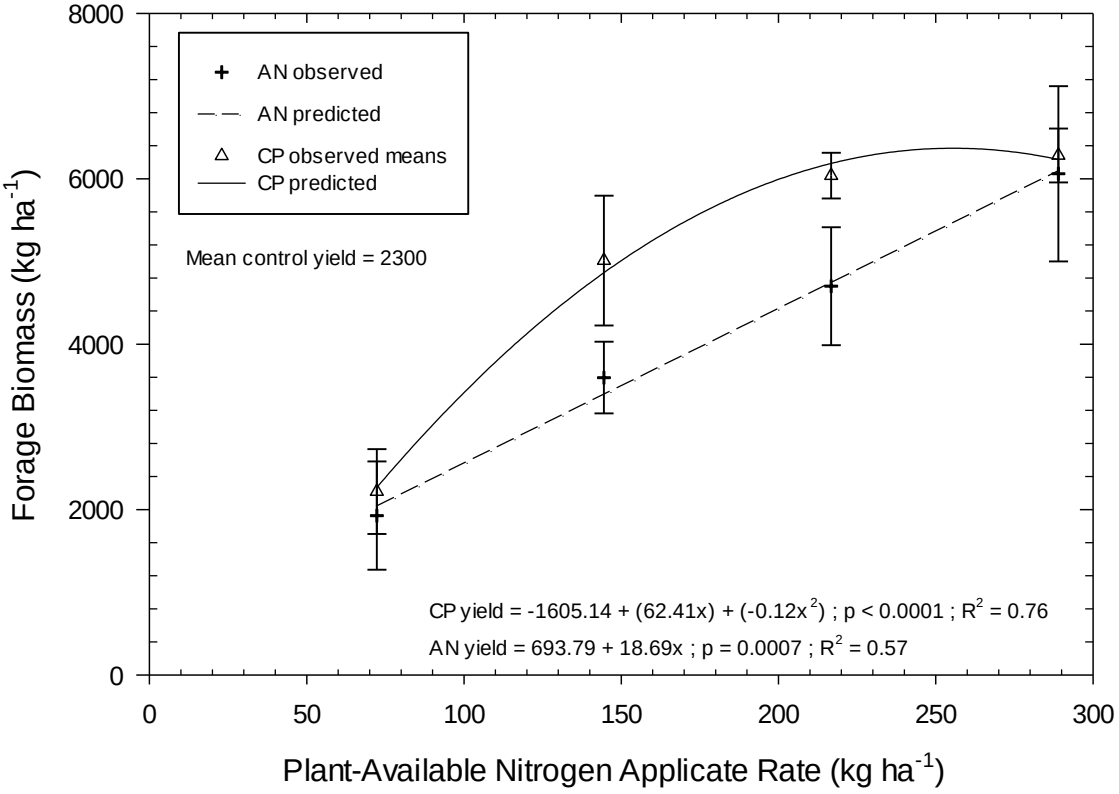

Figure 6.2. Linear regression of the simples effects of PAN application rate of Cary pellet (CP) and NH_4NO_3 (AN) vs. yield response. Amendments applied in the fall of 2010 and harvested in the summer of 2011. Average yield of the control plots were subtracted from the amended plots. Error bars represent the standard error of the individual means.

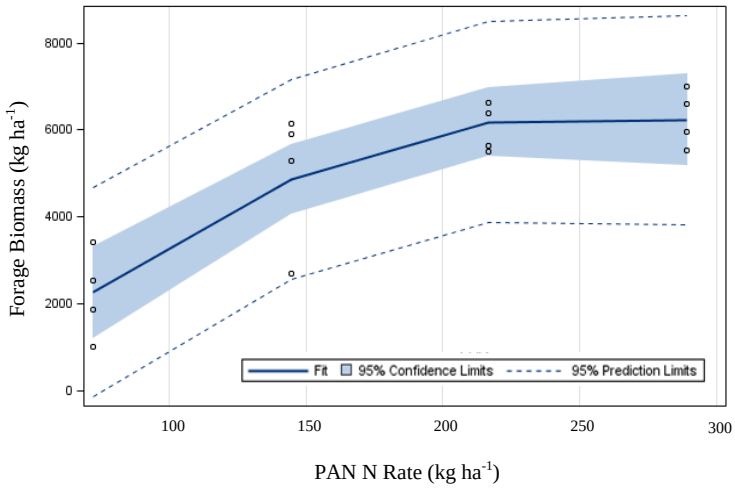

Figure 6.3a. Quadratic regression of the main effect of PAN application rate vs. yield response with 95% confidence limits. Amendments applied in the fall of 2011 and harvested in the summer of 2011. Average yield of the control plots were subtracted from the amended plots. Error bars represent the standard error of the individual means.

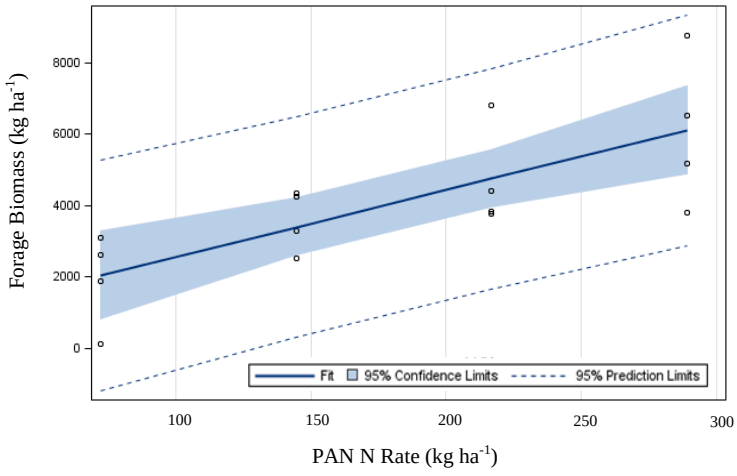

Figure 6.3b. Linear regression of the main effect of PAN application rate vs. yield response with 95% confidence limits. Amendments applied in the fall of 2010 and harvested in the summer of 2011. Average yield of the control plots were subtracted from the amended plots. Error bars represent the standard error of the individual means.

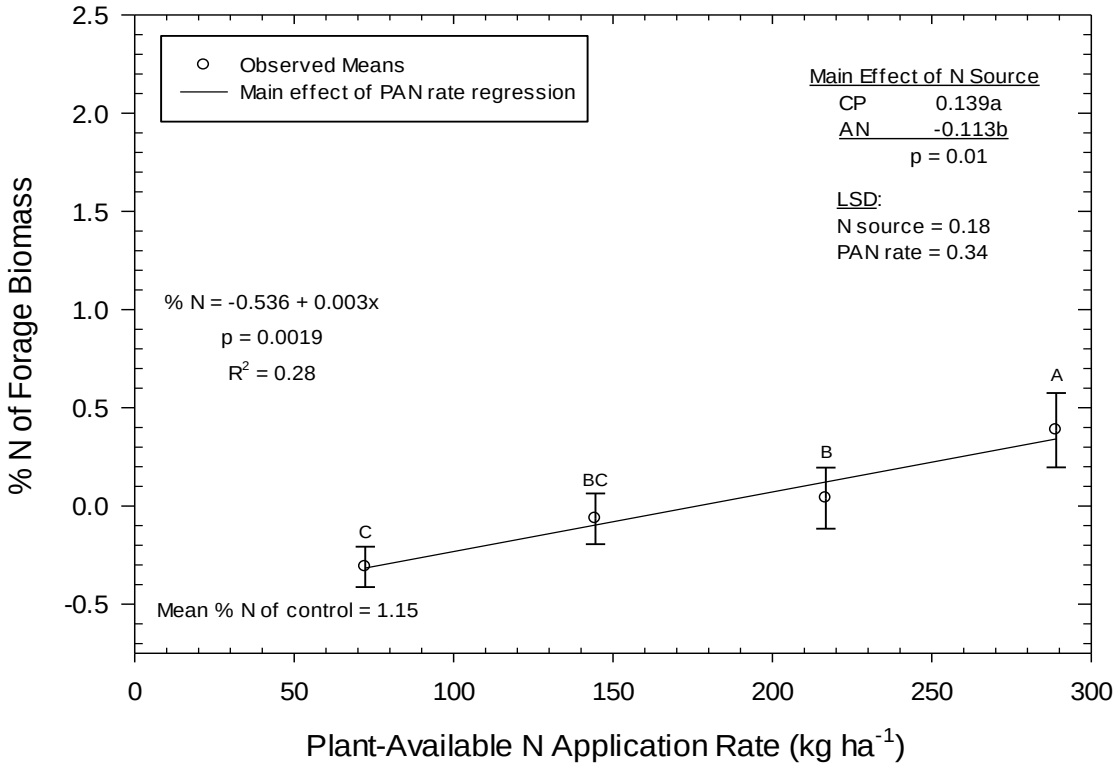

Figure 6.4. Linear regression of the main effect of PAN application rate and the main effect of N source vs. N concentration response. Amendments applied in the fall of 2010 and harvested in the summer of 2011. Average N concentration of the control plots were subtracted from the amended plots. Error bars represent the standard error of the individual means. Main effect means followed by the same capital letter (N rate) or lowercase letter (N source) are not significantly different by Fisher's Protected LSD, $\alpha = 0.05$.

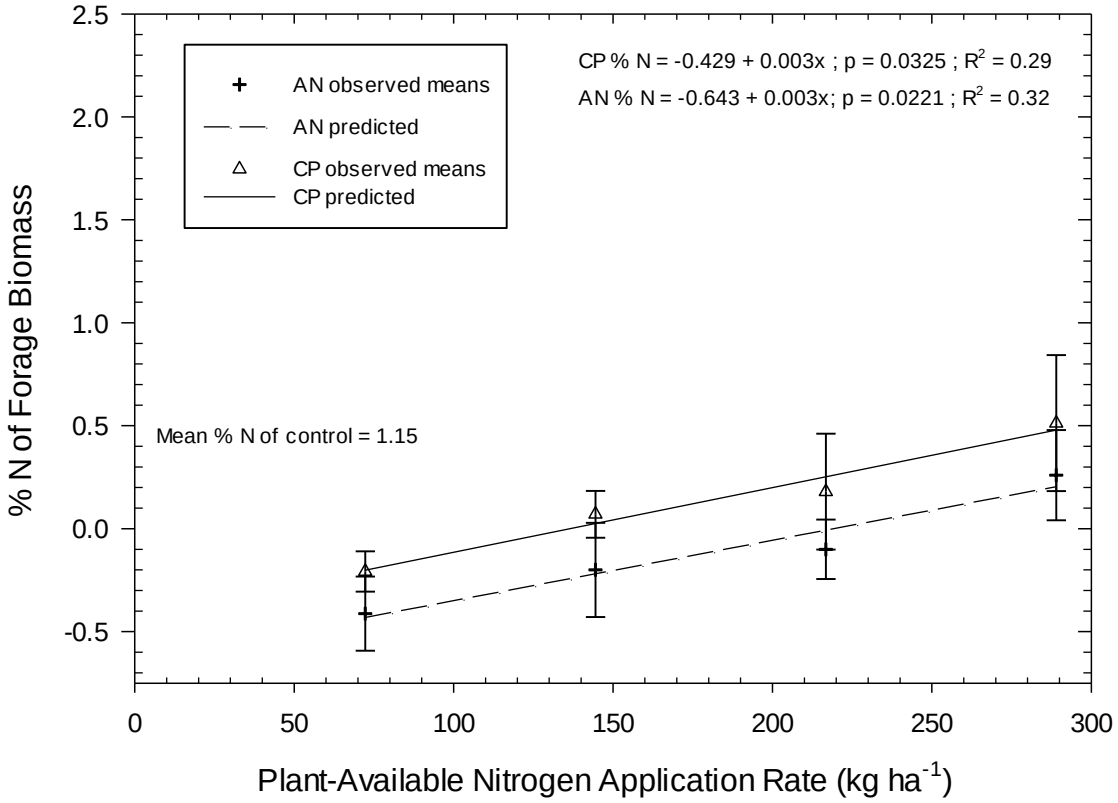

Figure 6.5. Linear regression of the simples effects of PAN application rate of Cary pellet (CP) and NH_4NO_3 (AN) vs. N concentration response. Amendments applied in the fall of 2010 and harvested in the summer of 2011. Average N concentration of the control plots were subtracted from the amended plots. Error bars represent the standard error of the individual means.

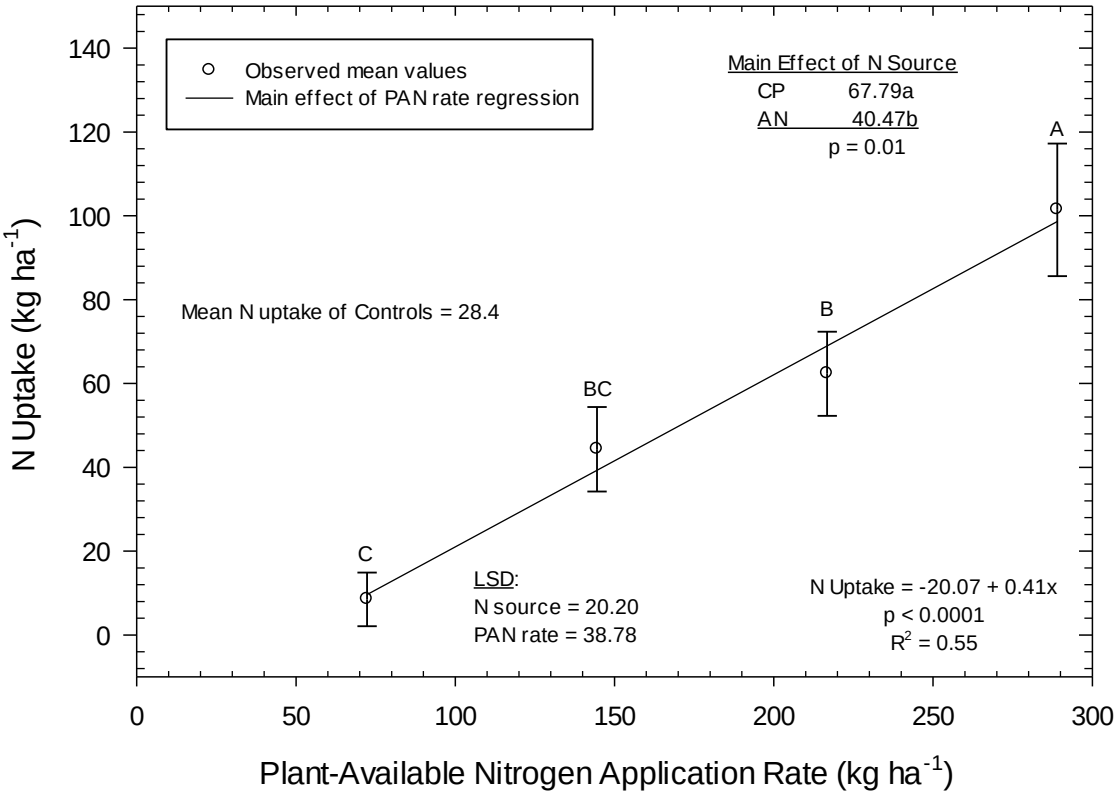

Figure 6.6. Linear regression of the main effect of PAN application rate and the main effect of N source vs. N uptake response. Amendments applied in the fall of 2010 and harvested in the summer of 2011. Average N uptake of the control plots were subtracted from the amended plots. Error bars represent the standard error of the individual means. Main effect means followed by the same capital letter (N rate) or lowercase letter (N source) are not significantly different by Fisher's Protected LSD, $\alpha = 0.05$.

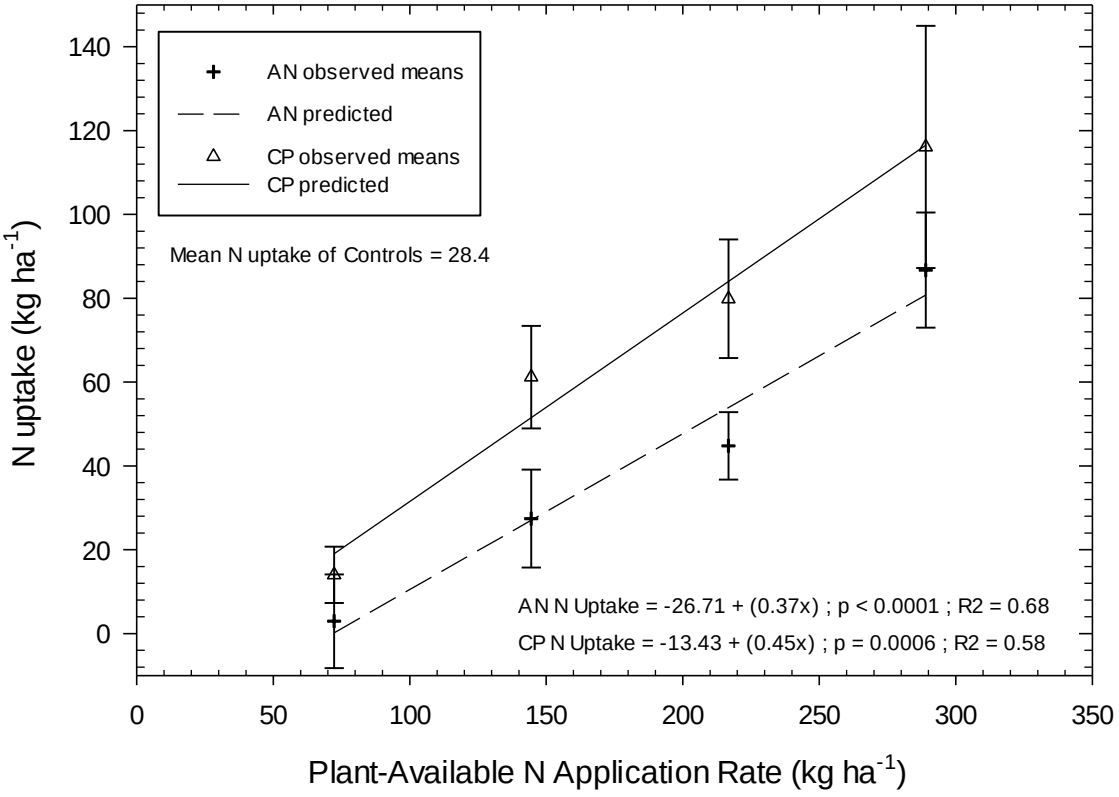

Figure 6.7. Linear regression of the simples effects of PAN application rate of Cary pellet (CP) and NH_4NO_3 (AN) vs. N uptake response. Amendments applied in the fall of 2010 and harvested in the summer of 2011. Average N concentration of the control plots were subtracted from the amended plots. Error bars represent the standard error of the individual means.

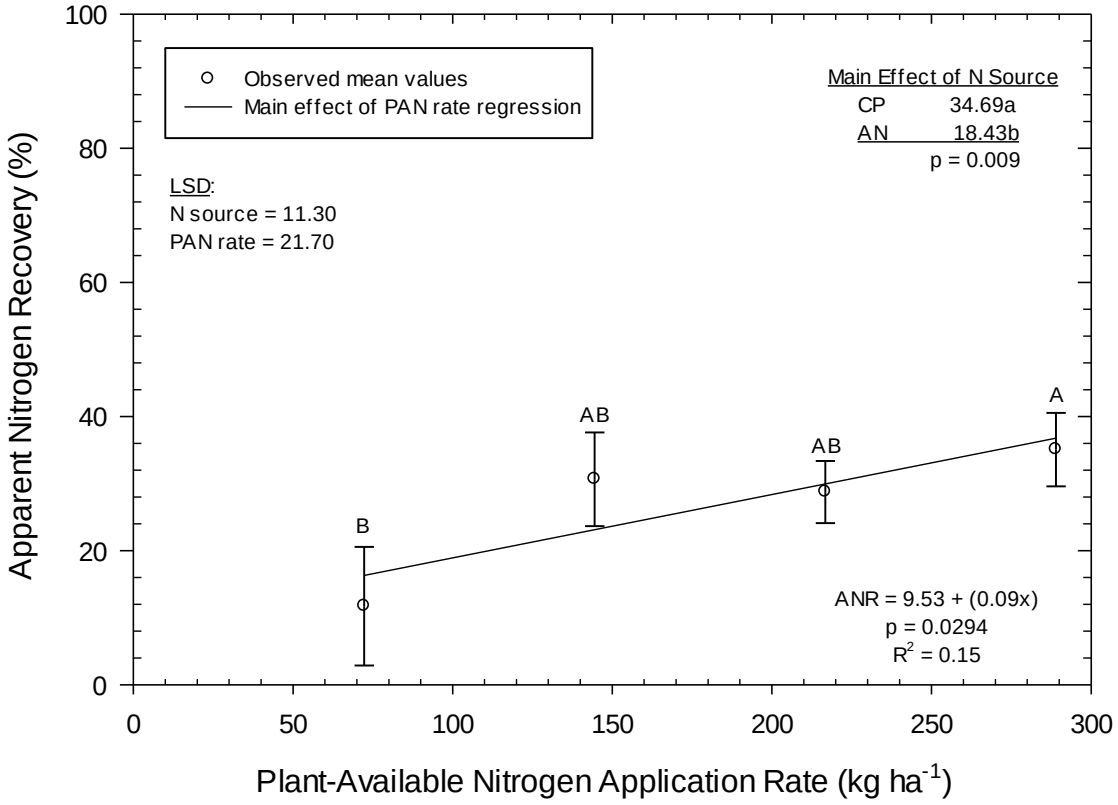

Figure 6.8. Linear regression of the main effect of PAN application rate and the main effect of N source vs. apparent N recovery (ANR). Amendments applied in the fall of 2010 and harvested in the summer of 2011. Error bars represent the standard error of the individual means. Main effect means followed by the same capital letter (N rate) or lowercase letter (N source) are not significantly different by Fisher's Protected LSD, $\alpha = 0.05$.

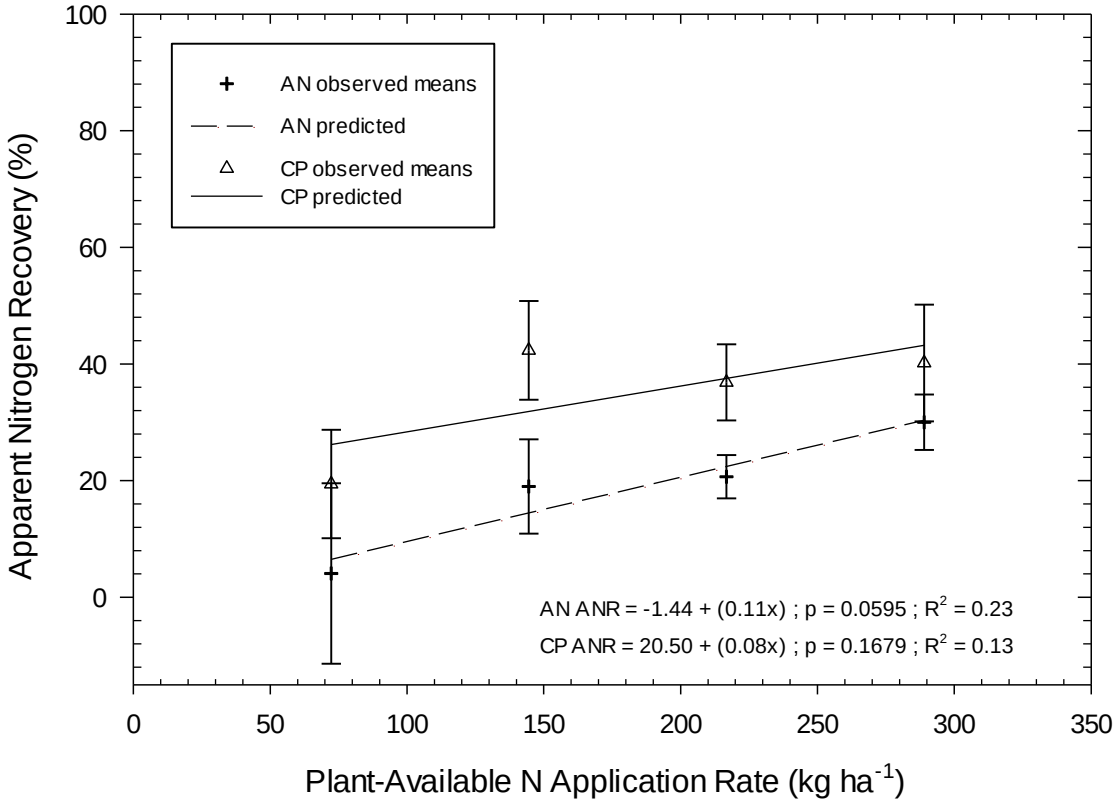

Figure 6.9. Linear regression of the simples effects of PAN application rate of Cary pellet (CP) and NH_4NO_3 (AN) vs. apparent N recovery (ANR). Amendments applied in the fall of 2010 and harvested in the summer of 2011. Average N concentration of the control plots were subtracted from the amended plots. Error bars represent the standard error of the individual means.

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CHAPTER 7: AEROBIC INCUBATION

7.0 Aerobic Incubation Total Inorganic N (Net Inorganic N Mineralization)

7.0.0 Unamended Soil Controls

Throughout this chapter, aerobic incubation total inorganic N is synonymous with net inorganic N mineralization, or net mineralization, and both are used interchangeably. The results of the aerobic incubation total inorganic N vs. incubation time are illustrated in Figure 7.1. Points represent the observed means of each soil at a particular incubation time (days) and each point is connected by a line. Across all incubation days, the Vance sandy clay loam had the highest aerobic incubation N of the four soils tested. The aerobic incubation N concentration was approximately 44 mg kg^{-1} at Day 0 and increased to approximately 80 mg kg^{-1} at Day 112. The Wedowee sandy loam had the second highest aerobic incubation N of the four soils tested, and its standard error bars overlapped with the Vance sandy clay loam at all days with the exception of Day 7. The Noboco loamy sand had the third highest aerobic incubation N content of the four soils tested, and appeared to be statistically significantly lower than the Vance and Wedowee soils, as the error bars did not overlap at most incubation intervals. The aerobic incubation N content of the Noboco soil was approximately 10 mg kg^{-1} at Day 0, and increased to an apparent maximum of 36 mg kg^{-1} at Day 56. The Norfolk loamy sand soil had the lowest aerobic incubation N content of the four soils tested, although the error bars overlapped with the Noboco soil at all incubation intervals with the exception of Day 56. The Norfolk soil appeared to mineralize organic N through Day 112.

The HM content of the four soils (Table 3.2) was investigated as a potential explanation of the differences in aerobic incubation N content, as described previously, but

the results were not as hypothesized. The soils with the highest HM content, Noboco and Norfolk, mineralized the least N. Therefore, it appeared as if great HM content did not lead to great N mineralization. Although no data was available on inherent C or N contents of the soils, it was possible that the organic matter in the Vance and Wedowee soils had lower C/N ratios, and had a greater ability to mineralize organic N than the Noboco and Norfolk soils. Other potential explanations include inherent differences in these soils' ability to mineralize organic matter, likely due to differing soil chemical, physical, and/or microbiological properties, which could have important implications for mineralization of biosolids added to these soils.

7.0.1 Analysis of Variance of Amended Soils

The average aerobic incubation total inorganic N of the unamended-soil controls was subtracted from the amended samples at each incubation interval so that the effect of the amendments alone could be identified (otherwise known as net inorganic N mineralization). The analysis of variance of all treatment factors tested is shown in Table 7.1. Some interactions were not statistically significant ($\alpha = 0.05$), and due to the complexity of the analysis of variance table, non-significant interactions were removed for easier viewing (Table 7.2). However, the ANOVA table was still very complex, and in an effort to simplify the analysis, the data were divided up by moisture: 80% field capacity (Table 7.3) or air dried soil (not shown). Only the moist soils are included hereafter in tables and figures. For soils that had their moisture content adjusted to an estimation of 80% of field capacity (Table 7.3), the analysis of variance showed that all interactions and main effects were statistically

significant, with the exception of the N source X RYE rate X Soil X Time, RYE rate X Soil X Time, N source X RYE rate X Soil, and RYE rate X Soil.

The next step in the analysis involved dividing the data set up by soil type, as done in the anaerobic incubation and the ASNT. The results of the analysis of variance are shown in Table 7.4. All effects of the Noboco soil were statistically significant except for the RYE rate*Time interaction. All effects of the Norfolk soil were statistically significant. All effects of the Vance soil were statistically significant except for the RYE rate*Time interaction. All effects of the Wedowee soil were statistically significant except for the N source*RYE rate*Time and RYE rate*Time interactions. The data was not broken down any further.

7.0.2 Plots of All Simple Effects: Noboco loamy sand

Due to the statistical significance of most interactions of the data when broken down by moisture adjusted to an estimate of field capacity and soil type, all simple effects were plotted, and the results of the Noboco loamy sand soil are illustrated in Figure 7.2. The average aerobic incubation total inorganic N of the unamended-soil controls was subtracted from the amended samples at each incubation interval so that the effects of the amendments alone could be identified. The results of the simple effects of RYE rate and time for AN are illustrated in Figure 7.2A. The maximum amount of aerobic N was found at Day 0 and the lines remained somewhat flat across all incubation days, which was expected since the N in AN was inorganic and in the forms of NH_4 and NO_3 upon dissolution of the prills. There was an initial decrease from Day 0 to Day 7, and likely was a result of initial microbial

immobilization as the inorganic N was dissolving. The amount of aerobic N from AN varied with RYE rate, as expected, and ranged from approximately 35 mg kg^{-1} to 132 mg kg^{-1} for the 0.5 and 2.0 RYE rates, respectively.

The results of the simple effects of RYE rate and time for CP are illustrated in Figure 7.2B. Nitrogen mineralization appeared to have occurred in a quadratic plateau fashion, and leveled off at Day 56. All RYE rates had approximately the same aerobic N content at Day 0, likely because little mineralization of the organic N had occurred yet, which suggested that the inorganic N concentration of CP had little effect on the aerobic N content (Table 3.1). The 0.5 RYE rate aerobic N content reached a maximum of approximately 30 mg kg^{-1}, and the 2.0 RYE rate reached a maximum of approximately 130 mg kg^{-1}. Compared to the AN plot (Fig. 7.2A), similar amounts of N were released/mineralized between the two N sources, which suggested that the NAC used for CP was satisfactory.

The results of the simple effects of RYE rate and time for R+ are illustrated in Figure 7.2C. As illustrated previously, the behavior of R+ was unexpected. Little to no N mineralization occurred across most incubation days, and there was some evidence of N immobilization in all RYE rates except for 0.5 RYE. Interestingly, the lowest RYE rate of 0.5 mineralized the most aerobic N across most incubation days. The 1.0 RYE rate showed the second most aerobic N, followed by the 1.5 and 2.0 RYE rates, which were coincident and suggested microbial N immobilization. The unexpected behavior was difficult to explain, but the unpredictable nature of the R+ biosolids was noticed throughout all laboratory tests used in this research, and is likely a result of the advanced N removal processes used to generate those biosolids. Also, the magnitude of the aerobic N content of

R+ was substantially lower than that of AN, and suggested that the NAC used overestimated the amount of PAN from those biosolids. Again, this was likely due to the advanced N removal used at the Raleigh wastewater treatment plant, a factor that current NCDA&CS NAC do not take into account. Similar results were found in the anaerobic incubation and the ASNT.

The results of the simple effects of RYE rate and time for OWC are illustrated in Figure 7.2D. It appeared as if little N mineralization occurred in the 0.5 and 1.0 RYE rates between Day 0 and Day 56. The 1.5 and 2.0 RYE rates reached a maximum aerobic N content at Day 56. The aerobic N contents ranged from approximately 48 mg kg^{-1} to 125 mg kg^{-1} for the 0.5 and 2.0 RYE rates, respectively. Maximum aerobic N contents were approximately 40 mg kg^{-1} to 155 mg kg^{-1} for the 0.5 and 2.0 RYE rates, respectively. It appeared as if the organic N in the OWC took longer to mineralize than the organic N in the CP because aerobic N content was relatively constant until Day 28, when net N mineralization rate appeared to increase. CP yielded a substantial amount of aerobic N between Day 0 and Day 14 (Fig. 7.2B). Additionally, the magnitude of aerobic N from the OWC was larger than that of AN, which suggested that the NAC used underestimated the amount of PAN from those biosolids, and the differences appeared to be of agronomic importance as the 2.0 RYE rate of OWC yielded approximately 150 kg aerobic N ha^{-1} more than the 2.0 RYE rate of AN.

7.0.3 Plots of All Simple Effects: Norfolk loamy sand

The results of the simple effects of RYE rate and time from the Norfolk loamy sand soil are illustrated in Figure 7.3. The results of aerobic N from AN are illustrated in Figure 7.3A. The maximum amount of aerobic N was generally found at Day 0, and decreased slightly among incubation days. The aerobic N values were approximately 28 mg kg^{-1} to 105 mg kg^{-1} for the 0.5 to 2.0 RYE rates. The immediate availability of the aerobic N from AN was expected given the properties of AN, but the slight decrease in aerobic N suggested some N loss over time. This may have been due to volatilization of NH_4 and/or N assimilation into the biomass of the soil microbial community.

The results of the simple effects of RYE rate for CP are illustrated in Figure 7.3B. Similarly to the Noboco soil, N mineralization of CP in the Norfolk soil appeared to follow a quadratic plateau model. All rates yielded approximately the same amount of aerobic N at Day 0 (2 mg kg^{-1}), and N mineralization proceeded quickly to a maximum around Day 28. The maximum amounts of N mineralization were approximately 30 mg kg^{-1} to 120 mg kg^{-1} for the 0.5 to 2.0 RYE rates, respectively. The 0.5 and 1.0 RYE plots had amounts of aerobic N similar to those of AN, but the 1.5 and 2.0 RYE rates had larger aerobic N values than AN. That suggested that the precision of the NAC used for CP in the Norfolk soil depended on rate. The approximate differences in aerobic N between AN and CP at the highest RYE rates were 45 kg PAN ha^{-1}, which would likely be agronomically important when land-applying those N sources.

The results of the simple effects of RYE rate for R+ are illustrated in Figure 7.3C. As was the case in the Noboco soil, the N mineralization behavior of R+ was unexpected. The

0.5 RYE rate produced the most aerobic N, and the other rates appeared to undergo microbial N immobilization, as previously described. Moreover, the aerobic N content of R+ for most RYE rates was much less than that of AN, which suggested that the NAC used for R+ overestimated the PAN from those biosolids. The 0.5 RYE rate reached a maximum aerobic N content of approximately 40 mg kg^{-1} at Day 112, and the other rates yielded approximately -20 mg kg^{-1}. These results were likely due to the treatment methods of the R+ biosolids.

The results of the simple effects of RYE rate for OWC are illustrated in Figure 7.3D. As was the case in the Noboco soil, substantial amounts of aerobic N from OWC were immediately available, which was likely a result of the inorganic N fraction of OWC (Table 3.1). The inorganic N fraction influence was not noticeable in Norfolk soil amended with CP, despite the fact that CP had a higher inorganic N content. Generally, N mineralization reached a maximum around Day 7 and remained constant throughout the remaining incubation days. The ranges of the approximate maximums were 30 mg kg^{-1} to 160 mg kg^{-1} for the 0.5 to 2.0 RYE rates. Additionally, the aerobic N values were substantially higher than that of AN, which suggested that the NAC used for OWC underestimated the amount of PAN from those biosolids.

7.0.4 Plots of All Simple Effects: Vance sandy clay loam

The results of the simple effects of RYE rate and time from the Vance sandy clay loam are illustrated in Figure 7.4. The results of aerobic N from AN specifically are illustrated in Figure 7.4A. Ammonium nitrate generally behaved the same in the Vance soil

as it did in the Noboco and Norfolk soils. The maximum aerobic N was reached by Day 3 or earlier, and either remained constant throughout the remaining incubation days, or slightly decreased with time. The maximum aerobic N values ranged from approximately 46 mg kg^{-1} to 120 mg kg^{-1} for the 0.5 and 2.0 RYE rates. As described previously, the slight decrease in aerobic N may have been due to N loss or microbial biomass assimilation.

The results of the simple effects of RYE rate for CP are illustrated in Figure 7.4B. The results were similar to those of the Noboco and Norfolk soils. The aerobic N content at Day 0 was similar for all rates, and was approximately 0 mg kg^{-1}. N mineralization proceeded in a quadratic plateau fashion to a maximum around Day 14 or 28, depending on RYE rate. The maximum aerobic N values ranged from approximately 30 mg kg^{-1} to 100 mg kg^{-1} for the 0.5 to 2.0 RYE rates. Once again, CP appeared to mineralize the organic N quickly, and the magnitudes of aerobic N were similar to that of AN, which suggested that the NAC used were satisfactory. The apparent rapid N mineralization of CP likely meant that the organic N from those biosolids was relatively young and easily decomposable vs. an older, more recalcitrant organic N pool. Understanding the N mineralization dynamics of biosolids is important to land-application planning in order to maximize crop N uptake and minimize N loss and environmental pollution.

The results of the simple effects of RYE rate for R+ are illustrated in Figure 7.4C. As was the case in the Noboco and Norfolk soils, the behavior of N mineralization of R+ was unexpected. The lowest RYE rate of 0.5 yielded the most aerobic N and the other rates either yielded a net N mineralization of approximately 0 mg kg^{-1}, or the N was immobilized by the soil microbial community. The maximum aerobic N content of the 0.5 RYE rate was

approximately 48 mg kg^{-1}. The magnitude of aerobic N was also substantially lower than that of AN, which suggested the NAC overestimated the amount of PAN from R+. As previously described, the unexpected behavior of R+ is likely due to the treatment process to produce those biosolids.

The results of the simple effects of RYE rate for OWC are illustrated in Figure 7.4D. The behavior of OWC was similar in this soil as in the Noboco and Norfolk soils. There were substantial differences in the aerobic N contents of different rates at Day 0, and ranged from 30 mg kg^{-1} to 110 mg kg^{-1}, respectively. Mineralization of the organic N generally appeared to follow a quadratic plateau model, and there was no increase in net N mineralization after Day 28 or 56, depending upon RYE rate. The maximum aerobic N contents ranged from approximately 50 mg kg^{-1} to 130 mg kg^{-1} for the 0.5 to 2.0 RYE rates. Moreover, the magnitude of the aerobic N appeared to be greater than that of AN, although the differences varied with rate. That suggested that the NAC used for OWC slightly underestimated the amount of PAN from those biosolids.

7.0.5 Plots of All Simple Effects: Wedowee sandy loam

The results of the simple effects of RYE rate from the Wedowee sandy loam soil are illustrated in Figure 7.5. The results of the aerobic N from AN specifically are illustrated in Figure 7.5A. Ammonium nitrate generally behaved the same in the Wedowee soil as it did in the previous three soils. The maximum aerobic N was reached by Day 3 or earlier, and either remained constant throughout the remaining incubation days, or slightly decreased with time. The maximum aerobic N values ranged from approximately 43 mg kg^{-1} to 115 mg kg^{-1} for

the 0.5 to 2.0 RYE rates. As described previously, the slight decrease in aerobic N may have been due to N loss or microbial biomass assimilation.

The results of the simple effects of RYE rate for CP are illustrated in Figure 7.5B. The approximate N mineralization rate and model appeared to be similar in the Wedowee soil as in the other three soils, but the magnitude of aerobic N was substantially lower in the Wedowee soil. The aerobic N content at Day 0 for all RYE rates was approximately 10 mg kg^{-1} which was likely from the inorganic N fraction of CP, and net N mineralization reached a maximum around Day 14. The maximum aerobic N values ranged from approximately 20 mg kg^{-1} to 70 mg kg^{-1} for the 0.5 to 2.0 RYE rates. The overall magnitude of aerobic N was lower than that of AN, which suggested that the NAC used for CP in this soil overestimated the amount of PAN from those biosolids. In the previous three soils, the NAC for CP appeared to be satisfactory, so the difference in the Wedowee soil suggested that soil type affected the N mineralization of the biosolids.

The results of the simple effects of RYE rate for R+ are illustrated in Figure 7.5C. There results were similar to those of the other three soils with the exception of the magnitude of the 0.5 RYE rate aerobic N. The maximum aerobic N of that RYE rate was approximately 18 mg kg^{-1}, which was less than in the other soils. At Day 0, all RYE rates had similar aerobic N contents, but all RYE rates except 0.5 appeared to immobilize the aerobic N throughout the incubation days. Additionally, the magnitude of aerobic N was substantially lower than that of AN, which suggested the NAC used for R+ overestimated the PAN from R+, which was likely due to the treatment methods used to produce those biosolids.

7.0.6 Summary of Aerobic Incubation

The behavior of the four N sources was generally similar among different soils, but there were some differences. The maximum amount of aerobic N from AN was generally present at Day 0, and the aerobic N content either stayed the same throughout the incubation days or slightly decreased likely due to some microbial immobilization and or other forms of N loss. The aerobic N response from CP generally appeared to have followed a quadratic plateau model and reached a maximum aerobic N content at Day 28 or 56, and remained constant through Day 112. The aerobic N content of R+ was consistently very different from the other N sources, and those results were expected based on R+ biosolids treatment processes and results from the anaerobic incubation and ASNT. The lowest RYE rate yielded the most aerobic N, and the other rates appeared to have immobilized the PAN. The aerobic N of the OWC generally mineralized quickly, reaching a maximum at Day 7 or beyond, but the response depended on soil type. As expected, the 2.0 RYE rate yielded the largest aerobic N content among all N sources, and the other rates followed accordingly in order of magnitude. Some biosolids NAC appeared to be reasonably correct, while others did not. For example, CP aerobic N content was generally coincident with that of AN, although it did vary with soil type. Raleigh plus aerobic N was consistently and substantially below that of AN, and suggested that the NAC overestimated the PAN from those biosolids. The aerobic N content from OWC was consistently higher than that of AN, which suggested that the NAC from those biosolids underestimated the amount of PAN from those biosolids. Soil type affected the aerobic N content both in terms of the rate of mineralization and the relative magnitude.

Table 7.1. Analysis of variance showing all treatment effects for aerobic incubation total inorganic N from four N sources (3 biosolids, NH_4NO_3) applied at four rates to four soils and at two different moisture contents over 112 days. Average N content of the unamended soil controls were subtracted from the amended samples within each replication and incubation day.

Effect	Numerator DF	Denominator DF	F Value	Pr > F
N source	3	381	1652.92	<0.0001
Soil	3	381	10.27	<0.0001
RYE rate	3	381	493.02	<0.0001
Moisture	1	381	59.83	<0.0001
Time	6	2304	34.38	<0.0001
N source*Moisture	3	381	189.65	<0.0001
N source*RYE rate	9	381	131.17	<0.0001
Moisture*RYE rate	3	381	1.92	0.1256
N source*Soil	9	381	4.04	<0.0001
Moisture*Soil	3	381	1.09	0.3523
RYE rate*Soil	9	381	1.12	0.3440
N source*Time	18	2304	23.10	<0.0001
Soil*Time	18	2304	7.30	<0.0001
Moisture*Time	6	2304	6.11	<0.0001
RYE rate*Time	18	2304	5.08	<0.0001
Moisture*RYE rate*Soil	9	381	0.21	0.9931
N source*Moisture*Soil	9	381	3.95	<0.0001
N source*Moisture*RYE rate	9	381	16.88	<0.0001
N source*RYE rate*Soil	27	381	1.01	0.4501
N source*Moisture*Time	18	2304	21.50	<0.0001
N source*RYE rate*Time	54	2304	4.62	<0.0001
Moisture*RYE rate*Time	18	2304	2.33	0.0012
N source*Soil*Time	54	2304	2.52	<0.0001
Moisture*Soil*Time	18	2304	8.42	<0.0001
RYE rate*Soil*Time	54	2304	1.13	0.2359
N source*Moisture*Soil*Time	54	2304	1.77	0.0005
N source*Moisture*RYE rate*Time	54	2304	2.14	<0.0001
N source*Moisture*RYE rate*Soil	27	381	1.26	0.1775
N source*RYE rate*Soil*Time	162	2304	0.71	0.9975
Moisture*RYE rate*Soil*Time	54	2304	0.64	0.9796
N source*Moisture*RYE rate*Soil*Time	162	2304	0.39	1.0000

Table 7.2. Analysis of variance showing only statistically significant treatment effects for aerobic incubation total inorganic N from four N sources (3 biosolids, NH_4NO_3) applied at four rates to four soils and at two different moisture contents over 112 days. Average N content of the unamended soil controls were subtracted from the amended samples within each replication and incubation day.

Effect	Numerator DF	Denominator DF	F Value	Pr > F
N source	3	381	1652.92	<0.0001
Soil	3	381	10.27	<0.0001
RYE rate	3	381	493.02	<0.0001
Moisture	1	381	59.83	<0.0001
Time	6	2304	34.38	<0.0001
N source*Moisture	3	381	189.65	<0.0001
N source*RYE rate	9	381	131.17	<0.0001
N source*Soil	9	381	4.04	<0.0001
N source*Time	18	2304	23.10	<0.0001
Soil*Time	18	2304	7.30	<0.0001
Moisture*Time	6	2304	6.11	<0.0001
RYE rate*Time	18	2304	5.08	<0.0001
N source*Moisture*Soil	9	381	3.95	<0.0001
N source*Moisture*RYE rate	9	381	16.88	<0.0001
N source*Moisture*Time	18	2304	21.50	<0.0001
N source*RYE rate*Time	54	2304	4.62	<0.0001
Moisture*RYE rate*Time	18	2304	2.33	0.0012
N source*Soil*Time	54	2304	2.52	<0.0001
Moisture*Soil*Time	18	2304	8.42	<0.0001
N source*Moisture*Soil*Time	54	2304	1.77	0.0005
N source*Moisture*RYE rate*Time	54	2304	2.14	<0.0001

Table 7.3. Analysis of variance showing only soils that were adjusted to 80% of field capacity for aerobic incubation total inorganic N from four N sources (3 biosolids, NH_4NO_3) applied at four rates to four soils and at two different moisture contents over 112 days. Average N content of the unamended soil controls were subtracted from the amended samples within each replication and incubation day.

Effect	Numerator DF	Denominator DF	F Value	Pr > F
N source	3	189	596.30	<0.0001
Soil	3	189	6.89	0.0002
RYE rate	3	189	206.30	<0.0001
Time	6	1152	26.72	<0.0001
N source*RYE rate	9	189	58.52	<0.0001
N source*Soil	9	189	4.17	<0.0001
N source*Time	18	1152	35.09	<0.0001
Soil*Time	18	1152	6.94	<0.0001
RYE rate*Time	18	1152	2.39	0.0009
RYE rate*Soil	9	189	0.68	0.7283
N source*RYE rate*Soil	27	189	1.00	0.4769
N source*RYE rate*Time	54	1152	5.34	<0.0001
N source*Soil*Time	54	1152	1.70	0.0015
RYE rate*Soil*Time	54	1152	0.77	0.8916
N source*RYE rate*Soil*Time	162	1152	0.60	1.0000

Table 7.4. Analysis of variance showing only soils that were adjusted to 80% of field capacity for aerobic incubation total inorganic N from four N sources (3 biosolids, NH_4NO_3) applied at four rates to four soils and at two different moisture contents over 112 days, by soil type. Average N content of the unamended soil controls were subtracted from the amended samples within each replication and incubation day.

Effect	Numerator DF	Denominator DF	F Value	Pr > F
	Noboco loamy sand			
N source	3	45	177.22	<0.0001
RYE rate	3	45	74.47	<0.0001
Time	6	288	20.94	<0.0001
N source*RYE rate	9	45	22.72	<0.0001
N source*Time	18	288	13.34	<0.0001
RYE rate*Time	18	288	1.10	0.3541
N source*RYE rate*Time	54	288	2.69	<0.0001
	Norfolk loamy sand			
N source	3	45	223.92	<0.0001
RYE rate	3	45	85.86	<0.0001
Time	6	288	21.10	<0.0001
N source*RYE rate	9	45	19.22	<0.0001
N source*Time	18	288	21.84	<0.0001
RYE rate*Time	18	288	3.76	<0.0001
N source*RYE rate*Time	54	288	2.99	<0.0001
	Vance sandy clay loam			
N source	3	45	141.44	<0.0001
RYE rate	3	45	43.32	<0.0001
Time	6	288	8.70	<0.0001
N source*RYE rate	9	45	16.27	<0.0001
N source*Time	18	288	7.69	<0.0001
RYE rate*Time	18	288	0.51	0.9509
N source*RYE rate*Time	54	288	1.44	0.0317
	Wedowee sandy loam			
N source	3	45	178.98	<0.0001
RYE rate	3	45	48.63	<0.0001
Time	6	288	4.43	0.0003
N source*RYE rate	9	45	14.81	<0.0001
N source*Time	18	288	4.62	<0.0001
RYE rate*Time	18	288	0.79	0.7099
N source*RYE rate*Time	54	288	0.92	0.6289

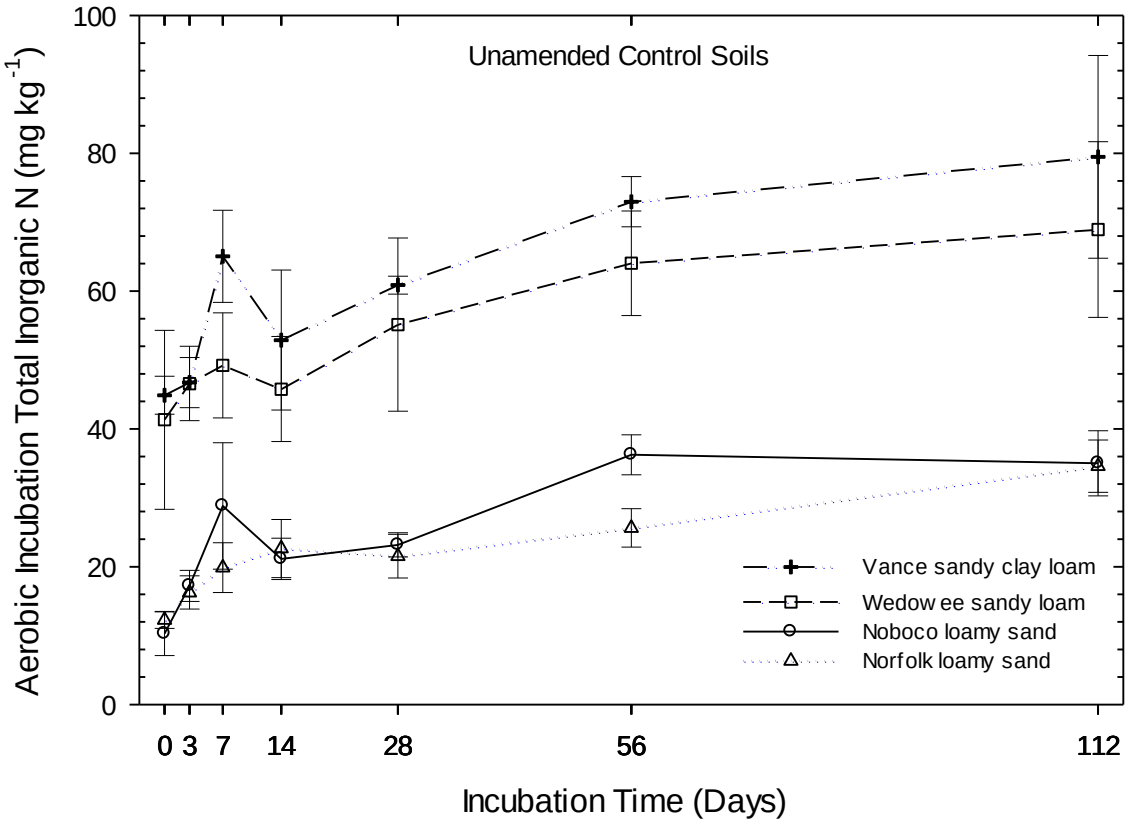

Figure 7.1. Aerobic Incubation Total Inorganic N (net N mineralization) vs. incubation time for unamended soil controls adjusted to 80% of field capacity. Plot shows the observed means of each soil type at each incubation day connected by a line from a 112-day aerobic incubation. Four soils were used, two representative coastal plain soils: Noboco loamy sand and Norfolk loamy sand, and two representative piedmont soils: Vance sandy clay loam and Wedowee sandy loam. Aerobic Incubation Total Inorganic N = NH_4 + NO_3. Error bars represent the standard error of the individual means.

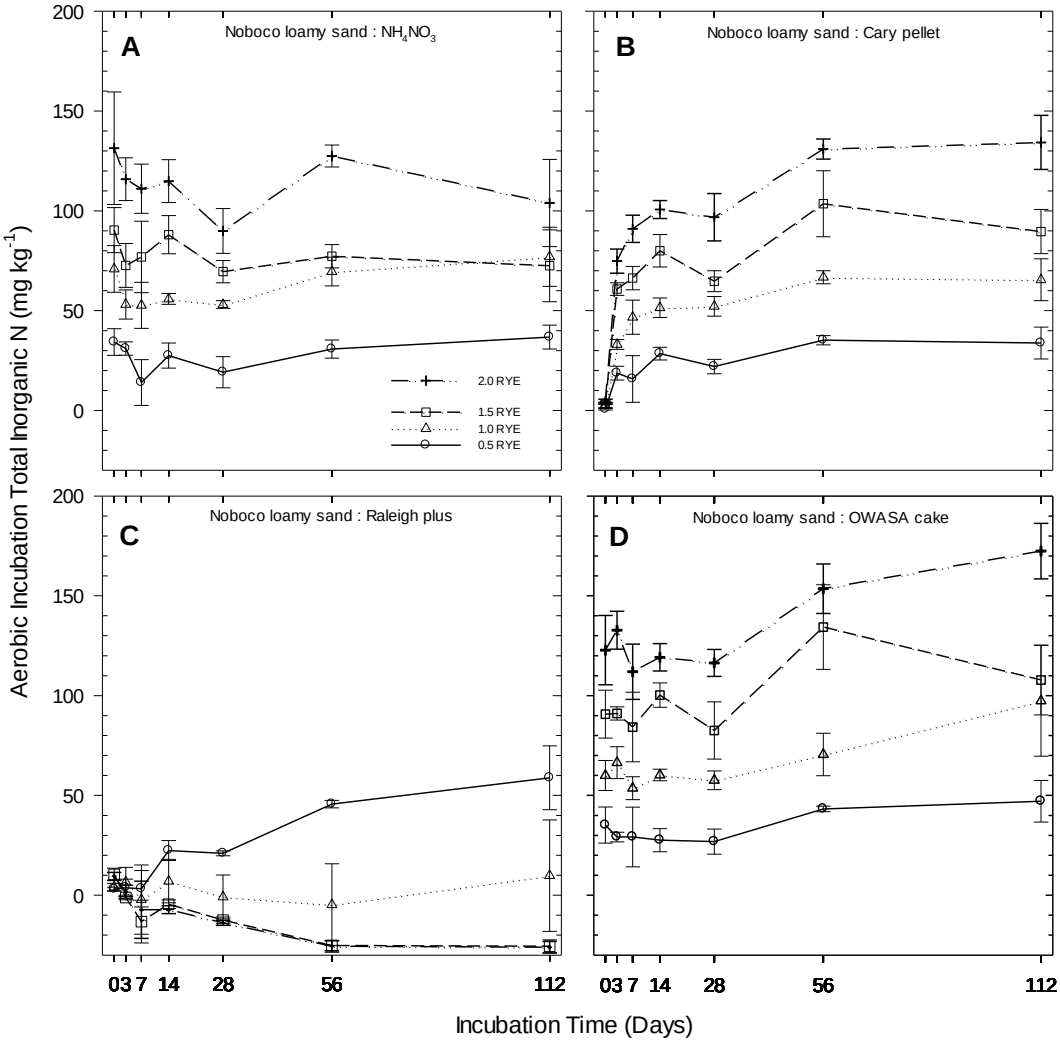

Figure 7.2. Aerobic Incubation Total Inorganic N (net mineralization) vs. Incubation time for the Noboco loamy sand soil adjusted to 80% of field capacity. Plot shows the observed means of each RYE rate for each N source at each incubation day connected by a line from a 112-day aerobic incubation. Nitrogen sources included three biosolids and NH_4NO_3, each mixed at five rates. Nitrogen rates were determined as 0, 0.5 X, 1.0 X, 1.5 X, and 2.0 X the North Carolina Realistic Yield Expectation Database (North Carolina Nutrient Management Workgroup) N rate for Fescue on a Wedowee coarse sandy loam soil. The 1.0 RYE rates were 144.5 kg ha^{-1} for NH_4NO_3 and 127 kg ha^{-1} for the three biosolids; the differences were due to a calculation error, but were grouped by RYE interval in the above figure. Aerobic Incubation Total Inorganic N = NH_4 + NO_3. Error bars represent the standard error of the individual means.

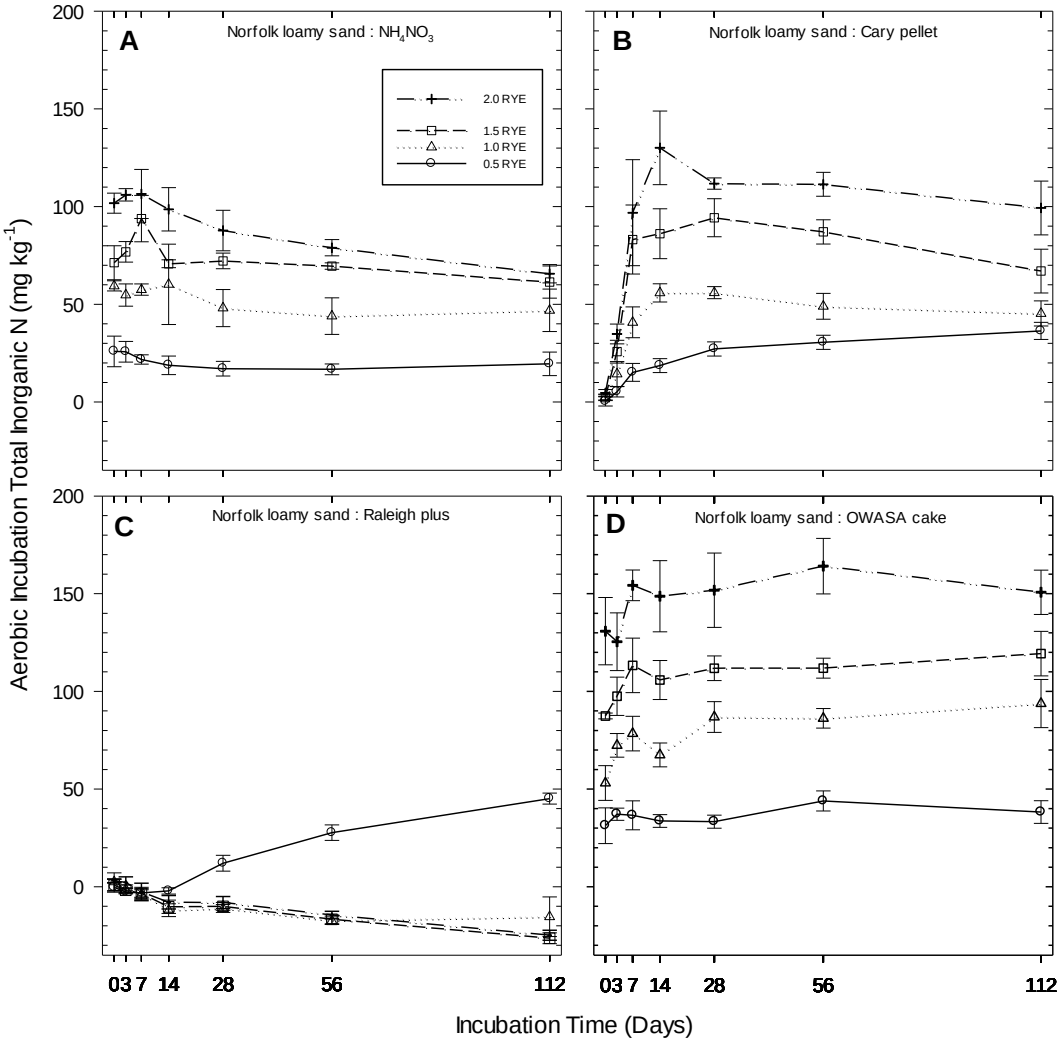

Figure 7.3. Aerobic Incubation Total Inorganic N (net mineralization) vs. Incubation time for the Norfolk loamy sand soil adjusted to 80% of field capacity. Plot shows the observed means of each RYE rate for each N source at each incubation day connected by a line from a 112-day aerobic incubation. Nitrogen sources included three biosolids and NH_4NO_3, each mixed at five rates. Nitrogen rates were determined as 0, 0.5 X, 1.0 X, 1.5 X, and 2.0 X the North Carolina Realistic Yield Expectation Database (North Carolina Nutrient Management Workgroup) N rate for Fescue on a Wedowee coarse sandy loam soil. The 1.0 RYE rates were 144.5 kg ha^{-1} for NH_4NO_3 and 127 kg ha^{-1} for the three biosolids; the differences were due to a calculation error, but were grouped by RYE interval in the above figure. Aerobic Incubation Total Inorganic N = NH_4 + NO_3. Error bars represent the standard error of the individual means.

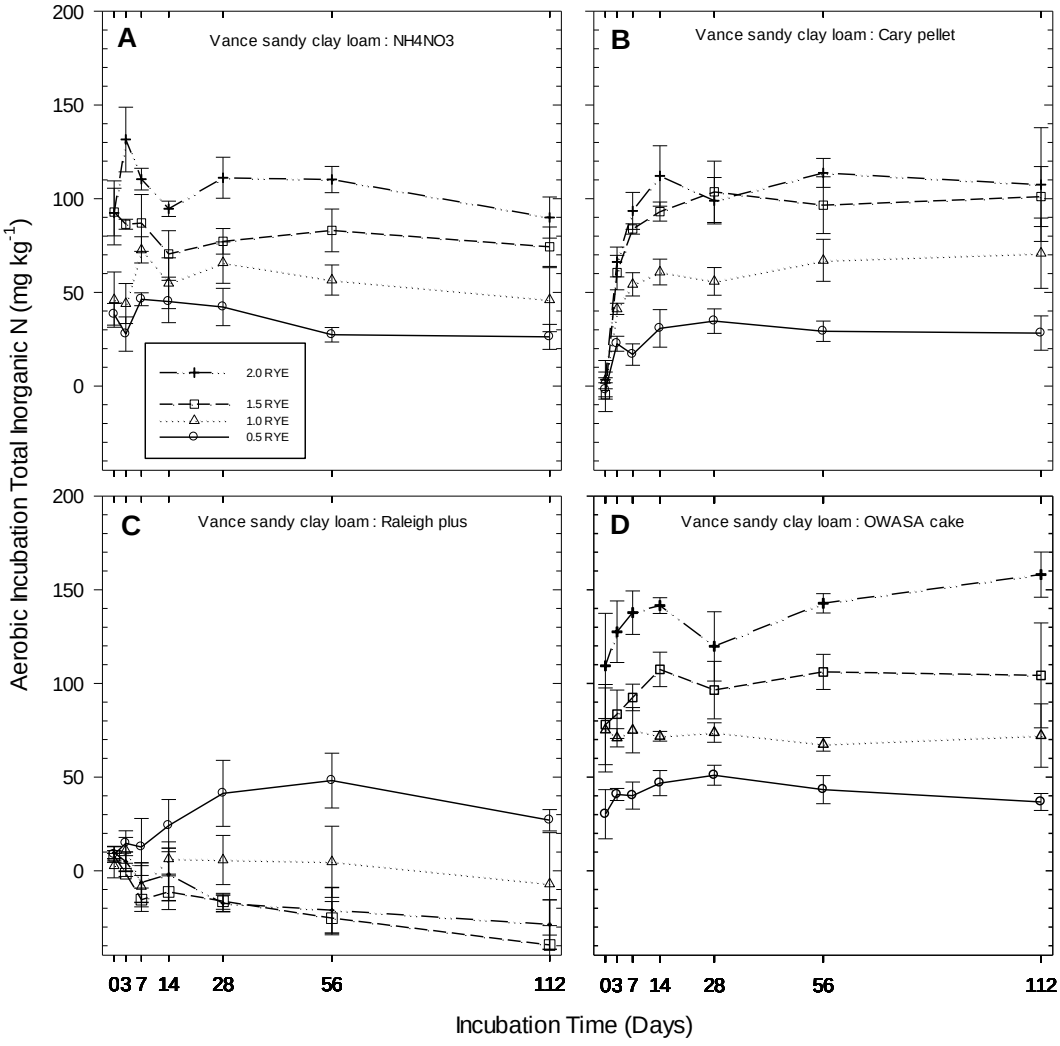

Figure 7.4. Aerobic Incubation Total Inorganic N (net mineralization) vs. Incubation time for the Vance sandy clay loam soil adjusted to 80% of field capacity. Plot shows the observed means of each RYE rate for each N source at each incubation day connected by a line from a 112-day aerobic incubation. Nitrogen sources included three biosolids and NH_4NO_3, each mixed at five rates. Nitrogen rates were determined as 0, 0.5 X, 1.0 X, 1.5 X, and 2.0 X the North Carolina Realistic Yield Expectation Database (North Carolina Nutrient Management Workgroup) N rate for Fescue on a Wedowee coarse sandy loam soil. The 1.0 RYE rates were 144.5 kg ha^{-1} for NH_4NO_3 and 127 kg ha^{-1} for the three biosolids; the differences were due to a calculation error, but were grouped by RYE interval in the above figure. Aerobic Incubation Total Inorganic N = NH_4 + NO_3. Error bars represent the standard error of the individual means.

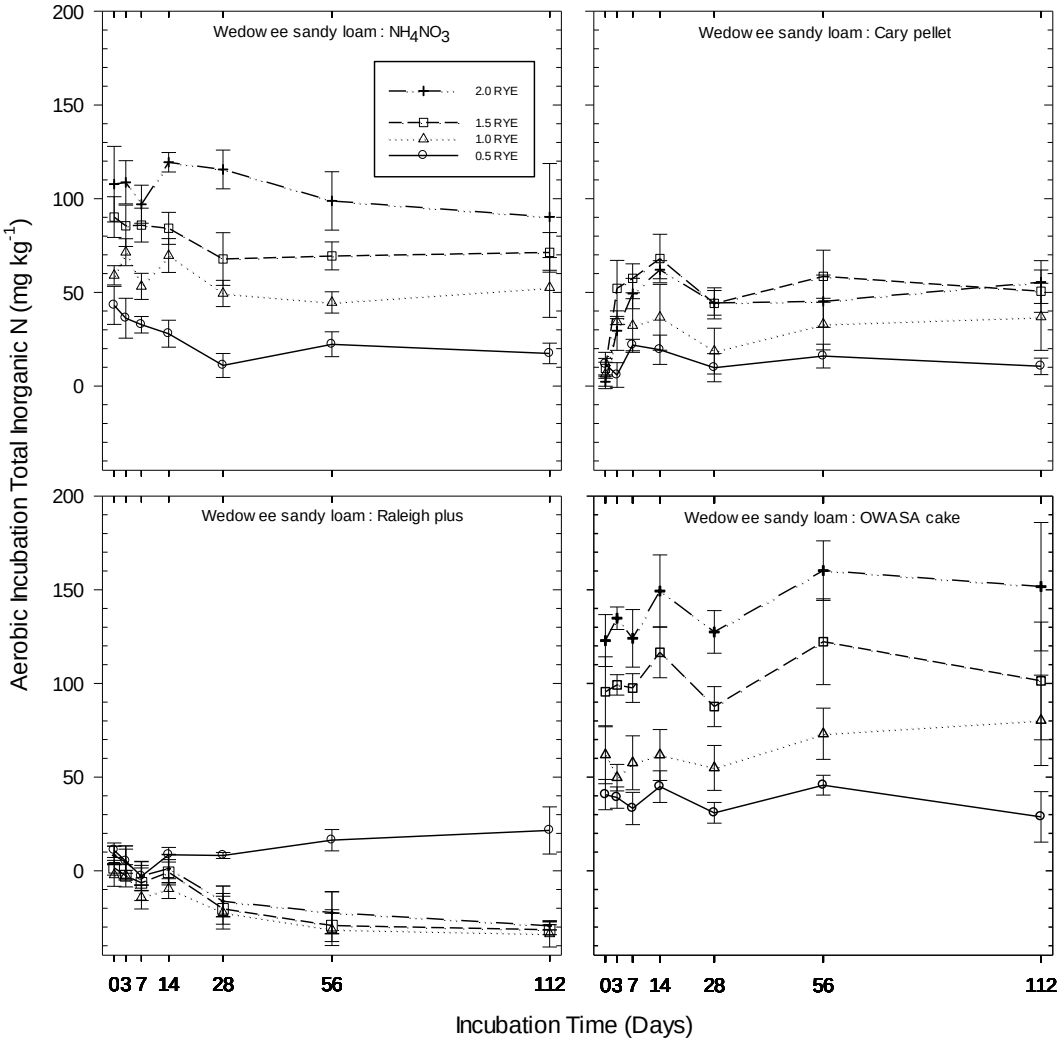

Figure 7.5. Aerobic Incubation Total Inorganic N (net mineralization) vs. Incubation time for the Wedowee sandy loam soil adjusted to 80% of field capacity. Plot shows the observed means of each RYE rate for each N source at each incubation day connected by a line from a 112-day aerobic incubation. Nitrogen sources included three biosolids and NH_4NO_3, each mixed at five rates. Nitrogen rates were determined as 0, 0.5 X, 1.0 X, 1.5 X, and 2.0 X the North Carolina Realistic Yield Expectation Database (North Carolina Nutrient Management Workgroup) N rate for Fescue on a Wedowee coarse sandy loam soil. The 1.0 RYE rates were 144.5 kg ha^{-1} for $NH_4NO_3$3 and 127 kg ha^{-1} for the three biosolids; the differences were due to a calculation error, but were grouped by RYE interval in the above figure. Aerobic Incubation Total Inorganic N = NH_4 + NO_3. Error bars represent the standard error of the individual means.

CHAPTER 8: SUMMARY AND CONCLUSIONS

Nitrogen availability coefficients are used to determine proper agronomic rates of land-application of municipal biosolids by estimating the percentage of total N that will become available to plants the first year. The basis and origin of the current NCDA&CS NAC are not well known, and they may have been derived for sewage sludge rather than for biosolids conforming to EPA water-quality rules promulgated in 1993. In addition, the coefficients only account for differences in some waste water treatment methods and not for varying soil types nor application rates. This may lead to improper fertilization with adverse agronomic, economic, and environmental consequences. Three laboratory tests and a growth response field trial were conducted to evaluate PAN and the NAC of three regional biosolids and were compared to AN. The effect of soil type and application rate was also evaluated, and the tests were compared among themselves, under the presumption that the tests were good indicators of PAN mineralized from biosolids. A comparison of all tests that were evaluated is illustrated in Table 8.1. The anaerobic incubation suggested that the current biosolids-specific NAC tested (Table 2.1) did not consistently estimate PAN from the biosolids. The NAC underestimated PAN from CP and OWC, and overestimated for R+. The magnitudes of the differences were judged agronomically important (differences greater than 20 kg N ha^{-1}). There were also differences across soil type and application rate. The ASNT-N from OWC was higher than all other N sources across all soils. Cary pellet ASNT-N was coincident with AN, and R+ was lower than all four N sources. Results suggested that the NAC did not properly estimate PAN from some biosolids. There were also differences among soil type and rate. The aerobic incubation showed that CP aerobic N was generally

coincident with AN, R+ was substantially lower, and OWC was slightly higher. The response of some N sources varied across different soils and rates, which suggested that those factors should be considered when estimating PAN from biosolids, perhaps by basing rates on RYE N need determinations. A growth response field trial in fescue showed that CP resulted in higher yields, N concentration and uptake, and apparent N recovery. Those results also suggested that the other nutrients in the biosolids facilitated N uptake or that the NAC for CP did not properly estimate PAN, if there were no other limiting nutrients that were satisfied by the biosolids.

Additionally, the accuracy of the NAC varied among different tests for CP and R+ (Table 8.1). The anaerobic and aerobic incubation for CP showed that N mineralized depended on soil type, but the ASNT did not. All tests showed that CP N mineralization depended on rate. All tests indicated that the NAC for OWC consistently underestimated PAN. The anaerobic and ASNT showed that N mineralization of OWC depended on soil type, but that the aerobic incubation did not. All tests showed that OWC mineralization depended on rate. For R+, the anaerobic and aerobic incubations indicated that NAC overestimated PAN; in contrast, the ASNT indicated that the NAC underestimated PAN. For R+, only the anaerobic incubation depended on soil type, while both the anaerobic and aerobic tests depended on rate (Table 8.1). It was expected that the anaerobic and aerobic incubations would produce similar results for all parameters tested, but these results showed that was not always the case, as the results were variable. The ASNT showed no consistent relationship to the other tests used. As a result, it was postulated that one or more of these tests was not an adequate predictor of N mineralization from the biosolids used, but further

comparisons are needed. If some or all of the laboratory tests represent satisfactory estimates of PAN, then the NCDA&CS availability coefficients did not consistently estimate PAN from the biosolids studied. As a result, laboratory analyses might better estimate biosolids PAN than established availability coefficients. Overall, it was concluded that different biosolids mineralized N differently in different soils and when applied at different rates. The magnitude of some of the differences observed was judged to be agronomically important.

8.1 Future Research

Improved understanding of N mineralization from municipal biosolids would result in better biosolids application rate recommendations. Subsequently, this would lead to better N-use efficiency, crop yield and reduced N loss, thereby protecting environmental and water quality. Ultimately, a reduced risk of negative environmental consequences would allow for better utilization of a plentiful organic resource with the potential to benefit both biosolids producers and growers. However, in order to obtain more definitive information on N mineralization of biosolids, further research is needed. More calibration of lab results with field response in NC is needed in addition to the work done by King (1984) and Gilmour et al, (2003), and would require more field trials, receiver crops, biosolids, locations, and time. More calibration is needed, in part, because the results of this research indicate that factors such as biosolids and soil characteristics, in addition to rate deserve greater consideration. Conducting such research would be a massive undertaking and would involve many logistical obstacles. Greenhouse studies would likely be useful to bridge the gap between the

laboratory and field trials. Ultimately, if this information were available, it could be integrated into nutrient management software to guide land application.

Additional work is also needed with the data collected in this research. In the ASNT, further investigation and calibration of the validity of the test to estimate PAN from biosolids is needed. In the aerobic incubation, further statistical analysis is needed to fully understand the data. In the field trial, additional years of data need to be analyzed and compared across years. Moreover, further comparison among all laboratory tests is needed (Table 8.1).

Table 8.1. A comparison of certain important factors among all tests that were evaluated in this research. Specific factors that are shown include whether the established nitrogen availability coefficients (NAC) over- (+) or underestimated (-) the test parameter or if they were equal (=), and whether or not the test parameter depended on soil type and/or rate, answered with "yes" (y) or "no" (n). If a factor was not applicable, it was labeled as "n/a."

	Biosolids								
	---------- Cary Pellets ----------			---------- OWASA Cake ----------			---------- Raleigh plus ----------		
Test	NAC	Depended on Soil Type	Depended on Rate	NAC	Depended on Soil Type	Depended on Rate	NAC	Depended on Soil Type	Depended on Rate
Anaerobic	-	y	y	-	y	y	+	y	y
ASNT	=	n	y	-	y	y	-	n	n
Field	-	n/a	y	n/a	n/a	n/a	n/a	n/a	n/a
Aerobic†	=	y	y	-	n	y	+	n	y

† Data has not been statistically analyzed.

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