ABSTRACT

WILLIAMS, JARED DEWEY. Soil Tests for Corn Nitrogen Recommendations and Their Relationships with Soil and Landscape Properties. (Under the direction of Drs. Carl Crozier and David Crouse)

An accurate soil nitrogen (N) test is needed to improve corn (*Zea mays* L.) production, profitability, and reduce environmental concerns of increasing nitrate (NO_3) levels in groundwater. The objectives of this study were to (i) compare four soil N tests: amino sugar N (ASN), gas pressure (GP), pre-plant NO_3 (PPNT), and incubation and residual N (IRN) for practicality, precision, and ability to predict corn response parameters; (ii) examine relationships between economic optimum N rates (EONR) and ASN levels to develop a fertilizer recommendation; and (iii) examine the spatial relationships of ASN with soil and landscape properties to develop a more efficient sampling strategy. Small-plot N-response trials (NRT) were conducted in 2001, 2002, 2003, and 2004 at 31 sites. Field scale (FS) ASN variability was measured in 2003 and 2004 at five sites located in the North Carolina Piedmont and Coastal Plain.

The ASN and PPNT tests were determined to be the most practical tests because they were easier to perform and could be completed in a day. The ASN test had lower coefficients of variation than the GP, PPNT, and IRN tests (10 versus 15, 30, 52%, respectively). Each tests was correlated with economic optimum N rates from NRT sites, but coefficients of determination were low for the PPNT, IRN, and GP tests. The ASN test had the strongest correlation with EONR, but only when sites were classified as well ($r^2 = 0.85$) or poorly ($r^2 = 0.78$) drained. A comparison of

ASN and EONR regression models across years showed no year effect. Additionally, the ASN test's ability to predict EONR was not affected when different corn price and fertilizer cost ratios were used.

Amino sugar N was positively correlated with humic matter (HM; r^2 = 0.25 to 0.71) at each FS site. Elevation was negatively correlated with ASN at the Lower and Middle Coastal Plain (r^2 = 0.44 and 0.11, respectively). At the two sites in the Piedmont, ASN was negatively correlated with slope gradient (r^2 = 0.10 and 0.04). Amino sugar N was positively correlated with percent clay for the Middle Coastal Plain (r^2 = 0.56) but negatively correlated for a site in the Piedmont (r^2 = 0.04). These correlations can be used to develop management zones for ASN soil sampling.

These results show that the ASN test was the best test for predicting economical optimum N rates and could be used to develop a soil test based fertilizer recommendation. Amino sugar N is spatially sensitive to changes in soil and landscape properties and soil sampling zones may be created using HM, soil texture, and soil type to reduce sampling costs and time.

SOIL TESTS FOR CORN NITROGEN RECOMMENDATIONS AND THEIR RELATIONSHIPS WITH SOIL AND LANDSCAPE PROPERTIES

by

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DEDICATION

I would like to dedicate this dissertation to my late mother, Debra Jane Williams, who encouraged me to obtain as much education as possible. It is her memory that has inspired me to reach for greater heights. She told me that I would be her “doctor” some day, and I only hope that in heaven she has a good sense of humor about me becoming a soil doctor instead of a medical doctor. I would also like to dedicate this dissertation to my wife, Katrina Williams, and my children, Debra, Tyler, and Kade whose support was paramount to my completion of this dissertation. I would like to dedicate this dissertation to my father, John D. Williams who raised me on a farm and taught me how to work hard. Finally, I would like to dedicate this dissertation to my stepmother who started me on my collegiate career by registering me for classes at private junior college.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

Current corn N recommendations in warm, humid areas often do not use a diagnostic test to determine corn N need. Soil tests have been either inaccurate in determining corn N (Schroeder et al., 2000) or require too much time to be used effectively for a pre-season or in-season fertilizer application. Nitrogen diagnostic tests based on crop parameters, such as tissue samples or aerial photography, only assess the N health of the crop at the time of measurement. These tests do not indicate whether adequate N is available in the soil for proper growth through maturity. Studies have shown the potential of three tests to predict N fertilizer response in humid regions of the United States (Meisinger and Bundy, 1994; Khan et al., 2001; Picone et al., 2002). These tests are the Illinois soil N or amino sugar N test (ASN, Mulvaney et al., 2001 and Khan et al., 2001), the gas pressure test (GP; Picone et al., 2002), and the incubation and residual N test (IRN, Bundy and Meisinger, 1994). These soil N tests may have the ability to accurately identify N fertilizer responsiveness and predict crop response factors such as economic optimal N rate (EONR), check yield (no N fertilizer applied), maximum yield, and/or delta yield (maximum yield - check yield). Crop response to N can be highly variable across fields (Scharf et al., 2005), which is the result of soil properties and landscape attributes. An understanding of how soil properties and landscape attributes affect N transformations, and a soil N test that determines plant-available N are necessary to predict N fertilizer need in corn.

This review will: (i) examine corn N need as the crop grows to determine usefulness of N diagnostic tests for fertilizer application at different corn growth

stages; (ii) discuss advantages and disadvantages of diagnostic tests for assessing crop N need; and (iii) discuss how soil properties and landscape attributes influence N transformation in soils and ways they might aid in developing N fertilizer recommendations using a diagnostic test.

Corn Nitrogen Requirement for Different Growth Stages

Understanding corn growth stages and the N need at each stage is critical in developing types and timings of diagnostic tests for accurate assessment of crop N health (Hanaway, 1962). Corn plants need N from the soil after seedling emergence and N uptake continues until after silking (R4; Fig. 1.1; Ritchie et al., 1989). Fertilization strategies for corn include numerous combinations of N fertilizer applications before planting, at planting, or during the growing season. In-season N fertilization is typically done using an early season (V2-5) or late-season side-dress application (V10-VT; Smeal and Zhang, 1994; Waskom et al., 1996). Nitrogen fertilization timing in humid regions is based on N diagnostic test accuracy and corn N need. For example, in the Northeast United States a pre-sidedress nitrate (NO_3) test has been used to accurately predict corn N need (Magdoff et al., 1984). The accuracy of this test is a result of waiting until the corn N demand is greater and avoiding measuring NO_3 that maybe lost to denitrification or leaching earlier in the growing season.

Crop Nitrogen Need and Fertilization at Vegetative Growth Stages

The rate of N uptake is constant until the plant reaches the V6 growth stage when N uptake accelerates (Fig. 1.1). Although side-dress fertilization before V5 does improve yields, waiting until at least the V5 growth stage has an even greater

effect on increasing yields (Ritchie et al., 1989; Evanylo, 1990 and 1991). Applying N at the V5-6 growth stage synchronizes it with the beginning of increased plant N need (Fig. 1.1). At this stage, risk of N loss due to NO_3 leaching and denitrification is lessened while insuring that N deprivation does not limit yield potential (Olson and Kurtz, 1982; Evanylo, 1990). Thus, diagnostic tools to assess N need at V5-6 are aimed at identifying situations where soil N contributions are minimal and often uncertain.

Corn continues to take N up at an accelerated rate during the mid and late vegetative growth stages (Fig. 1.1). A late vegetative N application during the period of greatest N uptake can increase N use efficiency and grain yield (Mengel and Barber, 1974; Bigeriego et al. 1979; Russelle et al., 1983). The advantage of N application during the V12 growth stage is that N diagnostic tools are more accurate (Sripada, 2005). Accuracy of N diagnostic tools improves with crop maturity because the influences on yield from soil moisture, disease and pest infestation, and limitations of soil N are more pronounced (Evanylo, 1991). While late vegetative N fertilizer application is best synchronized with higher rates of plant N uptake, corn does need N in the earlier growth stages, and if sufficient N is unavailable, grain yields may be irreversibly reduced (Scharf and Lory, 2002). Additionally, producers may be unable to wait this late in the season because of the unavailability of high clearance applicators and other unpredictable risks (e.g., wet soils). For irrigated land, N fertigation has been used to minimize some of the risk of late season N applications and allow producers to spoon feed N to corn in late growth stages (Blackmer and Schepers, 1995).

Crop Nitrogen Need and Fertilization at Reproductive Growth Stages

Corn continues to take up significant amounts of N throughout the reproductive growth stages (Fig. 1.1; Barber et al., 1992). Fertilizer application during the reproductive stages has been shown to increase kernel weight (Barber et al., 1992) and yield because 37-54% of total N uptake takes place during the reproductive growth stages (Ritchie et al., 1989; Fig. 1.1). Leaf and crop color diagnostic tools are very accurate in predicting corn N health and yield during R3-5 growth stages (Blackmer and Schepers, 1996). The disadvantage of fertilizing during the reproductive growth stages is most applicators lack the height to clear the crop and an earlier N fertilizer application is necessary to avoid N deficiencies at earlier growth stages. Fertigation could also be used to continue fertilization of a corn crop late into the reproductive stages. Nitrogen diagnostic tools, in general, are the most accurate at reproductive stages for assessing N status of a corn crop, but this may be too late for an effective fertilizer application (Piekielek and Fox 1992; Waskom et al., 1996).

In summary, a healthy corn crop needs significant amounts of N between the V5 and reproductive stages to achieve maximum grain yields (Fig. 1.1). In early vegetative growth stages, crop N diagnostic tools have not been very accurate in detecting corn N status, because soil N is susceptible to loss through denitrification and leaching (Schroeder et al., 2000). Diagnostic tools that can be used to predict corn N requirements during the V5-VT growth stages could be used to develop variable N fertilizer maps and allow for an in-season N fertilizer application.

Diagnostic Tools for Determining Corn Nitrogen Need

Diagnostic tools for determining crop N need are based on plant tissue N, crop color, or soil N. Each tool has advantages and disadvantages for assessing a corn crop's N need. For example, soil N tests measure the available N pool in the soil, whereas plant tissue and crop color diagnostic tools examine the amount of N already in the plant. As such, plant tissue and crop color tests ignore soil mineral N not yet taken up by the roots (Binford et al., 1992b; Schroder et al., 2000). Also, crop color diagnostic tools are unable to detect excess N uptake because plants exhibit little or no visible effects of excess N (Bundy and Andraski, 1993; Dwyer et al., 1995). Soil diagnostic tests can detect when the amount of available soil N exceeds the amount of N need for crop growth, but cannot measure the amount of N in the plant. Soil tests usually measure specific forms of N in the soil and often leave out organic N, which when mineralized becomes available for plant uptake (Bundy et al., 1999). This section will review the different plant tissue, crop color, and soil N diagnostic tools for predicting plant-available N. Additionally, the pros and cons of each tool, relative to its potential for developing in-season variable-rate fertilizer maps, will be given.

Plant Tissue Nitrogen Diagnostic Tools

Plant tissue tests were developed to measure the amount of NO_3 or total N concentration within the plant (Bundy et al., 1992). These tests examine NO_3 or total N content in a leaf or part of the corn stalk during or after the growing season. The tissue tests are destructive because the plant part being tested must be removed from the plant to be processed. The tests are laborious and time consuming

because a large number of samples must be acquired, dried, ground, and evenly mixed in order to achieve an accurate N assessment in the crop over a large area (Binford et al., 1990a). Plant tissue tests only assess the state of the plant at the time of sampling, and therefore, provide no indication of how much soil N is available or will become available during the remaining growing season.

Plant tissue $N0_3^-$ tests have been developed to measure concentration in different plant parts at different stages of plant growth. The validity of a plant NO_3 tissue test for corn is questionable because NO_3 is not an end-product in the plant (Schroeder et al., 2000). After plant uptake, NO_3 is transported to leaves and stems then quickly reduced and assimilated into numerous amino acids (Hanaway, 1962). Thus, differing levels of NO_3 have been found in different plant parts, with stalks generally having higher levels than leaf blades and upper leafs (Hanaway, 1962; Iversen et al., 1985a). Temporal changes in NO_3 concentration also occur. Low NO_3 concentrations are observed in the early afternoon and on sunny days because of ample availability of photosynthates (Iversen et al., 1985b; Schroder, 1999). In spite of these factors, a NO_3 plant tissue test of corn at the V6 growth stage showed that corn N health status could be determined using a 5-cm stalk sample taken from the base of the plant (Ritchie et al., 1989). More recent studies contradicted these findings and provided evidence that many factors play a role in plant NO_3 concentration and thus give mixed test results (Fox et al., 1989; Bundy and Andraski, 1993).

Another N tissue test for analyzing crop N health measures the amount of total N concentration from a leaf or stalk tissue sample. Total N concentration of

young plants changes in response to N in the soil; as a result a positive relationship between total N concentration and yield has been established (Binford et al., 1992). A problem with measuring total N concentration is that plants can take up N in amounts greater than physiological needed (i.e., luxury consumption). The test is unable to distinguish between sufficient and luxury amounts of N in the plant (Cerrato and Blackmer, 1991; Binford et al., 1992; Soltanpour et al., 1995). An additional problem is that initially, the test's statistics were incorrectly developed and have resulted in an over estimation of the critical N concentration (Schroder et al., 2000). The total N test has a wide variation within and across sites which has been judged to be too large for practical use for assessing N health of the crop (Cerrato and Blackmer, 1991).

Early season NO_3 and total N concentration diagnostic tests have not proven consistent enough to develop fertilizer recommendations for corn. However, these tests have been refined and are valuable tools for certain crops where N affects crop quality (e.g., potatoes and sugar beets). These tests are also destructive to the crop and acquiring the large number of samples necessary for a representative assessment is not plausible. Finally, interpretations of these tests are often complicated. Relationships have been established between plant tissue NO_3 concentration and available soil N, but the relationships are not linear and fail to detect when soil N is in excess of crop need (Binford et al., 1990). Tissue N tests do not lend themselves to be used for site-specific management or development of variable-rate N fertilizer maps for corn because of the large amount of samples needed to accurately and spatially assess corn N need.

Crop and Leaf Color as a Nitrogen Diagnostic Tool

Crop and leaf color tests for determining corn N health are based on the expression of types and amounts of plant pigments such as chlorophyll. Chlorophyll is a pigment that is very efficient at absorbing visible light, but it absorbs unequal amounts of light in the red (R), green (G), and blue (B) wavelengths (Walburg et al., 1982; Gardner, 1985; Hinzman et al., 1986; Blackmer et al., 1994). Plant leaves appear G because chlorophyll reflects more G than R or B light energy. The G brightness of the leaf is determined by the amount of chlorophyll in the leaf. As chlorophyll content becomes denser in the leaf the more light is absorbed especially in the R and B bands, but also the G. The increasing amounts of absorbed light results in the leaf becoming a darker green. Leaf chlorophyll content is positively correlated with N concentration (Wolfe et al., 1988).

Many tools have been developed to measure individual leaf or crop canopy color and quantify these differences between plants. These tools measure spectral properties through reflectance or transmittance from the visible and near infrared spectral bands. Aerial photographs have been used to detect crop N status at early and late vegetative stages. In photographs where the corn canopy is not closed, soil reflectance is a major obstacle in analyzing imagery, e.g. between the V5 and VT corn growth stages (Bausch and Duke, 1996; Scharf and Lory, 2002). Instruments used to measure differences in leaf color and predict corn N need through transmittance are typically hand-held instruments like the Minolta SPAD chlorophyll meter, which uses an active light source transmitted through the leaf (Blackmer and Schepers, 1995; Bullock and Anderson, 1998).

Remotely sensed data such as that obtained via aerial photography has an advantage over other N diagnostic tools because it allows for a thorough analysis of an entire field area by its ability to record large amounts of data relatively inexpensively (Blackmer and Schepers, 1996). However, all crop color tests are subject to error because visible expressions of insect or pathogen damage and nutrient and water deficiencies may be similar to N deficiency, and thus ground truthing with *in situ* measurements is needed for correct diagnosis causes of stress (Wildman, 1982). Aerial photographs taken from different fields and hybrids may be incomparable because of different soil and hybrid spectral signatures.

Remote sensing is a potential new N management aid, because strong relationship between corn color and corn N need between the V11 and R4 growth stages could be used to develop a late season N diagnostic test for a remedial N application (Scharf and Lory, 2002). Remote sensing of a late vegetative or reproductive growth stage corn crop may also be an aid for future N management where some of the N fertilizer is applied earlier in the season and remote sensing is used later in the season to assess corn N health and insure the crop has sufficient N (Bausch and Duke, 1996).

The SPAD chlorophyll meter has been used to determine the N health of a corn plant, but several disadvantages have impeded its use. Collecting numerous measurements required to achieve a good representation of a crop's N health status is laborious and expensive, and is therefore not practical for producers. Furthermore, the chlorophyll meter has not been able to accurately predict how much N fertilizer will be needed for the current growing season (Blackmer and

Schepers, 1995; Bullock and Anderson, 1998). The SPAD chlorophyll meter is also insensitive to NO_3 in the leaf and thus is unable to detect luxury consumption of N by the crop (Blackmer and Schepers, 1995). Luxury consumption is N taken up in excess of max plant demand.

Crop color N diagnostic tests are appealing because results from these tools are quickly obtained, and in the case of aerial photography, large amounts of data can be collected. One concern with crop color N diagnostic tests is that they can only accurately predict the N requirement late in the growing season, which is too late for an initial N fertilizer application (Scharf et al., 2002). Additionally, other corn stress problems are visually similar and crop color tests may not be able to distinguish between N deficiency and other crop stresses. Crop color N diagnostic tests may not replace current nutrient management tools, but likely will be an aid to these tools in improving optimal rates of N fertilization within fields (Blackmer and White, 1996).

Tools for Determining Soil Nitrogen

Soil Nitrate Tests

Soil NO_3 tests are widely used to determine soil N availability and for determining fertilizer needs. A number of concerns have been identified with soil NO_3 tests. Soil NO_3 tests may result in a N fertility recommendation that under predicts crop N need because of precipitation, soil permeability, and climatic conditions that alter N availability after the soil sample is taken (Magdoff, 1991). The tests have shown varying results when performed pre-plant, pre-sidedress, and post-harvest. The varying results of the NO_3 tests are largely because in some

climates NO_3 is subject to denitrification and leaching, thereby rendering it unavailable for plant use. Additionally, organic N can mineralize and nitrify increasing NO_3 levels after sampling (Schroeder et al., 2000).

Nitrate tests do not include testing for ammonium (NH_4) which is also a form of N that is also available for plant uptake (Schroeder et al., 2000). Ammonium usually is converted into NO_3 within days of being released into the soil, but some soil situations exist where exclusion of NH_4 from soil N tests can result in excessive N fertilizer recommendations. Despite the concerns with soil NO_3 tests, they have helped reduce over application of N fertilizer and non-point source pollution (Vanotti and Bundy, 1994).

Pre-plant soil NO_3 tests have been most successful in determining plant-available N in the semiarid regions of the southwestern and great plain states of the U.S. (Hergert, 1987). The tests have had limited success in more humid, higher rainfall areas (midwest and eastern states), where higher rates of denitrification and leaching reduce overall success (Bundy and Andraski, 1995). Further, the tests fail to account for N mineralization during the growing season which can result in over-application of N.

Pre-plant soil tests have been used to estimate residual N left from the past growing season in a continuous corn cropping rotation (Bundy and Andraski, 1995). Often excess inorganic N not used by the previous crop can remain in the soil under dry and cold conditions, and if weather conditions permit, will be available for crop uptake the following year (Bundy and Malone, 1988; Bundy et al., 1992; Vanotti and Bundy, 1994). Fertilizer recommendations can be adjusted by the amount of

residual N. The downside is that pre-plant NO_3 tests are unable to predict N loss after soil sampling due to denitrification or leaching nor additional N from mineralization, which gives uncertainty to fertilizer recommendations (Schmitt and Randall, 1994).

Pre-sidedress soil NO_3 tests (PSNT) have been found to be more useful than the pre-plant soil NO_3 test in the northeast states of the U.S. which are humid and have high rainfall. These tests are performed when corn is from 15 to 30 cm tall (V2-V4), typically 60 to 90 d after pre-plant NO_3 testing (Magdoff et al., 1984). Success of this test, in part, has been accredited to the fact that assessment occurs at a time when a more accurate evaluation of the capacity of the soil to supply N to the crop can be made (Schmitt and Randall, 1994). Later within-season testing allows more time for N mineralization, and therefore greater accuracy in predicting the additional amount of N needed to insure healthy crop growth through maturity.

Pre-sidedress NO_3 tests have also been shown to be sensitive to plant response to applied pre-plant N fertilizer (Bundy and Andraski, 1995). The test has been shown to effectively predict corn response to applied N fertilizer over a wide geographical area (Magdoff et al., 1984; Blackmer et al., 1989; Magdoff et al., 1990; Magdoff, 1991). At the same time, concerns about the test have risen after results on sandy and poorly drained soils showed a poor relationship between grain yields and the PSNT (Fox et al., 1989; Meisinger et al., 1992). Other studies have shown a strong correlation between the PSNT and lack of corn response to additional N application (Meisinger et al., 1992). The PSNT has been most useful for predicting non-responsive areas, which can help prevent over-fertilization (Blackmer et al.,

1989).

Soil NO_3 tests are not considered a feasible solution for developing in-season variable-rate N fertilizer maps. Soil NO_3 tests are laborious and time consuming. In order to create variable-rate N fertilization maps with soil NO_3 tests, a large number of samples would be needed to obtain an accurate assessment. Therefore, the tests are not quick enough or affordable for developing variable-rate N fertilizer maps. Further, the soil NO_3 tests give inconsistent results, mainly because they do not detect potential mineralizable N nor do they account for N loss when wet weather patterns persist (Schroder, 1999).

Soil Nitrogen mineralization Tests

Nitrogen fertilizer recommendations need to consider the soil organic N fraction that will be mineralized during the growing season and taken up by the crop. Predicting the fraction of soil organic N that will become available for crop uptake is difficult due to the effects of moisture and temperature on the N-cycle (Mulvaney et al., 2001). Different soil N mineralization tests have been developed based on how they determine the portion of soil organic N that will become available for crop uptake. Nitrogen mineralization test can be divided into biological and chemical tests (Bundy and Meisinger, 1994).

Biological tests attempt to imitate in the laboratory the biological processes that make soil organic N available under field conditions (Bundy and Meisinger, 1994). Many different methods exist for biologically estimating N mineralization. Long term (30-60 d) soil N mineralization tests under aerobic conditions have been the most accurate in predicting crop N need, but the incubation period makes the

test impractical for in-season N management (Keeney, 1982; Stanford, 1982). A short-term (7 d) anaerobic incubation test for determining available N was developed for in-season N recommendations (Waring and Bremmer, 1964, Stanford et al., 1974). The short-term test has had limited success in determining crop N need (Keeney, 1982), due to problems with sample handling and pretreatment (Bundy and Meisinger, 1994). The sources of mineralized N and the active microbial communities differ between the aerobic and anaerobic N mineralization tests, with the aerobic incubation more representative of field conditions (Boone, 1990). The anaerobic test is performed by water logging the soil and measuring the amount of NH_4 after a 7 d incubation. A concern with anaerobic incubation tests is they may have inflated results because aerobic microbes are killed in the anaerobic conditions and provide additionally N (Boone, 1990; Bundy and Meisinger, 1994). Additional error in measuring available N using an anaerobic biological test results because the tests do not consider soil NO_3 which is also available for plant uptake.

Chemical “N mineralization” tests are designed to estimate available N from soil organic N by using rapid and convenient chemical reactions. Chemical tests have had limited success because of low correlation with N mineralization and crop N need (Khan et al., 2001). Nevertheless, these tests have had limited uses as N availability indices for soils with differing past management (Bundy and Meisinger, 1994). Chemical N mineralization tests include hydrolysis with 2 *M* KCl at 100° C to estimate NH_4 (Gianello and Bremner, 1986), steam distillation with a pH 11.2 phosphate-borate buffer for measuring NH_4 (Gianello and Bremner, 1988), and UV absorbency at 200 nm of a $NaHCO_3$ soil extract which measures both NO_3 and

organic matter (Hong et al., 1990). These tests have shown correlations with biological N mineralization tests, but have been poorly correlated with available N in the field. Recently two chemical N mineralization tests, the hydrolyzable amino sugar N (Khan et al., 2001) and the gas pressure (Picone et al., 2002) tests, were developed and showed potential to be related to plant-available N in the field.

Amino Sugar Nitrogen Test

The ASN test was developed by examining the different components of organic N liberated through acid hydrolysis (soil hydrolysate) and NaOH alkalization (Mulvaney and Khan; 2001). The major components of the liberated organic N are total hydrolyzable N, hydrolyzable NH_4, amino acid N, and ASN (Mulvaney and Khan; 2001). A study of these components showed that hydrolyzable ASN was related to corn yield response to N fertilization (Mulvaney et al., 2001). Although, determining hydrolyzable ASN using a distillation method is tedious and not practical for routine laboratory analysis (Mulvaney et al., 2001; Khan et al., 2001). The alkalization (addition of NaOH) can be preformed directly on the soil, rather than on the soil hydrolysate. The modified test estimates hydrolyzable ASN by measuring alkali-hydrolyzable ASN. Hydrolyzable ASN and alkali-hydrolyzable ASN are well correlated ($r^2 = 0.82$). Soils high in alkali-hydrolyzable ASN were found to be non-responsive to fertilizer while sites with low alkali-hydrolyzable ASN were responsive (Khan et al., 2001). The trend between ASN and fertilizer response demonstrates potential for developing a N fertilizer recommendation with the ASN test (modified, alkali-hydrolyzable). The ASN soil N test does not consider soil NO_3, but the test might be improved by combining it with a soil NO_3 test for making a N fertilizer

recommendation for corn.

Gas Pressure Test

The GP test was originally developed for measuring NH_4 content in manure slurries (Chescheir et al., 1985; Piccinini and Bortone, 1991). The GP test is a measurement of pressure developed when a strong oxidizing reagent, $Ca(ClO)_2$, oxidizes NH_4 and organic matter to N_2 and CO_2 gas in a closed vessel. Ammonium is estimated from the pressure of the released gas which is proportional to the NH_4 in the manure. The $Ca(ClO)_2$ also oxidizes readily mineralizable forms of organic N in addition to oxidizing NH_4 (Cheschier et al. 1985). The GP test has been used to determine the amount of available N in poultry litter and soils, and the test was well correlated with other plant-available N tests such as a 24-d N mineralization incubation test ($r^2 = 0.77$; Picone et al., 2002). The greatest advantage to the GP test is its easy setup and rapid analysis time (25 min) which would make this test simple to implement into a routine laboratory procedure (Picone et al., 2002).

Soil and Landscape Properties' Influence on Nitrogen Mineralization

Site specific N need (i.e., variable-rate N fertilizer strategy) would be difficult to determine using a soil N tests because of the large number of samples needed. If available soil organic N is influenced by soil properties or landscape attributes, it may be possible to create management zones for soil sampling and N fertilization. Nitrogen fertilizer rates could be based on soil test values for each sampling zone, with application of variable-rate fertilizer across the field.

Soil Properties

The spatial variability of N in the soil is due to the rate of N cycle process

which are directly influenced by moisture, aeration and temperature and indirectly by soil properties. For example, mineralization of N from an organic form tends to be more rapid in loamy soils than clay soils because of better aeration (Delin and Linden, 2000; Schroder et al., 2000; Kitchen and Goulding, 2001). Soil properties have been used to understand N in the soil as it pertains to availability to the crop. Areas of the field which are susceptible to a high rate of N mineralization may need less N fertilizer. Soil with a high clay content may be susceptible to denitrification and soils with a high sand content have high leaching potential. High clay or sand content soils may need more N fertilizer than soils with a loamy texture. Some soil properties that may act as surrogates for measuring N mineralization and denitrification and aid in developing an in-season variable-rate fertilizer recommendation are soil organic matter (Schmidt et al., 2002), soil EC_a (Fritz et al., 1999), soil texture (Delin and Linden, 2000), and soil series (Blackmer and White, 1996).

Nitrogen is made available for plant uptake from organic matter through N mineralization. Relationships have been established between the amount of soil organic matter and N mineralization potential (Delin and Linden, 2000; Hatfield and Prueger, 2001; Schmidt et al., 2002). Soil organic N could be used to estimate N mineralization rates of the soil and be used to modify variable-rate application of N fertilizer (Schmidt et al., 2002).

Apparent soil electrical conductivity (soil EC_a) of bulk soil is a measurement of the soil's ability to transmit an electrical charge and is affected by soil salinity, cation exchange capacity, organic matter, volumetric water content, soil porosity, soil

temperature, and soil texture (McNeill, 1992). Soil EC_a has been related to soil conditions that interact with soil mineral N (Fritz et al., 1999). Fritz et al. (1999) suggested using soil EC_a to predict NO_3 concentrations in the soil. Two methods of measuring soil EC_a are electromagnetic induction (e.g., EM38 by Geonics Ltd.) and direct sensing with rolling coulters (e.g., Veris 3100 by Veris Inc.) A comparison of the EM-38 and Veris 3100 sensor cart for accuracy in predicting soil NO_3 concentrations showed a correlation with soil NO_3, but the authors indicate that further studies would be needed to confirm their results (Fritz et al., 1999). Soil EC_a measurements have also been found to correlate with grain yield under some circumstances, but the strength of the correlation is dependent on growing season precipitation and other factors (Jaynes et al., 1995; Kitchen et al., 1999). These studies suggest a possible relationship between soil EC_a and N mineralization and denitrification rates.

Soil texture, especially percent clay, influences the rate of denitrification and N mineralization (Delin and Linden, 2000). Soils with a fine texture or high clay content have been associated with high rates of denitrification and slow N mineralization rates. In contrast, soil textures which possess good aeration, such as soils that have a loamy attribute, have slower denitrification rates and faster N mineralization rates (Delin and Linden, 2000). Soils with high sand content or coarse texture are susceptible to N leaching. These relationships between soil texture and N mineralization and denitrification should aid in developing an in-season variable-rate N fertilizer recommendation (Schroder et al., 2000; Kitchen and Goulding, 2001).

Soil survey maps have been used to develop N management strategies (Blackmer and White, 1996; Ferguson et al., 1997). Different soil series interact differently with applied N fertilizer and have different rates of mineralization, denitrification, immobilization, and volatilization. Research has found N treatment plots that receive the same amount of N can have different N responses. These N responses may be related to soil characteristics captured in soil survey maps. Therefore, soil surveys may be helpful in developing a preseason, at-plant, or in-season variable-rate N fertilizer strategy (Blackmer and White. 1996).

Measuring N mineralization and denitrification can be expensive, laborious, and destructive, therefore soil properties may help in determining where to collect samples for measuring mineralizable N. Soil organic matter, soil EC_a, and soil texture are properties that might aid in predicting mineralization and denitrification of N in the soil, because they indirectly measure soil properties (soil moisture, aeration, and temperature) that control rates of microbial processes. Soil survey maps and bare soil images (Blackmer and White, 1996) might also be used to identify areas that have similar N mineralization and denitrification rates.

Landscape and Topography

The amount of plant-available N in the soil is indirectly affected by topography and landscape through surface and profile water movement (Franzen et al., 1996 Stevenson, 1982; Delin and Linden, 2000). The moisture content of the soil greatly influences mineralization and denitrification rates of N (Delin and Linden, 2000). Topographic indices have been created to model water movement across land surfaces (Wilson and Galant, 2000). Upslope catchment area (area above a defined

length of contour that contributes flow across the contour), specific catchment area (ratio of the contributing area to the defined contour length), and topographical wetness index (TWI; specific catchment area divided by slope) have been used to model water movement and predict the “wetness” or potential soil moisture of a soil. Topographic indices may aid in predicting N transformation rates due to their relationships with soil wetness.

Studies of residual N and landscape attributes have shown different spatial patterns. A study in Minnesota showed the effect of topography wherein high areas had higher amounts of residual N and low areas had low levels of residual N (Holland, 1996). Kitchen and Goulding (2001) demonstrated that landscape position influences plant-available N in Missouri. They showed that footslope positions have faster N mineralization rates than upslope positions with the exception of poor drainage and ponding in the footslope (Kitchen and Goulding, 2001). In North Dakota, Franzen et al. (1996) studied the effect of landscape position and concluded that similar landscapes had similar NO_3 levels. Solohub et al. (1996) showed that in Saskatchewan, Canada lower level and depression areas of the field had no yield response to N, but that shoulder and upper level positions did. Researchers have applied this knowledge and developed effective topographically based N rate maps (Solohub et al., 1996; Ferguson et al., 1997). Therefore, topographical and landscape data can help develop a pre-plant, at-planting, or in-season variable-rate N fertilizer recommendation by identifying N mineralization rates for different areas of the field.

Conclusion

The most effective time for N fertilizer application is during early corn growth stages (V5-V7; Magdoff 1991). A tissue N test can be used to accurately predict crop N health at the early growth stage, but a variable-rate N recommendation is impractical using tissue N tests (Fox et al., 1989; Bundy and Andraski, 1993). Remote sensing has been used to accurately predict corn N need later in growing season (V10-VT), but a variable-rate N application late in the growing season may be too late and irreversible yield losses may have already occurred (Scharf and Lory, 2002). Soil N tests have not been accurate in predicting available N during the growing season with the exception of biological N mineralization tests, but these tests are impractical for in-season N management because of 30 or 60 d incubations (Bundy and Meisinger, 1994). Short period (7 d) incubation tests have had some success (Waring and Bremner, 1964; Stanford et al., 1974). Additionally, the recently developed chemical N mineralization tests, ASN (Khan and Mulvaney, 2001) and GP (Picone et al., 2002), may be able to predict plant-available N during the growing season. Soil N tests, like tissue N tests, would be difficult to use for developing a variable N-rate strategy unless soil sampling zones could be created from soil properties and landscape attributes. Soil texture (Delin and Linden, 2000), moisture, and OM (Schmidt et al., 2002) influence N mineralization rates; and soil EC_a (Fritz et al., 1999) may be a quick method to measure those soil properties. Topography and landscape influence water movement and soil moisture and could be used to understand the spatial distribution of plant-available N.

The objectives of this study were to (i) compare practicality, precision, and

crop response predictability of four soil N tests, ASN, GP, PPNT, and IRN soil tests, (ii) examine the relationship between economic optimum nitrogen rates and the ASN test for developing a fertilizer recommendation, and (iii) examine the spatial relationships of ASN with soil and landscape properties to develop a site-specific, variable-rate N management strategy for North Carolina piedmont and coastal plain soils.

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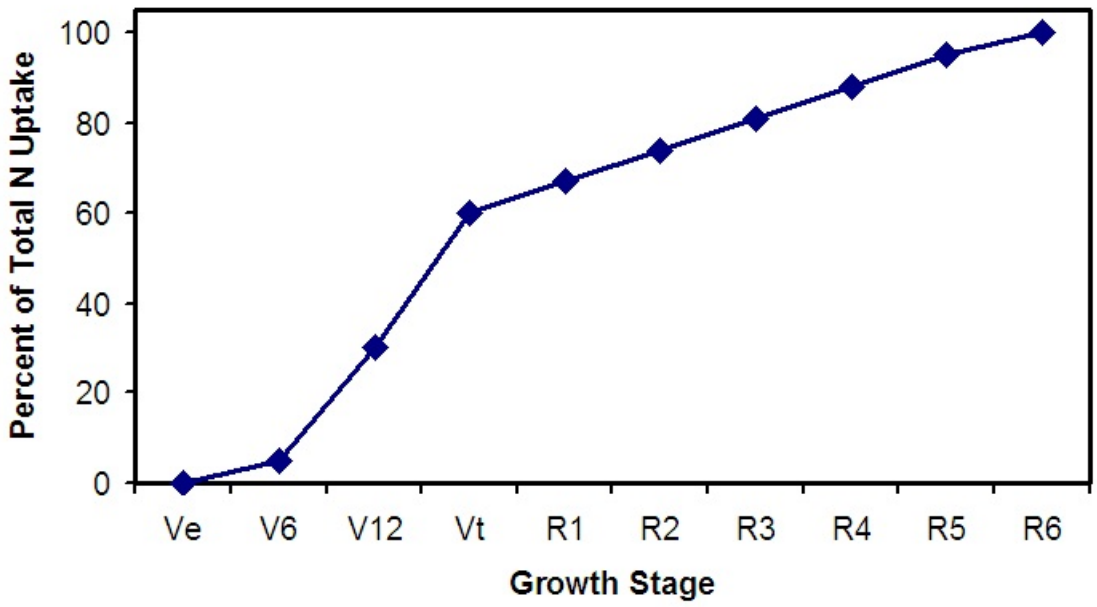

Figure 1.1. Corn growth and associated N need (modified from Ritchie et al. 1989).

CHAPTER II

COMPARISON OF FOUR SOIL NITROGEN TESTS FOR DETERMINING CORN NITROGEN NEED IN NORTH CAROLINA

Abstract

Environmental concerns of increasing nitrate (NO_3) levels in North Carolina watersheds indicate the need for better nitrogen (N) fertilizer management. Nitrate levels might be reduced if N rates could be adjusted based on knowledge of corn (*Zea mays* L.) response parameters to N fertilization. This study was conducted to compare four soil N tests: amino sugar N (ASN), gas pressure (GP), pre-plant NO_3 (PPNT), and incubation and residual N (IRN), for practicality, precision, and ability to correlate with corn response parameters (i.e., fertilizer response and economic optimum N rate). Soil samples and data were analyzed for 16 N response trials from 2001-2003 at sites that were expected to have different residual N levels. The ASN and PPNT tests were determined to be the most practical tests because they were easier to perform and could be completed in a day. The ASN test had better precision (lower coefficient of variation) than the GP, PPNT, and IRN tests (10 versus 15, 30, and 52%, respectively). The ASN test was significantly correlated with delta yield (maximum yield - check yield) and had an extrapolated non-responsive threshold of 145 mg ASN kg^{-1} soil. The ASN, PPNT, IRN, and GP tests were all correlated with economic optimum N rates, but ASN had the strongest correlation ($r^2 = 0.92$). These results show that the ASN test is the best soil N test among those tested and could be used to account for residual N levels and modify current N recommendations with the potential for a more accurate determination of crop N need.

Introduction

Currently recommended N management in North Carolina is based on realistic yield expectations by field or soil type and does not account for residual or potentially mineralizable N. This can result in over- or under-application of N fertilizer. Soils under certain management practices (e.g., manure applications or legume cover crops) or soils with high organic matter content may have sufficient residual and potentially mineralizable N for healthy corn growth and need little or no N fertilizer. Over-application of N fertilizer has been linked to high levels of NO_3 in shallow groundwater underneath North Carolina agricultural fields (Gambrell et al., 1974; Jacobs and Gilliam, 1985). The Neuse River Basin in North Carolina has experienced fish kills linked to non-point sources of NO_3 pollution (NC Division of Water Quality, 1996). Agricultural N pollution could be reduced by creating a more effective way of determining accurate fertilizer rates so less N is susceptible to leaching and runoff (Khan et al., 2001). This could be accomplished by using a soil N test to predict corn N need or corn responsiveness to N fertilizer. The soil N test could be used to adjust fertilizer recommendations. The challenge is developing a soil N test that is accurate, timely, and cost effective for predicting corn yield response to fertilizer or corn need for fertilizer N.

North Carolina's climate complicates soil N test development because of warm temperatures and high rainfall, which exceeds evapotranspiration, resulting in rapid mineralization, immobilization, leaching, and denitrification. The pre-plant NO_3 test (PPNT) and pre-sidedress NO_3 test (PSNT) have had limited success in estimating corn N need in the humid southern United States (Bundy et al., 1992;

Grove, 1992). Most soil N tests that measure mineralizable N are not practical for predicting yield response to fertilizer because they require a long incubation period (30 - 60 d; Bundy and Meisinger, 1994). Possible soil tests for predicting fertilizer response in corn would be the PPNT, an incubation and residual N (IRN) test (Bundy and Meisinger, 1994), the amino sugar N test (ASN, Khan et al., 2001), and the gas pressure test (GP; Picone et al., 2002).

Pre-plant Nitrate Test

The PPNT is a measurement of the amount of KCl-extractable NO_3 from a soil sample taken before corn planting. Pre-plant NO_3 soil tests have been used to estimate residual N (Bundy and Andraski, 1995). Excess inorganic N not used by the previous crop can remain in the soil under cold, dry conditions (e.g. arid western United States) and will be available for crop uptake the following year (Bundy and Malone, 1988; Bundy et al., 1992; Vanotti and Bundy, 1994). Fertilizer recommendations can be adjusted by the amount of residual N. The disadvantage of the PPNT is that it cannot predict N losses from denitrification or leaching or additions from mineralization after soil sampling, which gives uncertainty to predicting fertilizer response and corn N need (Schmitt and Randall, 1994).

Incubation and Residual Nitrogen Test

The incubation and residual N (IRN) test combines both residual NO_3 (PPNT; Bundy and Andraski, 1995) and NH_4 and mineralized N from a short-term (7 d) waterlogged incubation (Waring and Bremmer, 1964) to predict N that will be available for crop growth. The waterlogged incubation tests is a measurement of KCl-extractable NH_4 after a 7-d incubation from a soil sample taken before corn

planting. Independently, the PPNT and waterlogged incubation tests have had limited success in predicting of corn N need or fertilizer response (Meisinger and Bundy et al., 1994). The inability for these tests to predict corn N need and fertilizer response is because N provided to the crop by the soil comes from both the residual and mineralizable N pools (Bundy et al., 1992). Accounting for both inorganic and mineralizable N may better predict corn yield response to N fertilizer.

Amino Sugar Nitrogen Test

The ASN test was developed by examining the different components of organic N liberated through making a soil hydrolysate (acid hydrolysis) and alkalizing the soil hydrolysate with NaOH (Mulvaney and Khan, 2001). The major components of the liberated organic N are total hydrolyzable N, hydrolyzable NH_4, hydrolyzable amino acid N, and hydrolyzable ASN (Mulvaney and Khan, 2001). A study of the components of hydrolyzable soil organic N showed that hydrolyzable ASN was best correlated to corn yield response to N fertilization (Mulvaney et al., 2001). Determining hydrolyzable ASN using a distillation method is tedious and not practical for routine laboratory analysis (Mulvaney et al., 2001; Khan et al., 2001). An alternative method for determining ASN was developed by performing the alkalization directly on the soil, rather than using the soil hydrolysate, and measuring alkali-hydrolyzable ASN. Hydrolyzable ASN and alkali-hydrolyzable ASN are well correlated ($r^2 = 0.82$), and alkali-hydrolyzable ASN was accurately able to identify responsive and non-responsive sites in Illinois (Khan et al., 2001). The ASN threshold for differentiating fertilizer responsive and non-responsive sites warrants consideration for developing a North Carolina calibration.

Gas Pressure Nitrogen Test

The GP test was developed for measuring NH_4 content in manure slurries (Piccinini and Bortone, 1991). It measures pressure developed when the strong oxidizing reagent $Ca(ClO)_2$ oxidizes NH_4 to N_2 gas and reduced C to CO_2 in a closed vessel. Nitrogen mineralization is estimated by the increase in pressure (Cheschier et al. 1985). The GP test has been used to determine the amount of available N in poultry litter and soils (Picone et al., 2002). The GP test used with soils in Georgia was well correlated with other plant-available N tests values, e.g., the 24-d N mineralization incubation test ($r^2 = 0.77$) and soil microbial biomass C test ($r^2 = 0.90$; Picone et al., 2002). The correlation between the GP test and other plant-available N tests suggests it may also be correlated with corn yield response to N fertilization. The greatest advantage to the GP test is its rapid analysis time (25 min) which would make this test simple to implement into a routine laboratory procedure (Picone et al., 2002).

These soil tests could be performed before corn planting to modify N fertilizer recommendations based on realistic yield expectations. The objectives of this study were to: i) evaluate and compare the laboratory practicality and precision of the ASN, GP, IRN, and PPNT and ii) determine and compare the correlations of soil N test values and with crop N response parameters.

Materials and Methods

This research was conducted in the major row crop areas of North Carolina including the Piedmont and Middle and Lower Coastal Plain. Soils located in the Piedmont tend to have high clay content and are derived primarily from saprolyte.

Middle coastal plain soils tend to have a high sand content and are derived primarily from alluvial, marine, and marsh deposits. The Lower Coastal Plain has predominately sandy soils with high organic matter located near sea level, resulting in high water tables and poor drainage. Although soil parent material and genesis differ among the three regions, the dominant soil order is Ultisols (Table 2.1). The exceptions are the high water table and poor drainage areas of the Lower Coastal Plain where organic (Histosols) or mineral soils (Ultisols and Inceptisols) with high organic matter content have developed (Table 2.1).

Nitrogen response trials were conducted for 21 site-years in 2001, 2002, and 2003. Drought conditions in 2002 resulted in water stress at five sites and those sites were not analyzed as part of this study, resulting in 16 site-years being analyzed. Previous crop, irrigation, and tillage data for each site are presented in table 2.1. Soil samples were taken immediately before planting in April and May from each replication of each site-year. Soil samples were composites of six soil cores randomly taken from within each replication of a site to a depth of 20 cm. Samples were air dried and ground to pass through a 2-mm sieve. Nitrogen fertilizer was applied between the V4 and V10 growth stages, but typically at V7, at four to seven rates in a randomized complete block design with four to six replications (Table 2.2). The range in sidedress fertilizer application was the result of sampling soils from nitrogen response studies conducted by others. Nitrogen treatments were 4-rows wide and 10-m long and the experimental area was approximately 0.1 ha. Some sites received 12 to 112 kg N ha^{-1} at planting which was added to the side-dress N to calculate total N rates (Table 2.2). Grain yield data were collected by

hand (3 m) or machine harvesting (10 m) the middle two rows of each four row plot. All yield data were adjusted to15.5% moisture content. Soil humic matter (HM) was determined by the North Carolina Dept. of Agricutlure and Consumer Services Soil Testing Laboratory by extraction with 0.2 *M* NaOH + 0.02 *M* DTPA and percent HM determined colorimetrically (Mehlich, 1984).

Soil Nitrogen Test Methods

Amino Sugar Nitrogen Test

The ASN test was performed using a modification of the method of Khan et al. (2001). The modification was the use of an incubator instead of heating plates. The ASN test was performed by measuring 1.00 g of air-dried soil into a 0.47-L (1 pint) mason jar and adding 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 heated to 48-50˚C in an incubator for five hours. Thermometers were positioned randomly inside the incubator to verify temperature was within ± 1° C of 49° C. After the 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 (Khan et al., 2001; Univ. of Ill., 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, Univ. of Ill., 2004).

Gas Pressure Test

The GP test was conducted according to the Picone et al. (2002) method by placing 5.00 grams of air-dried soil and 5 ml of distilled water into a 125-ml serum bottle. An apparatus was created to suspend $Ca(ClO)_2$ above the soil slurry by placing a short piece of Tygon tubing in a septum then filling the Tygon tubing with 0.3 g of $Ca(ClO)_2$ and stopping it with a plastic cap. A hypodermic needle was used to push the plastic cap out of the Tygon tubing and allow the $Ca(ClO)_2$ to mix with soil slurry. The hypodermic needle was left in the septum for 3 s to allow pressure inside the bottle to equalize with the atmosphere. Since the needle sometimes became plugged with $Ca(ClO)_2$ and did not allow pressure to equalize, we modified the Picone et al. (2002) method by measuring initial pressure with a Tensimeter (Soil Measurements, Tucson, AZ) immediately after removing the hypodermic needle. The bottles were then placed on an orbital shaker at 250 rpm and agitated for 25 min. The pressure was measured immediately after shaking and pressure change was calculated by subtracting initial pressure from final pressure.

Pre-plant Nitrate Test

The PPNT test was performed by adding 5.00 g of soil, 20 ml of distilled water, and 20 ml of 2.0 *M* KCl to a plastic extraction cup. The samples were than agitated on a oscillating shaker for one hour and filtered with Whatman #2 paper into plastic scintillation vials. The vials were frozen until analysis for NO_3 could be conducted colorimetrically on a Lachat Autoanalyzer (Lachat Instruments, Loveland, CO).

Incubation and Residual Nitrogen test

The IRN test is a combination of a 7-d waterlogged incubation test for measuring residual NH_4 plus mineralizable N, and the PPNT test for measuring residual NO_3. The waterlogged incubation was performed by adding 5.00 g of soil and 20 ml of distilled water to a plastic extraction cup. The cups were capped and placed in an oven to incubate for 7 d at 35 - 40° C. After the incubation period, the samples were extracted by adding 20 ml of 2 *M* KCl to each sample and agitating on an oscillating shaker for one hour. The samples were filtered using Whatman #2 filter paper into scintillation vials (filtering was repeated if samples were not clear). Vials were frozen until NH_4 concentration could be determined colorimetrically on a Lachat Autoanalyzer (usually 7 d; Lachat Instruments, Loveland, CO). The IRN test values were calculated by adding the mg kg^{-1} NH_4-N from the incubated samples to the mg kg^{-1} NO_3-N concentration from the PPNT soil N test.

Statistical Analysis

Modified Coefficient of Variation

A modified CV was developed to compare the precision of soil N tests across different number of replications. The coefficient of variation was modified by replacing the observed mean (μ_{nx}, if $x < 6$) with the mean for the highest number of replications which was six (Equation 2.1). The modified CV shows the variation of each number of replications (2, 3, 4, 5 replications) compared to the mean of the six replications which was presumed to be the best predicted mean. Modified CV were calculated for every possible combination of two through five replications for each soil sample. A mean modified CV value was determined for each number (2, 3, 4, 5,

6) of replications for each soil N test.

$$\text{Modified C.V.} = \frac{\sqrt{\frac{\Sigma(X_i - \mu_{n6})^2}{n - 1}}}{\mu_{n6}} \times 100 \qquad [2.1]$$

where x_i = individual observation

n = number of observations (i.e., 2, 3, 4, 5, or 6)

μ_{N6} = mean of six observations, used in place of mean of 2, 3, 4, or 5 observations

Descriptive statistics were performed using PROC Mean in SAS version 8.3, and differences among soil N tests were determined using Tukey's means separation test in PROC GLM (alpha = 0.05; SAS Institute, Cary, NC).

Crop Response Parameters

Corn grain yield response to N fertilizer was modeled using a quadratic-plateau function in PROC NLIN in SAS version 8.3 (SAS Institute, Cary, NC). Check yield was determined as the corn yield from plots that did not receive N fertilizer, and for sites that did not have a 0 kg N ha^{-1} treatment check yield was extrapolated using the quadratic-plateau function. Economic optimum N rates (EONR) were derived using the first derivative of the quadratic plateau model and a price ratio of \$0.50 kg^{-1} N to \$100 Mg^{-1} corn (\$0.23 lb^{-1} N to \$2.50 bu^{-1} corn; Cerrato and Blackmer, 1990). If the EONR exceeded the highest applied N rate for a site, the highest applied N rate was used in place of EONR. If a response did not fit a quadratic-plateau function

(alpha = 0.05), treatment means were compared using a Tukey's means test to determine the optimum N level. Linear regression models of soil N test levels versus EONR were calculated using PROC REG in SAS version 8.3 (SAS Institute, Cary, NC). Economic optimum yield was determined as the yield at economic optimum N by either the quadratic-plateau function or Tukey's means test. Corn yield response to N fertilizer (fertilizer response) was calculated as 100 × (optimum yield - check-plot yield)/check-plot yield (Khan et al., 2001). Delta yield (yield response) was calculated by subtracting check yield from optimum yield. A N-sufficient threshold was represented by the x-intercept of the ASN versus EONR or delta yield function (0 kg ha^{-1} EONR or delta yield).

Regression analysis of soil N tests versus crop response parameters was performed using PROC Reg in SAS version 8.3 (SAS Institute, Cary, NC). Because the GP test could not be used on organic soils, analysis of soil N tests and crop response parameters were done for all (n = 16) sites and for mineral soil sites alone (n = 13).

Results and Discussion

Practicality of Lab Procedure

Soil tests were compared for lab procedure practicality based on the time required to analyze one sample, special equipment, hazardous waste, and whether the test can be used for mineral, organic, or both soil types (Table 2.3). The quickest tests were the GP and PPNT (1 and 1.5 h, respectively; Table 2.3). The time to process one sample for the ASN test was 5.5 h which was longer than the GP and PPNT, but still less than one day. The IRN test had the longest time to

process one sample (7.5 d), because it requires an incubation period. The ASN and GP tests required apparatus to be constructed and other special equipment (Table 2.3). The special apparatus for the GP test was disassembled as part of the test procedure and had to be reassembled each time the test was performed. The special apparatus for the ASN test (boric acid trap) was assembled once as part of the lab setup. Nitrate and NH_4^+ (part of the IRN and PPNT tests) were determined colorimetrically and required a colorimetric analyzer which is commonly found in soil testing laboratories. All the tests produced some waste, but the $Ca(ClO)_2$ in the GP test was the most hazardous because it had to be handled under a fume hood (Table 2.3). Another hazard of the GP test is the use of hypodermic needles which must be used carefully and disposed of properly. The GP test did not work on soils with high organic matter because the soils absorbed the water and did not form a slurry, thus did not allow the reagent to mix with the soil. Adjusting the ratio by increasing the amount of water might be appropriate for organic soils, but would decrease the sensitivity of the test, because some of the gas released by the reaction would be dissolved in the water (Picone et al., 2002).

The tests that we found most practical were the ASN and PPNT tests because they were the simplest to perform, could be completed in one day, and waste was easy to manage (Table 2.3). The GP test could be performed in the shortest amount of time, but the special apparatus was difficult to construct and the test could not be used for organic soils (Table 2.3). The IRN test was the most impractical because it required the longest amount of time to complete.

Precision of Soil Nitrogen Tests

Duplicates of each sample appeared sufficient, with little or no decrease in modified CV observed with additional replications (Fig. 2.1). The ASN test was determined to have the lowest modified CV for duplicate samples of the four test studied (Fig. 2.1). A technical note distributed by University of Illinois (Univ. of Ill., 2004) stated that CV should not exceed 2% if using an auto titrator instead of a manual color endpoint. The gas pressure test had the second lowest modified CV (Fig. 2.1) and the source of variation may be due in part to repeated piercing of the septa which may have created holes large enough to allow gas to escape. Sometimes the $Ca(ClO)_2$ would stick to the apparatus and would not fall and mix with the soil slurry. Picone et al. (2002) reported a decrease in pressure of 3 kPa when 0.20 g of $Ca(ClO)_2$ were used instead of 0.30 g. The PPNT and IRN soil test had the highest modified CV (Fig. 2.1). The large variation for the PPNT and IRN tests could be due to contamination with NO_3 from containers, filter paper, and water, even though blanks were used to correct for possible contamination. The IRN test had highest modified CV values because it included both the PPNT and waterlogged incubated ammonium test, and the test value is calculated as the sum of these two measurements, each with its associated errors. The waterlogged incubation itself could be variable because it is dependent on microbial activity.

Soil Nitrogen Tests to Predict Crop Response Parameters

Fertilizer Response

The yield response to N fertilization ranged between 27 and 395% (Table 2.4). In contrast to the Illinois study (Khan et al., 2001), no direct observation of

threshold level could be made due to the absence of non-responsive sites. Regression analyses of fertilizer response versus soil tests for all sites were not significant (Table 2.5). These results were perplexing especially for the ASN test because ASN for TOI2 (220 mg kg^{-1}), TCF (254 mg kg^{-1}), and GDA (488 mg kg^{-1}) were similar to or higher than the Illinois non-responsive threshold (225 to 235 mg kg^{-1}), but were responsive to fertilizer (Table 2.4). The TOI2, TCF, and GDA sites had organic soils which were poorly drained Histosols or very poorly drained Inceptisols with histic epipedons (Table 2.1). They had higher amounts of HM (> 10%) and ASN (220 - 248 mg kg^{-1}) than the mineral sites (HM = 0.3 - 4.3%, ASN = 21 - 73 mg kg^{-1}), but organic and mineral sites had similar ranges of fertilizer responses (Table 2.4). These findings suggest that correlation between fertilizer response and ASN may differ for mineral and organic soils, but no significant correlation was found between ASN and fertilizer response for mineral soil sites.

Check Yield

Check yield was not correlated with soil N tests (Table 2.5) which may be the result of the large diversity of soil productivity among sites. For example, corn grown in the piedmont region of the state on clay loam soils had an average corn yield of 6 to 9 Mg ha^{-1}, while corn grown in the Coastal Plain on loamy sand soils had an average yield of 10 to 12 Mg ha^{-1}. The differences in soil productivity resulted in different check yields and likely also influenced fertilizer response.

Delta Yield

Linear regression analysis of delta yield versus soil N tests showed only the ASN test from mineral sites being correlated with delta yield (r^2 = 0.41; Table 2.5 and

Fig. 2.2). An extrapolated yield-response threshold for determining the ASN for mineral soils at which delta yield equals zero was 145 mg ASN kg^{-1} (Fig. 2.2). The correlation between delta yield and ASN concentration for mineral sites might be used to modify N recommendations whereby sites with ASN at or above the yield response threshold would receive no N fertilizer, although no sites were above the threshold in this study.

Economic Optimum Nitrogen Rate

Regression analysis of EONR versus soil N tests showed only the PPNT test being correlated with EONR (r^2 = 0.47) for all sites (Table 2.5 and Fig. 2.3). When analyzing only mineral sites, regression models between EONR and each soil test were significant (Table 2.5 and Fig. 2.4). The best test for predicting EONR was the ASN test, but only for mineral sites (Table 2.5 and Fig. 2.4). The strong correlation between EONR and ASN shows potential for developing a N recommendation, but according to our results, its application would be limited to mineral soils. An analysis of ASN and EONR for organic soils was not explored because of insufficient organic sites, and a separate correlation may need to be developed. A possible explanation for the difference between mineral and organic soils is that the mineral soils are better drained and more susceptible to leaching and high rates of mineralization while the organic soils are poorly drained and susceptible to denitrification (Fox and Piekielek, 1984).

The PPNT test had an advantage over the other soil N tests, because it was able to predict EONR regardless of mineral or organic soil type (Table 2.5 and Fig. 2.3). Nevertheless, the PPNT regression coefficients of determination for predicting

EONR for all and mineral sites were low and would limit its usefulness for developing a fertilizer recommendation. The correlation between PPNT and EONR was stronger for all sites (r^2 = 0.47) than for the mineral sites (r^2 = 0.41; Table 2.5). The GP and IRN tests were correlated with EONR for the mineral sites, but their low coefficients of determination indicate a lack of potential for these tests being used for a N recommendation. Additionally, the GP test would have limited use in North Carolina, because it cannot be used with organic soils.

Conclusions

The best soil N test among those tested was ASN because it had a strong correlation with EONR, it was the simplest to perform, could be completed in one day, and hazardous waste was easy to manage. The ASN test was the most precise test in this study and might be improved by using an auto-titrator instead of color-determinated to an endpoint. The tests were not correlated with fertilizer response or check yield, but the ASN test was correlated with delta yield for the mineral soil sites. Economic optimum N rates may be a better crop response variable for developing N fertilizer recommendation, because it is a function of how much N is needed to obtain economically maximum yield. The ASN, PPNT, IRN, and GP tests were correlated with EONR for mineral sites, and ASN had the strongest correlation. The regression models using ASN to predict delta yield and EONR for the mineral soils had extrapolated non-responsive thresholds of 145 and 125 mg ASN kg^{-1}, respectively. The extrapolated threshold values could be used to alter N rate determinations by recommending no N fertilizer when ASN is above the thresholds. Additionally, the relationship between EONR and the ASN test could be

used to develop a N recommendation for corn on mineral soils. The correlations of EONR with the PPNT and GP tests were weak and unlikely to be useful to make N recommendations.

The ASN test was the best test of the four soil N tests included in this study based on logistics, precision, and correlation with corn N response parameters. Further studies need to be conducted to understand the influence of mineral and organic soil properties, year, region, and site on the relationship of ASN and EONR in order to refine N recommendations for corn.

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Table 2.1. Detailed soil information for research plots.

Year	Site	County	Soil Series	Soil Taxonomic Classification	Previous Crop	Irrigation[†]	Tillage[‡]
				Piedmont			
2003	CLE	Cleveland	Cecil clay loam	Fine, kaolinitic, thermic Typic Kanhapludults	corn	N	NT
2003	SAL3	Rowan	Hiwassee clay loam	Fine, kaolinitic, thermic Typic Rhodudults	soybean	N	CT
				Middle Coastal Plain			
2001	PBI1	Bertie	Norfolk loamy sand	Fine-loamy, siliceous, thermic Typic Paleudults	wheat-sorghum	N	CT
2001	PBI2	Bertie	Norfolk loamy sand	Fine-loamy, siliceous, thermic Typic Paleudults	wheat-sorghum	I	CT
2003	PBI3	Bertie	Norfolk loamy sand	Fine-loamy, siliceous, thermic Typic Paleudults	corn	I	CT
				Lower Coastal Plain			
2001	TI1a	Washington	Cape Fear loam	Clayey, mixed, thermic Typic Umbraquults	corn	I	CT
2001	TI1b	Washington	Cape Fear loam	Clayey, mixed, thermic Typic Umbraquults	corn	I	CT
2002	TMI2	Washington	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	corn	I	CT
2002	TOI2	Washington	Belhaven muck	Loamy, mixed, dysic, thermic Terric Medisaprists	corn	I	CT
2003	GDA	Washington	Wasda muck	Fine-loamy, mixed, semiactive, acid, thermic Histic Humaquepts	soybean	N	NT
2003	TCF	Tyrrell	Weeksville silt loam	Coarse-silty, mixed, semiactive, acid, thermic Typic Humaquepts	wheat-soybean	N	NT
2003	TE3	Washington	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	soybean	N	NT
2003	TMI3	Washington	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	soybean	I	NT
2003	TMN3	Washington	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	soybean	N	CT
2003	TOI3	Washington	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	soybean	I	CT
2003	TON3	Washington	Hyde loam	Fine-silt, mixed, thermic Typic Umbraquults	soybean	N	CT

[†]I, irrigated; N, not irrigated

[‡] CT, conventional tillage; NT, no-till

Table 2.2. Replication and fertilizer information for research plots.

Sites	Replications	At-planting N treatments (kg ha^{-1})	Sidedress N treatments (kg ha^{-1})
		Piedmont	
CLE	6	0	0, 56, 112, 168, 224
SAL3	6	26	0, 56, 112, 168, 224
		Middle Coastal Plain	
PBI1	3	0, 112	0, 112, 168, 224
PBI2	3	0, 112	0, 112, 168, 224
PBI3	3	0, 56	0, 112, 168, 224, 280
		Lower Coastal Plain	
TI1a	3	0	0, 112, 168, 224
TI1b	3	0	0, 112, 168, 224
TMI2	4	0	0, 112, 168, 224
TOI2	4	0	0, 112, 168, 224
GDA	6	24	0, 34, 68, 100, 134, 168, 202
TCF	6	56	0, 34, 68, 100, 134, 168, 202
TE3	6	28	0, 34, 68, 100, 134, 168, 202
TMI3	4	0, 56	0, 112, 168, 224, 280
TMN3	4	0, 56	0, 112, 168, 224, 280
TOI3	4	0, 56	0, 112, 168, 224, 280
TON3	4	0, 56	0, 112, 168, 224, 280

Table 2.3. Comparison of four soil nitrogen tests procedures.

Test	Time†	Equipment ¶	Hazardous waste §	Mineral and organic soils‡
Amino sugar nitrogen	5.5 h	mason jars boric acid trap microburette (5 mL) incubator	Boric acid	both
Gas pressure	1 h	Serum bottles (125 mL) Rubber septa $Ca(ClO)_2$ apparatus tensimeter rotary shaker	$Ca(ClO)_2$	mineral
Pre-plant nitrate	1.5 h	oscillating shaker extraction vials colorimetric autoanalyzer	Sulfamilanide NEDD NH_4Cl	both
Incubation and residual nitrogen	7.5 d	oscillating shaker incubator extraction vials colorimetric autoanalyzer	Same as pre-plant nitrate Salicylate-nitroprusside Na_2EDTA	both

† amount of time needed to complete analysis of one sample
¶ Equipment needed to perform test
§ Hazardous waste created as part of performing tests
‡ Can test be used on mineral and/or organic soils

Table 2.4. Soil nitrogen tests results and fertilizer response.

Site	ASN	GP	IRN	PPNT	Fertilizer Response	Delta Yield	Check Yield	EONR
	mg kg^{-1}	kPa	mg kg^{-1}	mg kg^{-1}	%	------ Mg ha^{-1} ------		kg ha^{-1}
				Piedmont				
CLE	58	2.6	55.0	4.6	154	6.0	3.9	205
SAL3	48	2.2	13.1	8.0	288	9.8	3.4	212
				Middle Coastal Plain				
PBI1	26	1.7	17.6	0	215	8.8	4.1	311
PBI2	21	0.6	14.8	0	212	8.5	4.2	336
PBI3	14	0	1.4	0.6	241	6.5	2.7	336
				Lower Coastal Plain				
TI1a	66	8.2	22.9	4.0	64	3.3	5.8	195
TI1b	67	8.0	23.4	1.5	110	4.3	3.9	168
TMI2	73	6.5	22.1	2.9	27	2.0	7.4	113
TOI2[†]	220	--	54.5	17.6	35	2.6	7.4	89
GDA[†]	488	--	97.4	5.5	395	8.3	2.1	201
TCF[†]	256	--	75.6	2.7	173	5.2	3	200
TE3	56	7.1	31.6	2.2	94	4.4	4.7	177
TMI3	74	9.2	36.7	6.2	126	5.4	4.3	178
TMN3	70	6.2	38.2	8.5	122	5.5	4.5	181
TOI3	68	6.6	29.0	6.7	183	5.5	3	178
TON3	82	6.3	35.1	6.4	233	5.6	2.4	140
				Descriptive statistics				
mean	105	5.0	35.5	4.8	167	5.7	4.2	201
st. dev.	121	3.1	24.7	4.4	97	2.2	1.6	71

[†]Organic sites

ASN, amino sugar nitrogen test; GP, gas pressure test; IRN, incubation and residual nitrogen test; PPNT, pre-plant nitrate test; EONR, economic optimum nitrogen rate

Table 2.5. Coefficients of determination from linear regression models of soil N tests results versus crop response parameters.

	Fertilizer Response		Check Yield		Delta Yield		EONR	
Soil N test	All	Mineral	All	Mineral	All	Mineral	All	Mineral
ASN	NS	NS	NS	NS	NS	0.41**	NS	0.92***
GP	--	NS	--	NS	--	NS	--	0.54**
PPNT	NS	NS	NS	NS	NS	NS	0.47**	0.41*
IRN	NS	NS	NS	NS	NS	NS	NS	0.31*

*,**,***, NS, significant at the 0.05, 0.01, 0.001 probability levels and not significant, respectively
ASN, amino sugar nitrogen test; GP, gas pressure test; IRN, incubation and residual nitrogen test; PPNT, pre-plant nitrate test; EONR, economic optimum nitrogen rate

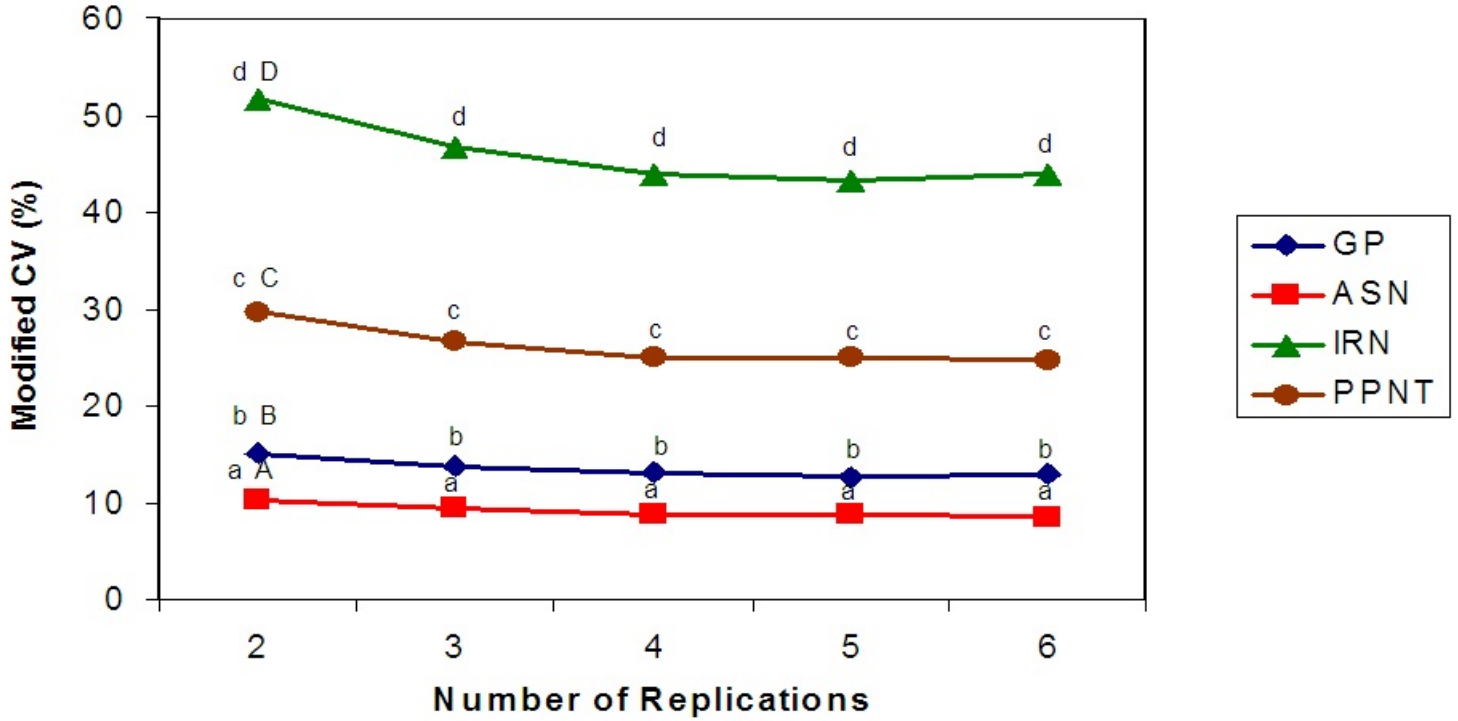

Figure 2.1. The influence of number of laboratory analysis replications on CV for each soil N test. Lower case letters denote statistical ($\alpha = 0.05$) difference among replication for each test and capital letters denote statistical difference among tests (two replications only).

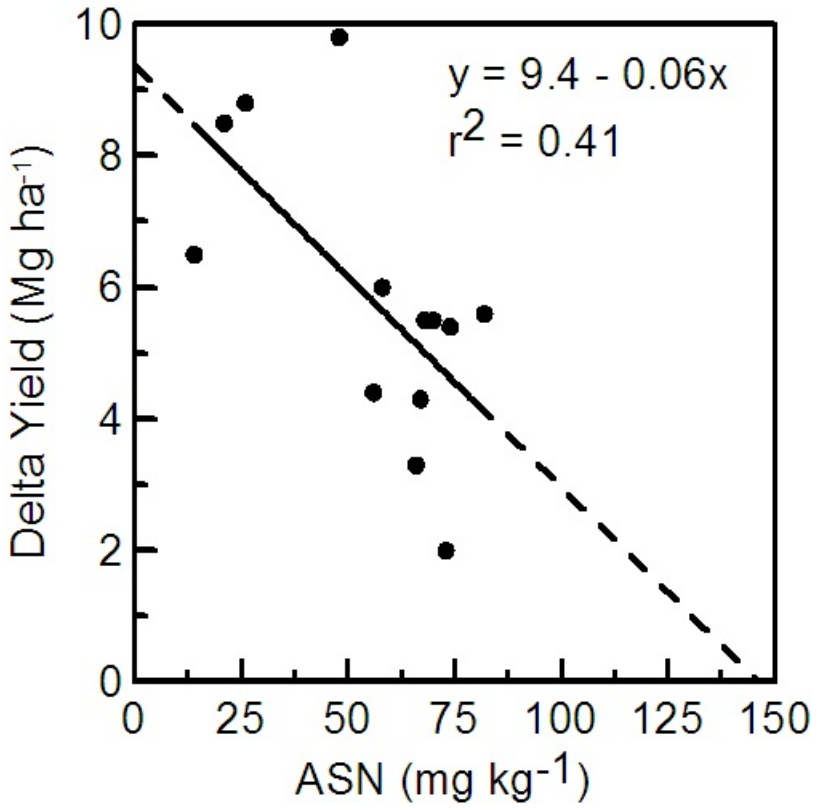

Figure 2.2. Delta yield as a function of amino sugar nitrogen (ASN) for mineral soil sites only. Extrapolation (dashed line) shows non-responsive threshold (145 mg kg^{-1}).

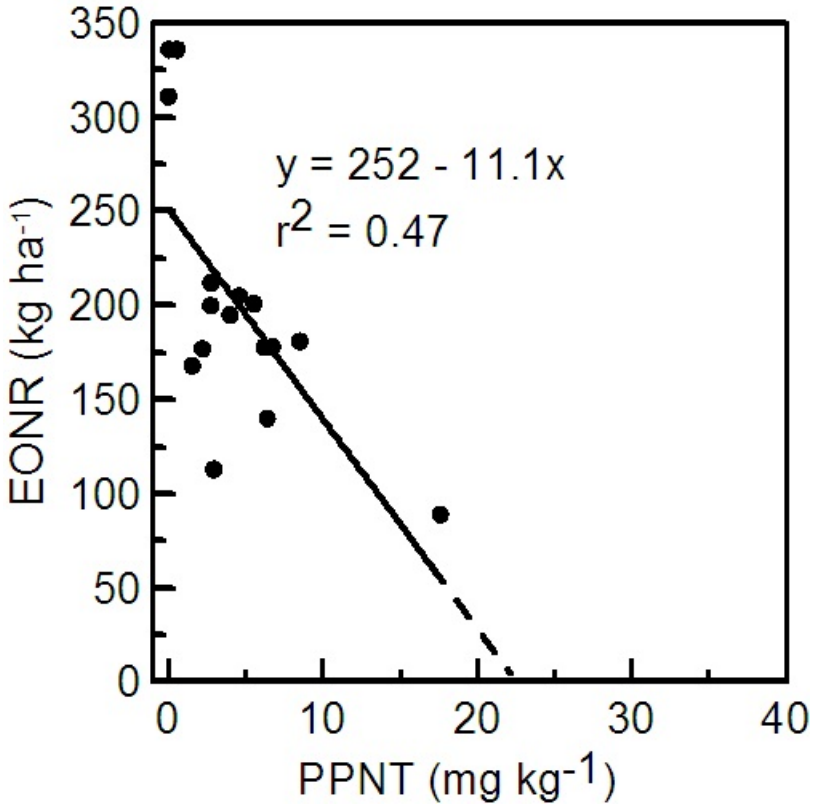

Figure 2.3. Economic optimum itrogen rate (EONR) as a function of pre-plant NO_3 (PPNT) concentration for all sites. Interpolation (dashed line) shows the threshold at which no fertilizer would be recommended based on PPNT.

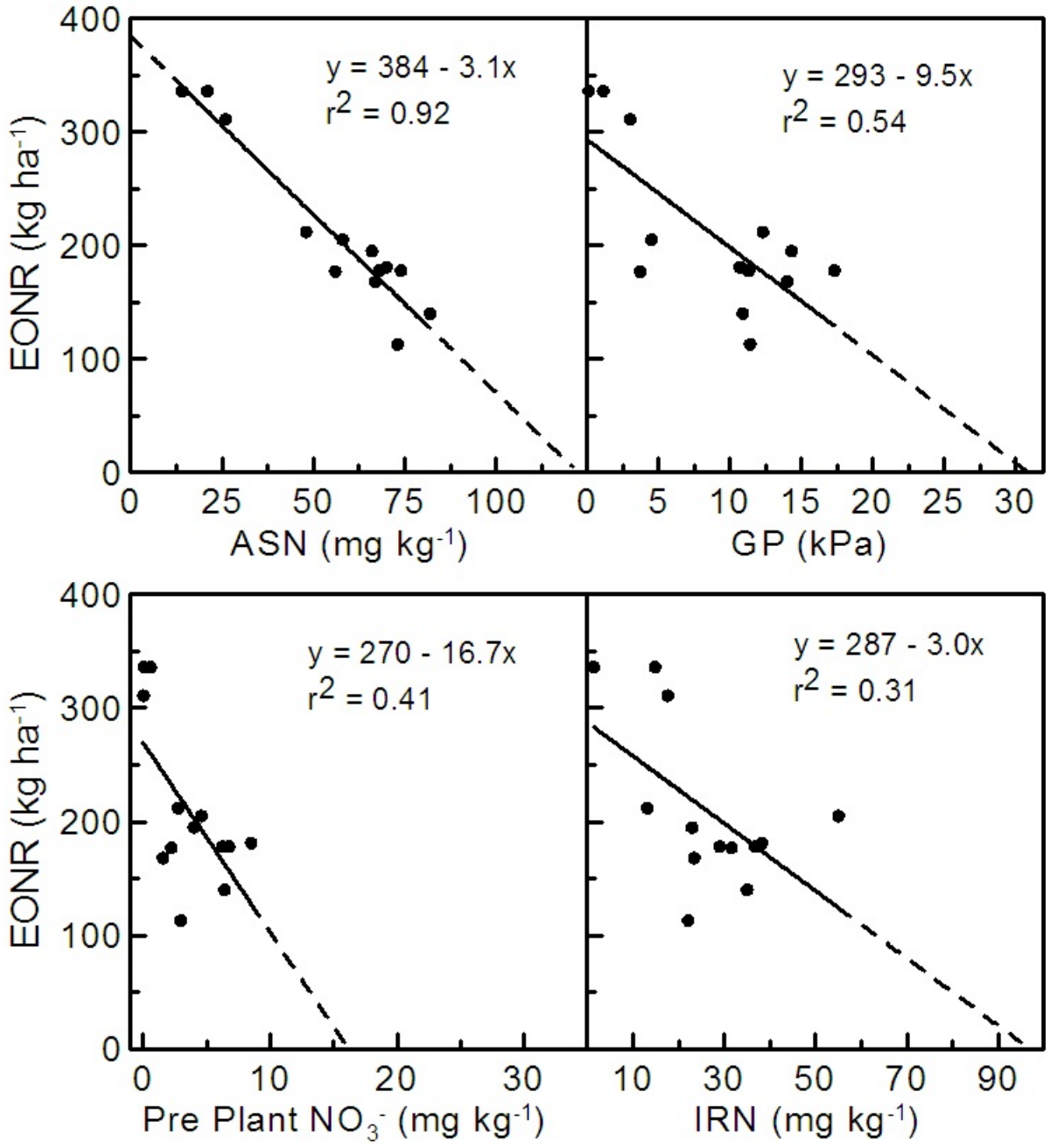

Figure 2.4. Economic optimum N rate (EONR) as a function of soil N tests (amino sugar N, ASN; gas pressure, GP; pre-plant NO_3; and incubation and residual N, IRN) for mineral soil sites. Extrapolation (dashed line) shows the threshold at which no fertilizer would be recommended based on soil N tests.

CHAPTER III

AMINO SUGAR NITROGEN TO PREDICT CORN ECONOMIC OPTIMUM NITROGEN FERTILIZER RATES

Abstract

An accurate and quick soil nitrogen (N) test is necessary for developing soil-based N fertilizer recommendations for corn (*Zea mays* L.) for the humid southeastern United States. The amino sugar N (ASN) test has been used to distinguish between fertilizer responsive and non-responsive sites in Illinois. This study was conducted to determine the relationships between economic optimum nitrogen rates (EONR) and amino sugar levels in support of a fertilizer recommendation. Soil samples were acquired from 31 N response trials located in the Piedmont and Middle and Lower Coastal Plains of North Carolina from 2001-2004. The ASN test was not correlated with EONR for all sites, but was strongly correlated for sites that were classified as well- or poorly drained (R^2 = 0.85 and 0.78, respectively). A comparison of regression models of EONR versus ASN across years showed no year effect, which suggests that ASN can predict EONR independently of environment, although drought-stressed sites were not analyzed as part of this study. The ASN test's ability to predict EONR was not affected when different corn price and fertilizer cost ratios were used. Because the relationship between ASN and EONR was robust across years and with different cost ratios, it could be used to modify or replace current N recommendation methods and foster appropriate fertilizer application.

Introduction

Nitrogen fertilizer management for corn is coming under increasing scrutiny because of environmental concerns. Agricultural application of N has been attributed to increasing levels of NO_3 and fish kills in the Mississippi River, the Gulf of Mexico (Cast, 1999), and in North Carolina's Neuse River (Gambrell et al., 1974; Jacobs and Gilliam, 1985; NC Division of Water Quality, 1996). Nitrogen fertilizer management based on realistic yield expectations (RYE) has reduced fertilizer inputs, but NO_3 levels remain too high. In North Carolina, RYE N recommendations are made from a database of yields by county and soil series or based on a grower's documented actual historic yields (the average of the best three yields over a five year period; North Carolina Nutrient Management Workgroup, 2003). The recommended N rate is calculated using the RYE and a soil and crop dependent N factor (18 to 22.5 kg N Mg^{-1} corn grain yield). Nitrogen fertilizer recommendations based on yields are accurate some of the time, but can result in under or over-application in part because they do not consider soil N. Over-applying N fertilizer can cause serious environmental concerns while under- or over-applying may have economic consequences for corn producers. A diagnostic tool is needed to refine estimation of plant-available N.

Nitrogen diagnostic tools can be categorized as plant- or soil-based. Plant-based tools include plant tissue and crop color tests. Early season plant tissue tests that measure NO_3 or total N concentration have not been consistent enough to develop fertilizer recommendations (Fox et al., 1989; Bundy and Andraski, 1995). Crop color tests are based on chlorophyll meters or aerial photographs, and neither

tool can be used to detect luxury consumption of N in corn (Blackmer and Schepers, 1995). Another concern with crop color tests is that they cannot accurately predict corn N need before the corn tasseling growth stage (Sripada, 2005).

Soil-based diagnostic tools can be categorized into residual and potential mineralizable N tests. Residual soil NO_3 tests such as the pre-plant NO_3 test (PPNT) and the pre-sidedress NO_3 test (PSNT) measure NO_3 remaining in the soil from previous crops, fertilizer applications, and mineralization reactions (Bundy et al., 1992; Magdoff et al., 1984). Residual NO_3 tests are widely used in the western United States to determine soil N availability for plant uptake and fertilizer needs (Bundy et al., 1992). A number of concerns have been identified with using residual NO_3 tests in North Carolina. Warm temperatures and high rainfall in North Carolina result in residual NO_3 being lost through leaching and denitrification. Residual NO_3 tests also do not measure N that may be mineralized after sampling, resulting in under-estimation of the soil N supply.

Excluding fertilizer, the largest source of N taken up by a corn crop in high rainfall areas like North Carolina tends to be N mineralized from organic matter (Crozier et al., 1994 and Crozier et al., 1998). Soil N tests that measure potentially mineralizable N may be better for predicting corn N requirements than residual NO_3 tests. Measuring the fraction of potentially mineralizable N that will become available for crop uptake during the growing season is difficult because of the effects of moisture and temperature on the N cycle (El-Sadek et al., 2002; Delgado et al., 2001). Soil N mineralization tests have been developed based on how they determine potentially mineralizable N and can be divided into biological and

chemical tests.

Biological N mineralization tests attempt in the laboratory to imitate biological processes that make soil organic N available under field conditions (Bundy and Meisinger, 1994). One concern with biological N mineralization tests is that they require an incubation period of 7 to 60 d (Keeney, 1982). The longer anaerobic tests can accurately predict mineralizable N, but are impractical because of the length of the incubation period (Standford, 1982). Short-term (7 d) anaerobic incubation tests may be more practical, but have proven to be poor predictors of corn N need (Fox and Piekielek, 1984; Williams, 2005).

Chemical N mineralization tests are designed to quantify specific chemical fractions present in soils. Chemical tests have not had widespread use because of low correlation with N mineralization and crop N need (Khan et al., 2001). Nevertheless, chemical tests have had limited use as N availability indices for soils with differing past management (Bundy and Meisinger, 1994). Recently, a chemical N mineralization test was developed by separating the different components of organic N liberated through acid hydrolysis: hydrolyzable NH_4, amino acid N, and amino sugar N (ASN; Mulvaney and Khan, 2001). A study of the hydrolyzable soil organic N components showed that ASN was related to corn yield response to fertilization in Illinois (Mulvaney et al., 2001; Khan et al., 2001). Williams (2005) developed response functions for ASN and crop response factors for corn that could be used to refine yield-based N recommendations. The results of the Illinois (Khan et al., 2001) and North Carolina (Williams, 2005) studies show potential for developing economic optimum N rate (EONR) recommendations for corn using the

ASN test.

Economic optimum N rates can be used to adjust N recommendations based on a cost ratio. Use of monetary values allow producers to calculate optimum N rates for any specific combination of fertilizers costs and corn grain value thereby maximize profits (Kelling and Bundy, 2001). Several different response models have been used to create optimum N rates, but a quadratic plateau model has been shown to best describe yield response (Cerrato and Blackmer et al., 1990).

The objectives of this study were to: i) examine the correlations between ASN and EONR for developing a soil test-based N recommendation; ii) evaluate the effects of soil, year, and seasonal temperature on ASN to predict EONR; and iii) compare the effect of different economic cost ratios on EONR as predicted by ASN.

Materials and Methods

This research was conducted in three major row-crop areas of North Carolina, the Piedmont and Middle and Lower Coastal Plains. Soil parent material and genesis are different among the three regions, but Ultisols predominate (Table 3.1). The exception is the high water table and poor drainage areas of the Lower Coastal Plain where organic (Histosols) or mineral soils (Ultisols and Inceptisols) with high organic matter content have developed (Table 3.1).

Soil Sampling

Soil samples were taken immediately before planting in April and May of 2001, 2002, 2003, and 2004 from 36 N response trials. Drought conditions in 2002 resulted in water stress and little or no yield response to N fertilizer at five sites, and those sites were not analyzed as part of this study resulting in 31 sites being

analyzed. Soil samples were composites of six soil cores randomly taken from within each replication of a site to a depth of 20 cm. Samples were air dried and ground to pass through a 2-mm sieve. Nitrogen fertilizer was applied between the V4 and V10 growth stages, but typically at V7, at four to seven rates in a randomized complete block design with four to six replications (Table 3.2). The range in sidedress fertilizer application is the result of using soil samples from nitrogen response studies conducted by others. Nitrogen treatment plots were 4-rows wide and 10-m long and experimental area was approximately 0.1 ha. Some sites received 12 to 112 kg N ha^{-1} at planting which was added to the side-dress N to calculate total N rates (Table 3.2). Grain yield data were collected by hand harvesting 3 m or machine harvesting 10 m of the middle two rows of each. All yield data were adjusted to 15.5% moisture content. Past cropping data for each site is located in table 3.1.

Drainage Type

Sites were categorized into three drainage types: well, poorly, and very poorly drained (Table 3.1). Criteria for delineating sites by drainage type were soil survey drainage classification, presence or absence of artificial drainage structure (tiles, ditches, and crownings), and field observations during the growing season. Well-drained sites were defined as having soils that were naturally well-drained or tile drained (Table 3.1). Poorly drained sites were defined as having either poorly drained soils without adequate artificial drainage (no tile, ditch, or crown drainage or observed poor drainage) or organic soils. Very poorly drained sites were defined as having a very poorly drained soil survey drainage classification and no artificial

drainage.

Soil Properties

Soil texture analyses were performed by using the hydrometer method to determine percent sand, silt, and clay. Hydrometer readings were taken at 30 s and 90 s to determine the sand fraction and at 6 h, and 16 h for the clay fraction (Gee and Bauder, 1979). Soil bulk density samples were taken at a depth of 10 cm at each site using a Lutz ring. Samples were oven dried and weighed to determine dry bulk density (Blake and Hartge, 1986). Soil humic matter (HM) was determined by the North Carolina Dept. of Agricutlure and Consumer Services Soil Testing Laboratory by extraction with 0.2 *M* NaOH + 0.02 *M* DTPA and determining percent HM colorimetrically (Mehlich, 1984). Soil HM was determined instead of SOM because it is part of routine soil analysis preformed by the NCDA&CS Laboratory; soil HM and SOM are highly correlated (r^2 = 0.95; Harrison et al, 1976).

Climate Data

Temperature and precipitation data were collected by the State Climate Office of North Carolina (Raleigh, NC) which has weather stations located on research stations and in most North Carolina counties. Data from the nearest weather station was used for sites not located on research stations. Average maximum, minimum, and mean temperatures for the growing season were calculated from 15 April to 31 August. Precipitation data were not included in statistical analysis because some sites were irrigated and amounts of irrigation water were not measured.

Amino Sugar N Test

The ASN test was performed on three replicates of each soil sample using a

modified Khan et al. (2001) method. The methods was modified by using an incubator in place of the commercial griddle used by Khan et al. (2001) in an attempt to provide more even heating of the samples. Soil samples were thoroughly mixed and 1.00 g of air-dried soil was placed in a 0.47-L (1 pint) mason jars. The soil was treated with10 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 heated to 48-50˚C in an incubator for five hours. Thermometers were positioned randomly inside the incubator to verify temperature was within ± 1° C of 49° C. After the incubation, 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 (approximately 0.01 *M*) H_2SO_4 solution to an endpoint established on the basis of color (Khan et al., 2001; Univ. of Ill., 2004). Soil test concentrations (ASN_c; 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}) of H_2SO_4 (Khan et al, 2001, Univ. of Ill., 2004). Test levels were converted to kilograms ASN per hectare furrow slice (ASN_{hfs}; 100 m x 100 m x 0.2 m; kg ha^{-1}) using bulk density data at each site (equation 3.1).

$$\frac{\mu g\ ASN}{g\ soil} \times \frac{g\ soil}{cm^3\ soil} \times \frac{2 \times 10^9\ cm^3\ soil}{hfs} \times \frac{kg\ ASN}{1 \times 10^9\ \mu g\ ASN} = \frac{kg\ ASN}{hfs} \qquad [3.1]$$

Statistical Analysis

Regression, multiple regression, and stepwise (best r-square) regression

analysis were performed using PROC Reg in SAS version 8.3 (SAS Institute, Cary, NC). Pairwise correlations were calculated using JMP 5.1 (SAS Institute, Cary, NC). Linear regression models for ASN versus HM and EONR versus ASN by drainage type, year, and cost ratios were compared using a multiple regression model with an indicator variable for testing differences among intercepts and/or interaction variables for testing differences among slopes.

Economic Optimum N Rates

Corn grain yield responses to N fertilizer were modeled using a quadratic-plateau function in PROC NLIN in SAS version 8.3 (SAS Institute, Cary, NC). Economic optimum N rates were calculated using the first derivative of the quadratic plateau model and three different price ratios (Cerrato and Blackmer, 1990). Since future price fluctuations cannot be predicted, correlation between ASN and EONR across a reasonable range of fertilizer:corn price ratios must be determined. Three different economic optimum N rates were calculated to represent the extremes of inexpensive fertilizer and expensive grain, expensive fertilizer and inexpensive grain average, and an average cost of fertilizer and grain (Table 3.3). Price ratios (corn:N) were calculated as an EONR maximum (2.75:1; $EONR_{max}$), average (5:1; $EONR_{avg}$), and minimum (8.25:1; $EONR_{min}$) using three different fertilizer costs and corn prices (Table 3.3). If the EONR exceeded the highest applied N rate for a site, the highest applied N rate was used in place of the EONR. In any case where a response did not fit a quadratic-plateau function (alpha = 0.05), treatment means were compared using a Tukey's means test to determine the optimum N level. Regression models of EONR versus soil N tests were calculated using PROC REG in SAS version 8.3

(SAS Institute, Cary, NC). A N-sufficient threshold was represented by the x-intercept of the ASN versus EONR function (0 kg ha^{-1} EONR).

Results and Discussion

Amino Sugar Nitrogen to Predict Economic Optimum N Rates

The mean ASN_c for the sites in this study was 99 mg kg^{-1} with a range of 14 to 488 mg kg^{-1} (Table 3.4). Economic optimum N rates (average) for the sites of this study had a range of 24 to 336 kg ha^{-1} with a mean of 194 kg ha^{-1} (Table 3.4). A regression analysis of ASN versus $EONR_{avg}$ including all sites showed no significant correlation between ASN_c and $EONR_{avg}$ (Table 3.5). A study by Williams (2005) showed EONR predicted by ASN_c was different for mineral (lower ASN_c) and organic (higher ASN_c) soils. Fox and Piekielek (1984) showed that correlation of anaerobically mineralized N and chemical indexes with N availability to corn improved when sites were divided into drainage classes, so we divided sites into three soil drainage types: well, poorly, and very poorly drained (Table 3.4). The GDA and TCF sites were the only very poorly drained sites, and both sites had high ASN_c (Table 3.4). Based on ASN_c, GDA and TCF were expected to have low $EONR_{avg}$, but both sites had slightly above average EONR (Table 3.4). The GDA and TCF sites were the only sites to have HM content above the detection level (+10%; Table 3.4). The high HM matter and very poorly drained soils at the GDA and TCF sites suggest frequent saturation and a tendency for slow mineralization and rapid denitrification. The GDA and TCF sites may have a relatively high EONR despite high ASN because N mineralized during the growing season may have been denitrified before plant uptake. The two very poorly drained sites were eliminated

from further analysis with ASN_c, because of these anomalies.

Amino sugar N concentration was correlated with $EONR_{avg}$ for the well- and poorly drained sites together, but the coefficient of determination was low (Table 3.5). Separate linear regression analysis of the well- and poorly drained sites showed strong correlations (Table 3.5 and Fig. 3.1). Regression analysis of ASN_c versus $EONR_{avg}$ for the well-drained site showed a strong negative correlation (r^2 = 0.85) with a low standard deviation (16 kg N ha^{-1}), and an extrapolated N-sufficient threshold of 123 mg ASN kg^{-1} (Fig. 3.1). Regression analysis of ASN_c versus $EONR_{avg}$ for the poorly drained sites displayed a linear negative correlation (r^2 = 0.78; Fig. 3.1) and an extrapolated N-sufficient threshold of 340 mg ASN kg^{-1} (Fig. 3.1). The strong correlations between ASN_c and $EONR_{avg}$ for the well- and poorly drained sites showed potential for developing N recommendations.

Comparison of Well- and Poorly Drained Models

The slopes for the well- and poorly drained site models were significantly different, and the well-drained sites were more sensitive to changes in ASN_c (Fig. 3.1). For example, an increase of 1 mg ASN kg^{-1} would result in a decrease of EONR by 2.99 kg ha^{-1} for the well-drained sites, but only 0.92 kg ha^{-1} for the poorly drained sites. The N-sufficient thresholds for the well- (123 mg ASN kg^{-1}) and poorly (340 mg ASN kg^{-1}) drained sites were also significantly different. The well-drained sites had significantly less HM content than did the poorly drained sites. Regression analysis of HM versus ASN showed that the well- and poorly drained sites had different intercepts, but the same slope (Fig. 3.2). The different intercepts suggest that the poorly drained sites have more ASN per percent HM than the well-drained

sites (Fig. 3.2). The differences in the N-sufficient thresholds and HM content between the well- and poorly drained soil sites may be a reflection of different rates of N mineralization and denitrification. The lower N-sufficient threshold and HM content for the well-drained soil sites suggests that N may be mineralized at faster rates than in poorly drained soils. Additionally, the higher N-sufficient threshold for the poorly drained soil sites might be because of higher denitrification rates resulting in higher N loss than at the well-drained sites. The higher denitrification and lower N mineralization rates for the poorly drained soil sites may be caused by the soils being wetter and more frequently saturated than the well-drained site soils (Fox and Piekielek, 1984; Delgado et al., 2001).

The different correlations of ASN_c and $EONR_{avg}$ for the well- and poorly drained sites may also be a consequence of soil texture, because soil moisture is effected by soil texture. However, analysis of ASN_c and soil texture (sand, silt, clay) showed no correlations for either the well- or poorly drained sites. Soil texture may not have been correlated with ASN_c because of the heterogeneity of soils and landscapes among sites in this study.

Effect of Year and Temperature on EONR Prediction by ASN

Limitations of soil N tests include changes in corn N demand among years and within growing seasons (Magdoff, 1991; Mulvaney et al., 2001). Corn N demand is influenced by the effects of weather and temperature on crop growth. Ideally, fertilizer recommendation based on ASN_c would accurately predict $EONR_{avg}$ independent of environment and temperature conditions. Drought conditions would be an exception because water-stressed corn takes up less N and has a suppressed

response to N fertilizer. Weather conditions were different for each year of this study. For example during the growing season (Apr 15 to Aug 31), 2001 had less than normal rainfall, 2002 had drought conditions, 2003 had above normal rainfall, and 2004 had average rainfall (Table 3.6). Average daily temperatures were higher than the 30 year average for all years and regions except the Piedmont 2003 (Table 3.6). Although, average daily temperatures did fluctuate among years and regions, and 2002 and 2004 had higher temperatures than 2001 and 2003 (Table 3.6).

Linear regression models of ASN_c versus $EONR_{avg}$ for the well-drained sites were compared for a year effect among 2001, 2003, and 2004; but 2002 was not included in the analysis because only one well-drained 2002 site existed. Regression model slopes and intercepts for individual years were not statistically different suggesting that the well-drained site model was robust across years (Table 3.7). The influence of temperature data on ASN_c prediction of the $EONR_{avg}$ was tested using a stepwise model that included average seasonal maximum, minimum, and mean temperatures. Temperature data were not significant in the stepwise model (data not shown), thus prediction of $EONR_{avg}$ using ASN was not influenced by temperature. Data for poorly drained sites are not presented because of insufficient sites to fit models to individual years.

Comparison of ASN Concentration and Mass Per Unit Volume to Predict EONR

Relationships between soil N tests and $EONR_{avg}$ might be improved by calculating ASN on a mass per unit volume basis, because it accounts for differences in soil bulk density among sites. For example, the LW and TE4 sites both had ASN_c of 93 mg kg^{-1} (Table 3.4), but LW had a higher bulk density and thus,

more kilograms of ASN_{hfs} than TE4 (279 and 246 kg ASN ha^{-1} respectively). Amino sugar N concentrations on a mass:mass basis were not correlated with $EONR_{avg}$ for all sites together (including the very poorly drained sites), but ASN on a kilograms:hectare furrow slice basis (ASN_{hfs}) were significantly correlated with $EONR_{avg}$ (r^2 = 0.21; Table 3.5 and Fig. 3.3). The use of mass per unit volume may have accounted for differences in bulk densities between mineral and organic soils, but the data still suggests two different relationships (well- and poorly drained sites) and correlations (Table 3.5 and Fig. 3.3). The coefficients of determination for the linear regression models ASN_c and ASN_{hfs} versus $EONR_{avg}$ by well- and poorly drained sites were similar (Table 3.5) and thus there was no benefit to additional work of determining soil bulk density.

Effect of Different Cost Ratios on Predicting EONR with ASN

The means for the three EONR were 179 kg ha^{-1} for $EONR_{min}$, 194 kg ha^{-1} for $EONR_{avg}$, and 204 kg ha^{-1} for $EONR_{max.}$ (Table 3.4). Regression models of ASN_c and the three EONR for the well-drained sites were all significant with similar coefficients of determination and N sufficient thresholds (Table 3.8 and Fig. 3.4). Nevertheless, the coefficients of determinations for the regression models between ASN_c and $EONR_{min}$ was slightly lower than for $EONR_{avg}$ and $EONR_{max}$. A comparison of the slopes and intercepts showed that they were not statistically different (Table 3.8). The regression models for predicting the three EONR with ASN_c for the poorly drained sites were linear and had similar coefficient of determination and N sufficient thresholds (Table 3.8 and Fig. 3.4). A comparison of the slopes and intercepts for the poorly drained sites showed no statistical difference (Table 3.8). Because the

linear regression models were not different and coefficients of determination were similar for both well- and poorly drained sites, the ASN_c and EONR regression models suggest that N fertilizer recommendations based on ASN_c would be robust across a reasonable range of fertilizer costs ($0.33 - $0.66 kg^{-1} N) and corn prices ($80 - $120 Mg^{-1} corn).

One concern with making N recommendations based on the ASN_c versus $EONR_{avg}$ models developed in this study is that some sites had high predicted EONR. For example, $EONR_{avg}$ was 336 kg N ha^{-1} for two Peanutbelt Research Station sites, but they also had the lowest ASN_c (PBI2 and PBI3; Table 3.4). The high EONR for these sites and other sites in the Lower Coastal Plain of North Carolina may be the result of continuous grass crop rotations (wheat/sorghum prior to corn) or other high N-demanding crop rotations on sandy soils that receive high rainfall. Additionally, corn yields on small plots tend to be higher than in production fields because of better management (e.g., irrigation). Although some sites had high predicted N rates, averaged across sites, the ratio of $EONR_{avg}$ and economic optimum yield was 20.9 kg N Mg^{-1} corn (1.15 lbs N bu^{-1} corn grain yield) which is within the recommended range of 18 to 22.5 kg N Mg^{-1} corn grain yield (1 to 1.25 lbs N bu^{-1} corn grain yield; Crozier, 2002). One advantage of using the ASN_c-$EONR_{avg}$ models is that they predicted the sites with highest EONR based on low ASN values.

Conclusions

Amino sugar N was negatively and strongly correlated to EONR for sites classified as well- and poorly drained. The reason for different models for different drainage classes probably reflect difference in HM content and rates of N

mineralization, denitrification, and immobilization. Amino sugar N concentration was able to predict $EONR_{avg}$ values regardless of moderate weather variation among years, soil bulk density differences, and cost ratio variability. The ASN_c versus EONR models predicted high N rates (>300 kg ha^{-1}) for some sites, but these sites were correctly identified by the ASN test (i.e., they had the lowest measured ASN_c of our study). Predicted optimum N rates averaged across site-years were within the range currently recommended for corn in North Carolina. The high predicted N rates for specific fields appeared to be related to crop rotation and soil conditions (i.e., repeated grass crops on irrigated coarse soils.

These findings indicated that the well- and poorly drained soil ASN models for predicting EONR are robust and can probably be used to create N recommendations. Further research is needed to calibrate and validate the ASN_c $EONR_{avg}$ relationship for field conditions. The future application of the ASN test could be to modify current yield-based N fertilizer recommendations or to develop a new recommendation based on ASN. A possible strategy for using ASN to predict corn N need could have farmers; i) take a pre-plant soil sample for ASN analysis, ii) apply a small amount of N at planting, iii) and apply additional N between V4-V10 based on results of the ASN soil test.

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Table 3.1. Detailed soil information for research plots.

Year	Site	County	Soil Series	Soil Taxonomic Classification	Drainage¶	Tillage†	Previous Crop‡
				Piedmont			
2003	CLE	Cleveland	Cecil clay loam	Fine, kaolinitic, thermic Typic Kanhapludults	Well	NT	Co
2003	SAL3	Rowan	Hiwassee clay loam	Fine, kaolinitic, thermic Typic Rhodudults	Well	NT	WS
2004	LW	Wake	Appling gravelly sandy loam	Fine, kaolinitic, thermic Typic Kanhapludults	Well	NT	Cr
2004	SAL4	Rowan	Hiwassee clay loam	Fine, kaolinitic, thermic Typic Rhodudults	Well	NT	WS
				Coastal Plain			
2001	PBN1	Bertie	Norfolk loamy sand	Fine-loamy, siliceous, thermic Typic Paleudults	Well	CT	WSo
2001	PBI1	Bertie	Norfolk loamy sand	Fine-loamy, siliceous, thermic Typic Paleudults	Well	CT	WSo
2001	PBI2	Bertie	Norfolk loamy sand	Fine-loamy, siliceous, thermic Typic Paleudults	Well	CT	WSo
2003	PBI3	Bertie	Norfolk loamy sand	Fine-loamy, siliceous, thermic Typic Paleudults	Well	CT	Co
2004	KIN	Lenior	Goldsboro loamy sand	Fine-loamy, siliceous, thermic Aquic Paleudults	Well	CT	WS
2004	PBN4	Bertie	Norfolk loamy sand	Fine-loamy, siliceous, thermic Typic Paleudults	Well	CT	CP
2004	PBI4	Bertie	Norfolk loamy sand	Fine-loamy, siliceous, thermic Typic Paleudults	Well	CT	Ct
2004	SD	Moore	Candor sand	Sandy, siliceous, thermic Arenic Paleudults	Well	CT	H
				Tidewater			
2001	H1	Beaufort	Ponzer muck	Loamy, mixed, dysic, thermic Terric Haplosaprists	Poorly	NT	S
2001	TI1a	Washington	Cape Fear loam	Clayey, mixed, thermic Typic Umbraquults	Well	CT	Co
2001	TI1b	Washington	Cape Fear loam	Clayey, mixed, thermic Typic Umbraquults	Well	CT	Co
2002	EP	Hyde	Hydeland silt loam	Fine-silty, mixed, semiactive, thermic Typic Umbraqualfs	Poorly	NT	S
2002	POB	Washington	Belhaven muck	Loamy, mixed, dysic, thermic Terric Medisaprists	Poorly	NT	S
2002	TMI2	Washington	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	Well	CT	Co
2002	TOI2	Washington	Belhaven muck	Loamy, mixed, dysic, thermic Terric Medisaprists	Well	CT	Co
2003	GDA	Washington	Wasda muck	Fine-loamy, mixed, semiactive, acid, thermic Histic Humaquepts	Very poorly	NT	S
2003	TCF	Tyrrell	Weeksville silt loam	Coarse-silty, mixed, semiactive, acid, thermic Typic Humaquepts	Very poorly	NT	S
2003	TE3	Washington	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	Well	NT	S

2003	TMI3	Washington	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	Well	CT	S
2003	TMN3	Washington	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	Well	CT	S
2003	TOI3	Washington	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	Well	CT	S
2003	TON3	Washington	Hyde loam	Fine-silt, mixed, thermic Typic Umbraquults	Well	CT	S
2004	HEP	Hyde	Acredale silt loam	Fine-silty, mixed, active, thermic Typic Endoaqualfs	Poorly	NT	S
2004	PNB	Washington	Belhaven muck	Loamy, mixed, dysic, thermic Terric Medisaprists	Poorly	CT	S
2004	TE4	Washington	Cape Fear loam	Clayey, mixed, thermic Typic Umbraquults	Well	NT	C
2004	TMN4	Washington	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	Well	CT	CS
2004	TON4	Washington	Cape Fear loam	Clayey, mixed, thermic Typic Umbraquults	Well	CT	CS

¶Poor, poorly drained mineral soils with no tile drainages or organic soil; Well, well-drained or artificial drained by tile drainage; Very poor, very poorly drained soils with a histic epipedon.

†CT, conventional tillage; NT, no-till

‡CP, corn and peanut; Co, Corn; Cr, Crimson; CS, corn and soybean; Ct, cotton; H, Horticulture crop; P, S, soybean; WS, double crop soybean and wheat; WSo, double crop sorghum and wheat

Table 3.2. Replication and fertilizer information for research plots.

Sites	Replications	At-planting N treatments (kg ha^{-1})	Sidedress N treatments (kg ha^{-1})
		Piedmont	
CLE	6	0	0, 56, 112, 168, 224
SAL3	6	26	0, 56, 112, 168, 224
LW	6	24	0, 56, 112, 168, 224, 280
SAL4	6	0	0, 56, 112, 168, 224, 280
		Middle Coastal Plain	
PBI1	3	0, 112	0, 112, 168, 224
PBI2	3	0, 112	0, 112, 168, 224
PBN1	3	0, 112	0, 112, 168, 224
PBI3	3	0, 56	0, 112, 168, 224
KIN	6	12	0, 56, 112, 168, 224, 280
PBI4	6	0, 56	0, 56, 112, 168, 224
PBN4	6	0, 56	0, 56, 112, 168, 224
SD	6	0	0, 34, 68, 100, 134, 168, 202
		Lower Coastal Plain	
H1	3	0	0, 112, 168, 224
TI1a	3	0	0, 112, 168, 224
TI1b	3	0	0, 112, 168, 224
EP	6	27	0, 34, 68, 100, 134, 168, 202
POB	6	55	0, 34, 68, 100, 134, 168, 202
TMI2	4	0	0, 112, 168, 224
TOI2	4	0	0, 112, 168, 224
GDA	6	24	0, 34, 68, 100, 134, 168, 202
TCF	6	56	0, 34, 68, 100, 134, 168, 202
TE3	6	28	0, 34, 68, 100, 134, 168, 202
TMI3	4	0, 56	0, 112, 168, 224
TMN3	4	0, 56	0, 112, 168, 224
TOI3	4	0, 56	0, 112, 168, 224
TON3	4	0, 56	0, 112, 168, 224
HEP	6	0	0, 34, 68, 100, 134, 168, 202
PNB	4	0	0, 39, 78, 118, 157, 196
TE4	4	0	0, 39, 78, 118, 157, 196
TMN4	6	0, 56	0, 56, 112, 168, 224
TON4	6	0, 56	0, 56, 112, 168, 224

Table 3.3. Ratios, corn prices, and fertilizer costs for different EONR for metric and English units.

		Metric Units		English Units	
EONR	Ratio	Nitrogen	Corn	Nitrogen	Corn
		kg^{-1}	kg^{-1}	lbs^{-1}	bu^{-1}
Maximum	2.75	$0.33	$0.12	$0.15	$3.00
Average	5.00	$0.50	$0.10	$0.23	$2.50
Minimum	8.25	$0.66	$0.08	$0.30	$2.00

Table. 3.4. Amino Sugar N concentrations, ASN_{hfs}, three different EONR calculations and HM data. Data sorted by drainage type and ranked by ASN_c.

Site	ASN		Bulk Density	HM	EONR Minimum	EONR Average	EONR Maximum
	mg kg^{-1}	kg ha^{-1}	g cm^{-3}	%	kg ha^{-1}		
			Well-drained sites				
PBI3	14	45	1.62	1.0	314	336	336
PBI2	21	72	1.72	0.6	329	336	336
PBI1	26	87	1.68	0.9	288	311	327
KIN	33	99	1.50	0.8	278	292	292
SD	33	112	1.70	0.7	218	236	248
PBN1	36	127	1.77	1.0	224	224	224
PBI4	37	112	1.52	1.0	208	262	299
PBN4	38	120	1.58	1.9	181	213	235
SAL4	44	132	1.5	0.3	202	234	257
SAL3	48	144	1.5	0.3	205	212	216
TMN4	51	131	1.28	3.4	167	194	213
TE3	56	171	1.53	3.9	163	177	188
CLE	58	174	1.50	0.5	192	205	215
TOI3	58	153	1.32	3.8	167	178	186
TMN3	60	158	1.32	2.9	170	181	189
TON4	60	154	1.28	3.4	147	188	216
TMI3	64	169	1.32	4.3	167	178	185
TI1a	66	199	1.51	3.6	172	195	211
TI1b	67	198	1.48	4.1	168	168	168
TON3	72	190	1.32	2.7	134	140	145
TMI2	73	184	1.26	1.2	98	113	123
LW	93	279	1.5	0.6	24	24	24
TE4	93	246	1.32	4.4	134	134	134
			Poorly drained sites				
EP	109	303	1.39	2.1	188	220	243
HEP	132	349	1.32	2.2	157	170	178
PNB	181	380	1.05	5.6	140	150	158
POB	198	400	1.01	5.8	156	174	187
TOI2	220	462	1.05	10.0	83	89	93
H1	233	396	0.85	10.0	81	91	98
			Very poorly drained sites				
TCF	256	456	0.89	10+	192	200	206
GDA	488	800	0.82	10+	192	201	207

Table 3.5. Evaluation of models and coefficients of determinations from $EONR_{avg}$ regression models with soil N tests concentration (mg kg^{-1}) or soil N test amount per soil volume (kg ha^{-1}) for all, well-, and poorly drained sites.

Sites		Concentration (mg kg^{-1})		Amount (kg ha^{-1})	
	n	Equation	r^2	Equation	r^2
All	31	NS		y = 240 - 0.20x	0.21***
Well and Poorly drained	29	y = 257 - 0.79x	0.43*	Y = 290 - 0.47x	0.52**
Well-drained	23	y = 367 - 2.99x	0.85***	y = 391 - 1.20x	0.87***
Poorly drained	6	y = 313 - 0.92x	0.78*	y = 456 - 0.80x	0.71*

*significant at the 0.05 probability level.
**significant at the 0.01 probability level.
***significant at the 0.001 probability level.

Table 3.6. Growing season (Apr 15 - Aug 31) precipitation (P) and daily average temperature (T°) data for the Piedmont and Middle and Lower Coastal Plains.

	2001		2002		2003		2004		30 yr average	
Regions	P (mm)	T° (c)	P (mm)	T° (c)	P (mm)	T° (c)	P (mm)	T° (c)	P (mm)	T° (c)
Piedmont	NA	NA	NA	NA	791	21.7	508	23.3	593	22.1
Middle Coastal Plain	561	22.8	NA	NA	815	23.3	686	23.9	660	21.8
Lower Coastal Plain	588	23.3	481	24.1	786	23.3	732	24.1	725	22.4

NA, no study sites.

Table 3.7. Regression parameters by year for ASN_c and $EONR_{avg}$ model.

		Regression Parameters		
year	n	Intercept	Slope	r^2
		kg ha^{-1}	(kg ha^{-1}) (mg kg^{-1})$^{-1}$	
2001	5	381 a†	- 3.10 a	0.86**
2003	8	358 a	- 2.67 a	0.92***
2004	9	358 a	- 2.99 a	0.81**

**significant at the 0.01 probability level.
***significant at the 0.001 probability level.
†Within a column, means followed by the same letter are not significantly different at alpha = 0.05

Table 3.8. Regression parameters for ASN_c and EONR with different cost ratios for the well and poorly drained sites and extrapolated N-sufficient thresholds (mg ASN kg^{-1}).

	Model parameters			N sufficient
EONR	Intercept	Slope	r^2	threshold
	kg ha^{-1}	(kg ha^{-1}) (mg kg^{-1})$^{-1}$		mg kg^{-1}
		Well-drained sites		
$EONR_{max}$	381 a†	-3.07 a	0.85	124
$EONR_{avg}$	367 a	-2.99 a	0.85	123
$EONR_{min}$	337 a	-2.74 a	0.79	123
		Poorly drained sites		
$EONR_{max}$	341 a	-1.01 a	0.76	337
$EONR_{avg}$	313 a	-0.92 a	0.78	340
$EONR_{min}$	273 a	-0.78 a	0.78	350

†Within a column, means followed by the same letter are not significantly different at alpha = 0.05

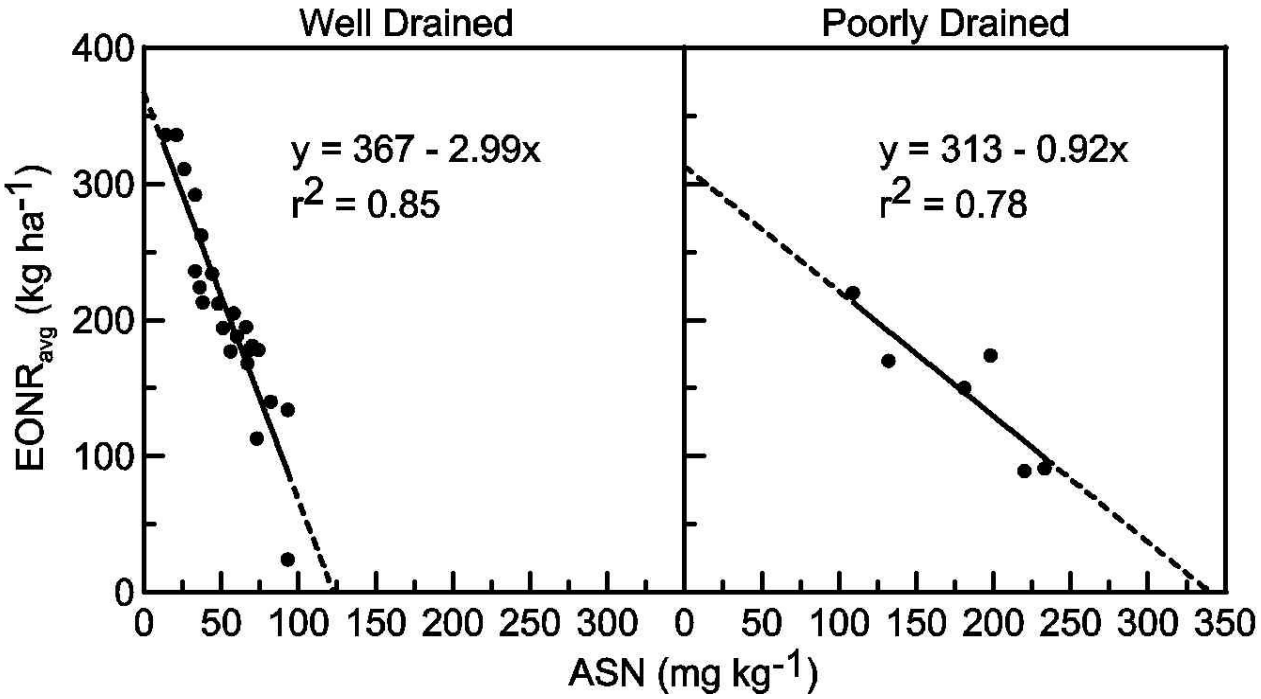

Figure 3.1. Economic optimum N rate (average) as a function of ASN_c for well and poorly drained sites. Extrapolations (dashed lines) suggests N sufficient thresholds.

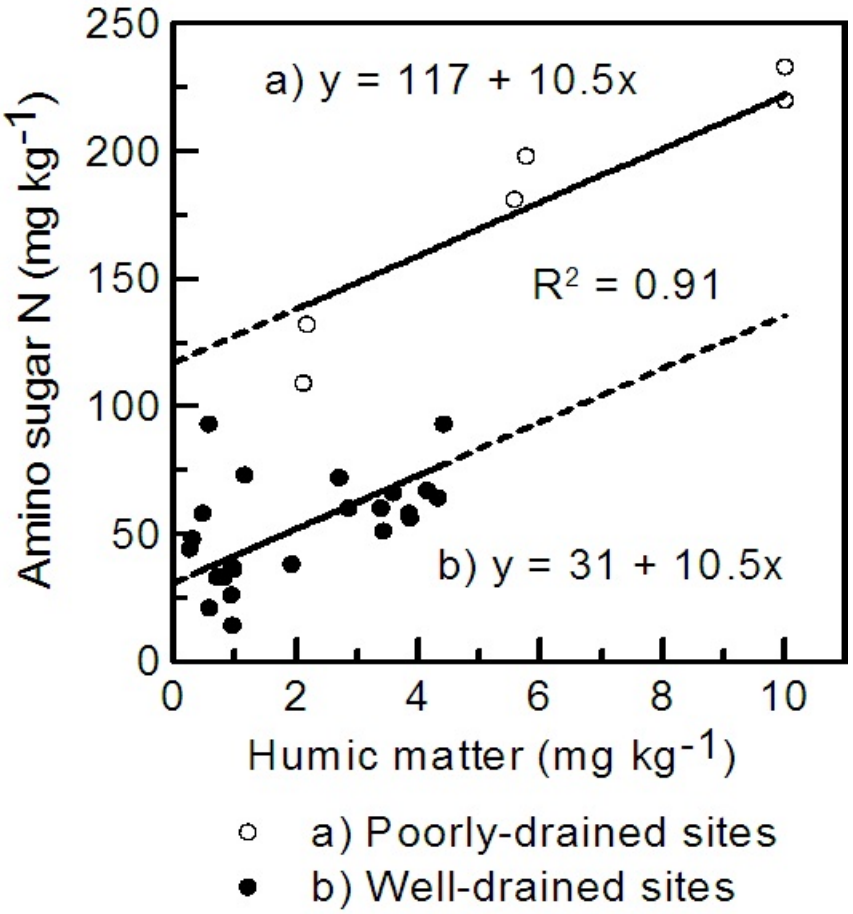

Figure 3.2. Amino sugar N (ASN) as a function of humic matter (HM) with different intercepts for the well- and poorly drained sites. Extrapolation (dashed line) shows the same increase in ASN between well- and poorly drained soil sites as HM increase.

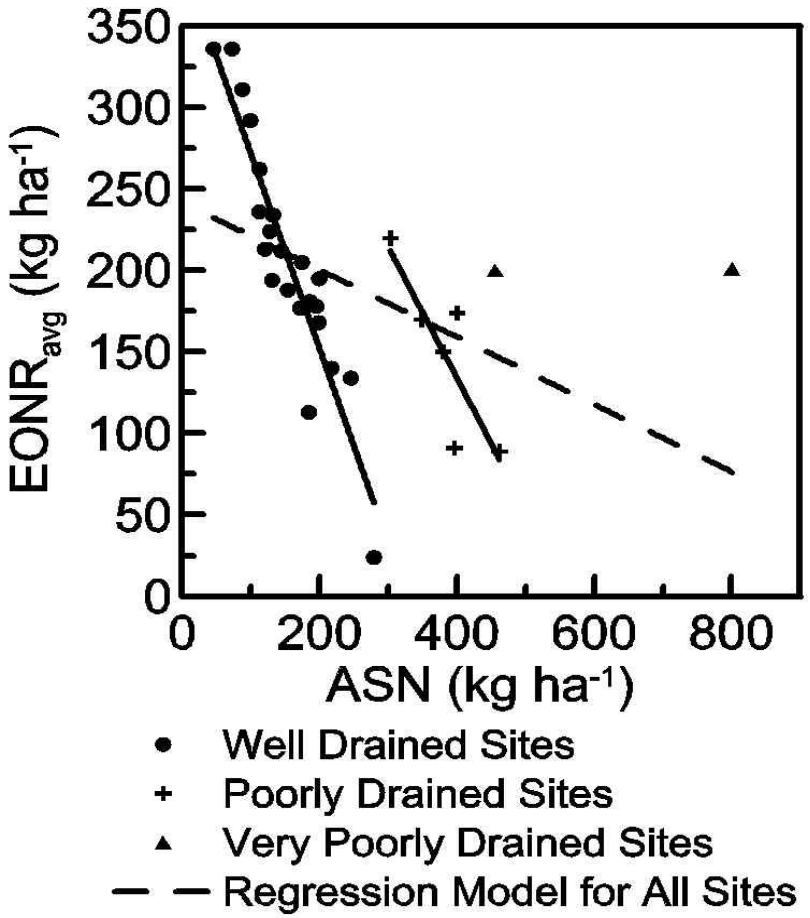

Figure 3.3. Economic optimum N rates (average) as a function ASN_{hfs} for all, well, and poorly drained sites.

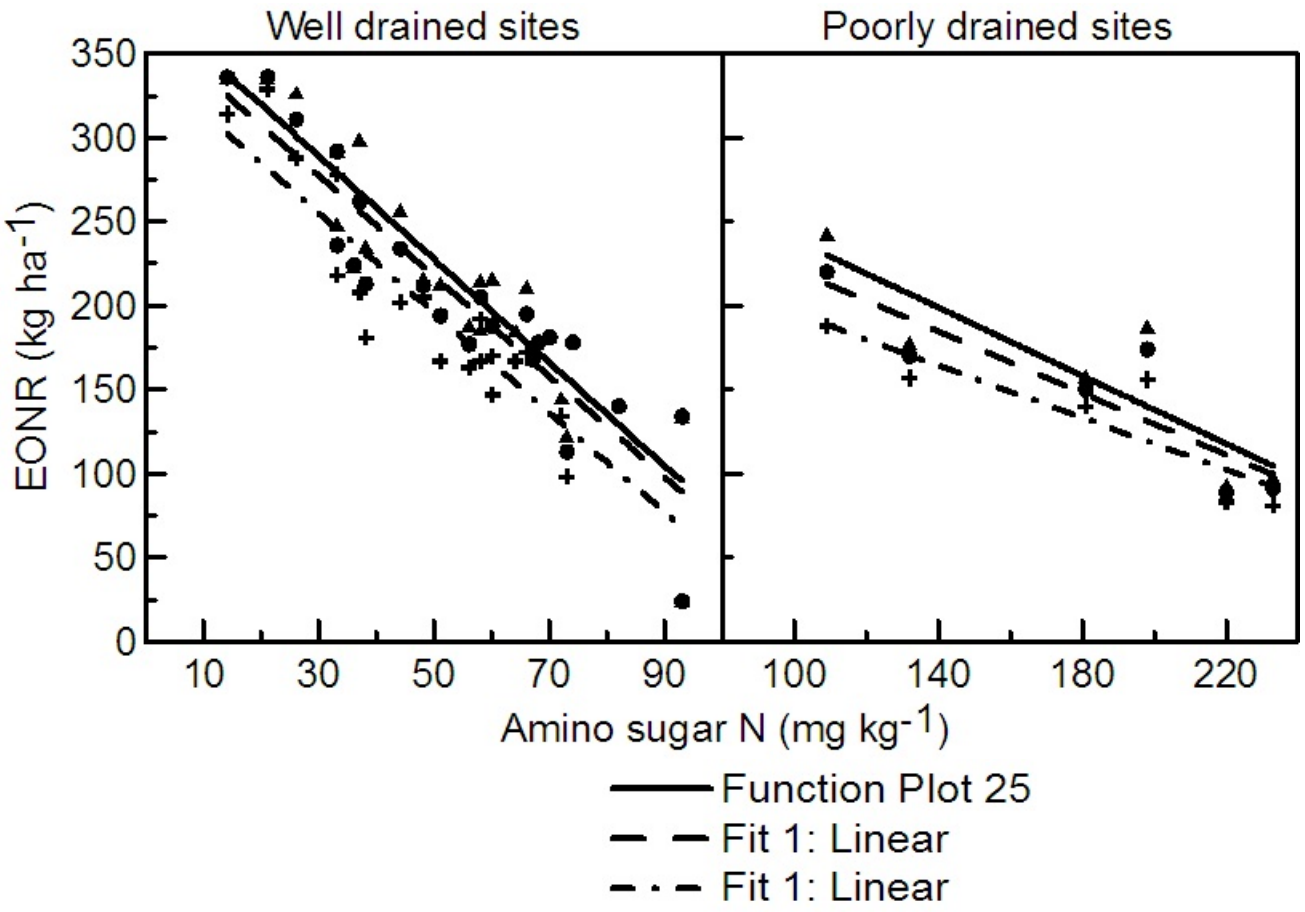

Figure 3.4. Economic optimum N rates with different cost ratios as a function of ASN_c for well and poorly drained sites.

CHAPTER IV

RELATIONSHIPS BETWEEN AMINO SUGAR NITROGEN AND SOIL AND LANDSCAPE PROPERTIES WITH IMPLICATIONS FOR IMPROVING NITROGEN MANAGEMENT FOR CORN

Abstract

The soil amino sugar nitrogen (ASN) test has been developed to predict corn (*Zea mays* L.) nitrogen (N) need, but ASN can vary widely within fields. This study examined the spatial relationships of ASN with soil and landscape properties to develop a variable-rate N and/or sampling zone management strategy. The study was conducted on four sites in the major row crop regions of North Carolina, USA: The Piedmont (clay loam and sandy loam), Middle Coastal Plain (loamy sand and sandy loam), and Lower Coastal Plain (sandy loam and muck). Soil samples were taken on a 20-m grid for ASN and humic matter (HM) and a 37-m grid for soil texture. Amino sugar N was correlated with HM (r = 0.50 - 0.84) for each site. Amino sugar N was negatively correlated with elevation for the coastal plain sites (r = -0.66 and -0.33, respectively). The sites in the Piedmont, which consists of rolling hills, showed ASN negatively correlated with slope gradient (r = -0.32 and -0.21). Amino sugar N was positively correlated with percent clay for the Middle Coastal Plain (r = 0.75) but negatively correlated for a site in the Piedmont region (r = -0.46). These correlations confirm that ASN is sensitive to spatial variability in soil and landscape properties that influence N availability, but correlations were different among sites. A comparison of different soil sampling grid scales showed a coarser grid (37-m) could be used to determine N fertilizer need with ASN. Amino sugar N soil sampling zones can also be created from maps of HM, soil texture, and soil series to reduce sampling costs and time.

Introduction

Producers in warm, humid climates do not have a functional soil nitrogen test for corn, and N fertilizer is typically applied at a uniform N rate across the field based on realistic yield expectations (RYE; Crozier, 2002). This fertilizer management strategy may result in both under- and over-fertilization of specific areas, resulting in possible yield and profit losses (economic inefficiency), and environmental pollution. Variable-rate N fertilization potentially satisfies site-specific crop N needs and reduces excess N fertilization and its negative impacts. The challenge is to develop accurate and cost-effective procedures and measurements to determine site-specific N rates. Researchers in Illinois, USA, have developed the amino sugar N (ASN) test that measures a readily available portion of soil organic N (Khan et al, 2001; Mulvaney et al, 2001). Work in North Carolina, USA, supports the validity of the ASN test for predicting corn N needs (Williams, 2005) in the southeast USA. Soil sampling for ASN at or before planting to develop a variable-rate N map could be the basis for a variable-rate side-dress application. Corn N fertilizer need varies widely within fields in part because of the spatial variability of soil and landscape properties (Blackmer and White, 1996; Franzen et al, 1996). Identifying relationships between ASN and soil or landscape properties may lead to more efficient sampling and variable-rate fertilization schemes.

Amino sugar N is probably influenced by soil and landscape factors, themselves related since topography is recognized as one of the major factors influencing soil formation. Landscape properties may affect ASN since they reflect

differences in parent material deposition and influence water and solute movement, microclimate, and plant productivity. Topographic indices such as the topographic wetness index (TWI) have been used to model water movement over the land surface and predict the “wetness” or potential moisture content of a soil (Wilson and Galant, 2000). The TWI models the spatial distribution and extent of water saturation as a function of upslope contributing area and slope gradient (Wilson and Galant, 2000). Soil texture also influences soil moisture which affects numerous processes that vary across fields, e.g., crop residue and soil organic matter production, breakdown, mineralization, and immobilization (Delin and Linden, 2000). Rates of these processes are also influenced by soil organic matter (SOM; Schmidt et al, 2002) and topography (Franzen et al, 1996). Amino sugar N concentrations may be correlated with soil organic matter concentrations since ASN is a labile N component of SOM. Amino sugar N may be influenced by soil texture via its influence on soil moisture. For example, plant residues and SOM may break down more rapidly in loamy textured soils than clayey soils (likely to be wetter and/or anaerobic) or sandy soils (likely to be excessively dry or leached; Delin and Linden, 2000). Therefore, loamy soils may have more ASN, because it is a partial hydrolysis product of SOM. Correlations of ASN with SOM, soil texture, and TWI may indicate that ASN is spatially influenced by N-cycle dynamics and that variable-rate N fertilizer maps could be created from ASN and or proxy, e.g., soil and landscape properties.

The expense and time of soil sampling and analyzing ASN for development of a variable-rate N map using a fine scaled grid may limit its implementation and a coarser grid could be used to reduce the number of samples. Additionally, soil sampling zones might be created based on soil and landscape properties that are correlated with ASN. Humic matter (HM), which is a component of SOM and should be correlated to ASN, is part of the routine North Carolina Dept. of Agriculture and Consumer Services (NCDA&CS) Agronomic Division Soil Testing Section laboratory analysis (Mehlich, 1984). Apparent soil electrical conductivity (EC_a) has been spatially related to soil conditions that interact with soil mineral N, presumably due to its dependence on water content, soil porosity, and soil texture (Fritz et al, 1999). Soil survey maps have been used to develop N management strategies because soil series may differ in productivity and crop response to N due to factors such as available water, mineralization, denitrification, immobilization, volatilization, and drainage (Blackmer and White, 1996). Topography is an important factor governing water movement within fields and thereby influences rates of soil N processes. LIDAR-derived digital elevation maps of some of the river basins in North Carolina are available to the public for free. Amino sugar N soil sampling costs might be reduced by developing soil sampling zones based on areas of the field with similar HM content, soil EC_a, topography, or soil map units.

This study investigated the relationships between soil ASN concentrations, selected soil properties, and landscape attributes to: i) verify that ASN is sensitive to spatial variability of soil and landscape properties, ii) compare different grid sizes for

predicting ASN using soil and landscape properties, and iii) determine which soil and landscape properties could be used to create ASN soil sampling zones.

Materials and Methods

This research was conducted in the Piedmont (Hall and Pump sites) and Middle (Kinston site) and Lower Coastal Plains (Tidewater site) of North Carolina. Studies were located at four sites with two years of observation at the Tidewater site. Site and cropping data are shown in Table 4.1. An area of 3.5 to 8.1 ha was chosen from within each site specifically for the purpose of having a range of different soil environments (e.g., soil series, landscape position, and slope). An exception was the Kinston site which was part of a four-year experiment that included three N management treatments. The treatment receiving variable N rate application was excluded from our study since we were interested in inherent properties associated with ASN variability that develop in spite of uniform management.

Soil Sampling

Soil sampling for ASN and HM for all sites was done prior to corn planting on a 20-m equilateral triangular grid (~ 25 samples ha^{-1}) by compositing eight cores taken within one meter of a grid point using a 20-mm diameter soil probe to a 20-cm depth (Fig. 4.1 - 4.4). At all sites except Kinston, soil textural analysis (percent sand, silt, and clay) was performed on one-quarter of the samples taken from the 20-m equilateral triangular grid (~ 37-m grid with 6 samples ha^{-1}; Fig. 4.1, 4.2, 4.4) because of expense.

Soil texture data for the Kinston site had been collected as part of a previous study that had 60 plots and a rectangular 60-m grid plus an additional sampling point that was placed randomly within the constraints of being an adequate distance from the plot center, outside a plot border-harvest buffer area, and inline from plot to plot parallel to crop rows to facilitate field operations (5 samples ha^{-1}; Fig. 4.3c). Samples were obtained using a 76-mm diameter probe to a depth of 15-cm. Soil texture data for the Kinston field were point kriged to a grid based on one-quarter of the samples taken from the 20-m equilateral triangular grid used for ASN and HM sampling (~ 37-m grid with 6 samples ha^{-1}). Kriging were done using an exponential semivariogram in the geostatistical extension of ArcGIS 8.3 (Fig. 4.3; ESRI, Redlands, CA).

Soil Properties and Landscape Attributes

The ASN test was performed on three replications of each soil sample using a modified Khan et al. (2001) method. The ASN test was modified by using an incubator instead of a commercial griddle to heat samples (Williams, 2005). Humic matter (HM) was determined by the North Carolina Dept. of Agriculture and Consumer Services Soil Testing Laboratory by extraction with 0.2 *M* NaOH + 0.02 *M* DTPA and determining percent HM colorimetrically (Mehlich, 1984). Soil HM was determined instead of SOM because it is part of routine soil analysis preformed by the NCDA&CS Laboratory; soil HM and SOM are highly correlated (r^2 = 0.95; Harrison et al, 1976). Soil textural analyses for Hall, Pump, and Tidewater sites were performed by Waters Agricultural Laboratory (Camilla, GA, USA) using the

hydrometer method (Gee and Bauder, 1979) to determine percent sand, silt, and clay. Soil texture analysis for the Kinston site was performed as part of a previous study using the hydrometer method and taking readings at 30-s and 90-s to determine the sand fraction and at 6-h, and 16-h for the clay fraction. Soil EC_a data were collected using a Geonics EM-38 (Mississauga, Ontario, Canada) in the horizontal dipole mode pulled behind a four-wheeler in a cart on continuous 10 m transects and data were logged approximately every 2 m. Soil EC_a data were kriged to the 20-m grid using exponential semivariograms except for the Tidewater site where soil EC_a was measured at each grid point.

Topographic attributes were derived using digital elevation models from either LIDAR data for Kinston and Tidewater or real-time kinematic global positioning system (RTK-GPS) elevation surveys for the Hall and Pump sites. Digital elevation models created from the LIDAR data had a 3-m resolution and a vertical accuracy of 150 mm (North Carolina Flood Mapping Program; www.ncfloodmaps.com). The RTK-GPS elevation surveys were performed using a four wheeler to drive continuous 10-m transects with data collected approximately every 2 m. Digital elevation models created from RTK-GPS elevation surveys had an 8-m resolution. Slope gradient and TWI were derived using the GRID function in ArcInfo 8.3 (ESRI, Redlands, CA, USA). Topographic wetness index was modified by adding 1.0 m^2 to upslope contributing area to account for areas of the field with little or no slope gradient (< 0.5%) and no flow direction (Wilson and Gallant, 2000). Soil series and map unit data were obtained from order one research station surveys for the

Tidewater (Kleiss et al., 1993) and Kinston (North Carolina Agricultural Experiment Station, 1977) sites and the Rowan County, North Carolina USDA-NRCS soil survey (Soil Survey Staff, 1995) for the Hall and Pump sites.

Statistical Analysis

Analysis of variance was performed using PROC GLM, means were compared using a Tukey's mean separation test, correlation analysis using PROC CORR, stepwise regression (best r-square) using PROC REG, and regression and spatial regression using PROC MIXED in SAS version 8.3 (SAS Institute, Cary, NC, USA). Spatial regression models were calculated using estimated restricted maximum likelihood spherical, exponential, and gaussian semivaroigrams. Variance inflation factors (VIF) were used to determine whether multi-collinearity problems existed in the stepwise models (Neter et al., 1996). Variables included in a stepwise model with a VIF less than 10 were considered not to have multi-collinearity with exception of quadratic terms (Neter et al., 1996). Interpolated maps for showing spatial variability were created using inverse distance squared weighting in ArcGIS version 9.0 (ESRI, Redland, CA), but the interpolated data were not used in the statistical analysis. Linear regression models for HM versus ASN by soil type for the Kinston and Tidewater sites were compared using a multiple regression model with indicator and interaction variables.

Results and Discussion

The 2003 and 2004 Tidewater sites were combined as one site (Tidewater), because samples were taken from the same locations. Soil texture, elevation, slope

gradient, TWI, and soil EC_a data were the same (collected once for both site-years). Additionally, a Tukey's mean separation test showed that ASN and HM were not different between years (data not shown). The combining of 2003 and 2004 Tidewater data resulted in an artificial increase in the number of replicate samples (two ASN and HM values for each data point).

The Tidewater site had high ASN and percent HM, the Pump and Kinston sites had low ASN and percent HM, and the Hall site had low HM but high ASN (Table 4.2). The Pump and Hall sites had high clay content (Table 4.2), but the Kinston and Tidewater sites had low clay content and high sand content (Table 4.2). The Pump and Hall sites were located in the Piedmont which consist of rolling hills and therefore had steeper slope gradients, than the Kinston and Tidewater sites which had relatively little relief and gradual slopes. The Tidewater, Hall, and Pump sites had high soil EC_a, but the Kinston site had a very low soil EC_a (Table 4.2).

Correlation and linear regression analyses of ASN with soil properties showed correlation with HM for each site (Table 4.3; Fig. 4.5 and 4.6). The correlation between ASN and HM for Hall and Pump were relatively weak and this may be a result of the detection level (0.05%) of the method used to measure HM and the limited range (0.15 - 0.85%; Table 4.3 and Fig 4.5). Amino sugar N and HM are components of SOM and thus should be related (Mulvaney et al, 2001), but not perfectly correlated, because ASN is a relatively labile form of SOM whereas HM is a more stable form of SOM (Mulvaney et al., 2001). Amino sugar N may be more rapidly mineralized, denitrified, and immobilized than HM. For example, if fields had

the same HM content, the field with faster rates of mineralization and immobilization would likely have less ASN than the field with slower rates. The correlation of ASN and HM show that ASN may be sensitive to the spatial variability of HM.

The Kinston and Hall sites had significant correlations between soil texture and ASN (Table 4.3 and Fig. 4.7). The ASN concentration at the Kinston site was positively correlated with silt and clay content, but negatively correlated with sand content (Table 4.3 and Fig. 4.7). The inverse relationship between percent sand and ASN is probably the result of soils with lower sand content (more clay and silt, i.e., loamy) having a higher soil moisture holding capacity. The Kinston site had loamy sand (Norfolk and Goldsboro) and sandy loam (Lynchburg) soils that had low percent HM. The Kinston site had topographical relief of 1.5 m and the sandy loam soils were located lower on the landscape than the loamy sand soils and ASN decreased as elevation increased (Table 4.4). The higher ASN for the sandy loam soils may be because they were wetter and had more HM accumulation because of drainage.

The Hall site had a clay loam soil that had low HM. Regression analysis of ASN and soil texture showed that silt had a weak positive correlation with ASN, but clay had a weak negative correlation (Table 4.3 and Fig. 4.7). The negative correlation between percent clay and ASN was opposite that of the Kinston site, but the textures, landscapes, and soil forming processes between the two sites were quite different (Table 4.1). The Hall site had a percent clay range of 40 - 60%, but the Kinston site had a range of 5 - 20%. Unlike the Kinston site where increased

clay content resulted in more ASN, the Hall site had less ASN as clay content increased. The Hall site may have had less ASN as clay content increases because higher clay content may reflect thinner topsoil and less HM.

The Tidewater did not have a correlation between ASN and soil texture, which may be because the site included a mineral (Portsmouth) and an organic (Belhaven) soil. According to ANOVA soil texture was not different between the Belhaven and Portsmouth soils (data not shown), but ASN (Fig. 4.8) and HM were different.

Amino sugar N was correlated with elevation for the Hall, Tidewater, and Kinston sites (Table 4.3), but not for the Pump site. The Hall site is located in the Piedmont, which receives less rainfall than the other regions. The positive correlation between elevation and TWI, which models soil wetness by flow direction and upslope catchment area, (Table 4.4) suggests that higher areas were more "wet" than lower areas (Wilson and Gallant, 2000). The reason the high areas might have been wetter was because of little relief high on the landscape and steeper slopes lower on the landscape. The Tidewater site is only 4 to 5 meters above sea level and the soils are poorly (Portsmouth) and very poorly (Belhaven) drained. Soils higher on the landscape drain onto soils lower on the landscape which become submerged or saturated. The lower areas may be saturated promoting denitrification and slower rates of plant decomposition than higher areas resulting in less ASN lower on the landscape. The result is mineral soils (less HM and ASN) in the higher landscape positions and organic soils (higher HM and ASN) in the lower-water collecting areas. The Kinston site had less ASN and HM in soils higher on the

landscape. The positive correlation of elevation and sand suggests that sandier soils occurred higher on the landscape (Table 4.4) probably because of original deposition, wind erosion, and weathering. The soils higher on the landscape could be subject to more rapid SOM decomposition due to better aeration. These soils would also be subject to rapid infiltration and subsurface loss of soluble N compounds as water flowed to lower, more poorly drained areas. The relationship between elevation and ASN for the Hall, Tidewater, and Kinston sites suggest that ASN is sensitive to elevational changes in HM, soil moisture, and soil texture which affect decomposition, N mineralization, and denitrification.

The Hall and Pump sites showed negative correlations between slope gradient and ASN, but the Kinston and Tidewater sites showed no correlation (Table 4.3). The steeper slopes on the Hall and Pump sites have less ASN, probably because the soils are eroded and have thinner topsoil (i.e., more clay than Kinston and Tidewater), less HM, and less water infiltration than areas with shallow or no slope. Slope gradient and ASN for the Kinston and Tidewater sites were probably not related because the relief in these sites is only approximately 1.5 m and the slope is between 0 to 2% resulting in little topographic variability across the site. Micro-topography may influence ASN, but probably at a finer scale than the 20-m grid examined in this study.

Spatial correlation of ASN versus soil and landscape properties may exist. The implications of spatial correlation on the relationships between ASN and soil and

landscape properties would not alter the goodness of fit (Pearson's correlation coefficient), but might likely inflated the significance of the correlation.

Stepwise regression of soil and landscape properties to predict ASN for the 20- and 37-m grids were compared to determine if ASN spatial variability within a field could be captured with a coarser grid. Because soil texture was sampled on a 37-m grid and could not be interpolated for the 20-m grid (large error), stepwise models did not include soil texture. Because some soil and landscape properties were highly correlated among themselves, multi-collinearity was a concern. The VIF for variables included in the stepwise regression models were low (<3) and indicated multi-collinearity had little or no effect on the models (Neter et al., 1996).

Topography and landscape attributes contributed little to the overall stepwise regression models for predicting ASN (Table 4.5). Topographic variables found significant and included in the stepwise model for predicting ASN at the 20-m grid were elevation for the Hall (partial r^2 = 0.03) and Kinston (r^2 = 0.03) sites and slope for the Pump site (partial r^2 = 0.04; Table 4.5). The topographic variables for Hall and Pump sites were not significant in the 37-m grid stepwise regression model (Table 4.5), but for the Kinston site the significance of elevation did not change. Humic matter was the most significant variable for predicting ASN for all sites using the stepwise model, and, except for Pump, individual sites had similar partial coefficients of determination regardless of grid scale (Table 4.5). Soil EC_a was not significant for any site or grid scale (Table 4.5). Overall, coefficients of determination were similar between grid scales despite topography and landscape attributes being

less significant at the 37-m grid as compared to the 20-m grid (Table 4.5). A coarser grid than the 20-m grid such as the 37-m grid could be used to reduce the number of ASN soil samples needed for developing a variable-rate fertilizer map. Spatial correlation was a concern, but semi-variograms that were calculated as part of spatial regression models for the variables found to be significant in stepwise models were not significant with exception of the Kinston site.

The number of samples for developing a variable-rate fertilizer map based on ASN might be further reduced by creating soil sampling zones. Soil sampling zones might be created using soil and landscape properties that are strongly correlated to ASN (i.e., HM and soil texture) or soil series. Maps of all the sites with soil series boundaries showed general spatial trends between ASN and HM (Fig. 4.1 - 4.4). Maps of the Kinston site indicated that the Lynchburg soil might have higher ASN, HM, and clay content than the Goldsboro or Norfolk soils (Fig. 4.3 and 4.9). Statistical comparison of the soils showed that the Lynchburg soil had more ASN than Goldsboro and Norfolk (91 versus 70 and 62 mg kg^{-1}, respectively Fig 4.9). The Tidewater site soils had different amounts of ASN, with the organic Belhaven soil having higher amounts than the mineral Portsmouth soil (196 versus 129 mg kg^{-1}, respectively; Fig. 4.4 and 4.8). Amino sugar N and HM for the Cecil and Pacolet soil at the Pump site might not be a different according to the maps (Fig. 4.2). Comparison of the Cecil and Pacolet soils showed ASN was not different between them (79 and 77 mg kg^{-1}, respectively). The Hall site included only one soil map unit and was not included in this analysis (Fig. 4.1). Correlation and regression analysis

of HM (Table 4.3; Fig. 4.5 and 4.6) and soil texture (Table 4.3 and Fig 4.7) indicated that ASN soil sampling zones might be created using HM, soil texture, and/or soil series for some sites.

The correlation between ASN and HM could be used to delineate sampling zones for all sites (Table 4.3). Sampling zones could be created for areas of the field that had similar HM content, although different criteria would be needed for each site in this study because of different ranges of HM (e.g. Pump 0.2-0.6% versus Tidewater 1- 10%; Fig. 4.5 and 4.6). Multi-variate regression models predicting ASN for Kinston and Pump showed both HM and clay contributed to the model (Table 4.6). Amino sugar N sampling zones might be based on areas of the field with similar HM content and the areas of similar HM content subdivided by percent clay.

Amino sugar N soil sampling zones can probably be delineated using soil series at some sites. The differences between soil series for the Kinston and Tidewater sites suggest that soil series can be used to delineate ASN sampling zones for these sites. Although ASN was statistically different among soil series for the Kinston and Tidewater sites, the range of ASN values within soil series was large (Fig. 4.8 and 4.9). Amino sugar N sampling zones might be further improved by using HM and soil texture by soil series.

The range of ASN within soil series for the Kinston and Tidewater sites might be managed by creating sub-zones based on HM. Regression models of HM and ASN were not different by soil series at the Kinston site. Regression models of HM

and ASN for the Tidewater soils showed that the ASN and HM models had the same intercept, but statistically different slopes for the Belhaven and Portsmouth soils (Fig. 4.10). The different slopes for HM and ASN for the Belhaven and Portsmouth soils could be used to create ASN sampling zones based on soil series and HM. Regression models of ASN versus soil texture (percent sand, silt, and clay) by soil series for the Kinston site were not different (data not shown) and therefore would not improve sampling zone delineation.

Conclusions

Our results show that ASN is sensitive to the spatial variability of HM content, and that ASN and HM variability are related to soil properties and landscape attributes that influence rates of organic matter production, decomposition, and redistribution. A variable-rate N strategy based on the ASN soil test would likely improve N management in warm, humid regions which currently do not have a functional soil N test. Amino sugar N would be sampled at or before corn planting and a variable-rate N fertilizer map would be created for side-dress N application. Based on models developed by Williams (2005), variable-rate N fertilizer application maps for corn would call for 0 - 65 kg N ha^{-1} for the Hall site, 30 - 150 kg N ha^{-1} for the Pump site, 0 - 180 kg N ha^{-1} for the Kinston site, and 0 - 200 kg N ha^{-1} for the Tidewater site. Additionally, the expense of soil sampling may be reduced by using a coarser grid (37-m) or by creating sampling zones. Soil sampling zones could be delineated for sites using maps of HM, soil textures, or soil series. The best soil sampling strategies for some sites would be maps created using HM and soil series.

Amino sugar N soil sampling zones would reduce sampling costs, but allow for a variable-rate N application which would meet corn need. Sampling zones for ASN do need to be validated experimentally before implementation into a N management strategy.

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Table 4.1. Detailed soil and climate information for study sites.

Site Locations	Hall 2003	Pump 2004	Kinston 2004	Tidewater 2003-2004
Region	Piedmont	Piedmont	Middle Coastal Plain	Lower Coastal Plain
County	Rowan	Rowan	Lenior	Washington
Annual Precipitation (mm)	1079	1079	1299	1321
Drainage	Well-drained	Well-drained	Well-drained with tile drainage	Poorly and very poorly drained with tile, ditch, and crowned drainage
Management	no-till, corn-soybean-wheat rotation	no-till, corn-soybean-wheat rotation	disc tillage prior to wheat, no-till double crop soybeans, corn rotation	chisel and disc tillage, continuous corn rotation
Research area (ha)	4.0	3.5	8.1	3.9
Predominant Soil Series and Taxonomic Names	Hiwasee clay loam Fine, kaolinitic, thermic Typic Rhodudults	Cecil clay loam Fine, kaolinitic, thermic Typic Kanhapludults	Goldsboro loamy sand Fine-loamy, siliceous, subactive, thermic Aquic Paleudults	Portsmouth fine sandy loam Fine-loamy, mixed, thermic Typic Umbraquults
		Pacolet sandy loam Fine, kaolinitic, thermic Typic Kanhapludults	Lynchburg sandy loam Fine-loamy, siliceous, semiactive, thermic Aeric Paleaquults	Belhaven Muck Loamy, mixed,dysic, thermic Terric Medisaprists
			Norfolk loamy sand Fine-loamy, siliceous, thermic Typic Kandiudults	

Table 4.2. Means and standard deviations for amino sugar N, soil properties, and landscape attributes.

		Amino Sugar N	Humic matter	Sand	Silt	Clay	Elevation	Slope gradient	TWI	Soil EC_a
sites		mg kg^{-1}	---------- % ----------				m	%	m m^{-1}	mS m^{-1}
Hall	mean	134	0.5	23	26	50	245.8	3.1	2.86	7.7
	SD	14	0.14	4	3	4	1.6	1.1	1.50	3.0
Pump	mean	79	0.4	42	21	38	224.7	5.7	2.73	7.5
	SD	13	0.1	5	3	5	3.4	1.4	1.03	3.6
Kinston	mean	76	1.1	62	28	10	20.1	2.0	2.41	1.8
	SD	19	0.6	9	6	3	0.3	1.1	1.41	0.6
Tidewater	mean	153	5.0	55	23	22	4.5	0.3	3.85	7.7
	SD	47	2.4	3	3	3	0.2	0.2	1.15	1.3

TWI, Topographic wetness index
Soil EC_a, Soil apparent electrical conductivity.
SD, Standard deviation

Table 4.3. Pairwise correlation coefficients (r) of amino sugar N with soil properties and landscape attributes.

Site	n	Humic matter	Sand	Silt	Clay	Elevation	Slope gradient	TWI	Soil EC_a
Hall	108	0.63***	NS	0.45*	-0.46*	0.26**	-0.32*	NS	-0.23*
Pump	99	0.50***	NS	NS	NS	NS	-0.21*	0.21*	NS
Kinston	174	0.76***	-0.78***	0.75***	0.75***	-0.33***	NS	NS	-0.25***
Tidewater	224	0.84***	NS	NS	NS	-0.66***	NS	-0.17**	NS

*significant at alpha = 0.05.
**significant at alpha = 0.01.
***significant at alpha = 0.001.
NS, not significant at alpha = 0.05.
TWI, topographic wetness index.
Soil EC_a, Soil apparent electrical conductivity.

Table 4.4. Pairwise correlation coefficients (r) of elevation with soil properties and landscape attributes.

	n	HM	Sand	Silt	Clay	TWI
Hall	108	NS	NS	NS	NS	0.52**
Pump	99	-0.37***	NS	NS	NS	-0.29**
Kinston	174	-0.37***	0.37**	-0.41**	NS	NS
Tidewater	224	-0.78***	-0.30*	NS	0.31*	NS

*significant at alpha = 0.05
**significant at alpha = 0.01
***significant at alpha = 0.001
NS, not significant at alpha = 0.05
HM, humic matter
TWI, topographic wetness index

Table 4.5. Soil and Landscape properties found to be significantly related to amino sugar N using a stepwise regression model for 20- and 37-m grids. Multi-collinearity of independent variables was not a concern because all VIF were less than three.

	Grid	Full model	Humic matter	Humic matter^2	Elevation	Slope	Soil EC_a
	m	R^2	---------- Partial R^2 ----------				
Hall	20	0.46	0.37***	0.06***	0.03*	NS	NS
	37	0.41	0.41***	NS	NS	NS	NS
Pump	20	0.31	0.27***	NS	NS	0.04*	NS
	37	0.38	0.38***	NS	NS	NS	NS
Kinston	20	0.66	0.58***	0.03***	0.02***	NS	NS
	37	0.63	0.57**	0.03***	0.03*	NS	NS
Tidewater	20	0.71	0.71***	NS	NS	NS	NS
	37	0.76	0.73***	NS	NS	NS	NS

*significant at a probability level = 0.05.
**significant at a probability level = 0.01.
***significant at a probability level = 0.001.
NS, not significant at a probability level of 0.05.
EC_a, apparent electrical conductivity.

Table 4.6. Soil properties found to be significantly related to amino sugar N using a multivariate regression model for 37-m grids. Multi-collinearity of independent variables was not a concern because all VIF were less than three.

	Full Model	Humic matter	Humic matter^2	Clay
	R^2	---------- Partial R^2 ----------		
Pump	0.50	0.38***	NS	0.12*
Kinston	0.70	0.57***	0.03*	0.10***

*significant at alpha = 0.05.
***significant at alpha = 0.001.
NS, not significant at a probability level of 0.05.

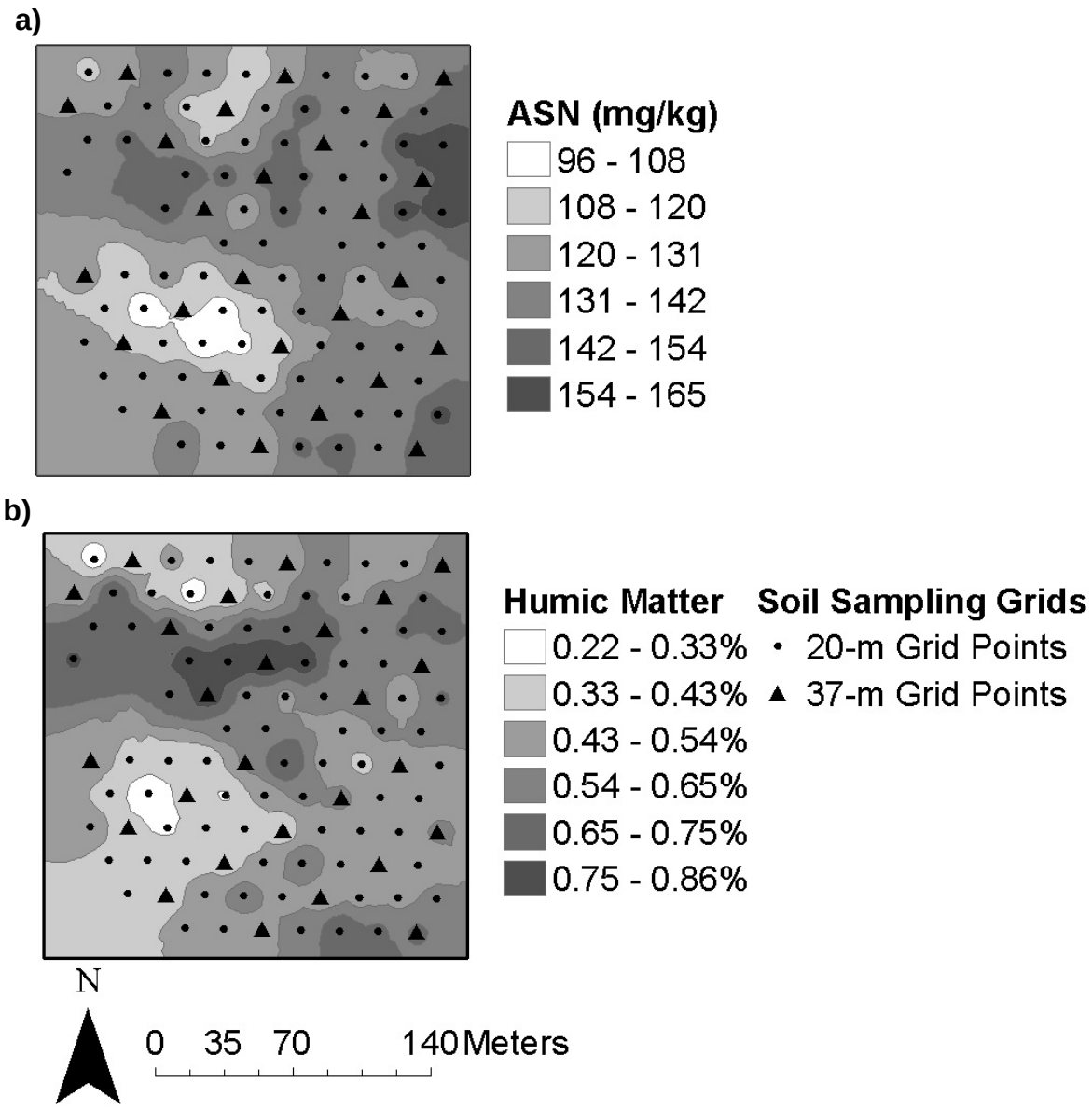

Figure 4.1. Interpolated maps of (a) ASN and (b) HM for the Hall site with grid points of areas sampled to demonstrate spatial variability. (Map is missing one data point.)

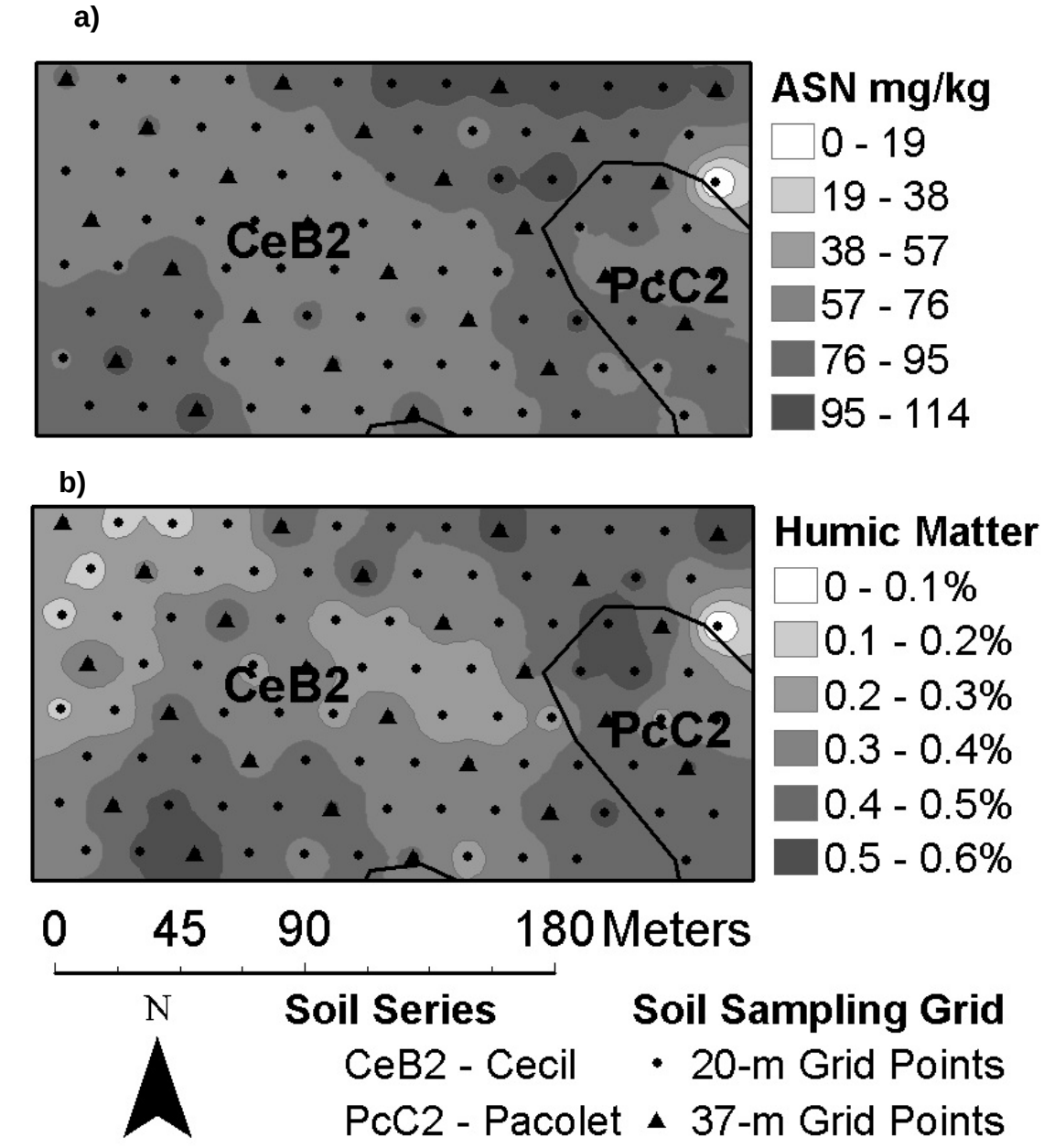

Figure 4.2. Interpolated maps of (a) ASN and (b) HM for the Pump site with soil series' boundaries and soil sampling grid points to demonstrate spatial variability.

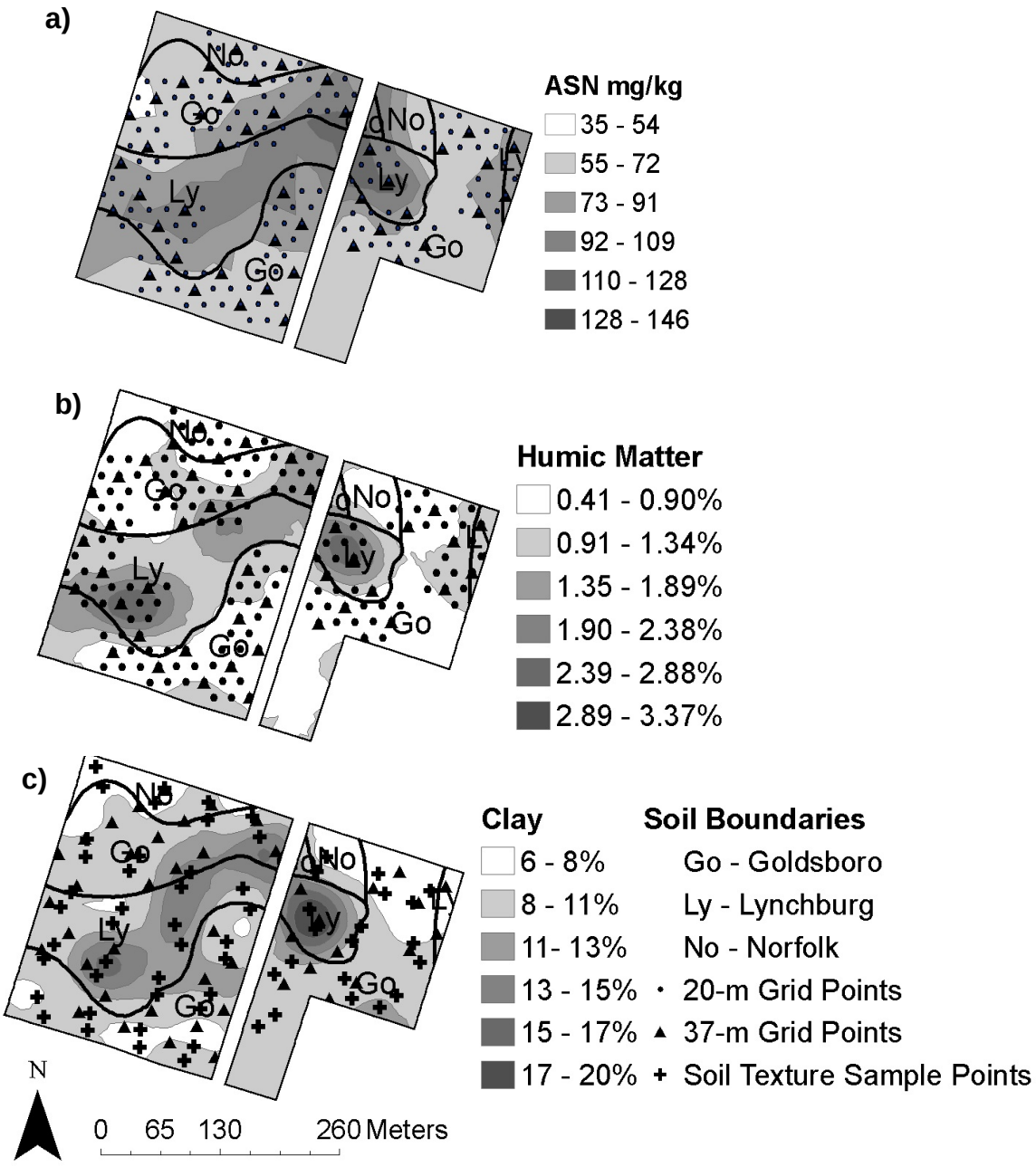

Figure 4.3. Interpolated maps of (a) ASN, (b) HM, and (c) percent clay for the Kinston site with soil series' boundaries and soil sampling grid points to demonstrate spatial variability.

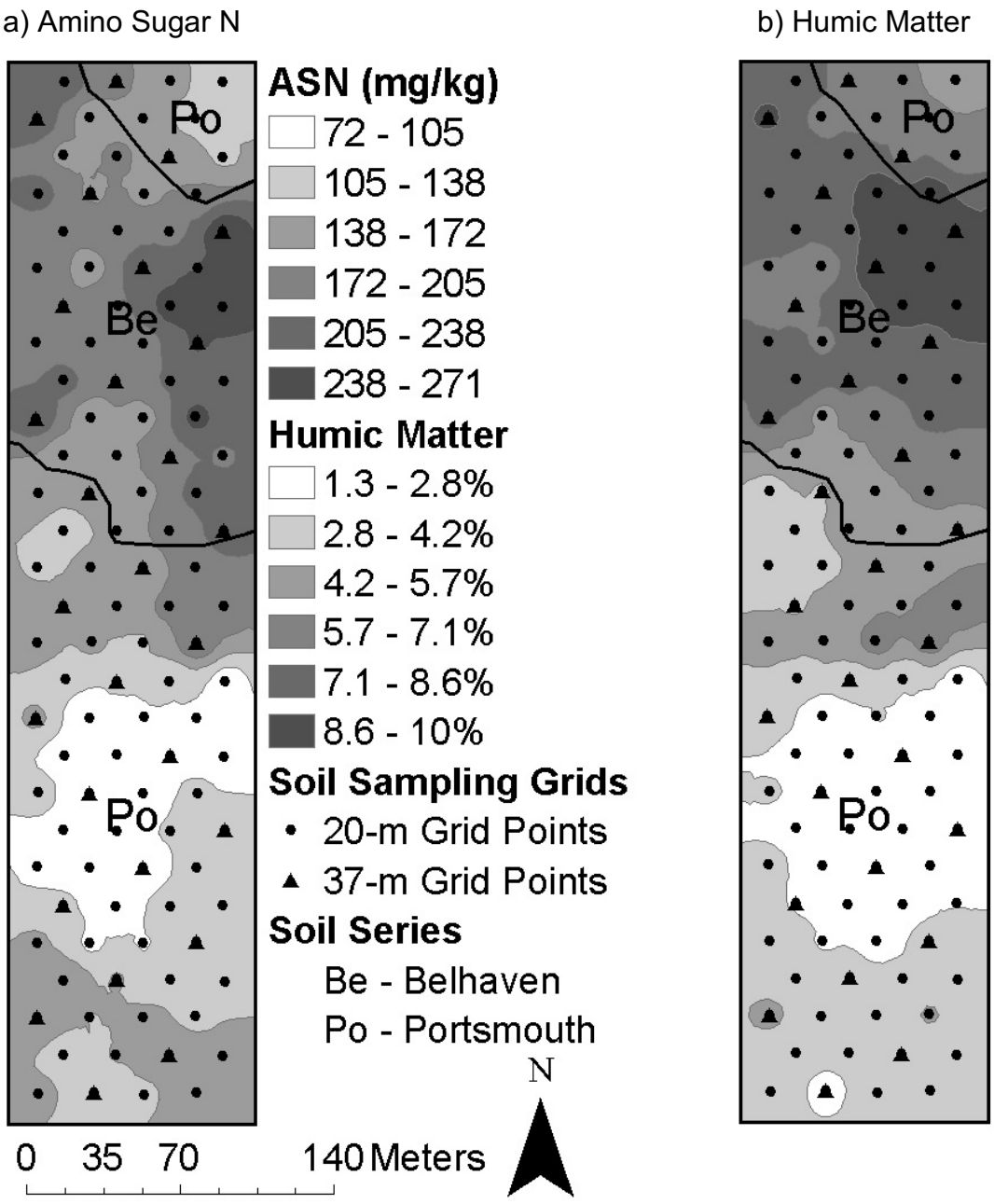

Figure 4.4. Interpolated maps of (a) amino sugar N (ASN) and (b) Humic matter (HM) for the Tidewater site with soil series' boundaries and soil sampling grid points to demonstrate spatial variability.

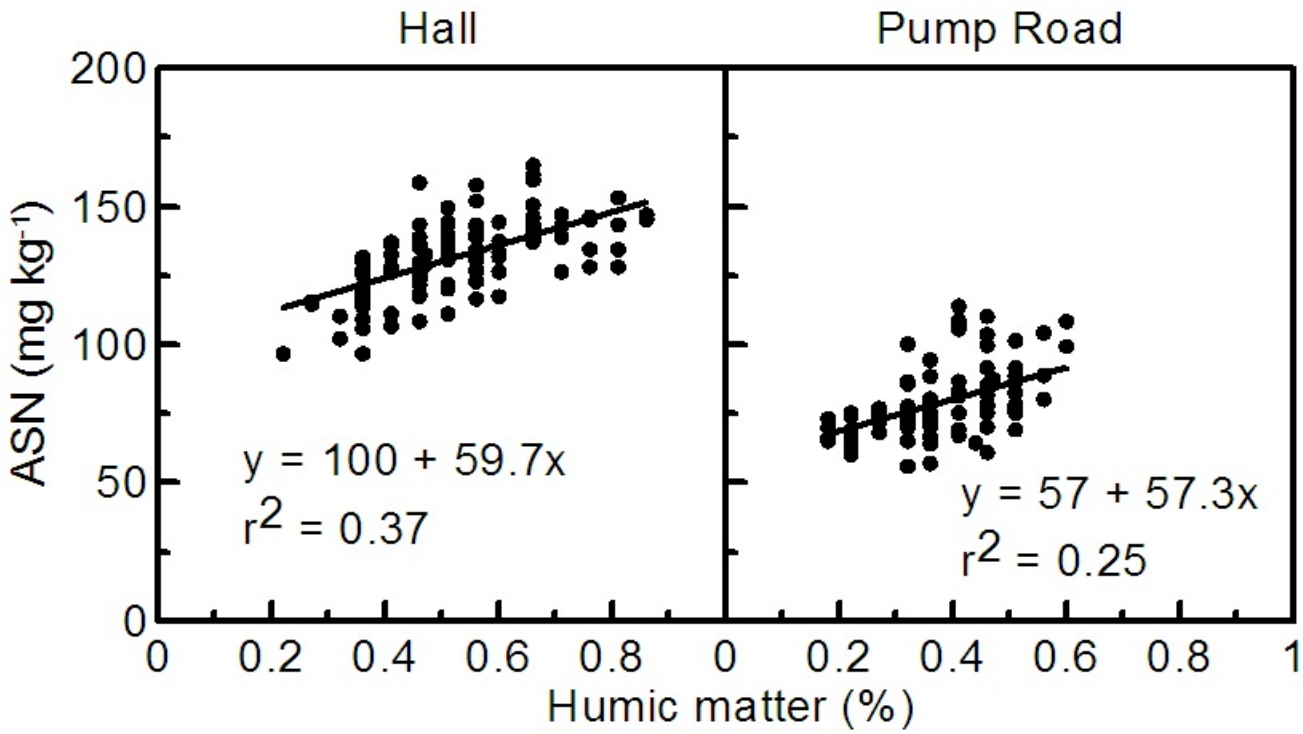

Figure 4.5. Amino sugar N (ASN)as a function of HM (range of 0.2 - 0.9%) for the Hall and Pump sites. Data appear to line up because of detection level (0.05%).

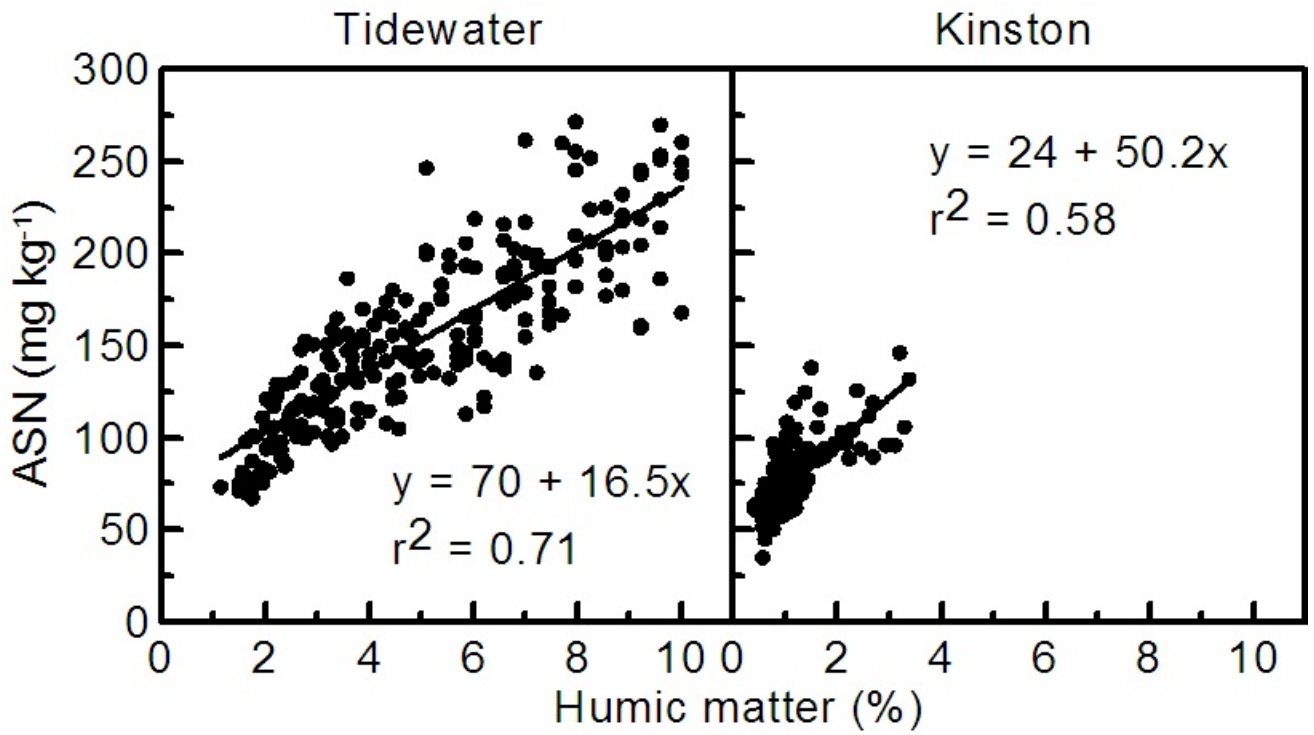

Figure 4.6. Amino sugar N (ASN) as a function of humic matter (HM; range of 0.5 - 10.0%) for the Plymouth and Kinston sites.

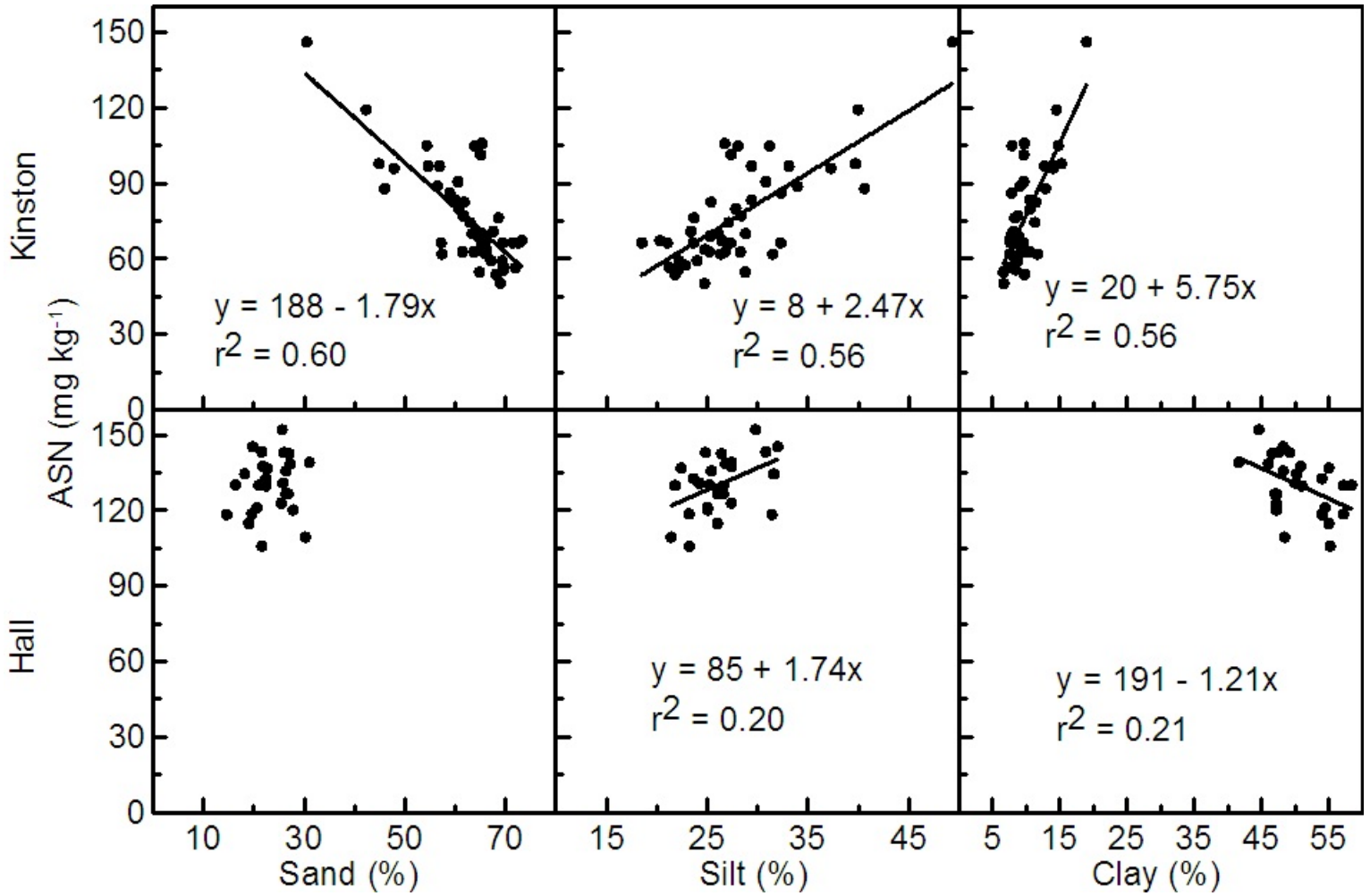

Figure 4.7. Amino sugar N as a function of soil texture (percent sand, silt, and clay) for the Kinston and Hall sites.

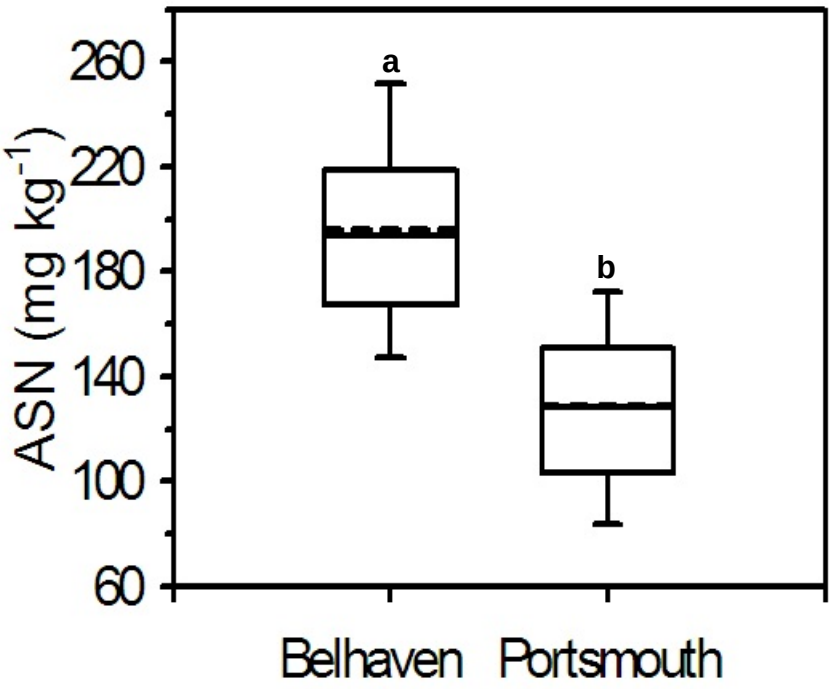

Figure 4.8. Box plot of ASN for Tidewater soils. Box indicates 25th, 50th (median), and 75th percentile, error bars indicate 10th and 90th percentile, dashed line indicates mean (if different from median), and letters denote statistical difference in mean using Tukey's mean comparison test with an alpha = 0.05).

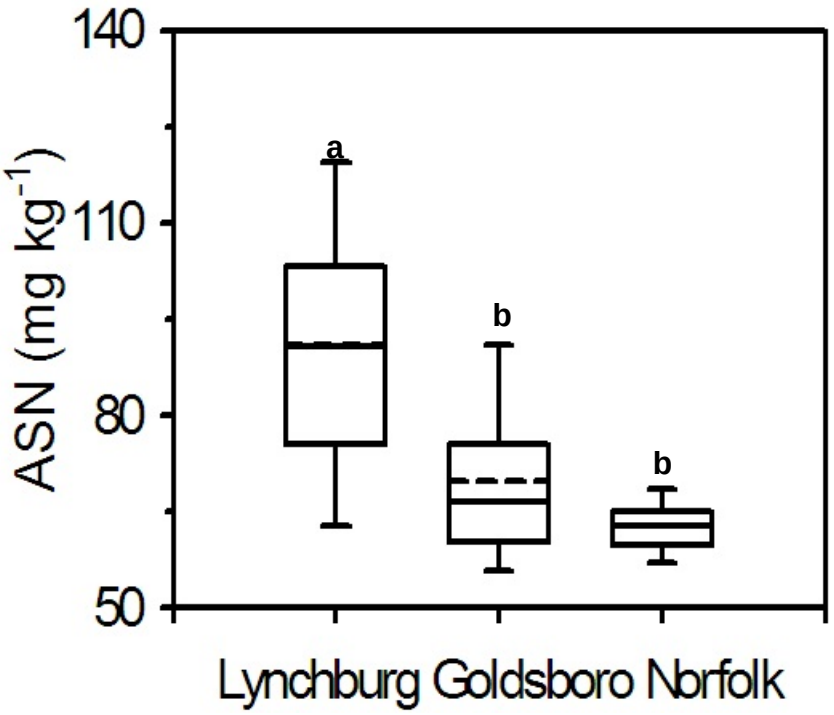

Figure 4.9. Box plot of ASN for Kinston site soils. Box indicates 25th, 50th (median), and 75th percentile, error bars indicate 10th and 90th percentile, dashed line indicates mean (if different from median), and letters denote statistical difference in mean using Tukey's mean comparison test with an alpha = 0.05).

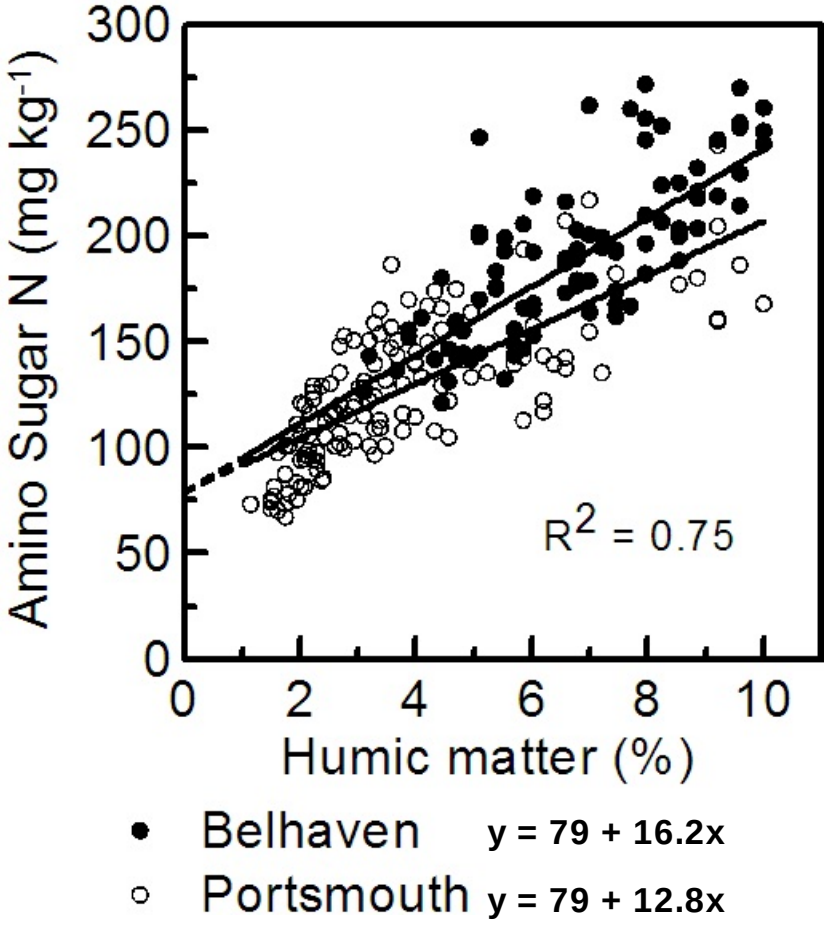

Figure 4.10. Amino sugar N as a function of HM for the Tidewater site soils.

APPENDICES

APPENDIX A

SPATIAL RELATIONSHIPS BETWEEN SOIL AMINO SUGAR NITROGEN, SOIL PROPERTIES, AND LANDSCAPE ATTRIBUTES

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Abstract

The soil amino sugar nitrogen (ASN) test has been developed to predict corn (*Zea mays* L.) nitrogen (N) need, but ASN varies widely within fields. This study examined the spatial relationships of ASN with soil and landscape properties to develop a better N management strategy. The study was conducted on a loamy sand field in North Carolina, USA. Amino sugar N was correlated with humic matter (HM; $r^2 = 0.61$) and soil texture ($r^2 = 0.55 – 0.63$). These correlations confirm that ASN can be used to create a variable rate N application, because it is spatially sensitive to soil properties that influence N availability. Amino sugar N soil sampling zones can be created from maps of HM, soil texture, and soil type to reduce sampling costs and time.

Keywords: nitrogen, nitrogen soil test, spatial variability, soil organic matter, soil texture

Introduction

Corn (*Zea mays* L.) nitrogen (N) fertilizer need varies widely within fields because of soil and landscape properties (Blackmer & White, 1996; Franzen et al, 1996). Producers in warm, humid climates do not have a functional soil N test, and N fertilizer is typically applied at a uniform N rate across the field based on realistic yield goal expectations. This fertilizer management strategy results in both under- and over-fertilization of specific areas, resulting in possible yield loss and environmental damage. Variable-rate N fertilization potentially satisfies site-specific

crop N needs and reduces excess N fertilization and its negative impacts. The challenge is to develop accurate and cost effective procedures and measurements to determine site-specific N rates. Researchers in Illinois, USA have developed the amino sugar N (ASN) test that measures a readily available portion of soil organic N (Khan et al, 2001; Mulvaney et al, 2001). Preliminary work in North Carolina, USA supports the validity of the ASN test for predicting corn N needs (Williams et al., 2004) in the southeast USA. A better N fertilizer strategy would be soil sampling before planting to develop a variable-rate N map based on ASN for side-dress application. But unlike small plots, fields have large spatial variation, and relationships of ASN with soil and landscape properties may provide verification that ASN is influenced by processes known to influence N availability.

Amino sugar N is probably influenced by soil and landscape factors, themselves related since topography is recognized as one of the major factors influencing soil formation. Landscape properties may affect ASN since they influence water and solute movement, microclimate, and plant productivity. Topographic indices such as topographic wetness index (TWI) have been used to model water movement over the land surface and predict the “wetness” or potential moisture content of a soil (Wilson & Galant, 2000). Soil texture also influences soil moisture which effects numerous processes e.g., crop residue and soil organic matter production, breakdown, mineralization, and immobilization (Delin & Linden, 2000), that vary across fields. Rates of these processes are also influenced by soil organic matter (SOM; Schmidt et al, 2002), and topography (Franzen et al, 1996).

Amino sugar N should be correlated with soil organic matter since ASN is a labile N component of SOM. Amino sugar N would be influenced by soil texture via soil moisture. For example, loamy textured soils may break down plant residues and SOM more rapidly than clayey (likely to be wetter and/or anaerobic) or sandy (likely to be excessively dry or leached) soils (Delin & Linden, 2000). Therefore, loamy soils would have more ASN, because it is a partial mineralization product of SOM. Correlations of ASN with SOM, soil texture, and TWI would verify that ASN is spatially influenced by N-cycle dynamics and an accurate variable rate N fertilizer map could be created from ASN.

Costs of soil sampling need to be minimized for ASN to be a practical variable rate N management tool. Soil sampling zones could be created based on soil and landscape properties that are correlated with ASN. Humic matter (HM), which is a component of SOM and should be correlated to ASN, is part of the routine North Carolina Dept. of Agriculture and Consumer Services (NCDA&CS) Agronomic Division Soil Testing Section laboratory analysis (Mehlich, 1984). Apparent soil electrical conductivity (EC_a) has been spatially related to soil conditions that interact with soil mineral N, presumably due to its dependence on water content, soil porosity, soil temperature, and soil texture (Fritz et al, 1999). Soil survey maps have been used to develop N management strategies because soil series differ in productivity and crop response to N due to factors such as available water, mineralization, denitrification, immobilization, and volatilization (Blackmer & White, 1996). Elevation influences water movement within fields and thereby influences

rates of soil N processes. Digital elevation maps are available to the public for free in North Carolina. Amino sugar N soil sampling costs could be reduced by developing soil sampling zones based on areas of the field with similar HM content, soil EC_a, topography, or soil map units.

This study investigated the relationships between ASN soil test values, soil properties, and landscape attributes to: 1) verify that ASN is sensitive to spatial variability of soil and landscape properties; and 2) determine which soil and landscape properties could be used to create ASN soil sampling zones. This study is a work-in-progress and this paper reports results from one of five fields currently being studied.

Materials and Methods

This research was conducted on 8.1 ha of a 12.1 ha corn field in the Coastal Plain of North Carolina, USA, the site of a four-year site-specific N management experiment. This four year experiment includes three N management treatments; the treatment receiving variable N rate application was excluded from our study since we were interested in inherent properties associated with ASN variability that develop in spite of uniform management. The field was selected because of its representative soil variability, and is mapped with three different soil series: Goldsboro loamy sand (Aquic Paleudults), Lynchburg sandy loam (Aeric Paleaquults), and Norfolk loamy sand (Typic Paleudults). The current management is a corn, wheat-double crop soybean rotation with conventional tillage.

Soil samples for determination of ASN and HM were taken on a 20-m equilateral triangular grid (Fig. A.2a, b) by compositing eight cores taken within 1 m of a grid point using a 20-mm diameter soil probe to a 20-cm depth. Sampling was done prior to corn planting in March 2004. Soil samples for textural analysis (percent sand, silt, and clay) were taken as part of the previous study on a regular 60-m grid plus an additional sampling point randomly located 20 m from each 60-m grid point (Fig. A.2c). Samples were obtained using a 76-mm diameter probe to a depth of 15-cm. Soil texture data were point kriged from the sample area to the 20-m equilateral triangular grid using an exponential semivariogram in the geostatistical extension of ArcGIS 8.3 (ESRI, Redlands, CA, USA).

The amino sugar N test was performed using the method described by Khan et al (2001) with exception of using an incubator instead of a commercial griddle for heating. Humic matter was determined by the NCDA&CS Soil Testing Section Laboratory using the method of Mehlich (1984), which determines HM colorimetrically. Soil HM was determined instead of SOM because it is part of routine soil analysis preformed by the NCDA&CS Laboratory; soil HM and SOM are highly correlated (r^2 = 0.95; Harrison et al, 1976). Soil texture analyses were performed by Waters Agricultural Laboratory (Camilla, GA, USA) using the hydrometer method to determine percent sand, silt, and clay. Horizontal soil EC_a was collected during a single day using the Geonics EM-38 (Mississauga, Ontario, Canada) on continuous transects spaced ~10 m apart and georeferenced using a differential global positioning system.

Topographic data were derived from a digital elevation model determined using LIDAR data with a horizontal posting distance of 1.0 m and a vertical accuracy of 150 mm (North Carolina Flood Mapping Program; www.ncfloodmaps.com). Slope and TWI were derived using the GRID function in ArcInfo (ESRI, Redlands, CA, USA). Topographic wetness index was modified by adding 1.0 m^2 to upslope contributing area to account for areas of the field with little slope (< 0.5%) and no flow direction (Wilson & Gallant, 2000).

Analysis of variance was performed using PROC GLM, correlation analysis using PROC CORR, and stepwise regression using PROC REG in SAS version 8.3 (SAS Institute, Cary, NC, USA).

Results and Discussion

Correlation analysis of ASN with soil properties showed relatively strong relationships with HM and soil texture (percent sand, silt, and clay; Table A.1). A regression model of ASN and HM showed a quadratic relationship (Fig. A.1). Amino sugar N and HM are components of SOM and thus should be related (Mulvaney et al, 2001), but not perfectly correlated. The variance between ASN and HM is probably because ASN is a relatively labile form of N whereas HM is a more stable form of SOM. Amino sugar N is more rapidly mineralized, denitrified, and immobilized than HM (Mehlich, 1984). For example, a field with the same HM percentage but different rates of N mineralization and immobilizations would have less ASN in areas with faster rates of mineralization and immobilization than areas

with slower rates. The relationship between ASN and HM verifies that ASN is sensitive to the spatial variability of HM.

Soil texture influences N mineralization, denitrification, and immobilization via soil moisture. If ASN is sensitive to the different rates of these soil N processes, then a correlation should exist between ASN and soil texture. Correlation and regression analysis of ASN versus silt and clay showed that ASN was positively correlated with silt and clay, but the correlation between ASN and sand was negative (Fig. A.1, Table A.1). The inverse relationship between percent sand and ASN is probably the result of soils with lower sand content (more clay and silt, i.e. loamy) having a higher soil moisture holding capacity. Relationships of ASN with percent sand, silt, and clay suggest that ASN is influenced by different rates of N mineralization, denitrification, and/or immobilization. No detailed N transformation studies have been performed to determine if better aeration increases N mineralization and leaching losses in sands, or if dryness restricts residue decomposition to yield labile organic N compounds in sands.

Among the topographic characteristics examined, only elevation was correlated, albeit weakly, with ASN (Table A.1). Sandier soils occurred higher on the landscape and could be the sites of more rapid decomposition due to better aeration. These soils would also be most subject to rapid infiltration and subsurface loss of soluble N compounds as water flowed to lower, more poorly drained areas. Topographic wetness index was not correlated with ASN (Table A.1), probably because the relief in the field is only approximately 1.5 m and the slope is between 0 to 2% resulting in

little topographic variability across the field. Micro-topography may influence ASN, but probably at a finer scale than the 20-m grid examined in this study.

Correlations of ASN with soil properties reflect N cycle processes that make N available for plant uptake. These results suggest ASN can be used for variable rate N application, but expense and time of sampling and analysis for developing a variable-rate N map may limit its implementation. Soil and landscape properties that are correlated with ASN can be used to create sampling zones. Some soil and landscape properties that can be inexpensively measured are HM, soil texture, soil EC_a, and elevation. For example, soil analysis in North Carolina is free at the state laboratory, and percent HM is part of the routine analysis. Soil texture would be expensive initially, but like soil elevation, it is relatively static and would only need to be measured once. Amino sugar sampling zones could be created by using one of these properties or a combination of them.

Maps of ASN and soil properties show potential for delineating ASN sampling zones (Fig. A.2). Maps of both ASN and HM indicated highest values in the more poorly drained Lynchburg soil map units (Fig. A.2a, b). Soil texture had similar spatial patterns, and a map of percent sand was chosen to represent soil texture because it was better correlated with ASN than silt and clay. The map of percent sand showed that areas of low sand content had high ASN (Fig. A.2a, c). These maps and regression analysis (Fig. A.1) indicate that ASN soil sampling zones could be created using either HM or soil texture.

Stepwise regression model was used to explore multi-variate possibilities for delineating ASN soil test sampling zones. Individually, HM and soil texture were strongly correlated with ASN, and a stepwise regression model of ASN predicted by HM, sand, and clay had an $R^2 = 0.71$. The contributions of HM, sand, and clay to predict ASN are not known for certain, because these variables are strongly correlated (Table A.1), and multi-collinearity exists in the model. Multi-collinearity was avoided by examining models for predicting ASN based on HM or sand individually with the additional covariates elevation and soil EC_a. The first model included HM (partial $R^2 = 0.58$), HM^2 (partial $R^2 = 0.03$), and elevation (partial R^2 =0.02) with a coefficient of determination of 0.63. The second model showed sand (partial $R^2 = 0.63$) and soil EC_a (partial $R^2 = 0.02$) significant with an overall R^2 = 0.65. Soil ECa and elevation contribution to the stepwise models is small and both variables are weakly correlated with ASN suggesting that although statistically significant they provide no practical improvement to the model. Thus, these multi-variate approaches for creating ASN sampling zones are not better than using either soil texture or HM alone. Spatial regression models will be investigated in the future to deal with multi-collinearity.

Soil type is another possible way to delineate ASN soil sampling zones. The study field included the Goldsboro, Lynchburg, and Norfolk soils series. Amino sugar N, soil texture, and HM were significantly different among soil types. The Lynchburg soil had a higher amount of ASN than Goldsboro and Norfolk (91 versus 70 and 62 mg kg^{-1}, respectively), suggesting soil type can be used to delineate ASN

sampling zones. Although ASN was statistical different among soil types, the range of ASN values within soil types was large. The Lynchburg had the largest range with ASN values between 60 and 138 mg kg^{-1} which overlapped both the Goldsboro soil (45 – 108 mg kg^{-1}) and the Norfolk (56 – 70 mg kg^{-1}, Fig. A.3). The range of ASN within soil series could be managed by creating sub-zones based on HM or soil texture. Regression models of HM and ASN by soil type were different with the exception of the Norfolk soil, for which there was no significant relationship (Fig. A.3). The range of ASN values was smallest for the Norfolk soil, and further delineation of ASN samples zones might not be necessary. The different models of HM and ASN for the Lynchburg and Goldsboro soils could be used to create ASN sampling zones by soil type and HM. Regression models of ASN versus soil texture (percent sand, silt, and clay) by soil series were not different and therefore would not improve sampling zone delineation.

Conclusions

Our results show that ASN is spatially sensitive to HM content, and that ASN and HM concentration variability are both related to soil texture and other landscape properties that influence the balance between organic matter production, decomposition, and redistribution. A variable-rate N strategy based on the ASN soil test would improve N management in warm, humid regions which currently do not have a functional soil N test. Amino sugar N would be sampled before corn planting and a variable-rate N fertilizer map would be created for side-dress N application. Additionally, soil sampling zones could be delineated for a field using maps of HM,

soil texture, or soil type, but the best strategy for creating sampling zones would be maps created using HM by soil type. Amino sugar N soil sampling zones would reduce sampling costs, but allow for a variable-rate N application which would meet crop need. A variable-rate N fertilizer application map for corn in this field in 2004 would have had a range of 75 – 250 kg N ha^{-1}. Results are preliminary and four additional sites are currently being analyzed which represent different cropping areas in North Carolina, USA.

Acknowledgements

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Table A.1. Pearson correlations coefficients (r) of amino sugar nitrogen, soil properties, and landscape attributes.

			Soil Texture				Topography	
	ASN	HM	Sand	Silt	Clay	Soil EC_a	Elevation	TWI
ASN	1							
HM	0.76***	1						
Sand	-0.79***	-0.79***	1					
Silt	0.76***	0.76***	-0.98***	1				
Clay	0.74***	0.74***	-0.86***	0.76	1			
Soil EC_a	-0.25**	-0.11	0.12	-0.13	-0.04	1		
Elevation	-0.33***	-0.20***	0.34***	-0.38***	-0.17*	0.54***	1	
TWI	0.06	0.08	-0.07	0.05	0.07	0.06	-0.04	1

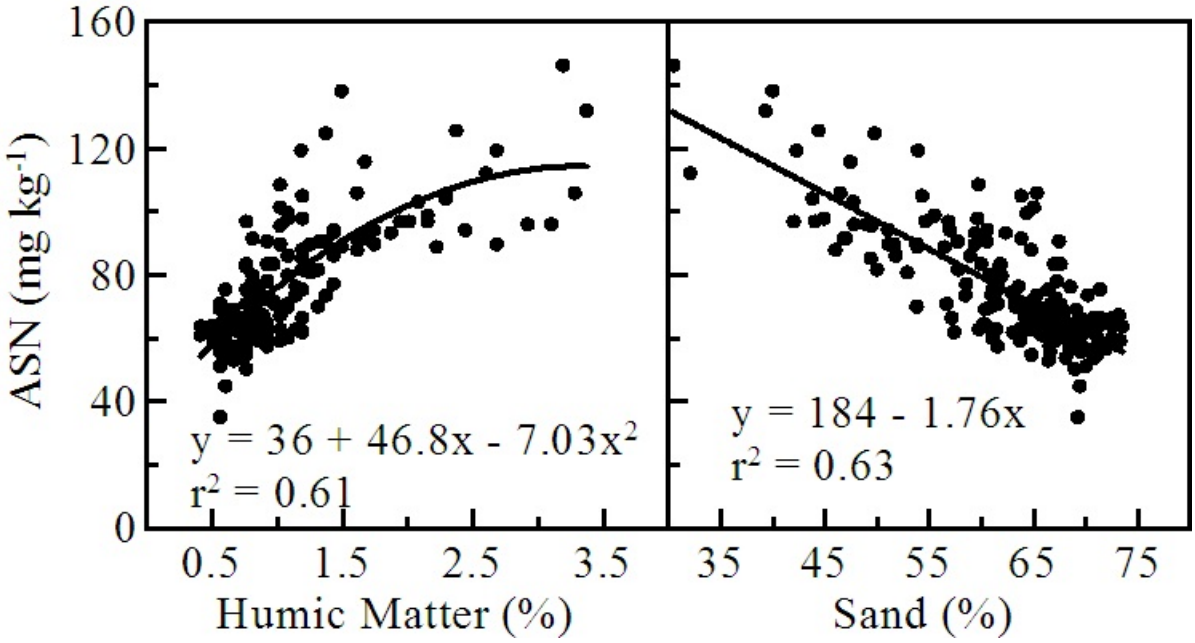


Figure A.1. Amino sugar N as a function of percent HM and sand ($p \leq 0.05$).

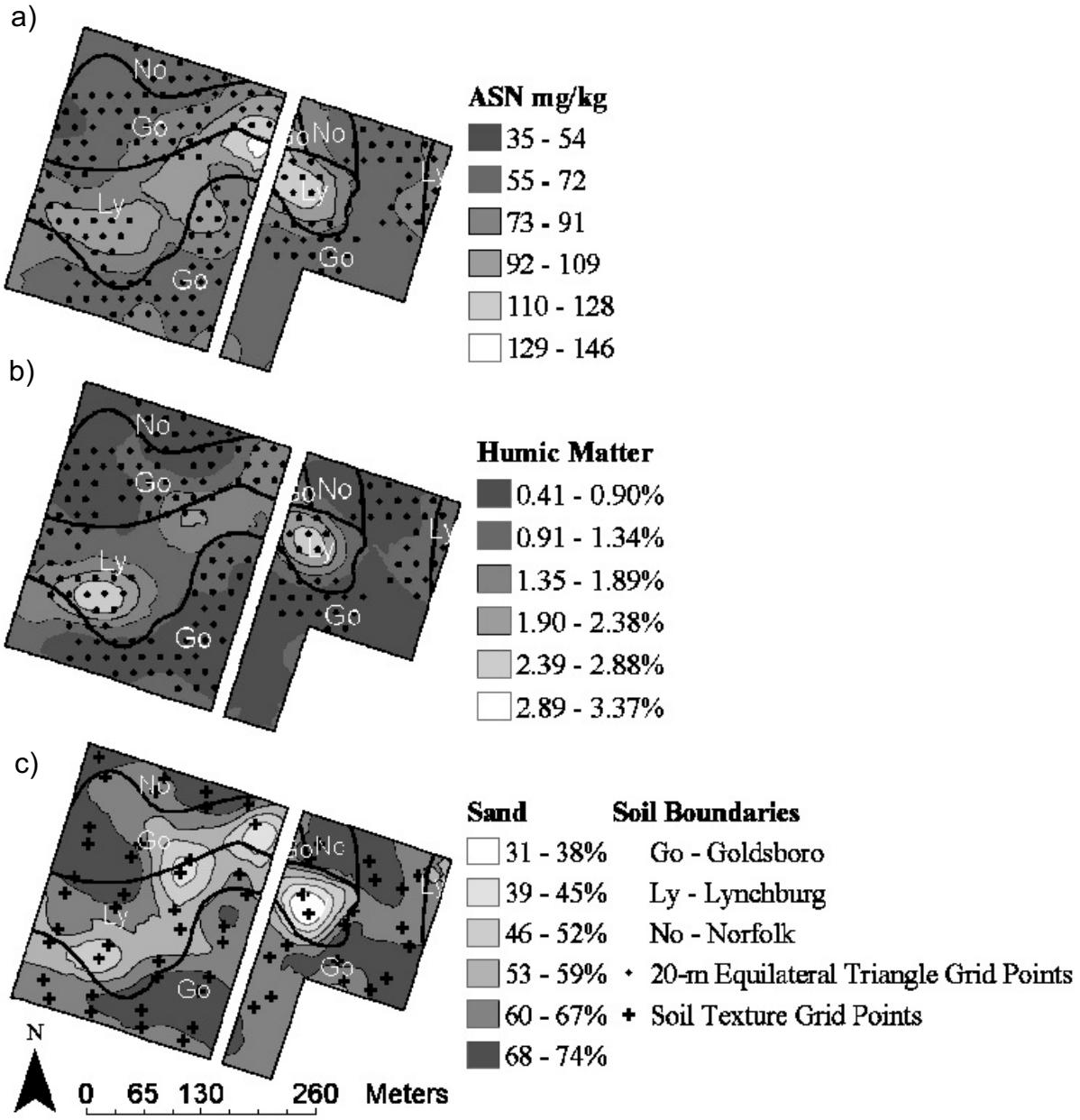


Figure A.2. Kriged maps of (a) ASN, (b) HM, (c) percents sand, soil types, and grid points of areas sampled to demonstrate spatial variability.

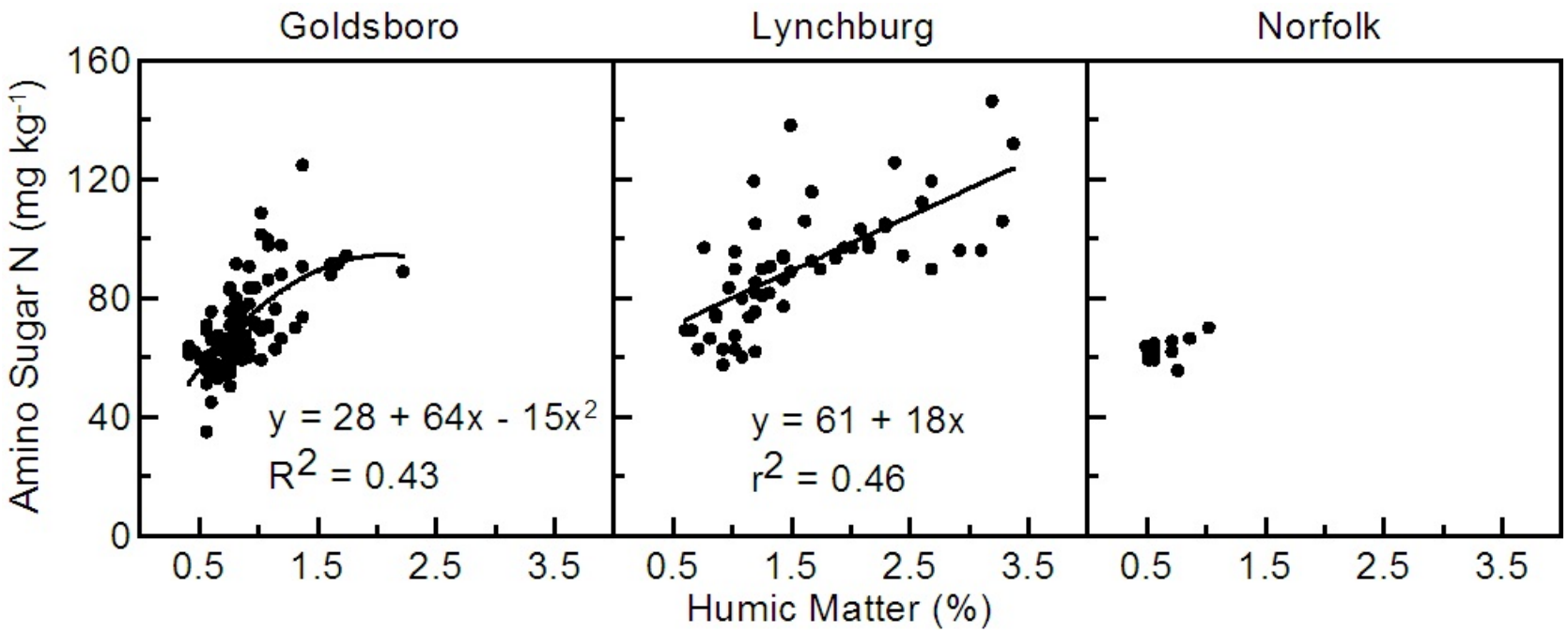


Figure A.3. Amino sugar N as a function of percent HM by soil order.

APPENDIX B

STANDARD LABORATORY OPERATING PROCEDURE FOR SOIL NITROGEN TESTS

Amino Sugar Nitrogen Test

Procedure:

1. Air dry soil sample and grind to pass a 2-mm sieve.

2. Preheat incubator between 48 and 50° C.

3. Place 1.00 ± 0.01 grams soil sample into one pint mason jar.

4. Make boric acid trap by attaching a petri dish to the jar lid with a cable tie and dispense 5 mL of H_3BO_3 indicator solution (formula listed below) into the dish. For detail instruction on how to make the boric acid trap apparatus consult University of Illinois Technical Note 02-01 (rev. f).

5. Treat the soil sample with 10 mL of 2 M NaOH and gently swirl the contents of the jar. Minimize soil adherence to the wall of the jar.

6. Allow to sit for about 15 to 30 seconds and place the lid on the jar and seal with screw band.

7. Place in an incubator at 48 to 50° C for five hours.

8. Place additional thermometers into incubator and check hourly to confirm constant and consistent temperature.

9. Shake H_2SO_4 well and standardize using 5 mL of Tris (Hydroxymethyl) Aminomethane (THAM) solution* and 5 mL of boric acid indicator solution before and after titrating samples. Calculate exact molarity of H_2SO_4 as 0.05/V, where V is the mean value for the milliliters of H_2SO_4 required to reach endpoint. Determine titer (μg N mL^{-1}) by multiplying the molarity by 28000.

10. Remove petri dish, dilute with 5 mL deionized water, and titrate with 0.01 M H_2SO_4.

11. Micrograms of liberated N is calculated as S x T, where S is the volume (milliliters) of H_2SO_4 used in titration and T is the titer of the titrant.

*THAM solution is made by dissolving 0.2430 g THAM with 100 mL deionized water.

Reagents and Chemicals:

1. 4% $H_3BO_3^-$ (Boric Acid)

2. 2 M NaOH
3. Deionized water
4. 0.01 M (0.02 N) H_2SO_4
5. Tris (Hydroxymethyl) Aminomethane granules (THAM; $C_4H_{11}NO_3$; Fischer catalog #T395-100)

Equipment List:

1. Scale
2. Measuring boats
3. Measuring utensil
4. Pint Mason Jars
5. Ball^R dome lid (86 mm)
6. Petri dish
7. Boric acid trap apparatus
8. Incubator
9. Beakers
10. Microburette (for titration)
11. Stir plate and stir bar
12. Pipet (5 and 10 mL)
13. Thermometer

Formula:

Boric Acid 4% with indicator Recipe (makes 20 L):

1. While stirring vigorously with a motorized stirrer, dissolved 800 g of reagent grade H_3BO_3 in approx. 18 L of deionized water.

2. Add 0.099 g of bromocresol green and 0.066 g of methyl red (as water soluble sodium salts).

3. Bring the volume up to 20 L with deionized water.

4. Stirring continuously, adjust the pH to 4.2 to 4.3 by adding single NaOH pellets

Sodium Hydroxide Solution (2 M; makes 1 L):

1. Dissolve 80 g of reagent grade NaOH pellets in approx. 800 mL of deionized water in a 1 L volumetric flask.

2. Dilute to 1 L after solution has cooled to room temperature

3. Mix thoroughly

4. Store in a tightly stoppered flask or bottle that prevents absorption of atmospheric CO_2

Sulfuric Acid Solution (0.01 M standard; makes 1 L):

1. Dilute 0.56 mL of concentrated H_2SO_4 to 1 L

2. Mix thoroughly

3. Standardize by titrating several 5 mL aliquots of THAM solution

Gas Pressure Test

Procedure:

1. Measure 5 g of air dried soil into 5 mL of distilled water in a 125 mL Serum bottle.

2. Add 0.3 g $Ca(ClO)_2$ to a small Tygon tube (stopped on one end using a gray polyvinyl chloride stopper) and insert tube into septum.

3. Place rubber septum on bottle.

4. Use a hypodermic needle to push the stopper out of the Tygon tube and remove needle.

5. Measure initially pressure inside the bottle.

6. Shake the mixture on an orbital shaker at 250 rpm for 25 minutes.

7. Immediately remove bottles and measure final pressure.

8. Calculate change in pressure by subtracting initial pressure from final pressure.

Reagent List:

1. $Ca(ClO)_2$ Calcium hypochlorite

Equipment List:

1. 125 ml serum bottle
2. Rubber septum
3. Tygon tube (8 mm o.d., 5 mm i.d., 30mm long)
4. Stopper
5. Hypodermic needle (18 gauge)
6. Scale (for weighing sample)
7. Orbital shaker
8. Tensimeter (Soil Measurement Systems, Tucson, AZ)

Pre-plant Nitrate Test

Procedure:

1. Air dry soil sample and grind to pass a 2 mm sieve.

2. Weigh out 5 g of dry soil and place in plastic extraction cup (record actual mass to 2 decimal places!).

   *Prepare at least one blank per 10 samples.

3. Add 40 ml of 1 N KCl to each sample.

4. Shake samples for 1 hour, filter with Whatman #2 paper into a plastic scintillation vial.

5. Determine NO_3 colorimetrically using the Lachat Autoanalyzer.

6. Correct the NO_3 concentration (mg N L^{-1}) in the extract from each tube by subtracting the mean concentration found in blanks.

7. Nitrate ammonium concentration per gram dry weight of soil: (corrected extract NO_3 mg N L^{-1}) x (1L/1000mL) x (40ml/1 sample) x (1sample/ ____ g) = ___ mg NO_3 /g soil

Reagents:

1. 2 N KCl

Equipment List:

1. Plastic beakers
2. Scale (for weighing sample)
3. Shaker
4. #2 Whatman filter paper
5. Plastic scintillation vial
6. Lachat autoanalyzer

Incubation Residual Nitrogen Test

Procedure:

1. Air dry soil sample and grind to pass a 2-mm sieve.

2. Weigh out sample into replicate sets of tubes, with approximately 5 g of dry soil per tube (record actual mass to 2 decimal places!).

   Prepare at least one blank per 10 samples.

3. Added 20 ml distilled water to each test tube.

4. Extract 1 set of tubes immediately,(fresh extract; add 20 ml of 2 N KCL, shake 1 hour, filter with Whatman #2 paper into a plastic scintillation vial, freeze).

5. Incubate 2nd set of tubes for 7 days (or longer) at 35-40C. Extract samples after one week period; add 20 ml of 2 N KCL, shake 1 hour, filter with Whatman #2 paper into a plastic scintillation vial, freeze.

6. Run sample on the Lachat to determine NH_4 levels

7. Correct the ammonium concentration (mg N L^{-1}) in the extract from each tube by subtracting the mean concentration found in blanks for this set of tubes.

8. Calculate the ammonium concentration per gram dry weight of soil: (corrected extracted NH_4-N mg N L^{-1}) x (1L/1000mL) x (40ml/1 sample) x (1sample/ ____ g) = ___ mg NH_4-N/g soil

9. Calculate N mineralization by subtracting ammonium concentration of the fresh extract from ammonium concentration of the incubated extract.

10. Calculate the incubated residual N by adding nitrate from the pre-plant nitrate test to the N mineralized amount.

Reagents:

1. 2 N KCl

Equipment List:

1. Plastic beakers
2. Scale (for weighing sample)
3. Shaker
4. #41 Whatman filter paper
5. Plastic scintillation vial
6. Freezer
7. Incubator (35-40° C constant)
8. Lachat autoanalyzer

APPENDIX C

DETAILED SOIL, CROP, AND CLIMATE INFORMATION FOR NITROGEN RESPONSE TRIALS

Table C.1. Detailed soil information for research plots.

Year	Site	Research Station	County	Soil Series	Soil Taxonomic Classification	Drainage¶
				Piedmont		
2003	CLE	Cleveland	Cleveland	Cecil clay loam	Fine, kaolinitic, thermic Typic Kanhapludults	Well
2003	SAL3	Piedmont	Rowan	Hiwassee clay loam	Fine, kaolinitic, thermic Typic Rhodudults	Well
2004	LW	Lake Wheeler	Wake	Appling gravelly sandy loam	Fine, kaolinitic, thermic Typic Kanhapludults	Well
2004	SAL4	Piedmont	Rowan	Hiwassee clay loam	Fine, kaolinitic, thermic Typic Rhodudults	Well
				Coastal Plain		
2001	PBI1	Peanut Belt	Bertie	Norfolk loamy sand	Fine-loamy, siliceous, thermic Typic Paleudults	Well
2001	PBI2	Peanut Belt	Bertie	Norfolk loamy sand	Fine-loamy, siliceous, thermic Typic Paleudults	Well
2001	PBN1	Peanut Belt	Bertie	Norfolk loamy sand	Fine-loamy, siliceous, thermic Typic Paleudults	Well
2003	PBI3	Peanut Belt	Bertie	Norfolk loamy sand	Fine-loamy, siliceous, thermic Typic Paleudults	Well
2004	KIN	Lower Coastal Plain	Lenior	Goldsboro loamy sand	Fine-loamy, siliceous, thermic Aquic Paleudults	Well
2004	PBI4	Peanut Belt	Bertie	Norfolk loamy sand	Fine-loamy, siliceous, thermic Typic Paleudults	Well
2004	PBN4	Peanut Belt	Bertie	Norfolk loamy sand	Fine-loamy, siliceous, thermic Typic Paleudults	Well
2004	SD	Sandhills	Moore	Candor sand	Sandy, siliceous, thermic Arenic Paleudults	Well
				Tidewater		
2001	H1	Smith Farms	Beaufort	Ponzer muck	Loamy, mixed, dysic, thermic Terric Haplosaprists	Poor
2001	TI1a	Tidewater	Washington	Cape Fear loam	Clayey, mixed, thermic Typic Umbraquults	Well
2001	TI1b	Tidewater	Washington	Cape Fear loam	Clayey, mixed, thermic Typic Umbraquults	Well
2002	EP	Earl Pugh Farms	Hyde	Hydeland silt loam	Fine-silty, mixed, semiactive, thermic Typic Umbraqualfs	Poor
2002	POB	Tidewater	Washington	Belhaven muck	Loamy, mixed, dysic, thermic Terric Medisaprists	Poor
2002	TMI2	Tidewater	Washington	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	Well
2002	TOI2	Tidewater	Washington	Belhaven muck	Loamy, mixed, dysic, thermic Terric Medisaprists	Poor
2003	GDA	Davenport Farms	Washington	Wasda muck	Fine-loamy, mixed, semiactive, acid, thermic Histic Humaquepts	Very poor

2003	TCF	Foster Farms	Tyrrell	Weeksville silt loam	Coarse-silty, mixed, semiactive, acid, thermic Typic Humaquepts	Very poor
2003	TE3	Tidewater	Washington	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	Well
2003	TMI3	Tidewater	Washington	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	Well
2003	TMN3	Tidewater	Washington	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	Well
2003	TOI3	Tidewater	Washington	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	Well
2003	TON3	Tidewater	Washington	Hyde loam	Fine-silt, mixed, thermic Typic Umbraquults	Well
2004	HEP	Earl Pugh Farms	Hyde	Acredale silt loam	Fine-silty, mixed, active, thermic Typic Endoaqualfs	Poor
2004	PNB	Tidewater	Washington	Belhaven muck	Loamy, mixed, dysic, thermic Terric Medisaprists	Poor
2004	TE4	Tidewater	Washington	Cape Fear loam	Clayey, mixed, thermic Typic Umbraquults	Well
2004	TMN4	Tidewater	Washington	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	Well
2004	TON4	Tidewater	Washington	Cape Fear loam	Clayey, mixed, thermic Typic Umbraquults	Well

¶Poor, poorly drained mineral soils with no tile drainages or organic soil; Well, well-drained or artificial drained by tile drainage; Very poor, very poorly drained soils with a histic epipedon.

Table C.2 Detailed climate and cropping information for research plots.

	Total precipitation (mm)		Mean temperature (°C)						
Site	Apr-Sept	Annual	Apr-Sept	Annual	Tillage*	Previous Crop	Irrigation†	Planting Date	Variety
					Piedmont				
CLE	626	1250	20.8	14.4	NT	corn	NI	17 Apr 2003	Pioneer 31B12
SAL3	543	1079	22.1	15.6	NT	wheat-soybeans	NI	17 Apr 2003	Dekalb C61-25
LW	609	1180	22.0	15.4	NT	crimson	NI	19 Apr 2004	Pioneer 31R88
SAL4	543	1079	22.1	15.6	NT	wheat-soybeans	IR	06 Apr 2004	Pioneer 3163
					Coastal Plain				
PBI1	621	1165	21.4	14.8	CT	wheat/sorghum	IR	4 Apr 2001	Pioneer 31G98
PBI2	621	1165	21.4	14.8	CT	wheat/sorghum	IR2	4 Apr 2001	Pioneer 31G98
PBN1	621	1165	21.4	14.8	CT	wheat/sorghum	NI	4 Apr 2001	Pioneer 31G98
PBI3	621	1165	21.4	14.8	CT	corn	IR	03 Apr 2003	Pioneer 31G98
KIN	740	1299	22.0	15.8	CT	wheat-soybeans	NI	04 Apr 2004	Pioneer 32R25
PBI4	621	1165	21.4	14.8	CT	cotton	IR	14 Apr 2004	Pioneer 31G98
PBN4	621	1165	21.4	14.8	CT	corn/peanuts	NI	14 Apr 2004	Pioneer 31G98
SD	629	1218	22.3	15.8		Horticulture	NI		Dekalb 69-71 RR
					Tidewater				
H1	701	1252	22.1	15.9	NT	soybean	NI	16 Apr 2001	Cargill 8327
TI1a	738	1321	22.7	16.6	CT	corn	IR	30 Apr 2001	Pioneer 31G98
TI1b	738	1321	22.7	16.6	CT	corn	IR2	30 Apr 2001	Pioneer 31G98
EP	731	1334	22.4	16.5	NT	soybean	NI		
POB	738	1321	22.7	16.6	NT	soybean	NI		Pioneer 31G98
TMI2	738	1321	22.7	16.6	CT	corn	IR	02 May 2002	Pioneer 31G98
TOI2	738	1321	22.7	16.6	CT	corn	IR	02 May 2002	Pioneer 31G98
GDA	738	1321	22.7	16.6	NT	soybean	NI		Pioneer 31G66
TCF	729	1218	22.3	16.2	NT	soybean	NI		
TE3	738	1321	22.7	16.6	NT	soybean	NI		Pioneer 32R25
TMI3	738	1321	22.7	16.6	CT	soybean	IR	01 May 2003	Pioneer 31G98

TMN3	738	1321	22.7	16.6	CT	soybean	NI	01 May 2003	Pioneer 31G98
TOI3	738	1321	22.7	16.6	CT	soybean	IR	01 May 2003	Pioneer 31G98
TON3	738	1321	22.7	16.6	CT	soybean	NI	01 May 2003	Pioneer 31G98
HEP	731	1334	22.4	16.5	NT		NI		
PNB	738	1321	22.7	16.6	CT	soybean	NI		Dekalb 69-71 RR
TE4	738	1321	22.7	16.6	NT	corn	NI		Pioneer 31G98
TMN4	738	1321	22.7	16.6	CT	corn/soybean	NI	30 Apr 2004	Pioneer 31G98
TON4	738	1321	22.7	16.6	CT	corn/soybean	NI	30 Apr 2004	Pioneer 31G98

*CT, conventional tillage; NT, no-till

†IR, irrigated; IR2, irrigated at twice the normal rate; NI, not irrigated

Table C.3. Experimental Design, fertilizer and harvesting information for research plots.

Site	Spacing (cm)	Length (m)	Reps	Trt	N fertilizer source*	Pre-plant N (kg ha^{-1})	Side-dress N rates applied (kg ha^{-1})	Fertilizer Application†	Harvesting method‡
					Piedmont				
CLE	76	8	6	5	NH_4NO_3	0	0, 56, 112, 168, 224	broadcast	hand/combine
SAL3	76	9	6	5	NH_4NO_3	26	0, 56, 112, 168, 224	broadcast	combine
LW	91	9	6	6	NH_4NO_3	24	0, 56, 112, 168, 224, 280	broadcast	hand/combine
SAL4	76	9	6	6	NH_4NO_3	0	0, 56, 112, 168, 224, 280	broadcast	combine
					Coastal Plain				
PBI1	91	9	3	4	UAN	0, 112	0, 112, 168, 224	broadcast	combine
PBI2	91	9	3	4	UAN	0, 112	0, 112, 168, 224	broadcast	combine
PBN1	91	9	3	4	UAN	0, 112	0, 112, 168, 224	broadcast	combine
PBI3	91	9	4	10	UAN	0, 56	0, 56, 112, 224, 280	broadcast	combine
KIN	97	9	6	6	NH_4NO_3	12	0, 56, 112, 168, 224, 280	broadcast	hand/combine
PBI4	91	9	6	10	UAN	0, 56	0, 56, 112, 168, 224	broadcast	combine
PBN4	91	9	6	10	UAN	0, 56	0, 56, 112, 168, 224	broadcast	combine
SD			6	7	UAN	0	0, 34, 68, 100, 134, 168, 202	bc, in, dr	hand
					Tidewater				
H1	91	9	3	4	UAN	0	0, 112, 168, 224	broadcast	combine
TI1a	97	9	3	4	UAN	0	0, 112, 168, 224	broadcast	combine
TI1b	97	9	3	4	UAN	0	0, 112, 168, 224	broadcast	combine
EP			6	7	UAN	27	0, 34, 68, 100, 134, 168, 202	bc, in, dr	hand
POB			6	7	UAN	55	0, 34, 68, 100, 134, 168, 202	bc, in, dr	hand
TMI2	97	9	4	4	UAN	0	0, 112, 168, 224	broadcast	combine
TOI2	97	9	4	4	UAN	0	0, 112, 168, 224	broadcast	combine

GDA			6	7	UAN	24	0, 34, 68, 100, 134, 168, 202	bc, in, dr	hand
TCF			6	7	UAN	56	0, 34, 68, 100, 134, 168, 202	bc, in, dr	hand
TE3			6	7	UAN	28	0, 34, 68, 100, 134, 168, 202	bc, in, dr	hand
TMI3	97	9	4	8	UAN	0, 56	0, 56, 112, 224, 280	broadcast	combine
TMN3	97	9	4	10	UAN	0, 56	0, 56, 112, 224, 280	broadcast	combine
TOI3	97	9	4	8	UAN	0, 56	0, 56, 112, 224, 280	broadcast	combine
TON3	97	9	4	8	UAN	0, 56	0, 56, 112, 224, 280	broadcast	combine
HEP			6	7	UAN	0	0, 34, 68, 100, 134, 168, 202	bc, in, dr	hand
PNB			4	6	UAN	0	0, 39, 78, 118, 157, 196		hand
TE4			4	6	UAN	0	0, 39, 78, 118, 157, 196		hand
TMN4	97	9	6	10	UAN	0, 56	0, 56, 112, 168, 224	broadcast	combine
TON4	97	9	6	10	UAN	0, 56	0, 56, 112, 168, 224	broadcast	combine

*UAN, 32% urea ammonium nitrate solution; NH_4NO_3, Ammonium nitrate

†bc, broadcast applied; in, incorporated into the soil; dr, dribbled onto the soil surface

‡combine, harvest the middle two rows with grain combine; hand, harvest 3 m of middle two rows by hand, weighed cobs, and shelled a subset; hand/combine, harvest 3 m of middle two rows by hand and threshed cobs in grain combine

APPENDIX D

PAIRWISE CORRELATION TABLES OF AMINO SUGAR NITROGEN, SOIL PROPERTIES, AND LANDSCAPE ATTRIBUTES BY FIELD AND GRID SIZE

Table D.1. Hall field pairwise correlation of ASN, soil properties, and landscape attributes for 20-m grid.

	ASN	HM	Soil EC_a	Slope	Elevation	TWI
ASN	1					
HM	0.61***	1				
Soil EC_a	-0.23*	NS	1			
Slope	-0.32***	-0.46***	-0.09	1		
Elevation	0.26**	NS	-0.79***	NS	1	
TWI	NS	NS	NS	-0.25*	NS	1

*significant at alpha = 0.05
**significant at alpha = 0.01
***significant at alpha = 0.001
NS, not significant at alpha = 0.05

Table D.2. Hall field pairwise correlation of ASN, soil properties (including soil texture), and landscape attribute for 37-m grid.

	ASN	HM	Soil EC_a	Slope	Elevation	TWI	Sand	Silt	Clay
ASN	1								
HM	0.56**	1							
Soil EC_a	NS	NS	1						
Slope	NS	NS	NS	1					
Elevation	NS	NS	-0.82***	NS	1				
TWI	NS	NS	NS	NS	0.52**	1			
Sand	NS	NS	-0.47*	NS	NS	NS	1		
Silt	0.44*	0.46*	NS	-0.41*	NS	NS	NS	1	
Clay	-0.46*	-0.53**	0.41*	NS	NS	NS	-0.74***	-0.40*	1

*significant at alpha = 0.05
**significant at alpha = 0.01
***significant at alpha = 0.001
NS, not significant at alpha = 0.05

Table D.3. Pump pairwise comparison of ASN, soil properties, and landscape attributes for 20-m grid.

	ASN	HM	Soil EC_a	slope	elev	TWI
ASN	1					
HM	0.50***	1				
Soil EC_a	NS	NS	1			
slope	-0.21*	NS	NS	1		
elev	NS	-0.37***	-0.43***	NS	1	
TWI	0.21*	NS	NS	-0.26**	-0.29**	1

*significant at alpha = 0.05
**significant at alpha = 0.01
***significant at alpha = 0.001
NS, not significant at alpha = 0.05

Table D.4. Pump field pairwise correlation of ASN, soil properties (including soil texture), and landscape attribute for 37-m grid.

	ASN	HM	Soil EC_a	Slope	Elevation	TWI	Sand	Silt	Clay
ASN	1								
HM	0.62***	1							
Soil EC_a	NS	NS	1						
Slope	NS	NS	NS	1					
Elevation	NS	NS	NS	NS	1				
TWI	NS	NS	-0.49*	NS	-0.47*	1			
Sand	NS	NS	NS	NS	NS	NS	1		
Silt	NS	NS	NS	NS	NS	NS	-0.48*	1	
Clay	NS	NS	NS	NS	NS	NS	-0.87***	NS	1

*significant at alpha = 0.05
***significant at alpha = 0.001
NS, not significant at alpha = 0.05

Table D.5. Plymouth field pairwise comparison of ASN, soil properties, and landscape attributes 20-m grid.

	ASN	HM	Soil EC_a	Slope	Elevation	TWI
ASN	1					
HM	0.84***	1				
Soil EC_a	NS	NS	1			
Slope	NS	NS	NS	1		
Elevation	-0.66***	-0.78***	-0.25***	NS	1	
TWI	-0.17*	-0.14*	NS	-0.43***	NS	1

*significant at alpha = 0.05
**significant at alpha = 0.01
***significant at alpha = 0.001
NS, not significant at alpha = 0.05

Table D.6. Plymouth field pairwise correlation of ASN, soil properties (including soil texture), and landscape attribute for 37-m grid.

	ASN	HM	Soil EC_a	Slope	Elevation	TWI	Sand	Silt	Clay
ASN	1								
HM	0.68***	1							
Soil EC_a	0.39**	0.40**	1						
Slope	NS	NS	NS	1					
Elevation	-0.65***	-0.67***	-0.39**	NS	1				
TWI	NS	NS	NS	-0.30*	NS	1			
Sand	NS	NS	NS	NS	-0.30*	NS	1		
Silt	NS	NS	0.33*	NS	NS	-0.27*	NS	1	
Clay	NS	-0.34**	NS	NS	0.31*	0.27*	-0.61***	-0.39**	1

*significant at alpha = 0.05
**significant at alpha = 0.01
***significant at alpha = 0.001
NS, not significant at alpha = 0.05

Table D.7. Kinston field pairwise correlation of ASN, soil properties, and landscape attributes for 20-m grid.

	ASN	HM	Soil EC_a	slope	elev	TWI
ASN	1					
HM	0.76***	1				
Soil EC_a	-0.25***	NS	1			
slope	NS	NS	NS	1		
elev	-0.33***	-0.20**	0.53***	-0.44***	1	
TWI	NS	NS	NS	-0.36***	NS	1

*significant at alpha = 0.05
**significant at alpha = 0.01
***significant at alpha = 0.001
NS, not significant at alpha = 0.05

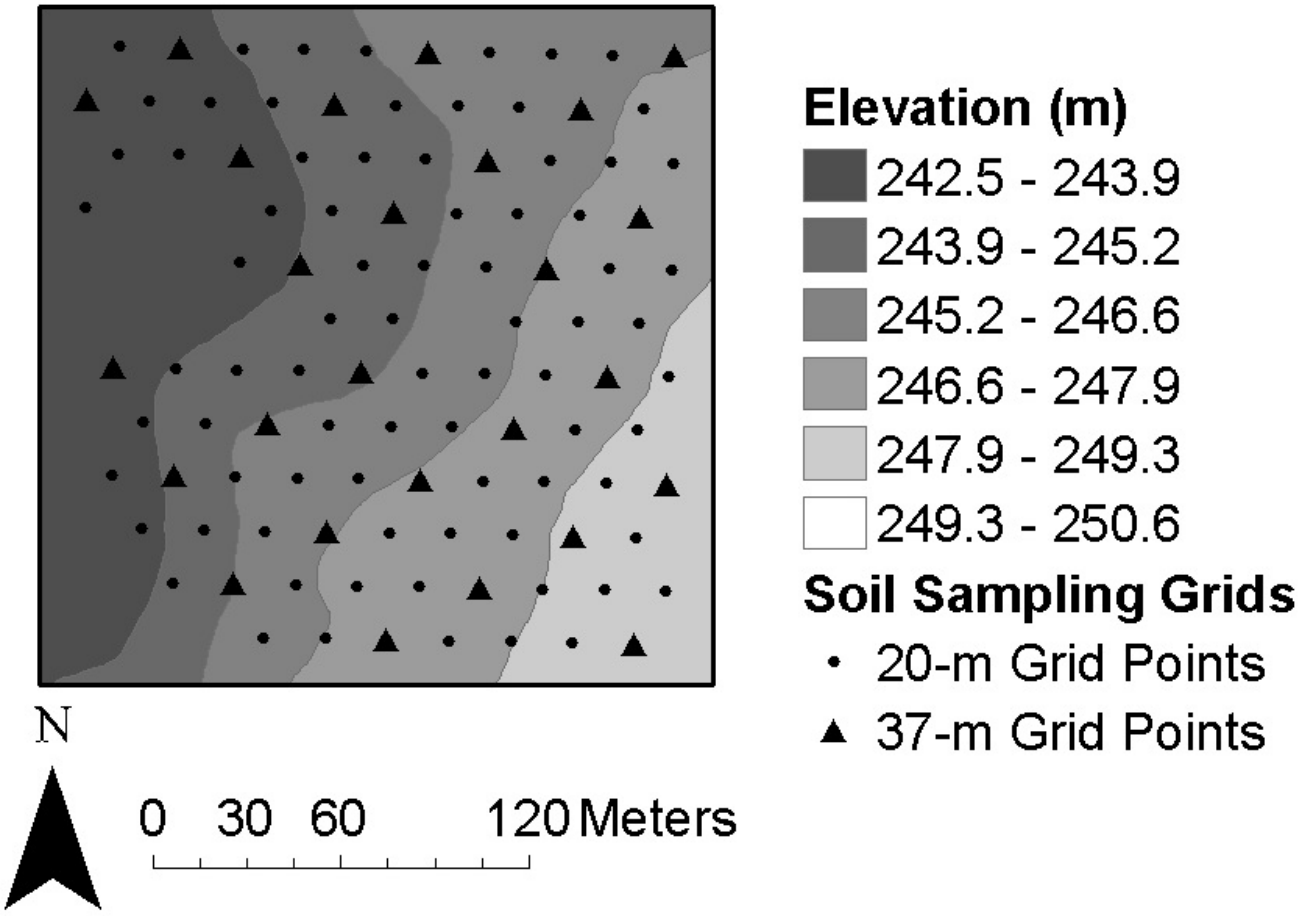
Table D.8. Kinston field pairwise correlation of ASN, soil properties (including soil texture), and landscape attribute for 37-m grid.

	ASN	HM	Soil EC_a	Slope	Elevation	TWI	Sand	Silt	Clay
ASN	1								
HM	0.75***	1							
Soil EC_a	NS	NS	1						
Slope	NS	NS	NS	1					
Elevation	-0.38**	NS	0.52***	-0.34*	1				
TWI	NS	NS	NS	-0.41**	NS	1			
Sand	-0.78***	-0.73***	NS	NS	0.37**	NS	1		
Silt	0.75***	0.70***	NS	NS	-0.41**	NS	-0.98***	1	
Clay	0.75***	0.70***	NS	NS	NS	NS	-0.87***	0.76***	1

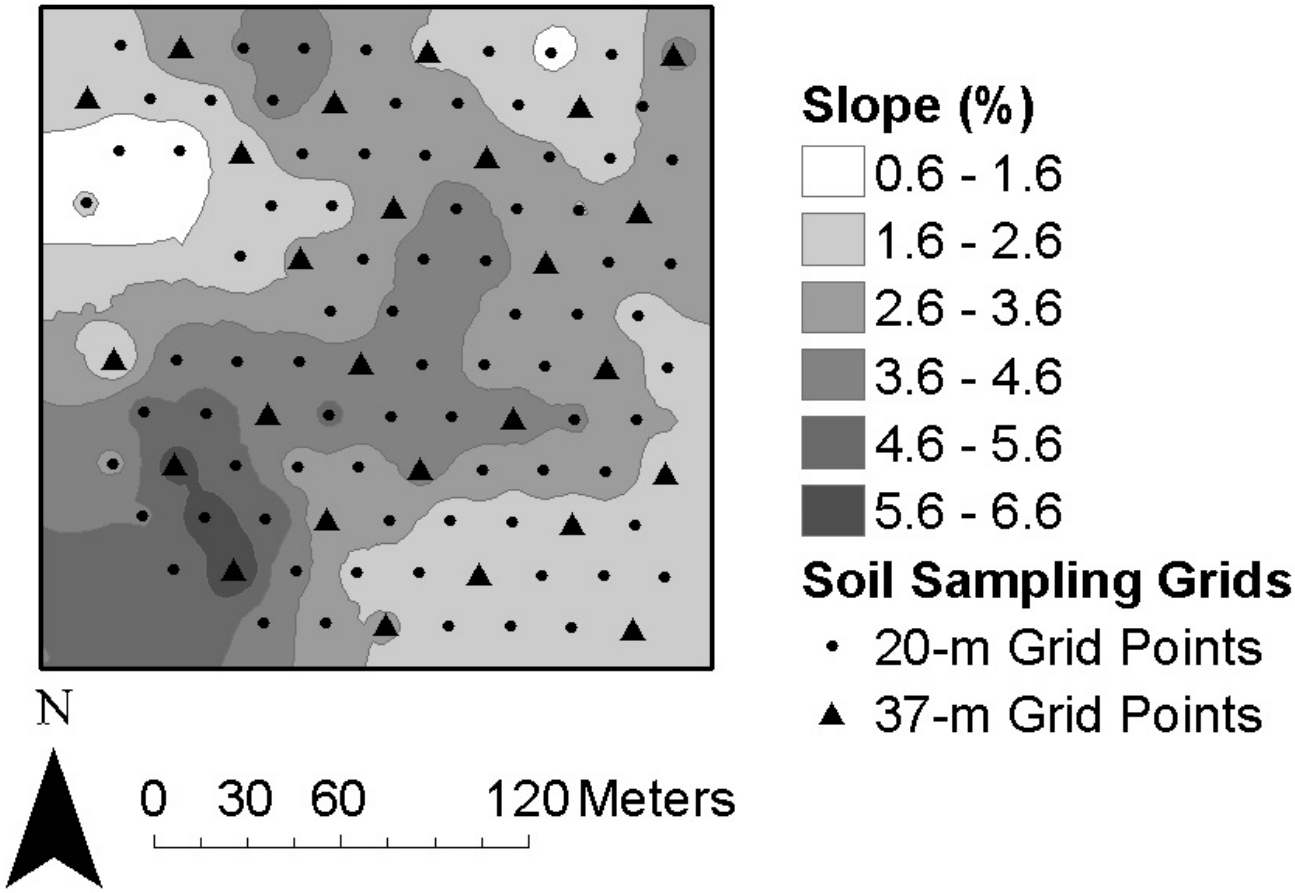
*significant at alpha = 0.05
**significant at alpha = 0.01
***significant at alpha = 0.001
NS, not significant at alpha = 0.05

APPENDIX E

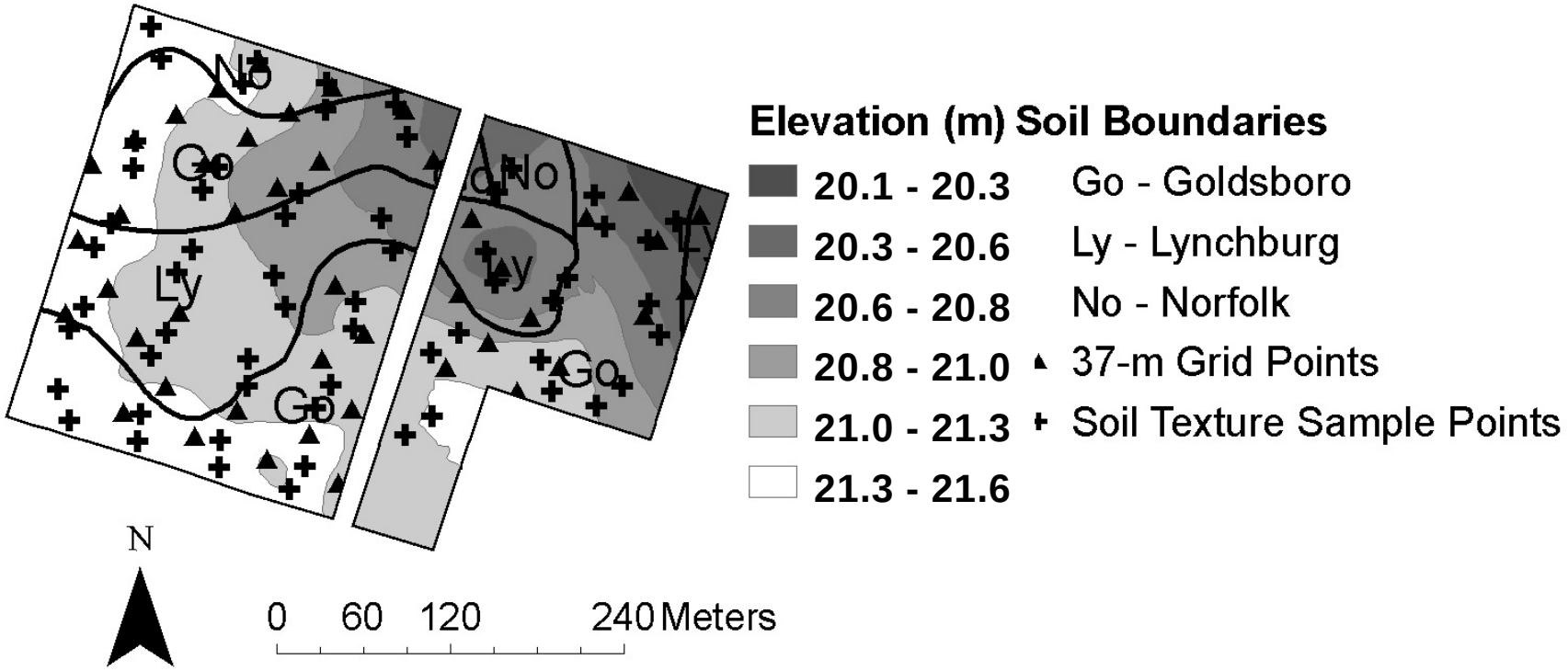
MAPS OF SOIL TEXTURE AND TOPOGRAPHY FOR SELECTED SITES



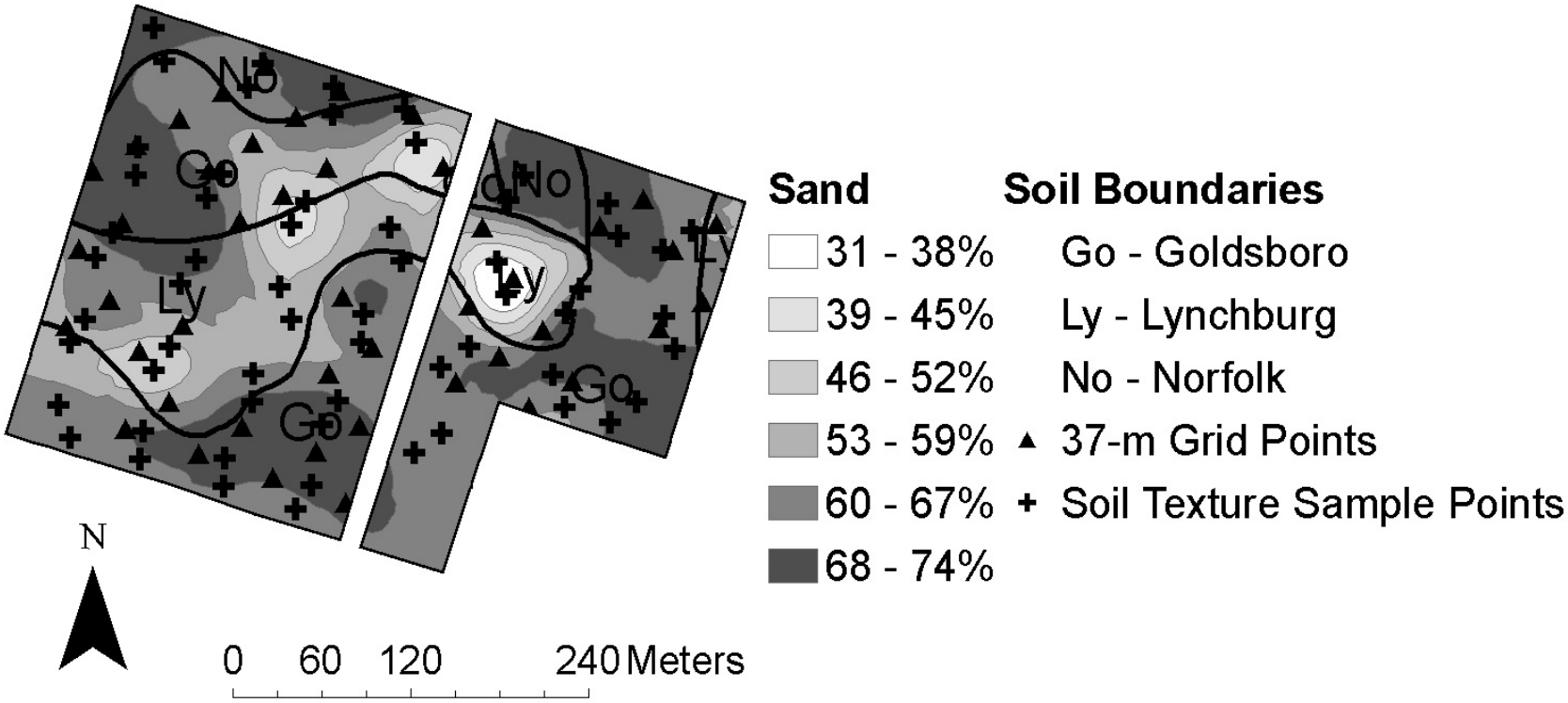
Map E.1. Interpolated map of elevation for the Hall site.



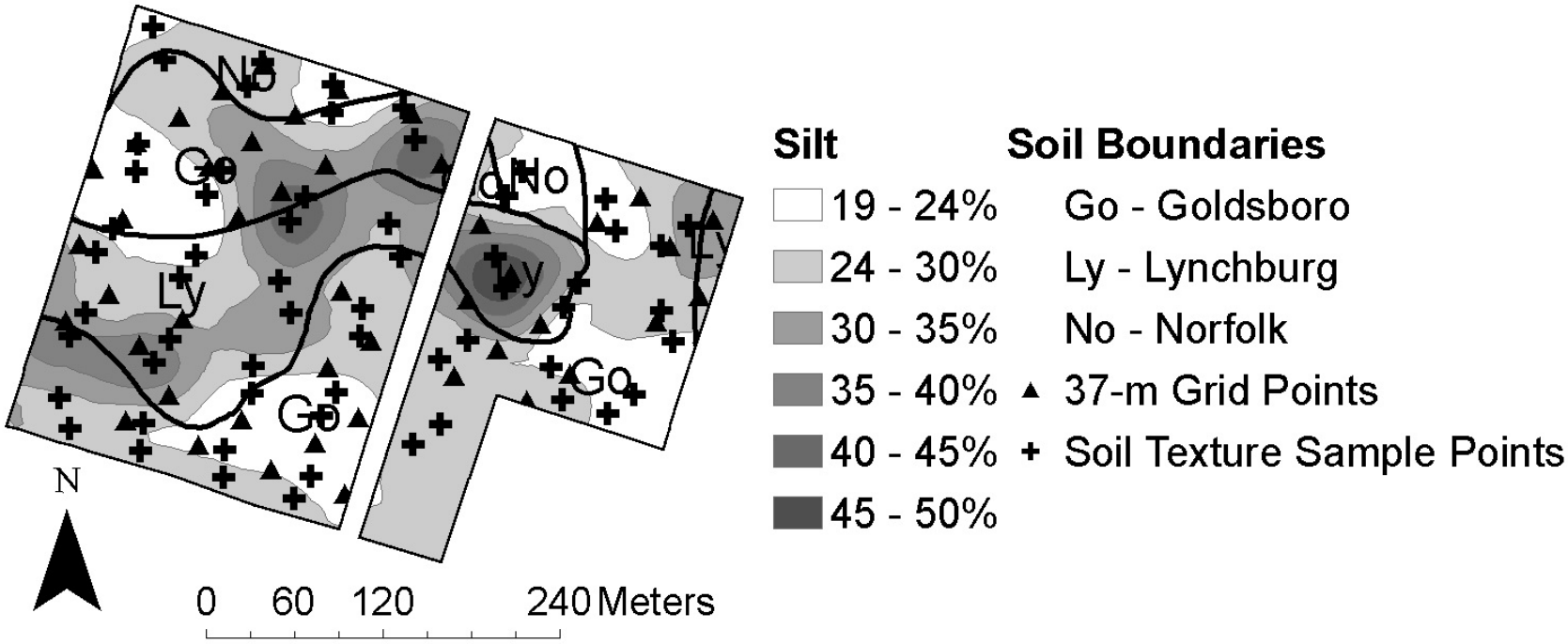
Map E.2. Interpolated map of slope for the Hall site.



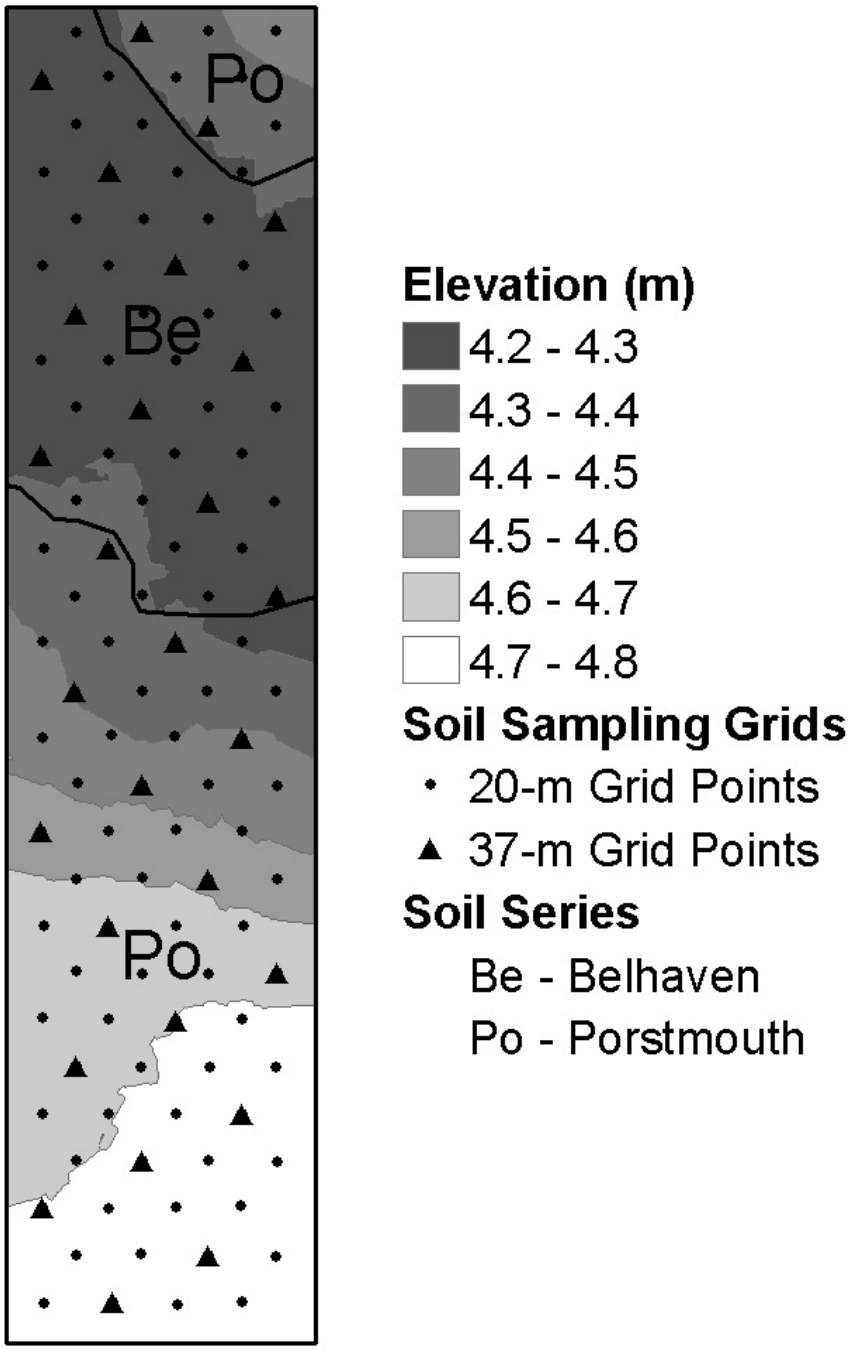
Map E.3. Interpolated map of elevation for the Kinston site.



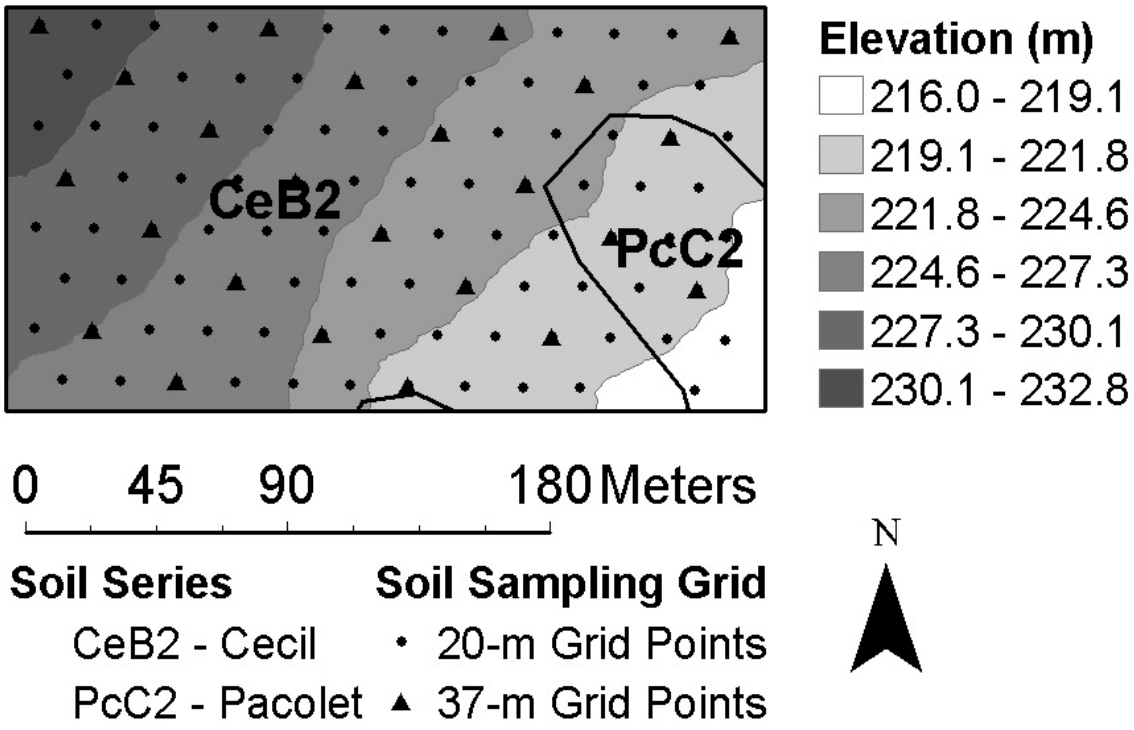
Map E.4. Interpolated map of sand for the Kinston site.



Map E.5. Interpolated map of percent silt for the Kinston site.



Map E.6. Interpolated map of elevation for the Tidewater site.



Map E.7. Interpolated map of elevation for the Pump site.