ABSTRACT

WALL, DAVID PETER. Soil Tests for Winter Wheat Nitrogen Management in the Southeastern USA. (Under the direction of Randy Weisz).

Developing a system where soil tests could be used for winter wheat (*Triticum aestivu*m L.) nitrogen (N) management and to determine N-fertilizer requirements in the southeastern USA would have many environmental and economic benefits. The objectives of this study were to: (i) evaluate the effects of sampling time, sampling depth, crop rotation, and N fertilizer application on the amino sugar-N test (ASNT); (ii) determine appropriate and reliable sampling depths for the ASNT and soil mineral N tests; (iii) explore the relationships between these soil N tests and winter wheat N uptake; (iv) calibrate these soil N tests to predict optimum spring N rates (OptN) for winter wheat in North Carolina.

Ten study sites were established, to assess changes in ASNT values with sampling depth, crop rotation, and N fertilizer application. A repeated measures experimental design was implemented whereby soil samples were taken from each of the 10 study sites at specific times and depths during the course of a 19-month period. Soil ASNT, NO_3-N, NH_4-N, and soil organic matter (SOM) levels were evaluated at each sampling.

At all sites, soil ASNT decreased with depth and showed significant variation over time. Soil ASNT was influenced by crop rotation and tillage but was not significantly affected by fertilizer applications. When all sites were considered together, soil ASNT was well correlated with SOM, however, they were not correlated across time within sites. This suggests that the ASNT is measuring a nitrogenous fraction of SOM that behaves somewhat independently over time. These results indicate that sampling to 30 cm at a specific sampling time will be necessary to capture temporal variability in ASNT.

Sixty-nine winter wheat N response trials were conducted for three growing seasons over a wide range of soils, drainage and previous crop management practices. A randomized complete block design with five replications and seven growth-stage 30 (GS30) N-fertilizer rates ranging from 0 to 168 kg ha^{-1} was used. Each February, soil samples were taken over three depths (0-10, 0-20, and 0-30cm) and analyzed for ASNT, NO_3-N, NH_4-N and SOM levels.

In the wheat N uptake study, the relationships between these soil N tests and N uptake from the unfertilized check plots (NUP_{Zero}) were evaluated. Considering all the experimental locations, the soil tests had poor relationships with NUP_{Zero}. When only the well-drained locations (n=58) were considered, the data aggregated into two distinct groups: 1) locations with low residual N, and 2) locations with high residual N and elevated levels of NH_4-N and ASNT. For locations with low residual N, the ASNT was able to predict NUP_{Zero} and grain yield without spring fertilizer applied (YLD_{Zero}) and was therefore a good predictor of soil N supply through mineralization. A combination of soil tests (NH_4-N, ASNT and SOM) was required to predict NUP_{Zero} for all well-drained locations (R^2 = 80).

In the N fertilizer response study, the ASNT by itself was a poor predictor of OptN for all well-drained locations, however the ASNT was correlated with N uptake at the optimum spring N fertilizer rate (NUP_{Opt}) and N uptake at GS30 (NUP_{GS30}) for locations with low residual N. The ASNT could not satisfactorily predict OptN for these low residual N locations, but could be used to predict the NUP_{Opt} and wheat yield at the optimum GS-30 N fertilizer rate (YLD_{Opt}) for well-drained fields. A combination of soil tests was required to predict OptN for all locations (i.e., NH_4-N and SOM with resultant R^2 = 0.59). Soil NO_3 tests showed no benefit for winter wheat N management in North Carolina.

Soil Tests for Winter Wheat Nitrogen Management in the Southeastern USA

by
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DEDICATION

Do mo thuismitheoirí

Máire agus Pádraig de Bhál

Is de bharr bhfur dtacaíocht, spreagadh agus treoir a éirigh liom an méid seo a bhaint amach.

To my parents

Marion and Patrick Wall

Your support, encouragement, and guidance have helped me achieve so much in life.

BIOGRAPHY

David Peter Wall grew up on dairy and tillage farm in Co Wexford, in Southeastern Ireland. He is the third of four children of Patrick and Marion Wall. While working closely with his father on the family farm from early childhood David gained a keen interest in agriculture. He received his B.AgSc. from University College Dublin, Ireland in 2002 majoring in animal and crop production. In 2004 he earned his M.AgSc., majoring in crop science also from University College Dublin. This work investigated the relationships between winter wheat nitrogen uptake and timing of nitrogen fertilizers and validated systems for prescribing N rates for winter wheat in Ireland. After graduating David worked as a field crop research manager gaining further agricultural research experience. In August 2005 he moved to Raleigh, NC to pursue Doctoral studies in the Department of Crop Science at North Carolina State University. He hopes to pursue a research career in the areas of crop and soil science.

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CHAPTER I

ASSESSING SOIL NITROGEN STATUS FOR WINTER WHEAT NITROGEN MANAGEMENT: A LITERATURE REVIEW

INTRODUCTION

Application of fertilizer nitrogen (N) is often necessary to supplement soil-supplied N in commercial cropping systems. Efficient N management utilizes soil N as much as possible, adding fertilizer N to enhance crop growth only when necessary. The amount of fertilizer N necessary will vary depending on yield desired, residual soil, N and the N-supplying capacity of the soil. Even with good management practices, N-use efficiency (NUE) is usually less than 50% for annual cultivated crops (Gilliam et al., 1985). This low NUE may result in large amounts of N being released into the environment and public concern about NO_3-N contamination of groundwater under agricultural land. Nitrogen fertilization in agricultural areas has been cited as a cause of high NO_3-N concentrations in shallow groundwater (Spruill et al., 1996) and shallow groundwater N concentrations in the North Carolina Coastal Plain have been detected at up to 20 mg NO_3-N L^{-1} (Osmond et al., 2002) which is well above the maximum contaminant level goal for drinking water set by the USEPA (2002). In the southeastern U.S. Coastal Plain, this has become a regulatory and social issue threatening regional crop production (Hong et al., 2006, 2007). Matching fertilizer N rates and application timing with crop N needs can reduce NO_3-N losses and minimize a potential source of surface and groundwater pollution (Ferguson et al. 2002; Hong et al., 2007). Additionally, there is growing concern among the farming community with regard to increasing N-fertilizer prices. Consequently, to minimize the potential for environmental pollution and to maintain crop profit margins growers need be able to accurately prescribe N-fertilizer rates to meet potential crop requirements.

Cereal crops take up N from soil and fertilizer sources primarily as NH_4-N and NO_3-N. Plants take up N from the soil and use it in the growth of vegetative organs. Then during grain fill much of this N is translocated to the reproductive organs (Dalling et al., 1976; Campbell et al., 1977). A deficiency of plant-available N early in the season could decrease yields, as important agronomic crops such as corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.) genetically express the potential number and size of kernels during the emergence of the growing point from the soil (Johnston and Fowler, 1991a, 1991b; Dou et al., 1995). A deficiency in soil mineral N during this differentiation period can result in a season-long drag on potential yields (Sweeney, 1993). Sylvester-Bradley et al. (2001) studied the dynamics of N capture in winter wheat without N fertilizer. They showed that N uptake at harvest related directly and was approximately similar to soil mineral-N levels in early spring.

Soil Mineral-Nitrogen Tests

Levels of soil mineral-N (NH_4-N and NO_3-N) can be highly variable over time. For example, mineral N can remain in the soil where the fertilizer N was in excess of the crop needs for that year. This situation is more pronounced where crop yields may have been lower than expected due to drought conditions and where dry and cold weather conditions aid winter carryover of this mineral-N. If weather conditions permit, this remaining N will be available for crop uptake the following year (Bundy et al., 1992; Vonotti and Bundy, 1994; Halvorson et al. 2001). Drought not only effects crop yield, but can also impact the balance of NH_4-N and NO_3-N levels. Salazar Sosa et al. (1998) reported that in very dry years when the soil water content decreased, NH_4-N tended to be higher than NO_3-N. Because NH_4-N is strongly held by soil colloids, NH_4-N is less likely to move than NO_3-N and may be present in the surface soil. Soon et al. (2001), found that exchangeable NH_4-N was low relative to NO_3-N (<3 kg N ha^{-1}) following winter wheat crops in most years, however in dry years soil NH_4-N concentrations could remain high. They attributed this relatively high exchangeable NH_4-N to inhibition of fertilizer N nitrification associated with early summer drought. Wang and Below (1992) reported that wheat N uptake was increased by 35% when one-quarter of the N was supplied as NH_4-N compared with all N as NO_3-N. Assimilation of NO_3-N requires up to 4 times the energy equivalent in ATP compared to NH_4-N assimilation (Salsac et al., 1987). This energy saving may lead to greater dry matter production in plants where NH_4-N is the sole N source (Huffman, 1989). As NH_4-N is less subject to leaching and denitrification losses, N maintained as NH_4-N in the soil should be available for uptake late into the growing season if conditions are unfavorable for NH_3 volatilization (Tsai et al., 1992). In years with high N carryover, there is potential for more of crop N needs to be satisfied from the soil N reserves thus reducing crop fertilizer N requirements.

Clearly, if it was possible to measure the amount of mineral N available for a given crop, this would help growers to better match N fertilizer rates with the crop's requirements. The pre-sidedress NO_3 test (PSNT) was developed to estimate soil NO_3-N levels available for uptake by a corn crop (Magdoff et al., 1984). The PSNT has been successfully adopted to predict N response to corn in the northeastern and north-central USA (Fox et al., 1989; Klausner et al., 1993; Bundy et al., 1999).With high average temperatures and rainfall in the southeastern USA, soil N dynamics and reactions occur at faster rates over longer periods relative to cooler regions, thus making quantification of soil N availability more difficult, especially when predicting N availability over a single growing season. Consequently, due to its transient nature, profile mineral N in the southeastern USA is generally assumed to be too transient for it to be used to predict crop response to N fertilization (Gilliam and Boswell, 1984; Bundy and Malone, 1988; Scharf and Alley, 1994). Where the PSNT has been more widely used it requires soil samples to be taken at a 0- to 30-cm depth (Magdoff et al., 1984, 1990; Klausner et al., 1993). In contrast, in the southeastern USA, Scharf and Alley (1994) observed substantial amounts of NO_3-N in the soil profile at the 30- to 120-cm depth in the Virginia Coastal Plain during the winter wheat growing season. They indicated that sampling to a depth of 120 cm may be most appropriate as this was the depth to which soil mineral N may be extracted by the wheat crop. In contrast Reddy et al. (1993) found that the amounts of NH_4-N and NO_3-N available for corn below 15-cm depth were negligible in North Carolina. They reported that approximately one-half of the residual N was recovered from the surface 0 to 15 cm. Vaughan et al. (1990) found that soil NH_4-N levels in spring explained more wheat yield variation than did NO_3-N, and sampling to depths greater than 60 cm did not improve the test. This diversity of research results demonstrates the difficulty in determining a sampling protocol for a soil NO_3-N test in the southeastern USA, and is consistent with mineral N pools being highly transient in this region.

While soil NO_3-N may be highly transient in southeastern USA soils, results from several studies in the eastern USA have shown that residual soil mineral-N can help satisify crop N requirements (Scharf and Alley, 1994; Alley et al., 1996). Meisinger et al. (1987) reported contribution of residual NO_3-N to the spring N uptake of wheat in a corn-wheat cropping system on the eastern shore of Maryland. Fox et al. (1989) reported 21 mg NO_3-N kg^{-1} within 0.3 m of the soil surface as a critical level for separating responsive from non-responsive sites for corn in Pennsylvania. Scharf and Alley (1994) showed that there was considerable variability in the amount of soil mineral-N available to wheat in spring following both corn and soybean. In their study, N being retained over winter ranged from 19 to 167 kg N ha^{-1} in a 0- to 1.2-m deep profile at nine locations cropped to winter wheat in the Virginia Coastal Plain. Alley et al. (1996) reported high levels (76-150 kg ha^{-1}) of soil mineral N in a 0- to 0.9-m-deep profile on typical coastal plain soils utilized for no-till grain sorghum (*Sorghum bicolor* [L.] Moench) in Virginia. These studies demonstrate the potential advantages and difficulties in developing a soil mineral N test for making crop N fertilizer recommendations in the humid southeastern USA. On one hand, soil mineral N can make a substantial contribution to crop N uptake, but the ephemeral nature of soil NO_3-N across both time and space makes it difficult to assess (Khan et al., 2007).

Soil Organic Matter and Nitrogen Mineralization

Another soil property that has been shown to have a big impact on yield and crop N uptake is soil organic matter (SOM) level. Johnson (1986) reported that crops grown in soils with higher organic matter may benefit from higher water holding capacity, better physical structure of the soil and availability of N in ways that cannot be mimicked by fertilizer N applications. Rasmussen et al. (1994) found that long-term decline in SOM, and specifically organic N, was tracked closely by a decline in wheat yields. Organic nitrogen compounds, an important component of SOM, make up almost 95% of the total N in soil (Mengel 1996).

The topsoil has been shown to have the greatest microbial biomass and activity, residue content, easily decomposable organic matter and therefore N mineralization (Woods 1989; Soudi et al., 1990). Research since the 1950s has provided growing support for the concept that SOM is not uniformly mineralizable, but consists primarily of a passive fraction accompanied by a less extensive pool of biologically active organic N associated with microbial biomass (Jansson, 1958; Mengel, 1996).

Nitrogen mineralization is the process by which microbes decompose organic N from SOM and crop residues to NH_4-N. The amount and rate of soil N mineralization are important components that can be used to evaluate the need for N fertilization during crop growth (Westermann et al., 1980). Net mineralization during the growing season can vary from 0.25 to 1.50 kg N ha^{-1} d^{-1}, depending on weather, soil conditions, and the nature and management of crop residues and cover crops (Greenwood et al., 1985; Magdoff, 1991; Bundy and Andraski, 1995; Schroder, 1999). Crops grown in previous years impact the amounts of residual nutrients available for subsequent plant growth (Gan et al., 2003). Grant et al. (2002) noted that crops differ substantially in the amount of N returned in the crop residue for use by subsequent crops primarily due to the quantity of crop residue and N concentration of residue. The level of N in the soil may increase due to N returned to the system from the previous crop (Grant et al., 2002), not taken up by the previous crop (either fertilizer or mineralized), and mineralized from SOM.

Researchers have long been aware that the small percentage of organic N mineralized in the course of the growing season can contribute significantly to N requirements of a crop

(Forth and Ellis, 1988; Power and Doran, 1984; Marumoto et al., 1982). However, predicting the net mineralization of plant-available N is complicated by the opposing processes of mineralization and immobilization and their interactions with seasonal temperature and soil moisture (Jansson and Persson, 1982). During mineralization-immobilization, soil microbial biomass itself temporarily serves as source and sink of easily mineralizable N (Smith, 1994). Soil microbial biomass predominantly assimilates NH_4-N during the immobilization process, but in the absence of NH_4-N and oxygen, microbes use NO_3-N as an energy source (Recous et al., 1988). The difference between gross rates of mineralization and immobilization is net mineralization. Net mineralization is strongly dependet on the C-to-N ratio of the organic matter (Whitmore and Groot, 1994; Neve et at. 1994). Given the complexity of these processes it is not surprising that growers find it difficult to correctly estimate the N contributed to the crop through mineralization. Such estimates become even more difficult when animal manure is a major source of N (Schroder et al., 2000).

The Amino Sugar-N Test

The ability to predict the amount of N actually mineralized from the soils potentially mineralizable soil-N pool may improve the accuracy of predicting N rates and increase NUE (Cabrera and Kissel, 1988). Accurate prediction is difficult, however, because the indigenous N supply may be highly variable in the same field over time, as well as in different fields within the same agroecological zone, even when the fields have similar soil type, management, and climatic conditions (Casmann et al., 2002). Even so, many attempts have been made to identify a labile pool of soil organic N through chemical fractionation of the soil N hydrolysates (Keeney and Bremner, 1964; Porter et al., 1964; Kahn, 1971), but with little success. Diffusion methods for fractionating the N in soil hydrolysates are far more accurate and specific than steam distillation, while also being much simpler and more convenient (Mulvaney and Khan, 2001b). The major components of the liberated organic N are total hydrolysable N, hydrolysable NH_4, amino-acid N, and amino sugar-N (ASN). After examining the different components of SOM liberated through acid hydrolysis (soil hydrolysate) followed by alkalization with NaOH, Mulvaney et al. (2001b) found that the ASN fraction was higher at corn sites that were non responsive to N fertilization. Based on these findings, a relatively simple soil test often called the Illinois soil N test but referred to here as the amino sugar-N test (ASNT) was developed (Khan et al. 2001). This test estimates ASN + NH_4-N without the need for acid hydrolysis which is time consuming and labor intensive. The ASN fraction is thought to be a relatively stable fraction of SOM, which can potentially be mineralized during the growing season. The ability of the ASNT to accurately predict mineralizable N likely to become available to a crop during the growing season could have important economic implications for future in-season N recommendations.

The success of the ASNT in research studies has been quiet variable. Initial studies in Illinois using the ASNT (Mulvaney et al., 2001a) showed that N fertilizer response in corn was related to ASNT as ASNT increased, corn N fertilizer response decreased to zero once a threshold soil ASNT value in the soil was reached. In North Carolina, Williams et al. (2007b) found strong correlations between ASN and responses to fertilizer N in corn. In this study an incubator was used during the test procedure (Williams et al., 2007a) rather than a hotplate as used in the original test procedure (Khan et al., 2001). Using 20-cm-depth soil samples

Williams et al. (2007a, 2007b) successfully calibrated the ASNT to predict economic optimum N rates (EONR) for corn and delta yield (yield at the yield maximizing N rate minus zero N control yield). Separating the data on the basis of soil drainage classes (well- and poorly-drained sites), the ASNT had the strongest relationship with EONR compared to other soil test methods evaluated in the study (Williams et al., 2007a). From this study it was suggested that regional differences (e.g., soil types) may require region-specific models to account for potential existence of unique relationships of ASNT values with EONR within each region. Klapwyk and Ketterings (2006) found that the ASNT may estimate a potentially available pool of mineralizable N, but that additional consideration of soil organic matter is needed for crediting contributions of that pool for crop N supply. They concluded that soil samples taken to a depth of 20 cm at planting and analyzed with the ASNT and for organic matter can be used to predict the need for additional N for corn beyond starter fertilizer in New York. In Arkansas Ross et al. (2005) showed that hydrolysable ASN and ASNT (based on 10-cm soil samples) were correlated with N uptake and grain yield in both wheat and rice (*Oryza sativa* L.).

Many studies of ASNT have, however, not been as successful as those cited above. Studies in the North Central region of the USA showed no relationship between ASNT and EONR for corn (Barker et al. 2006b; Osterhaus et al., 2005; Laboski et al., 2008). In a study conducted in Saskatchewan, Canada, Torrie et al. (2004) showed that there was a poor correlation between ASNT and N response in wheat. Laboski et al. (2008) found that the ASNT was not correlated with relative N uptake (RU_N, N uptake in the zero-N control treatments [NUP_{Zero}] divided by the N uptake in the highest N-rate treatment) and was poorly correlated to NUP_{Zero} (r = –0.37). They concluded that the negative correlations of ASNT with RU_N and NUP_{Zero} indicated that the ASNT was apparently not measuring a fraction of mineralizable and crop-available soil N. Based on results from their multi-state project, Laboski et al. (2008) concluded that the ASNT should not be used when adjusting corn N rates in the U.S. Corn Belt. In North Carolina, Williams et al. (2007a) found that the corn yield from the zero N control plots was not correlated with ASNT. They attributed this lack of relationship between control-plot yield and ASNT to the large diversity of soil productivity among sites and soil variability in retention and mineralization of N.

Russell et al. (2006), found that ASNT and other soil tests that measure N released from SOM were more effective than mineral-N tests in predicting soil-N supply. They estimated that tests for soil mineral N represented only about 15% of the amount available to the crop, whereas recoveries of mineralized N for crop production based on the ASNT were estimated to be up to 75% of that measured by gross anaerobic incubation at 40^{o}C for 21 d. However, the relationship between the ASNT and crop N uptake was poor. They concluded that the poor relationship between the ASNT and crop N uptake was because factors other than N may have been limiting crop growth. This suggests that while the ASNT may be useful in predicting mineralizable N available for crop uptake, even when combined with other soil tests like the PSNT, it may not allow useful estimations of fertilizer N requirements.

There are many factors that might explain the diversity in research findings concerning the ASNT. The success of relating ASNT to crop N uptake or EONR might be influenced by sampling depth, tillage history of the researched fields, crop rotation prior to sampling, or even weather and the time of year when the soil samples were taken. Any of these factors might confound attempts to develop the ASNT for crop management recommendations.

Though the ASNT may measure a relatively stable fraction of soil N, the nature of ASN should result in moderate fluctuations over the growing season due to N cycling (Klapwyk et al., 2006). Randall and Vetsch (2002) reported a decline in ASNT over the summer months in Minnesota. They found greater temporal variability at the 15- to 30-cm sampling depth than at 0 to 15 cm. Hoeft et al. (2005) also reported a decreasing trend in ASNT values over the summer from soil samples taken in the midwestern USA, however the changes in ASNT values were not consistent across locations. In Illinois, Ellsworth et al. (2005) found their highest ASNT values in early spring and late summer with an annual low in test values occurring roughly in mid-May. This pattern was consistent across all five of their test sites which had a mixture of different tillage, manure applications, and crop rotations. Their study supported previous conclusions by Mulvaney et al. (2003) that when using the ASNT for corn, soil sampling is best practiced after fall harvest or can be postponed until early spring. In New York, soil samples taken in the same field at corn planting, at sidedress, and at harvest had similar ASNT values (Klapwyk et al., 2006). Barker et al. (2006a) found that ASNT values were relatively consistent over time for soils in Iowa. The inconsistency in results among these studies indicates that the degree of temporal variability in ASNT differs across regions and needs to be assessed carefully before attempting to relate ASNT to crop N uptake or EONR.

There are a number of factors that might play a role in influencing temporal variability in ASNT. Crop rotation (both rotational intensity and the specific crops included in the rotation) could be one of these. Crops grown in rotation often produce more and different quality dry matter than those grown in continuous culture (Copeland and Crookston, 1992; Collins et al., 1992). Consequently, the greatest increases in soil organic carbon (SOC) are often observed in highly intensive rotational cropping systems (Wood et al., 1991a; Franzluebbers et al., 1994). Additionally, cropping systems with more crops per unit time have been shown to conserve SOC and N (Wood et al., 1991a). In the southeastern USA, Langdale et al. (1985) and Wood et al. (1992) showed that cropping systems of corn and soybean [*Glycine max* (L.) Merr.] maintained stability in SOM compared to continuous culture of either crop. It seems likely that soils under intensive rotation could differ in both the overall magnitude and degree of temporal variability of ASNT. The fertilizer-N requirement for optimum grain yield is often reduced for crops in rotation compared to continuous culture (Heichel and Barnes, 1984; Franzluebbers et al., 1994), which could be an indication that rotational intensity does indeed impact soil ASN pools.

The specific crop species included in the rotation could also impact soil ASN. Crop species play important roles in C sequestration because their residues vary in quantity and quality (e.g. lignin, phenolic content, C:N), which affect their turnover rates in soil (Martens, 2000). Soils under crop rotations including soybean have been shown to have less soil organic C than those with continuous corn (Havlin et al., 1990; Wood et al., 1991b). The higher C-to-N ratios of corn and wheat residues compared to soybean increases their resistance to decomposition (Parr and Papendick, 1987), leading to greater long-term soil C and N concentrations in corn and wheat rotations. Cereal crops grown after a legume often have a higher yield compared to those grown after a non-legume (Wani et al., 1991) because legume residues generally have a low C-to-N ratio and large fraction of readily available N. In Iowa, Blackmer et al. (1997) showed that soybean can credit up to 56 kg N ha^{-1} for a subsequent corn crop. Martens et al. (2006) demonstrated that total soil N decreased following corn growth and was enriched following soybean. As these factors influence soil C and N pools, it seems reasonable to expect them to also have an impact on soil ASN, but limited information is currently available on how ASN changes in response to rotation. Barker et al. (2006a) reported that corn with soybean in rotation had no significant effect on ASN levels, while corn in rotation with alfalfa (*Medicago sativa* L.) produced higher ASN levels.

Soil ASNT levels have been correlated with SOM content in a number of studies (Laboski et al., 2006; Williams et al., 2007b). Temporal variation in SOM in tilled soils is greatly influenced by the input and decomposability of organic substrates after harvest (Jensen et al., 1997). Consequently, ASN might also show temporal variability in response to tillage events.

The ASNT was originally developed in Illinois for analysis of soil samples collected to a depth of 30 cm in spring before corn planting (Khan et al., 2001). In New York, the 20-cm soil sampling depth was better when analyzing for ASN and SOM to predict if additional N was needed for corn beyond starter fertilizer (Klapwyk et al., 2006). Randall and Vetsch (2002) and Hoeft et al. (2005) reported greater ASNT values at their shallower 0- to 15-cm sampling depth than at 15 to 30 cm. Barker et al. (2006a) reported mixed results, with two out of four sites having greater ASNT values at 15- to 30-cm depth compared to 0 to 15 cm. These studies did not establish clear relationships for changes in the ASNT with depth, and consequently there is no defined soil sampling protocol for this test.

CONCLUSIONS AND RESEARCH OBJECTIVES

Nitrogen fertilization for winter wheat production has relied extensively on yield-goal- and/or tissue-N-based recommendations that were developed to represent regional averages. These recommendations are routinely applied to individual fields based on the assumption that fertilizer N is the main supply for crop N uptake. These methods can lead to inaccurate N recommendations, where N fertilizer either is oversupplied leading to pollution and crop injury or undersupplied leading to reduced yields and profits. Soils under certain management practices (e.g., manure applications or legume cover crops) or soils with high N reserves contained in SOM may have sufficient residual and potentially mineralizable N for optimal winter wheat growth with little need for additional N fertilizer. Therefore a soil N test capable of measuring the soil N contribution to plant-available N would be useful to winter wheat producers as they make N fertilizer rate decisions. Soil NO_3 tests have shown promise in the southeastern USA (Scharf and Alley, 1994), however, their use has been limited somewhat by the hot and humid conditions in this region. The ASNT, which measures potentially mineralizable soil N, also shows promise and has been calibrated successfully to determine N fertilizer rates for corn in North Carolina (Williams et al., 2007b).

The objectives of this research were to: 1) evaluate the temporal variability of the ASNT and how that variability might influence attempts to use it for making N fertilizer recommendations; 2) determine the best sampling depth for the ASNT; 3) determine if the ASNT and/or mineral-N soil tests can be used to predict wheat N uptake in the absence of fertilizer N; and 4) determine if the ASNT and/or mineral-N soil tests can predict EONR.

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CHAPTER II

VARIABILITY OF THE AMINO SUGAR-NITROGEN TEST

ACROSS TIME AND SAMPLING DEPTHS

IN THE HUMID SOUTHEASTERN USA

ABSTRACT

There is potential for using the amino sugar-N test (ASNT) to improve N fertilizer recommendations for crops in the southeastern USA. The ASNT has been previously calibrated to predict N rates for corn (*Zea mays* L.) in North Carolina. This study was conducted to evaluate the effects of sampling time, sampling depth, crop rotation, and fertilizer application on ASNT values over a two-year period in the humid coastal plain and piedmont regions of North Carolina. Ten sites were repeatedly sampled at 0- to 10-cm, 10- to 20-cm, and 20-to 30-cm depths in fall, mid-winter, and spring between Oct. 2006 and May 2007. Amino sugar-N (ASN), KCl-extractable soil NO_3-N and, NH_4-N, and soil organic matter (SOM) levels were evaluated at each sampling. Temporal changes in these soil parameters were evaluated for various crop rotations and N fertilizer applications. Soil ASNT levels decreased with depth and showed significant variation over time at all three depths at all sites. Soil ASNT was influenced by crop rotation and tillage but was not significantly affected by N fertilizer applications. When all sites were considered together, ASNT was well correlated with SOM, however, ASNT was not correlated with SOM across time within sites. This suggests that the ASNT is measuring a fraction of SOM that behaves somewhat independently over time. Large variations in SOM were observed at the no-till piedmont sites compared to the conventionally tilled coastal plain sites where SOM remained more constant. If the ASNT is to be used to make N-fertilizer recommendations in the southeastern USA, our results indicate that sampling to 30-cm depth will be necessary to capture temporal

variability in ASN, and that the ASNT will need to be calibrated for any specific sampling time.

Abbreviations: ASNT, amino sugar-N test; ASN, amino sugar-N, SOM, soil organic matter.

INTRODUCTION

The ability to predict the amount of N actually mineralized from potentially mineralizable soil-N may help the accuracy of predicting N fertilizer rates and increase crop N-use efficiency (NUE) (Cabrera and Kissel, 1988). After examining the different components of SOM liberated through acid hydrolysis (soil hydrolysate) followed by alkalization with NaOH, Mulvaney et al. (2001) found that the ASNT fraction was higher at corn (*Zea mays* L.) sites that were non responsive to N fertilization. Based on these findings, Khan et al. (2001a) developed a simple soil test called the amino sugar-N test (ASNT). This test estimates NH_4-N plus ASN without the need for acid hydrolysis. The ASN fraction is thought to be a relatively stable fraction of SOM, which can potentially be mineralized during the growing season. The ability of the ASNT to accurately predict mineralizable N likely to become available to a crop during the growing season could have important implications for pre-plant and in-season N recommendations.

The success of the ASNT in predicting crop N uptake or optimum N-fertilizer rates has, however, been quiet variable. For example, some studies have reported useful correlations between ASNT and crop responsiveness to N fertilizer (Mulvaney et al., 2001;

Williams et al. 2007a, 2007b; Ross et al., 2005; Klapwyk and Ketterings, 2006), while others (Torrie et al., 2004; Osterhaus, 2005; Barker et al. 2006b; Laboski, 2008) failed to find the ASNT useful in making crop N recommendations. Laboski et al. (2008) concluded that the ASNT should not be used to adjust corn (*Zae mays* L.) N-fertilizer rates in the U.S. Corn Belt. There are many factors that might explain the diversity in research findings concerning the ASNT. The success of relating ASNT to crop N uptake or optimal N-fertilizer rates might have been influenced by sampling depth, tillage history of the researched fields, crop rotation prior to sampling, or the time of year when soil samples were taken.

Though the ASNT measures a relatively stable fraction of soil N, the nature of ASN should result in moderate fluctuations over the growing season due to N cycling (Klapwyk et al., 2006). Randall and Vetsch (2002) reported a decline in ASNT values over the summer in Minnesota and found greater temporal variability at the 15- to 30-cm sampling depth than at 0 to 15 cm. Hoeft et al. (2005) also reported a decreasing trend in ASNT values over the summer in the Midwestern USA, however the changes in ASNT values were not consistent across locations. In Illinois, Ellsworth et al. (2005) found highest ASNT values in early spring and late summer with an annual low occurring roughly in mid-May. Their study supported the previous conclusion by Mulvaney et al. (2003) that when the ASNT is used for corn, soil sampling is best done after fall harvest or in early spring. In New York, soil samples taken in the same field at corn planting, at sidedress, and at harvest had similar ASNT values (Klapwyk et al., 2006a). Barker et al. (2006a) found that ASNT values were relatively consistent over time for soils in Iowa. The lack of consistency in these results indicate that the degree of temporal variability in ASNT differs among regions and needs to be assessed carefully before attempting to relate ASNT to crop N uptake or N-fertilizer requirements.

There are a number of factors that might influence temporal variability in ASN pool including crop rotational intensity and composition. Crops grown in rotation often produce more and different quality dry matter than those grown in continuous culture (Copeland and Crookston, 1992; Collins et al., 1992). Consequently, the greatest increases in soil organic C (SOC) are often observed in highly intensive cropping systems (Wood et al., 1991a; Franzluebbers et al., 1994). Additionally, cropping systems with more crops per unit time have been shown to conserve SOC and N (Wood et al., 1991). In the southeastern USA, Langdale et al. (1985) and Wood et al. (1992) showed that cropping systems rotating corn and soybean (*Glycine max* [L.] Merr.) maintained stability in SOM compared to continuous culture of either crop. It seems likely that soils under intensive rotation could differ in both the overall magnitude and degree of temporal variability of ASN.

The specific crop species in the rotation could impact soil ASN. In Iowa, Blackmer et al. (1997) showed that soybean can credit up to 56 kg N ha^{-1} for a subsequent corn crop. Martens et al. (2006) demonstrated that total soil N decreased following corn growth and was enriched following soybean. As these factors influence soil C and N pools, it seems reasonable to expect them to also have an impact on soil ASN, but limited information is currently available on how ASN changes in response to rotation. Barker et al. (2006a) reported that corn with soybean in rotation had no significant effect on ASN levels, while corn with alfalfa (*Medicago sativa* L.) in rotation produced higher ASN levels.

Soil ASNT levels have been shown to be correlated with SOM content (Laboski et al., 2008). Temporal variation in SOM in tilled soils is influenced by the input and decomposability of organic substrates after harvest (Jensen et al., 1997). Consequently, ASN might also show temporal variability in response to tillage events.

The ASNT was originally developed in Illinois for analysis of soil samples collected to a depth of 30 cm (Khan et al., 2001). In New York, the 20-cm soil sampling depth was better compared to 30 cm when analyzing for ASNT and SOM, and predicting if additional N was needed for corn (Klapwyk et al., 2006). Randall and Vetsch (2002) and Hoeft et al. (2005), reported greater ASNT values at their 0- to 15-cm sampling depth than at 15 to 30 cm. Barker et al. (2006a) reported mixed results, with two out of four sites having greater ASNT values at 15- to 30-cm depth compared to 0 to 15 cm. These studies did not establish clear relationships for changes in the ASNT with depth, which might explain, in part, differences among previously published research findings. Consequently, there is, as yet, no defined soil sampling protocol for this test.

Given the uncertainty about how soil ASNT varies with depth, time, rotation, and tillage, and how that variation might relate to crop N fertilizer requirements, there are many questions that must be answered before the ASNT can be practically applied. Our goal was to develop a better understanding of how ASNT values behave temporally and with sampling depth, crop rotation, and fertilizer N applications. Our specific objectives were to evaluate the effects of sampling time, sampling depth, crop rotation, and N-fertilizer application on ASNT values over a two-year period in the humid coastal plain and piedmont regions of the southeastern USA.

MATERIALS AND METHODS

Field Selection

Experiments were conducted from fall 2005 to May 2007 in ten 0.2-ha fields in North Carolina. Of these, five were long-term no-till piedmont fields on the Piedmont Research Station in Rowan County (35.7^{o} N, 80.6^{o} W). The remaining five were conventionally tilled coastal plain fields on the Cunningham Research Station in Lenoir County (35.3^{o} N, 77.6^{o} W). Therefore tillage was confounded with region. The fields at each research station were selected based on soil series and mapping unit so as to represent the main soils used for crop production in these regions of North Carolina (see Table 2.1 for soils, rotation, and tillage information for each site). The soils at each field had uniform surface texture to a depth of 30 cm (Table 2.1) with the exception of sites P4 (Hiwassee: clay loam 0-24 cm, clay 24-30 cm) and P5 (Cecil: loam 0-15 cm, clay loam 15-30 cm).

Experimental Design and Soil Sampling

In each field, a N-response experiment with seven N rates in a RCBD with five replications was initiated in Fall 2005. For the temporal soil sampling study described here, the plot to which 112 kg ha^{-1} N was applied was selected within each block for soil sampling. Six to eight 1.7-cm diameter soil cores were taken within a 1-m radius of the center of the plots to a depth of 30 cm. Each core was divided into depth increments: upper depth 0 to 10 cm; middle depth 10 to 20 cm; and lower depth 20 to 30 cm; which were bulked into a single sample for each depth-by-block. Consequently for each field on every sampling date, five bulked soil samples were collect at each of three sampling depths. The sampling positions within each field were geo-referenced in order to return to the same positions on each sampling date. This soil sampling protocol was repeated three times each year coinciding with winter wheat (*Triticum aestivum* L.) planting (October), in mid-winter prior to winter wheat topdress N application (February), and at double-cropped soybean planting or shortly after corn planting in spring (May) through May 2007.

Amino Sugar Nitrogen Test

Soil samples were air dried and ground to pass through a 2-mm mesh. The sieved soil samples were analyzed in triplicate for ASNT using the procedure described by Khan et al. (2001) and modified by Williams et al. (2007a). The modification consisted of using an incubator instead of heating plates (Williams et al., 2007a). The ASNT 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), which was heated to 48 to 50^{o}C in an incubator for 5 hr. Thermometers were positioned randomly inside the incubator to verify the temperature was within ±1°C of 49°C. After the incubation, samples were allowed to cool to room temperature, the petri dishes were removed from the jars, and the indicator solution was diluted with 5 mL of deionized H_2O. 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* was milliliters of H_2SO_4 used in titrating and *T* was 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).

Other Soil Analyses

Soil samples were also extracted with 2 *M* KCl (Keeney and Nelson, 1982) and analyzed for soil mineral N (NO_3- and NH_4- N). The NO_3-N in the soil extracts was determined colorimetrically with a QuikChem Automated Ion Analyzer (Lachat Instruments, Milwaukee, WI) according to QuikChem Method No. 12-107-04-1-B, a Griess-Ilosvay method (Keeney and Nelson, 1982). Ammonium in the soil extracts was also measured colorimetrically with the same instrument and QuikChem Method No. 12-107-06-2-A. Soil organic matter content was determined by loss on ignition (360^{o}C) (Schulte et al., 1991).

Data Analysis

To determine how ASNT changed over time and sampling depths within a field, repeated measures analysis was conducted using PROC MIXED in SAS Version 9 (SAS Institute, Cary, NC). For this analysis, six error covariate structures were modeled: a nontemporal covariance structure that assumed independent and identically distributed errors and five temporal covariance structures: compound symmetry, spatial spherical, spatial exponential, spatial gaussian, and spatial power. The best-fit covariance model was selected using the Akaike Information Criterion (Akaike, 1974), and that model was then used for analysis of sampling date and depth for that field. Additionally, a least-squares means procedure was used for means separation (Tukey's method) using the best-fit model. This procedure was repeated for analysis of residual soil NO_3- and NH_4-N over time and depths within each field.

RESULTS AND DISCUSSION

Coastal Plain Sites

Figure 2.1 shows the temporal pattern of ASNT, SOM, NO_3-N and NH_4-N over a 19-mo period for the five coastal plain sites (CP1-5). Sampling began in the fall of 2005 following corn for grain harvest just prior to wheat planting. The same wheat, double-crop soybean, and corn rotation ensued at each of these five sites with samples taken in the fall, mid-winter, and spring over the 19-mo sampling period.

Effect of Soil Sampling Depth

Amino sugar-N values were greater at shallower depths at all coastal plain sites (CP1-5) (Fig. 2.1). In these loam, sandy loam, and loamy sand soils, ASNT decreased to varying degrees with increasing depth on all dates, resulting in both the main effect of depth and the interaction of depth X time being significant for all sites except CP3, where there was no depth X time interaction (Table 2.2 and Fig. 2.1). The average ASNT for these five coastal plain sites over the entire sampling period were 69, 51, and 39 mg kg^{-1} for the upper, middle, and lower depths respectively. In the finer-textured Bayboro loam, Pantego fine sandy loam, and Lynchburg sandy loam sites (CP1, CP2, and CP3), the upper depth had significantly greater ASNT values than the middle depth which was significantly greater than the lower depth. In the coarser-textured Norfolk and Goldsboro loamy sands (CP4 and CP5) ASN was not significantly different between some depths on many dates. Thus soil texture appeared to directly influence the stratification of ASNT through the profiles of these Coastal Plain soils.

For SOM at all the coastal plain sites the interaction of depth X time was not significant, but the main effect of depth was significant and SOM was consistently greatest at the upper depth (Table 2.3 and Fig. 2.1). At CP1 and CP2, SOM was lowest at the lower depth, while at CP3, CP4, and CP5, there were no significant difference in SOM between the middle and lower depths.

Temporal Trends in Amino Sugar Nitrogen and Soil Organic Matter

The main effect of time for ASNT was significant at all five coastal plain sites (Table 2.2). Larger temporal fluctuations in ASNT occurred at shallower depths at CP1 and CP2. The temporal variations in ASN at a given depth and within sites ranged from 12.3 to 20.0 mg kg^{-1} throughout the 19-mo sampling period of this study (Fig. 2.1). There was a general trend for ASN values to be at their lowest levels over the mid-winter to spring months (sample times mid-winter 2006 and mid-winter 2007). This trend was less pronounced at deeper sampling depths where there tended to be less temporal variation throughout the study. Lower winter ASN values may be attributed to lower microbial activity during these cooler wetter months, thus less breakdown of stover and release of organic N into the ASN pool. Amino sugar-N values remained relatively stable from spring to fall 2006 at these coastal plain sites. During this period in 2006, soil microbial activity may have been more constant due to relatively steady soil temperatures and moisture compared to other periods where soil temperature and soil moisture were more variable (State Climate Office of North Carolina, 2008).

The main effect of time was significant for SOM at four of the Coastal Plain sites (CP2, CP3, CP4, and CP5) (Table 2.3) and not significant at CP1. Even though this effect was statistically significant at four sites, temporal variations in SOM at a given depth and within sites were relatively small (0.14 to 0.23 percentage points) throughout the 19-mo sampling period of this experiment (Fig. 2.1).

Crop Rotation and Tillage Effects

The same crop rotation was used at all five coastal plain sites preceding and during this study and there was no formal test conducted for differences in ASNT levels caused by tillage. However several important trends were seen in the data. Conventionally tilled corn for grain preceded the experiment, and soil sampling began just after these corn residues were tilled under and winter wheat was planted. Values of ASNT were highest at that initial sampling (Fig. 2.1) possibly due to the breakdown of the wheat and double-crop soybean residues tilled under prior to corn planting the previous spring or the corn stover incorporated in the fall before soil sampling and wheat planting. Over the following winter-wheat crop, ASN values tended to decrease and then remained constant at all sites from planting to harvest of the no-till double-cropped soybean. Tillage occurred just prior to corn planting in 2007, and ASNT in soils sampled just after that were once again higher. Hoyt et al. (2004) found that while the quantity of N available from legume cover crops depended on growing conditions and location, most of the N becomes available in the following three to four months. In our tests, ASNT did not increase in the 3 to 4 mo following winter wheat or double-cropped soybean, but instead appeared to increase only after a tillage event.

Crop rotation appeared to have very little effect on SOM levels at these coastal plain sites, with few statistically significant changes recorded over the 19-mo study (Fig. 2.1). Seasonal changes in active C and N pools of SOM may be more dependent upon tillage and the distribution of substrates within the surface soil than upon N fertilizer and the quantity and quality of different crop residues (Salinas-Garcia et al., 1997). During tillage the incorporation of crop residues hastens their breakdown and distribution within the surface soil leading to relatively stable C and N levels in the soil.

Fertilizer Application Effects

At all sites and on most sampling dates, there were differences in soil NO_3-N and NH_4-N over sampling depth or time (Table 2.4 and 2.5) but most of these were so small as to be agronomically meaningless. There were, however several important increases in soil NO_3-N and NH_4-N associated with N-fertilizer application (Fig. 2.1). Pre-plant fertilizer N (34 kg ha^{-1}, urea-ammonium nitrate solution, $CO[NH_2]_2$-NH_4NO_3: 30%N) was applied for winter wheat prior to sampling in fall 2005 and for corn after sampling in mid-winter 2007. Wheat top-dress fertilizer N was applied just after sampling in mid-winter 2006 and corn side-dress fertilizer N was applied just prior to sampling in spring 2007. This corn side-dress application caused a spike in NH_4-N at all sites and an increase in NO_3-N at three of the five sites (Fig. 2.1).

Soil NO_3-N decreased over the winter months following tillage for wheat but remained stable the following winter through the no-till soybean and winter fallow. These fluctuations and the spike prior to spring 2007 in NO_3-N and NH_4-N from fertilizer N applications did not significantly affect the ASNT values. This is consistent with the findings of Ruffo et al. (2005) who found that 1 yr of N fertilizer applications did not affect ASNT values in the Illinois fields they studied. Barker et al. (2006a) reported that long-term fertilization rate has small effects on ASNT and found a small but significant linear increase in ASN concentrations (< 20 mg kg^{-1}) with increasing N rate.

Piedmont Sites

Figure 2.2 shows the temporal pattern of ASNT, SOM, NO_3-N and NH_4-N over the 19-mo period for the five Piedmont sites (P1-5). Sampling began following different crop rotations but always prior to no-till winter wheat planting. Winter wheat and no-till double-crop soybean were grown at all five sites during the first 13 mo of this study. At P1, P2, and P3, the double-cropped soybean was followed by no-till cover-crop rye (*Secale cereale* L.) and then either no-till full-season soybeans or summer fallow (Fig. 2.2). Winter fallow and then no-till corn for grain followed the double-cropped soybean at P4 and P5.

Effect of Soil Sampling Depth

On all but one sampling date in one field, the upper depth had higher ASN values compared to the middle or lower depths and the average ASN values for these five piedmont sites over the entire sampling period were 96, 64, and 47 mg kg^{-1} for the upper, middle, and lower depths respectively. In the Pacolet and Hiwassee clays and the Davidson clay loam

(P1, P2, and P3), ASNT decreased to varying degrees with increasing depth on all dates (Fig. 2.2), resulting in the main effect of depth and the interaction of depth X time being significant (Table 2.2). In the finer-textured Pacolet and Hiwassee clay sites (P1 and P2), the upper depth had significantly higher ASNT values than the middle depth, which was significantly higher than the lower depth on all sampling dates. Overall in the Hiwassee clay loam and Cecil loam (P4 and P5), the main effects of depth and time were significant, but the interaction of depth X time was not. However in the coarser-textured clay loam and loam sites (P3, P4, and P5), the middle and lower depths did not differ significantly on many sampling dates. As in the coastal plain soils, soil texture appeared to influence the stratification of ASN in the soil profile. In both regions, the finer-textured soils had stronger ASN stratification within 30 cm of the surface, an exception being the Cecil loam (P5). At this site the shallowness of the start of the Bt horizon (clay) may have contributed to stratification in ASN. Texture strongly influences many important soil physical and chemical properties. Perhaps the most directly related textural soil characteristics involve the movement and aggregation of organic substrates in the surface soils. Needelman et al. (1999) found greater SOM and particulate organic matter stratification in fine-textured soils compared to coarser-textured soils.

For SOM at all sites, the main effects of depth and time were significant, while the interaction of depth X time was only significant in the Pacolet clay site (P1) (Table 2.3). Soil organic matter decreased with sampling depth at all piedmont sites (Fig. 2). The Cecil loam site (P5) and the Pacolet and Davidson clay loam sites (P1 and P3) had significantly higher SOM levels at the upper depth consistently on most sampling dates. At the other sites (P2 and P4), there were often no significant differences between adjacent sample depth increments.

Temporal Trends in Amino Sugar Nitrogen and Soil Organic Matter

The main effect of time was highly significant for ASN at all piedmont sites (Table 2.2). At four of the Piedmont sites (P1, P2, P4, and P5) ASN values were at or near their lowest level in the fall of 2005 at the start of sampling. The ASNT values at these four sites showed little temporal variation from the following spring until mid-winter 2007. While not always statistically significant, there was a tendency for ASNT to decrease in these four fields on the last one or two sampling dates.

The Davidson clay loam site (P3) behaved differently from the other four. The ASNT values were at their highest level in fall 2005 at the start of this study, fluctuated over the first three sampling dates, and continued in a downward trend thereafter. Unlike the other four sites that had been in long-term no-tillage production, P3 had been tilled 2 yr prior to the start of this study. This site received chisel and disc tillage treatments in 2003 prior to a corn grain crop, thus all surface SOM would have been redistributed within the plow layer creating narrower ranges of SOM and ASNT values across sampling depths and lowering the overall levels of SOM matter. In addition to this tillage operation this was the only piedmont site not to have a silage-corn crop in at least one of the previous two years. The possible implications of this different management history are discussed under rotation effects below.

For SOM the main effect of time was highly significant at all piedmont sites (Table 2.3). While there were large temporal changes in SOM at all sampling depths, the largest variability was in the upper depth where SOM varied by 0.5 percentage points or more among some sampling dates (Fig. 2.2). At four of the five sites (P1, P2, P4, and P5), SOM was at its lowest at the start of the study, and then increased through the winter-wheat and double-cropped soybean that followed (spring and fall 2006 sampling times). In 2006, higher-than-average winter wheat yields were reported throughout the piedmont region, and these high yields may have helped return greater quantities of crop residues to the soil. These higher SOM levels remained stable during the 2006 double-crop soybean season, and then decreased again during the following winter fallow or rye cover-crop. There was also a trend at all sites for SOM to increase between the mid-winter sampling and the following spring in both 2006 and 2007. These temporal changes in SOM were larger than expected but may be explained by the cropping history at these sites discussed below.

Crop Rotation Effects

As described above and shown in Fig. 2.2, while the first two crops at all five sites were no-till winter wheat followed by no-till double-cropped soybean, crop rotations at the end of the study in 2007 differed amongst fields. Additionally, the crop rotations implemented in the years prior to the first sampling were not the same for all fields. These differences in cropping history and rotation may account, at least in part, for some of the temporal patterns seen in ASNT and SOM.

Four sites (P1, P2, P4, and P5) had similar temporal patterns in ASNT and SOM (Fig. 2.2). At these sites, ASNT and SOM increased after the initial sampling through the first winter-wheat crop, generally reaching the highest level by the end of the following double-cropped soybean. This was especially true for the shallowest sampling depth. Each of these four sites had either one or two silage-corn crops in the rotation in the 2 yr prior to this study. This was in contrast to P3, where neither ASNT nor SOM increased during the first 14-mo of the study. In fact, ASNT decreased, and SOM, while dropping during the first winter, remained relatively constant through the first 14 mo. One notable difference between P3 and the other four sites was that instead of silage-corn, either corn for grain or full-season soybean were produced in this field in the years prior to this study. Consequently SOM and ASN may have been depleted in P1, P2, P4, and P5 due to the removal of all residues (corn silage) in the years prior to this study, and this may explain the low initial values at these four sites. The 2006 piedmont winter-wheat and soybean yields were unusually high (NCDA&CS, 2008), and these high yields may have contributed to the substantial increase in SOM, and to the smaller but statistically significant increases in ASN between fall 2005 and fall 2006.

At the 2007 mid-winter sampling, three of the Piedmont sites (P1, P2, and P3) were in cover-crop rye. It is interesting to note that ASNT decreased between mid-winter 2007 and the spring sampling that followed the burn down of the rye cover crop. This was in contrast to P4 and P5 that were in winter fallow during this period and did not show a statistically significant reduction in ASNT. It is possible that the cover-crop rye growth depleted soil N reserves during early spring 2007 growth and the rye residues had not yet decomposed to return some of these nitrogenous materials to the soil by the spring sampling.

Another possible effect of crop rotation was evident in the soil NO_3-N levels (Fig. 2.2). Soil NO_3-N was at its lowest at the 2006 spring sampling shortly after soybean planting. At all five sites there was a small increase in soil NO_3-N just after soybean harvest at the 2006 fall sampling. There was a significant increase in soil NO_3-N during the subsequent months resulting in even higher levels at the mid-winter 2007 sampling, possibly due to breakdown of the previous season's soybean residue. Interestingly, these increases in soil NO_3-N that may have been due to breakdown of soybean residues were not reflected in the ASN levels.

Fertilizer Application Effects

As in the coastal plain sites, most differences in soil NO_3-N and NH_4-N over both sampling depth and sampling time were agronomically insignificant (Fig. 2.2; Tables 2.4 and 2.5). There were, however several important fluctuations in these soil N pools that may have been related to either N fertilizer application or to crop rotation (as described above). At all the piedmont sites, pre-plant fertilizer N was applied just after soil sampling in fall 2005, and top-dress N was applied just after the mid-winter 2006 sampling for the winter wheat crop. At the Hiwassee clay loam and Cecil loam sites (P4 and P5) N was also applied after the mid-winter 2007 sampling. This corn N application explained the spike in NO_3-N in spring 2007 at these two sites. At the Hiwassee clay site (P2), a failed prior corn crop likely led to high levels of residual NO_3-N being present when sampling began (Fig 2.2.). For NO_3-N the interaction of depth X time was highly significant for the sites (P4 and P5) that received N applications for corn in their rotations (Table 2.4). The main effect of time for NO_3-N was highly significant at all these Piedmont sites, while the main effect of depth was significant at four of the sites (P1, P2, P4 and P5). In general during the period of the rotation when N fertilizer was applied, there was little difference in NO_3-N in the top 0 to 30 cm. The interaction of depth X time for NH_4-N was significant at one site (P4). While the main effects of depth and time for NH_4-N were statistically significant in most cases, these differences were agronomically negligible.

Relationship between Amino Sugar Nitrogen and Soil Organic Matter

As soil ASN has been shown to be strongly correlated to SOM across a wide range of soils (Laboski et al., 2006; Klapwyk et al., 2006; Williams et al., 2007b; Sawyer et al., 2003), researchers have suggested that the ASNT is simply measuring a constant fraction of total N and is not sensitive to the amount of N mineralized during the growing season (Laboski et al., 2006; Osterhaus et al., 2008). Soil organic matter or total N alone are not predictive of the N fertilizer needs of a crop because these parameters do not reflect the sizes of the readily mineralizable or available pools.

In this study we also found that ASNT and SOM were correlated when the data for all ten sites were considered together (Fig 2.3). This relationship between ASNT and SOM was strongest at the shallowest sampling depth (r^2 = 0.65) and decreased with increasing depth. However, when the relationship between ASNT and SOM was examined on a within-site-over-time basis there was no correlation at any of the 10 sites. This lack of within-site correlation is evident in Fig 2.3. The lack of a relationship between ASN and SOM at individual sites demonstrates that ASNT is behaving somewhat independently of SOM over time. This is possibly due to variations in the rates of decomposition of SOM during different periods of the year. Our findings indicate that the ASNT does not just estimate a constant fraction of total N but is, instead, dependent on the quantity and forms of nitrogenous compounds contained in the SOM fraction at the time of soil sampling.

Implications for Using the Amino Sugar Nitrogen Test to Predict Crop Nitrogen Fertilizer Requirements

Given the temporal changes in ASN, when should soil ASNT levels be sampled to predict N needs for winter wheat and/or corn in the southeastern USA? Taking the temporal variation in ASN and field crop management considerations into account, appropriate sampling windows for these crops need to be identified if the ASNT is to be successfully deployed.

To analyze the effects of different soil sampling periods, ASNT levels were compared between the mid-winter, spring, and fall sampling dates at individual sampling depths over all sites. The mid-winter vs. spring ASN values over all sites showed strong linear relationships in the shallower depth increments (Fig. 2.4). On the other hand, mid-winter ASN values were poorly correlated with those sampled in the fall (Fig. 2.5). Large differences in soil conditions prior to the fall and mid-winter samplings may have resulted in these poor relationships. Lower soil moisture and higher temperatures prior to the fall sampling may have differently affected the behavior of soil bacteria which in turn affected

ASN levels as opposed to the wetter and cooler conditions at the mid-winter sampling. The air temperatures and soil moisture levels in the months between the mid-winter and mid-spring sampling dates were more similar than those between fall and mid-winter samplings (State Climate Office of North Carolina, 2008), possibly leading to more similar ASN levels for the mid-winter to spring period. For both comparisons the strengths of the relationships between ASN measured at different sampling times decreased with sampling depth.

These relationships indicate that the ASNT will need to be calibrated based on a defined sampling period for the crop in question on a regional basis if it is to be used to guide N-fertilizer management. In the southeastern USA an appropriate sampling period for winter wheat would be during the winter. Taking soil samples during this time gives the farmer time to make decisions on purchasing and applying N fertilizer to the winter wheat crop based on the ASNT recommendations. Sampling during mid-winter also immediately precedes the period of rapid N uptake in winter wheat (Baethgen and Alley, 1989) and is therefore more representative of the soil N status at this critical time. Sampling for the ASNT in the fall in order to make winter wheat N fertilizer recommendations may not result in ASN values well related to conditions at rapid N uptake (Fig. 2.5). Klapwyk et al. (2006) reported that moderate fluctuations in ASN occurred over the corn growing season. They suggested that such fluctuations may have weakened the performance of the ASNT to predict corn response to sidedress N. The potential for fluctuation in soil ASNT levels over the winter would probably be greater over longer time periods especially in regions with relatively warm and humid winter months such as North Carolina. Therefore keeping a tighter time frame between sampling for ASNT and the start of rapid winter wheat N uptake may reduce potential error in assessing soil N availability.

If the ASNT is to be used to make N fertilizer recommendations for corn, the good relationship between mid-winter and spring values (Fig 2.4) indicates that producers may be able to sample for ASNT over a wider time period for a spring-planted crop like corn. In Iowa, Barker et al. (2006a) found a strong relationship between ASNT in early and late spring (r^2 = 0.89). The ASNT in late spring was, on average, 29 mg kg^{-1} higher than early spring levels. They attributed this elevation in soil ASNT to increased soil microbial activity and N processing in late spring as temperatures began to increase. However, due to the relatively consistent nature of ASNT over time, they concluded that any of these time periods would be appropriate for sampling.

Selecting an Appropriate Soil Sampling Depth for the Amino Sugar Nitrogen Test

In the coastal plain sites, the upper 0- to 10-cm sampling depth had the highest level of ASNT. The middle 10- to 20-cm and deepest 20- to 30-cm sampling depths had lower average ASNT levels, however their values were still 74 and 57% respectively of the average value in the upper sampling depth. The impact of sampling depth on the average ASN value was similar in the Piedmont where the average ASNT values of the middle and deepest sampling depths were 67 and 49% of that found in the upper 0 to 10 cm. While each increase in sampling depth resulted in a reduction in ASN, even at the 20- to 30-cm depth ASNT levels were still a significant fraction of those found on the soil surface. If the purpose of the ASNT is to capture the amount of ASN available to crop plants that can mine N to depths well below 30 cm, these data suggest that soil sampling should include the 20- to 30-cm depth. Furthermore, Figs. 2.1 and 2.2 demonstrated that statistically significant changes in ASNT even within a given field were occurring through time at this deepest sampling depth.

CONCLUSIONS

Overall

This study examined the effects of soil sampling time, soil sampling depth, crop rotation, and fertilizer application on ASNT values over a two-year period in the humid Coastal Plain and Piedmont of the Southeastern USA. This region-specific information on the temporal behavior of soil ASN should prove useful in exploring the usefulness of the ASNT for predicting crop N needs.

Soil ASNT levels decreased with depth in both the Coastal Plain and Piedmont. More variability in soil ASNT levels was observed at shallower depths. This may have been due to the concentration of SOM at the soil surface and the variability in the state of decomposition of the crop residues contributing to this component of the soil. In addition to changes with sampling depth, ASNT levels varied substantially over time at all sites. This suggests that soil ASN is influenced by cropping sequence and seasonal and other factors acting in the soil. Williams et al., (2007b) reported, for samples taken in April and May prior to corn planting, that an increase of 1 mg ASNT kg^{-1} resulted in decreases in economic optimum N rates (EONR) for corn of up to 2.9 kg ha^{-1} using their N prediction models. The temporal changes in ASNT reported in this current study (≤ 28 mg kg^{-1}) could have large effects on estimated EONR, therefore sampling time for the ASNT will need to be carefully considered if it is going to be used to make fertilizer N recommendations.

When all sites were considered together, soil ASNT levels were positively correlated with SOM however, within each site, ASNT levels were not related to SOM over time (Fig. 2.3). As soil ASNT is derived from a SOM fraction, it is not surprising that we found a correlation between ASNT and SOM across sites. However the lack of any temporal relationship between ASNT levels and SOM shows that soil ASNT levels do vary somewhat independently of SOM during the growing season. This independent fluctuation of soil ASNT levels may be attributed to changes in the state of decomposition of SOM and the quantity and availability of its ASNT fractions. Nitrogen mineralization and immobilization are highly dependent on the quantity and quality of C available for microbial utilization. This interaction likely affects the values of the ASNT especially following a recent incorporation of carbonaceous residues such as a non-leguminous cover crop (Mulvaney et al., 2006).

Nitrogen fertilization had no effect on ASNT values. Other researchers have reported similar findings (Barker et al., 2006a; Ruffo et al., 2005). Thus, when using the ASNT, the presence of residual NO_3-N and NH_4-N may need to be considered using other soil tests when making N fertilizer recommendations where residual mineral N carryover is expected.

Coastal Plain Sites

Tillage practice seemed to have a major impact on soil ASNT levels and its fluctuation over time. At these sites a tendency for soil ASNT to increase after tillage was evident. The highest ASNT values in the 0- to 10-cm depth were observed after corn residue was tilled under just before the first sampling. These values tended to decline through the following wheat, soybean, and fallow periods during which there was no tillage, but then increased after the pre-plant tillage for corn in spring 2007. This suggests that changes in ASNT over time may have been influenced by tillage events. Incorporation and burial of crop residues during tillage exposes them to microbial decomposition which may increase the ASNT pool.

Piedmont Sites

All crops grown at these sites during the study were produced with no-tillage. In four of the Piedmont sites (P1, P2, P4, and P5) initial SOM levels (fall 2005) were low, possibly due to limited corn residue returned in at least one of the previous two years when corn silage was harvested. In these fields, SOM levels increased during the following winter wheat season and remained stable during the double-crop soybean. At the fifth piedmont site (P3) corn residues were never removed following harvest in the years prior to this study. This site had narrower ranges of SOM and ASNT values across sampling depths. It appears that tillage events and removal of corn residues for silage can have significant impacts on SOM levels over a period of several years (Mann et al., 2002).

Following a winter-fallow period or a rye cover-crop, ASNT values (at the 0-10cm depth) decreased. Hoyt et al. (2004) observed reduced available soil N following rye cover crops in North Carolina, and concluded that rye acts as a net drain rather than a contributor of N to the system. Mulvaney et al. (2006) also found that the ASNT was less accurate in predicting a response to N fertilizer for corn, where rye had been grown as an over winter catch crop following soybean. They attributed the failure of the ASNT to N immobilization during the corn growing season promoted by the rye.

For the two deeper sampling depths (10- to 20- and 20- to 30-cm), changes in ASNT values over time were not as great as at the shallowest depth, however the ASNT values were highest at both these depths during the latter part of the 2006 wheat crop and into the double-crop soybean. This suggests that even without tillage, soil ASNT values at deeper profile depths responded to factors such as cropping sequence and season or environment.

Management Considerations

From an agronomic perspective, the results from this field investigation of ASNT indicated that this nitrogenous fraction of SOM is likely altered by a number of different management and environmental factors. The ASNT has been calibrated successfully to make corn fertilizer recommendations for the southeastern U.S.A. (Williams et al., 2007b), and further investigations are underway to try to calibrate it for other crops in this region.

The temporal changes in ASNT values shown here indicate that for a winter-wheat crop, the time of year that soil is sampled will be critically important. In this study, fall

ASNT values were poorly related to mid-winter values (Fig 2.5.). Therefore, this soil N test will need to be calibrated specifically to either of these soil-sampling periods. It may be more appropriate that the ASNT be calibrated for mid-winter soil samples, as the test values at this time will coincide with and likely relate better to the period of rapid N uptake for this crop.

For a corn crop, the timing of soil sampling may be less critical as long as soil samples are collected at or after mid-winter. Our results show that the mid-winter vs. spring ASNT values were strongly related to one another especially at shallower sampling depths (Fig 2.4). This may have been due in part to decreased soil microbial activity until temperatures began to increase in spring. Therefore, farmers and agronomists may have a wider window to sample when using the ASNT to make N recommendations for corn.

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FIGURE CAPTIONS

Fig. 2.1. Amino sugar-nitrogen test (ASNT) (mg kg^{-1}), soil organic matter (SOM) (%), NO_3-N (mg kg^{-1}), and NH_4-N (mg kg^{-1}) at three sampling depths (upper: 0 to 10 cm, middle: 10 to 20 cm and lower: 20 to 30 cm), versus sampling time for five conventionally tilled coastal plain sites (CP1 through CP5). For ASNT at sites where the interaction of sample depth X time was significant, means followed by the same lower case letter were not statistically different (α = 0.05). Different upper case letters in horizontal and vertical directions represent statistically significant differences for the main effects of time and depth respectively for ASNT and SOM (where the interaction of depth X time was not statistically significant). Crop rotation information is presented through the center of the table where; C_g = grain corn, W = winter wheat, S_d= double-cropped soybean, and F = fallow.

Fig. 2.2. Amino sugar-nitrogen test (ASNT) (mg kg^{-1}), soil organic matter (SOM) (%), NO_3-N (mg kg^{-1}) and NH_4-N (mg kg^{-1}) at three sampling depths (upper: 0 to 10 cm, middle: 10 to 20 cm and lower: 20 to 30 cm), versus sampling time for five piedmont sites (P1 through P5). For ASNT and SOM at sites where the interaction of sample depth X time was significant, means followed by the same lower case letter were not statistically different. Different upper case letters in horizontal and vertical directions

represent statistically significant differences for the main effects of time and depth respectively for ASNT and SOM (where the interaction of depth X time was not statistically significant). Crop rotation information is presented through the center of the table where; C_g = grain corn, C_s = silage corn, W= winter wheat, S_d= double-cropped soybean, S_f= full-season soybean, R= cover-crop rye, and F= fallow.

Fig. 2.3. Relationship between amino sugar-nitrogen test (ASNT) values (mg kg^{-1}) and soil organic matter (SOM) (%) at five coastal plain sites (CP1 through CP5), and five piedmont sites (P1 through P2) sampled six times between fall 2005 and spring 2007 at three sampling depths. Upper depth: 0- to 10-cm, middle depth: 10- to 20-cm and lower depth: 20- to 30-cm.

Fig. 2.4. Relationship between amino sugar-N test (ASNT) values (mg kg^{-1}) in mid-winter versus spring at ten sites over two growing seasons. Upper depth: 0- to 10-cm, middle depth: 10- to 20-cm and lower depth: 20- to 30-cm.

Fig. 2.5. Relationship between amino sugar-N test (ASNT) values (mg kg^{-1}) in mid-winter versus fall at ten sites over two growing seasons. Upper depth: 0- to 10-cm, middle depth: 10- to 20-cm and lower depth: 20- to 30-cm.

Table 2.1. The site abbreviation, region, soil series map unit, soil taxonomic classification, surface texture, and crop rotation throughout the experiment (C_g: grain corn, C_s: silage corn, W: winter wheat, S_d: double-cropped soybean, S_f: full-season soybean, R: cover-crop rye, F: fallow), for five coastal plain fields and five piedmont fields in a two-year study of the amino sugar-N test.

Site	Region	Soil series map unit	Soil taxonomic classification	Soil texture	Rotation
			Coastal Plains†		
CP1	Coastal Plain	Bayboro loam	Fine, mixed, thermic Umbric Paleaquults	loam	C_g-W-S_d-C_g
CP2	Coastal Plain	Pantego fine sandy loam	Fine loamy, silicious, thermic Umbric Paleaquults	sandy loam	C_g-W-S_d-C_g
CP3	Coastal Plain	Lynchburg sandy loam	Fine loamy, silicious, thermic Aeric Paleaquults	sandy loam	C_g-W-S_d-C_g
CP4	Coastal Plain	Goldsboro loamy sand	Fine loamy, silicious, thermic Aquic Paleudults	loamy sand	C_g-W-S_d-C_g
CP5	Coastal Plain	Norfolk loamy sand	Fine loamy, silicious, thermicTypicPaleudults	loamy sand	C_g-W-S_d-C_g
			Piedmont‡		
P1	Piedmont	Pacolet clay	Fine, kaolinitic, thermic Typic Kanhapludults	clay	S_d-W-S_d-R-F
P2	Piedmont	Hiwassee clay	Very fine, kaolinitic, thermic Rhodic Kanhapludults	clay	C_g-W-S_d-R-S_f
P3	Piedmont	Davidson clay loam	Fine, kaolinitic, thermic Rhodic Kanhapludults	clay loam	S_f-W-S_d-R-S_f
P4	Piedmont	Hiwassee clay loam	Fine, kaolinitic, thermic Typic Rhodudults	clay loam	S_f-W-S_d-C_g
P5	Piedmont	Cecil loam	Fine, kaolinitic, thermic Typic Kanhapludults	loam	C_s-W-S_d-C_g

† In the Coastal Plains corn and wheat were planted with conventional tillage, double-cropped soybeans was no-till.

‡ All crops in the Piedmont were planted with no-tillage.

Table 2.2. ANOVA results for amino sugar-N test (ASNT) at each of ten sites. The site abbreviation (Site), the best-fit repeated measures model, and significance for the main effects of soil sampling depth and sampling time and their interaction are shown.

Site	Best-Fit Model	Depth	Time	Depth X Time
		Coastal Plain Sites		
CP1	Spatial Spherical	***	***	**
CP2	Spatial Spherical	***	***	***
CP3	IID§	***	***	NS
CP4	IID§	***	***	***
CP5	Spatial Spherical	***	***	*
		Piedmont Sites		
P1	Spatial Gaussian	***	***	**
P2	IID§	***	***	***
P3	IID§	***	***	**
P4	Spatial Exponential	***	***	NS
P5	IID§	***	***	NS

§ Independent and identically distributed errors.
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

Table 2.3. ANOVA results for soil organic matter (SOM) at each of ten sites. The site abbreviation (Site), the best-fit repeated measures model, and significance for the main effects of soil sampling depth and sampling time and their interaction are shown.

Site	Best-Fit Model	Depth	Time	Depth X Time
		Coastal Plain Sites		
CP1	Spatial Exponential	***	NS	NS
CP2	Spatial Spherical	***	*	NS
CP3	Spatial Exponential	***	***	NS
CP4	IID§	**	***	NS
CP5	IID§	***	***	NS
		Piedmont Sites		
P1	Spatial Exponential	***	***	*
P2	IID§	*	***	NS
P3	IID§	**	***	NS
P4	Spatial Exponential	***	***	NS
P5	Spatial Exponential	***	***	NS

§ Independent and identically distributed errors.
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

Table 2.4. ANOVA results for NO_3-N at each of ten sites. The site abbreviation (Site), the best-fit repeated measures model, and significance for the main effects of soil sampling depth and sampling time and their interaction are shown.

Site	Best-Fit Model	Depth	Time	Depth X Time
		Coastal Plains Sites		
CP1	IID§	***	***	***
CP2	IID§	*	***	NS
CP3	Spatial Spherical	*	***	***
CP4	IID§	**	***	***
CP5	IID§	***	***	***
		Piedmont Sites		
P1	Compound Symmetry	**	***	NS
P2	Spatial Spherical	**	***	*
P3	IID§	NS	***	NS
P4	Spatial Exponential	**	***	***
P5	Spatial Spherical	**	***	***

§ Independent and identically distributed errors.
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

Table 2.5. ANOVA results for NH_4-N at each of ten sites. The site abbreviation (Site), the best-fit repeated measures model, and significance for the main effects of soil sampling depth and sampling time and their interaction are shown.

Site	Best-Fit Model	Depth	Time	Depth X Time
		Coastal Plains Sites		
CP1	IID§	***	***	***
CP2	IID§	***	***	***
CP3	Spatial Spherical	***	***	***
CP4	Spatial Spherical	***	***	***
CP5	Spatial Spherical	***	***	***
		Piedmont Sites		
P1	Spatial Spherical	*	***	NS
P2	Spatial Spherical	NS	**	NS
P3	Spatial Spherical	**	***	NS
P4	Spatial Spherical	***	***	*
P5	Spatial Spherical	NS	**	NS

§ Independent and identically distributed errors.
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

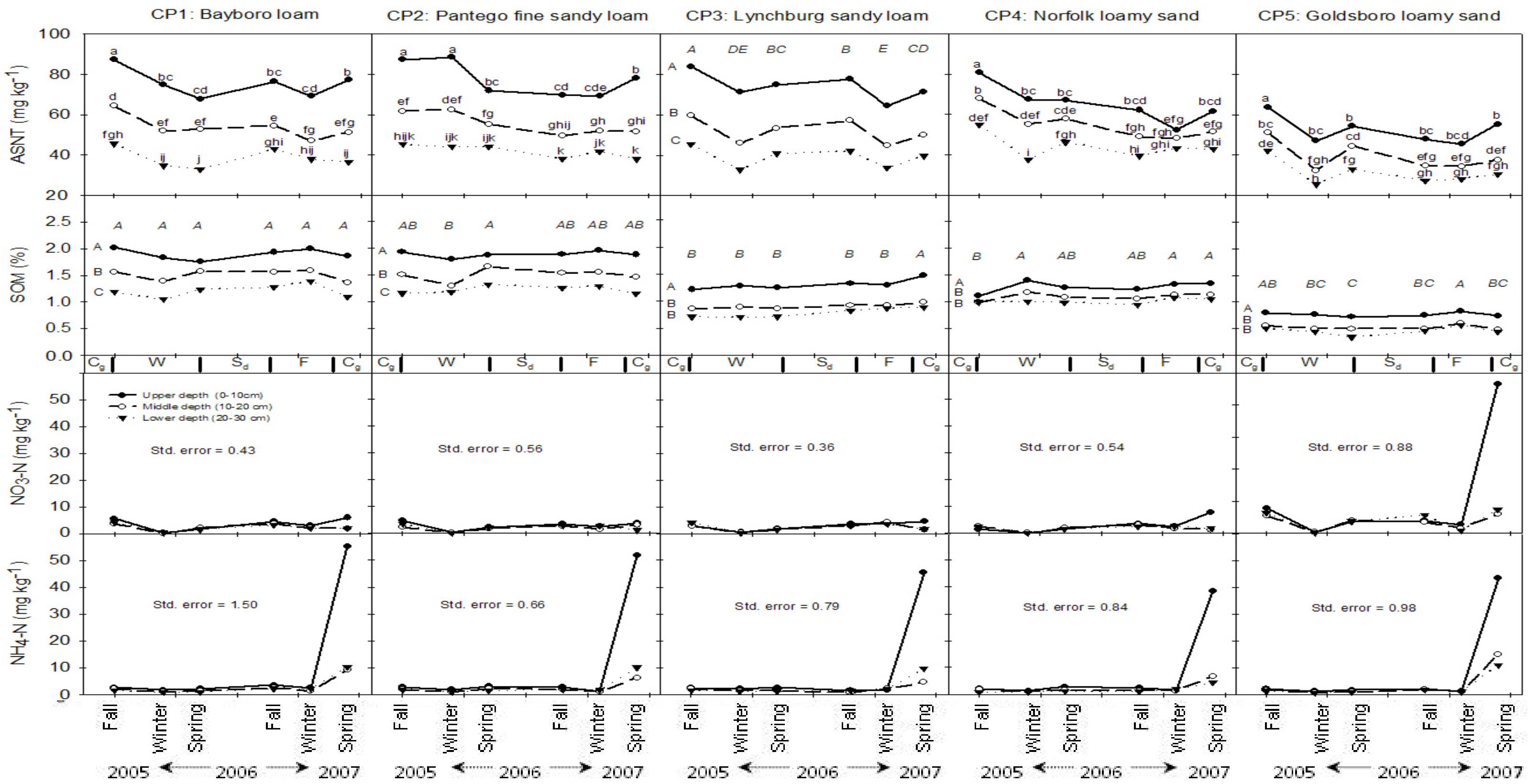
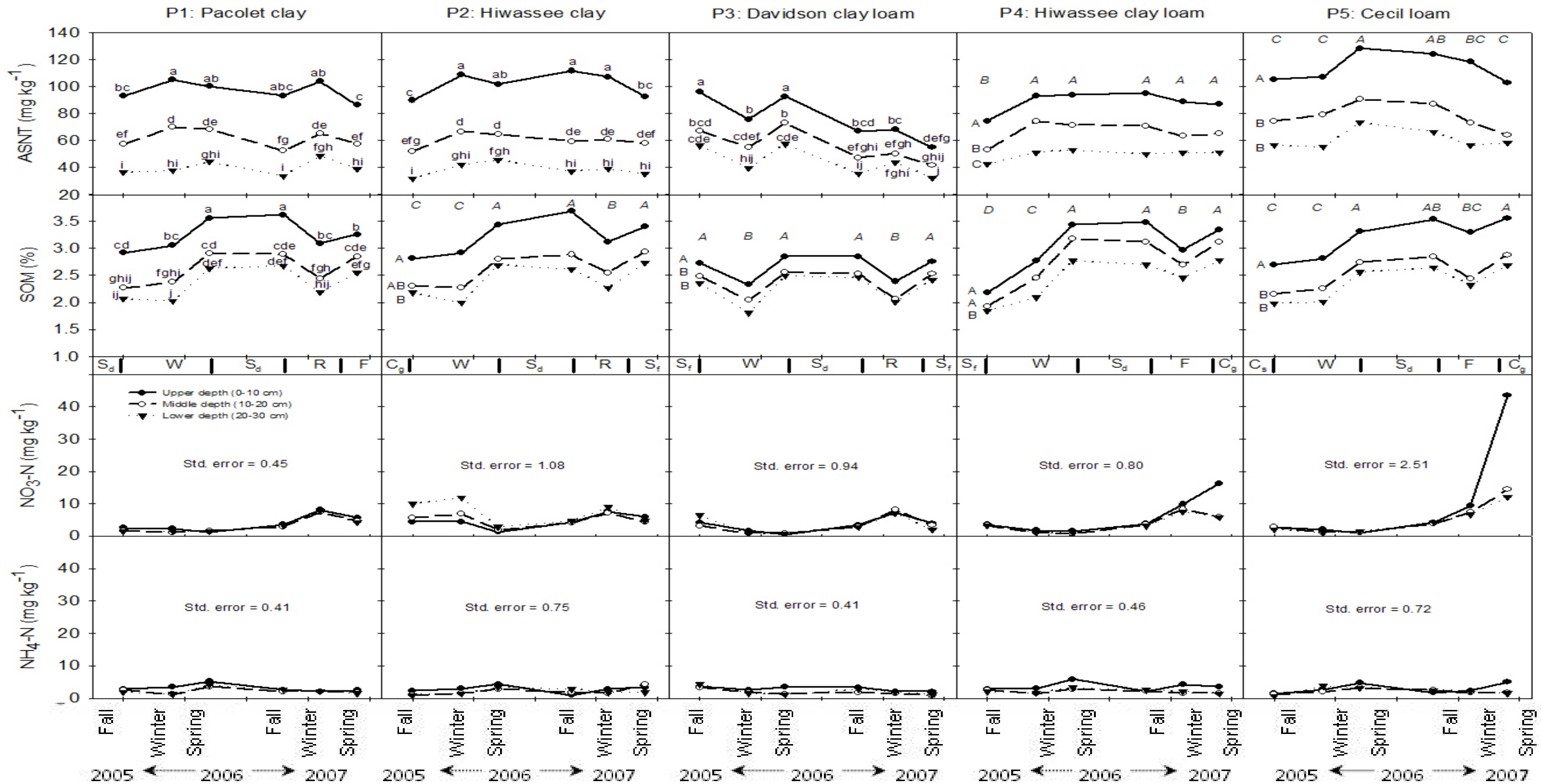


Fig. 2.1. Amino sugar-nitrogen test (ASNT) (mg kg^{-1}), soil organic matter (SOM) (%), NO_3-N (mg kg^{-1}), and NH_4-N (mg kg^{-1}) at three sampling depths (upper: 0 to 10 cm, middle: 10 to 20 cm and lower: 20 to 30 cm), versus sampling time for five conventionally tilled coastal plain sites (CP1 through CP5). For ASNT at sites where the interaction of sample depth X time was significant, means followed by the same lower case letter were not statistically different (α = 0.05). Different upper case letters in horizontal and vertical directions represent statistically significant differences for the main effects of time and depth, respectively, for ASNT and SOM, when the interaction of depth X time was not statistically significant. Crop rotation information is presented through the center of the table where: C_g = grain corn, W = winter wheat, S_d= double-cropped soybean, and F = fallow.



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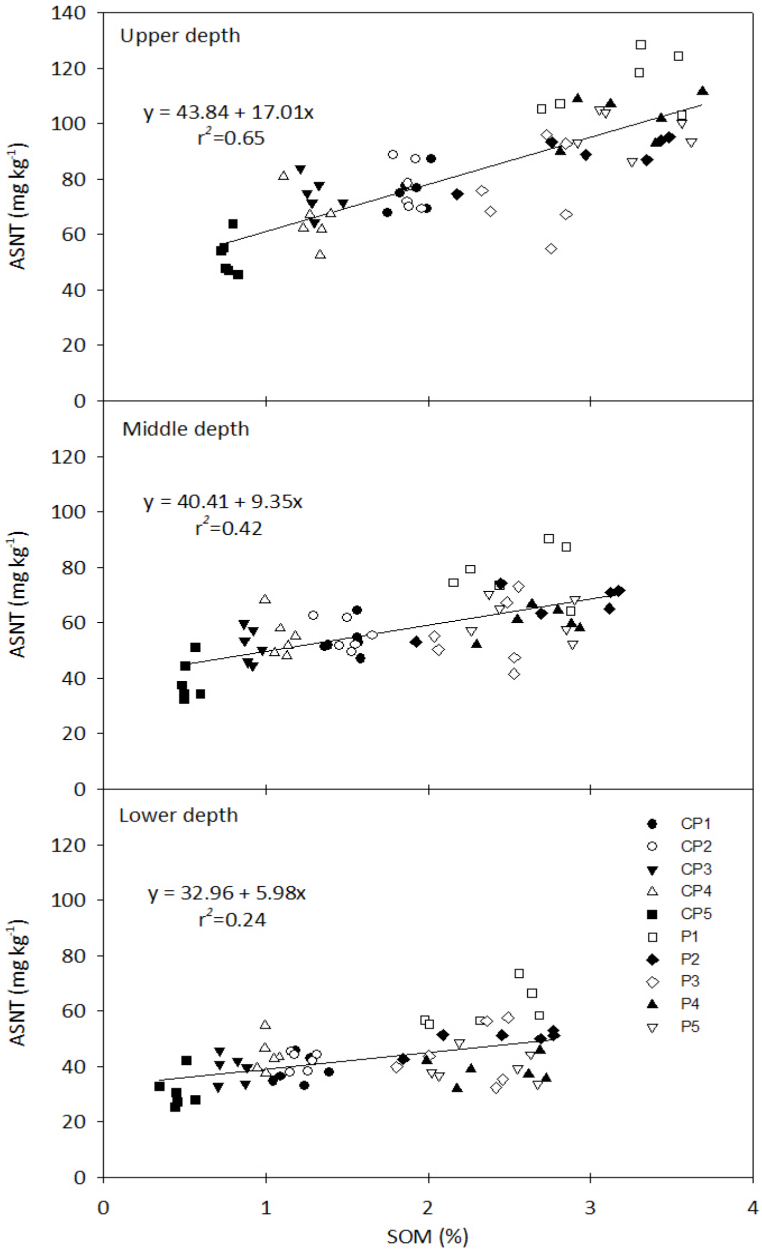

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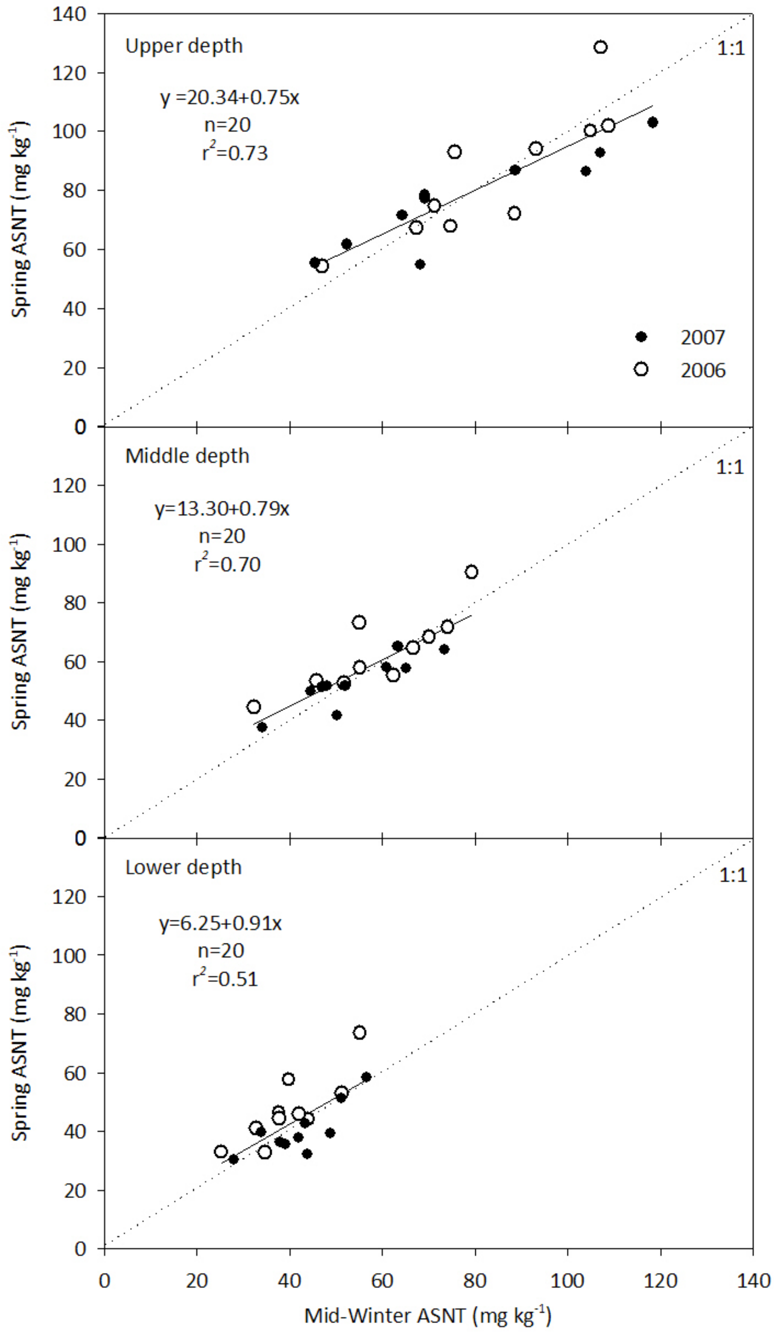

Fig. 2.4. Relationship between amino sugar-N test (ASNT) values (mg kg^{-1}) in mid-winter versus spring at ten sites over two growing seasons. Upper depth: 0- to 10-cm, middle depth: 10- to 20-cm and lower depth: 20- to 30-cm.

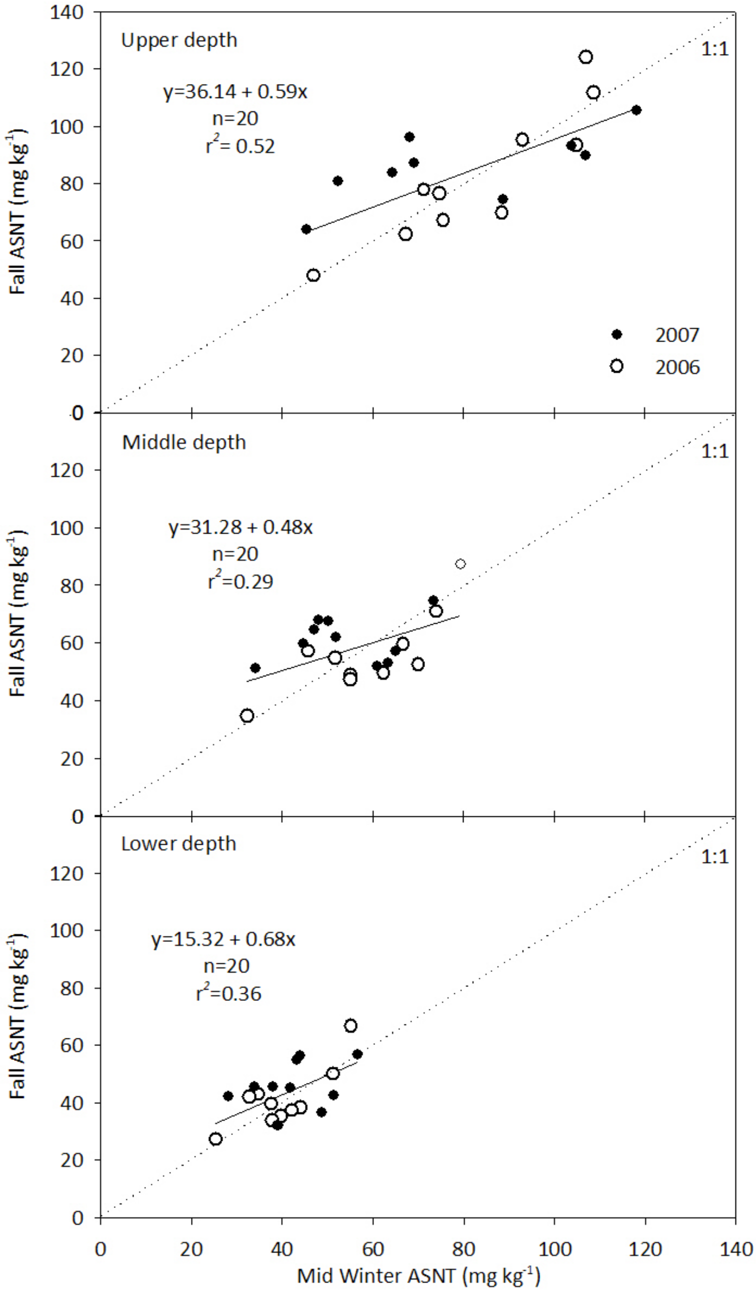

Fig. 2.5. Relationship between amino sugar-N test (ASNT) values (mg kg^{-1}) in mid-winter versus fall at ten sites over two growing seasons. Upper depth: 0- to 10-cm, middle depth: 10- to 20-cm and lower depth: 20- to 30-cm.

CHAPTER III

PREDICTING WHEAT NITROGEN UPTAKE AND YIELD IN THE ABSENCE OF SPRING NITROGEN FERTILIZER WITH THE AMINO SUGAR-N AND SOIL MINERAL NITROGEN TESTS

ABSTRACT

With N fertilizer prices on the increase, accurate measurement of N availability and supply from the soil is critical for better utilization of this valuable crop input. In the humid southeastern USA, the amino sugar-N test (ASNT) has shown potential to estimate N availability for corn (*Zae mays* L.) where soil mineral N tests have failed. The objectives of this study were to determine if shallower soil sampling depths (0 to 10 cm or 0 to 20 cm) could reliably predict ASNT levels compared to the 0- to 30-cm depth; to explore the relationships between the ASNT, KCl-extractable soil NH_4-N and NO_3-N, and soil organic matter (SOM); and to determine if these parameters could be used to predict N uptake by winter wheat (*Triticum aestivum* L.) without the addition of spring N fertilizer (NUP_{Zero}). The relationships between NUP_{Zero} and four soil tests (ASNT, NH_4-N, NO_3-N and SOM) were assessed using 69 winter wheat N response studies conducted during the 2006 to 2008 growing seasons on soils representing the main growing regions in North Carolina. The ASNT was correlated between different soil sampling depths, however, predictions of 0- to 30-cm ASNT values measured at shallower depths were less accurate at higher ASNT levels, therefore the 0- to 30-cm sampling depth was used for analyzing the data and was recommended for future use in the southeast USA. When all experimental sites were considered together, the soil tests had poor relationships with NUP_{Zero}. When only the well-drained sites were considered the data aggregated into two distinct groups: 1) sites with low residual N, and 2) sites with high residual N locations and elevated levels of NH_4-N and

ASNT at a given value of SOM. These groups could also be distinguished by past management. For sites with low residual soil N, the ASNT had a strong relationship with NUP_{Zero}. Across both groups, the ASNT was able to predict the minimum amount of N uptake expected from soil N supplies (i.e., NUP_{Zero}) and wheat grain yield without spring fertilizer applied (YLD_{Zero}) and was therefore a good predictor of soil N supply through mineralization. To best predict NUP_{zero} across all the well-drained sites, a combination of soil tests was required (i.e., NH_4, ASNT, and SOM, with resultant $R^2 = 0.80$). The results of this study indicate the potential of these soil N tests to account for mineralizable and residual soil N and improve N management for winter wheat in North Carolina.

Abbreviations: ASNT, amino sugar-N test; ASN, amino sugar-N; NUP_{Zero}, wheat N uptake in plots with no growth stage 30 N fertilizer applied; PSNT, pre-sidedress nitrate test; SOM, soil organic matter; YLD_{Zero}, grain yield in plots with no growth stage 30 N fertilizer applied.

INTRODUCTION

Fertilizer nitrogen (N) is often necessary to supplement soil-supplied N in commercial cropping systems. Efficient N management utilizes soil N as much as possible, adding fertilizer N to enhance crop growth only when necessary. Cereal crops take up N from the soil and fertilizer sources primarily as NH_4-N and NO_3-N and use it in the growth of vegetative organs. During grain fill, much of this N is translocated to reproductive organs (Dalling et al., 1976; Campbell et al., 1977). Sylvester-Bradley et al. (2001) studied the dynamics of N capture in winter wheat without N fertilizer. They showed that N uptake at harvest related directly and was approximately similar in quantity to soil mineral-N levels in early spring. Clearly, if it was possible to measure the amount of mineral N available for a given crop, this would help growers to better match N fertilizer rates with the crop's requirements.

The pre-sidedress NO_3 test (PSNT) was developed to estimate soil NO_3-N levels available for uptake by a corn crop (Magdoff et al., 1984). However, with high temperatures and rainfall in the southeastern USA, soil N dynamics and reactions occur at faster rates relative to cooler regions thus making quantification of soil mineral N availability more difficult. Consequently, profile mineral N in the southeastern USA is generally assumed to be too transient for it to be used to predict crop response to N fertilization (Gilliam and Boswell, 1984; Bundy and Malone, 1988; Scharf and Alley, 1994).

Another soil property that has been shown to have a big impact on yield and crop N uptake is soil organic matter (SOM) level. Organic N compounds an important component of SOM, make up almost 95% of the total N in soil (Mengel, 1996). The topsoil has been shown to have the greatest microbial biomass and activity, residue content, easily decomposable organic matter and therefore N mineralization (Woods, 1989; Soudi et al., 1990). Nitrogen mineralization is the process by which microbes decompose organic N from organic matter and crop residues to NH_4-N. The amount and rate of soil N mineralization are important factors that can be used to evaluate the need for N fertilization during crop growth (Westermann et al., 1980). Net mineralization during the growing season can vary from 0.25 to 1.50 kg N ha^{-1} d^{-1}, depending on weather, soil conditions and the nature and management of crop residues and cover crops (Greenwood et al., 1985; Magdoff, 1991; Bundy and Andraski, 1995; Schroder, 1999). The small percentage of organic N mineralized in the course of the growing season can contribute significantly to crop N uptake (Forth and Ellis, 1988; Power and Doran, 1984; Marumoto et al., 1982). However, predicting the formation of plant-available N is complicated by the opposing processes of mineralization and immobilization and their interactions with seasonal temperature and soil moisture (Jansson and Persson, 1982). The ability to predict net mineralization may improve the accuracy of predicting N fertilizer rates and increase N-fertilizer use efficiency (Cabrera and Kissel, 1988).

After examining the different components of SOM liberated through acid hydrolysis followed by alkalization with NaOH, Mulvaney et al. (2001) found that the amino sugar-N (ASN) fraction was higher at sites where corn was nonresponsive to N fertilization. The ASN fraction is thought to be a relatively stable fraction of SOM, which can potentially be mineralized during the growing season. Based on these findings Khan et al. (2001) developed the relatively simple Illinois Soil N Test (referred to in this paper as the amino sugar-N test, ASNT) was developed (Khan et al., 2001). The ability of the ASNT to accurately predict mineralizable N likely to become available to a crop during the growing season could have important implications for developing N fertilizer recommendations.

In Arkansas, Ross et al. (2005) showed that hydrolysable ASN and ASNT (in soil sampled to 10 cm) were correlated with N uptake and grain yield in wheat and rice (*Oryza sativa* L.). On the other hand, many studies have failed to find strong correlations between ASNT and crop N uptake. Laboski et al. (2008) found that the ASNT was not correlated with relative N uptake and was poorly correlated with N uptake of non-fertilized corn plots. They concluded that the ASNT is not measuring a fraction of mineralizable and crop-available soil N and should not be use in adjusting corn N-rate applications in the U.S. Corn Belt. In North Carolina Williams et al. (2007a) found that the corn yield from their zero-N control plots was not correlated with ASNT using 0- to 20-cm soil samples. Russell et al. (2006) found that ASNT and other soil tests that measured N released from organic matter were more effective than tests of initial mineral N (like the PSNT) in predicting soil-N supply. They estimated that tests of soil mineral N represented only about 15% of the amount available to rice, whereas recoveries of mineralized N for crop production were estimated to be up to 75% using the ASNT as compared to gross anaerobic incubation at 40^{o}C for 21 d. However, the relationship between ASNT and crop N uptake was poor. They concluded that the poor relationship may have been a result of crop N uptake responding to factors other than N.

There are many factors that might explain the diversity in research findings concerning the usefulness of the ASNT to predict crop N uptake. It is possible that success in relating ASNT to crop N uptake might be influenced by sampling depth. In the previous chapter, I showed that there was significant temporal variability in ASNT levels at the 20- to 30-cm depth in North Carolina piedmont and coastal plain soils. I also demonstrated that ASNT levels at this sampling depth were on average 49 to 57% of those found in the upper 0- to 10-cm, indicating that ASNT pools at the 20- to 30-cm depth could be important for crop N uptake in these regions. Consequently, the fact that Williams et al. (2007a) only sampled to 20 cm might explain, in part, why they found no relationship between ASNT and corn check yield in North Carolina. Another factor that might explain some of the differences among previous research findings could be the time of year when soil samples were taken. I showed in Chapter 2 that when sampling ASNT to estimate N fertilizer rates for winter wheat, the best time to take soil samples was in February prior to the end of tillering. Sampling in the fall before wheat planting resulted in ASNT values that were poorly related to those present when winter wheat began maximum N uptake. Consequently, the objectives of this study were to: (i) determine if soil sampling at 0- to 10-cm or 0- to 20-cm depths can reliably predict ASNT levels in the 0- to 30-cm soil profile, (ii) explore the relationships between the ASNT, and tests of mineral soil N, and (iii) determine if these soil N tests could predict NUP_{Zero} and YLD_{Zero} using soil sampled in February shortly before wheat begins maximum N uptake.

MATERIALS AND METHODS.

Field Selection

Research sites were selected so as to represent the three main wheat growing regions in North Carolina, the Piedmont, Coastal Plain and Tidewater. Experiments were conducted at 48 site-years during the 2006, 2007, and 2008 winter wheat growing seasons. The sites were located at the Piedmont Research Station (PRS) in Rowan County, at the Cunningham and Caswell Research Stations (CRS) in Lenoir County, and at the Tidewater Research Station (TRS) in Washington County. At each research station the trial site locations were selected based on soil series and mapping unit so as to represent the main soils used for crop production in these regions of North Carolina (see Table 3.1 for soil, rotation, tillage, and management information for each site).

Pre-plant N at 34 kg ha^{-1} was applied at all sites prior to winter wheat planting in the fall, based on North Carolina standard recommendations (Weisz et al., 2001) for early planted wheat. Phosphorus, K, S, lime and micronutrients were applied as needed at each site according to standard North Carolina recommendations (Hardy et al., 2002; Tucker et al., 1997) based on pre-season soil test results. Appropriate crop pesticides were applied as needed (Weisz et al., 2000) and weed, insect, and disease control was excellent at all sites.

Experimental Design and Soil Sampling

At each site, a RCBD N-response experiment with five replications and seven N-rates was initiated at winter wheat planting in the fall of each year. In the 2008 season at all sites a split-plot treatment consisting of two cultivars was incorporated into the experimental design

(Table 3.1). This resulted in 48 site-years, and because two cultivars were tested in 21 of those site-years, there were a total of 69 N-rate experiments. The main plot treatment was growth-stage 30 (GS30, Zadocks, 1974) N. Within each block one of seven N rates was assigned randomly at GS30 to each plot. The GS30 N rates were 0, 28, 56, 89, 112, 140, and 168 kg N ha^{-1} surface applied as urea-ammonium nitrate solution ($CO(NH_2)_2$–NH_4NO_3: 30% N) using a custom built spray applicator. Our objective in this present study was to determine if the ASNT or other soil tests for mineral N could predict NUP_{Zero} and YLD_{Zero}, thus only data from the 0 kg N ha^{-1} plots are reported here.

At each site, 1.7-cm diameter soil cores were taken to three depths (0- to 10-cm, 0- to 20-cm and 0- to-30 cm) in the first week of February when wheat was near GS25. For each depth within each block, a minimum of seven soil cores were taken and bulked to give a total of 5 composite samples for each sampling depth-by-site-year.

Grain Yield and Nitrogen Concentration

Plots were harvested with a Wintersteiger Delta combine (Wintersteiger, Salt Lake City, Utah) and the grain yield was measured with a HarvestMaster grain gauge (Juniper Systems, Inc., Logan, UT). Grain yield was adjusted to 135 g kg^{-1} moisture for analysis. At all sites in 2006 and 2007, grain yield was determined by harvesting the center 1.75-m-wide strip from each plot; at all sites in 2008, grain yield was determined by harvesting each 1.52-m-wide subplot. A 2.0-kg grain sample was collected from each plot, subsampled and analyzed for grain N concentration analysis using a CHN analyzer (McGeehan and Naylor, 1988). Grain N uptake (kg N ha^{-1}) was computed as the product of grain yield and N concentration.

Straw Yield and Nitrogen Concentration

After separation from the grain by the combine, the straw was collected from each harvested strip within the plot and weighed in the field. A ~100-g straw subsample was also weighed in the field, dried, then reweighed to determine moisture content. Average straw dry weight was then determined for each plot. For all plots and sites, a straw sample was analyzed for N concentration using a CHN analyzer (McGeehan and Naylor, 1988). Straw N uptake (kg N ha^{-1}) was computed as the product of straw yield and N concentration.

Amino Sugar Nitrogen Test

Soil samples were air dried, and ground to pass through a 2-mm mesh. The sieved soil samples were analyzed in triplicate for alkali hydrolysable amino sugar-N plus NH_4-N (ASNT) using the procedure described by Khan et al. (2001) and modified by Williams et al. (2007a). The modification consisted of using 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 the ensemble heated at 48 to 50^{o} C in an incubator for 5 hr. Thermometers were positioned randomly inside the incubator to verify the temperature was within ±1 °C of 49 °C. After the incubation, samples were allowed to cool to room temperature, the petri dishes were removed from the jars, and the indicator solution was diluted with 5 mL of deionized H_2O. 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* was milliliters of H_2SO_4 used in titrating and *T* was 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).

Other Soil Analyses

Soil samples were also extracted with 2 *M* KCl (Keeney and Nelson, 1982) and analyzed for soil mineral N (NO_3- and NH_4- N). Extract NO_3-N was determined colorimetrically with a QuikChem Automated Ion Analyzer (Lachat Instruments, Milwaukee, WI) according to QuikChem Method No. 12-107-04-1-B, a Griess-Ilosvay method (Keeney and Nelson, 1982). Extract NH_4-N was also measured colorimetrically with the same instrument and QuikChem Method No. 12-107-06-2-A. Soil organic matter content was determined by the loss on ignition (360 ^oC) (Schulte et al., 1991).

Data Analysis

Correlation, and linear regression analyses for NUP_{Zero} and YLD_{Zero} were conducted in SAS Version 9 and JMP 7.0 (SAS Institute, Cary, NC) A linear-plateau regression model for NUP_{Zero} was developed using PROC NLIN in SAS. Where appropriate, more specific linear regression models were developed for NUP_{Zero} and YLD_{Zero} after the data was split by site-drainage class and previous crop management history (Table 3.1).

RESULTS AND DISCUSSION

Soil Sampling Depth for the Amino Sugar Nitrogen Test

The ASNT was originally developed for analysis of soil samples collected to a depth of 30 cm (Khan et al., 2001; Mulvaney, 2006). The ASNT values have been shown to generally decrease with depth of sampling (Mulvaney et al., 2005; chapter 1 of this dissertation) and some researchers have reduced sampling depth to 20 cm (e.g. Williams et al., 2007ab; Kalpwyk and Ketterings, 2006). In the previous chapter, I showed ASNT values did decrease with depth in coastal plain and piedmont fields. Even so, ASNT levels at the 20- to 30-cm soil depth increment were still as high as 49 to 57% of those in the shallower 0- to 10-cm samples. Additionally, there were significant changes in ASNT levels in the deeper depth increment over time. From those findings I concluded that sampling to 30 cm was probably important in North Carolina soils. However, sampling to 30 cm is not always easy, especially in the Piedmont and studies have reported that the correlation between ASNT measured at shallower depths and that at the 0- to 30-cm depth were strong (Barker et al., 2006a; Laboski et al. 2008). Consequently, we wanted to know if the ASNT values from the shallower depths (0 to 10 cm and 0 to 20 cm) were well correlated with those from the 0- to 30-cm samples. If so, sampling at these shallower depths might be possible in North Carolina.

The mean ASNT levels for the 0- to 10-cm, 0- to 20-cm, and 0- to 30-cm depths were 95, 80, and 69 mg kg^{-1}, respectively. These ASNT values were lower than the values from northern U.S. regions. In Illinois, Khan et al. (2001) reported a mean ASNT of 171 mg kg^{-1}.

In Wisconsin Osterhaus et al. (2008) reported a mean of 194 mg kg^{-1} for sites sampled to a 30-cm depth. In New York, Klapwyk and Ketterings (2005) reported a mean of 223 mg kg^{-1} for all sites sampled to a 20-cm depth. Different types of SOM and SOM levels between our soils and those in these northern U.S. regions may explain some of these differences in ASNT levels. Additionally, some of these differences may have been due to the methods use to assay ASNT. Spargo et al. (2008) found that N recovery was lower when diffusion was performed in an incubator (as done in our experiments) relative to using griddles for the 5-h time period used in the original method. They reported an average N recovery of only 58% when using an incubator compared to using griddles. However, there was a near-perfect relationship (r^2 = 0.9996) between the two methods.

In the present study, there was a reasonably strong linear relationship between the 0- to 10-cm ASNT levels and those from 0 to 30 cm (Fig. 3.1A, r^2 = 0.74). However the model residuals were not randomly distributed (Fig. 3.1B). Instead, the relationship had the best fit at lower ASNT values, and had an increasingly poorer fit as ASNT levels in the 0- to 10-cm increment rose above about 80 mg kg^{-1}. At these higher ASNT values, soil testing only the 0- to 10-cm depth would not have well represented ASNT levels in the full 0- to 30-cm profile. The ASNT values in the 0- to 20-cm samples and those from 0 to 30 cm had a stronger linear relationship (Fig. 3.1C, r^2 = 0.95), but again, the model residuals were not randomly distributed (Fig. 3.1D) and demonstrated that the linear regression was less reliable at ASNT levels over about 90 mg kg^{-1}. At these higher ASNT levels, sampling only to 20 cm resulted in prediction errors ranging from plus or minus 20 mg kg^{-1}. Based on these data, and consistent with the findings I reported in Chapter 2, it appears that sampling for the ASNT in

North Carolina should be done to a depth of 30 cm. Consistent with this conclusion, the data reported below are only from the 0- to 30-cm soil samples.

Overall Relationships Between Crop Nitrogen Uptake and Soil Nitrogen Tests

Correlation was initially used to explore whether any of the soil N tests (ASNT, NH_4-N, NO_3-N, or SOM) were related to NUP_{Zero} or YLD_{Zero} (Table 3.2). The ASNT was weakly correlated to both NUP_{Zero} and YLD_{Zero} with r values of 0.25 and 0.26 respectively. Soil NH_4-N was somewhat more strongly correlated with both NUP_{Zero} and YLD_{Zero} with r values of 0.55 and 0.47, respectively. SOM and NO_3-N were not correlated to NUP_{Zero} or YLD_{Zero}. Soil NO_3-N levels were relatively low for all trials (< 7.4 mg kg^{-1}) and did not come close to approaching the PSNT NO_3-N critical range of 20 to 25 mg kg^{-1} for corn (Bundy and Meisinger,1994; Evanylo and Alley, 1998) and 20 mg kg^{-1} level reported for wheat (Guillaumes et al., 2006). These relationships between NUP_{Zero} and the four soil test levels are shown graphically in Fig. 3.2. The relationships were clearly weak and could not be used to practically predict NUP_{Zero}.

Previous research on North Carolina soils, (Williams et al. 2007a) found that the ability of the ASNT to predict corn crop N-use parameters was improved when site-years were separated by soil drainage classes. Consequently, we divided our data into well- and poorly-drained sites. Well-drained sites were defined as those that had soils that were naturally well drained, those that had functioning tile drainage, or sites assayed during the 2008 drought. These included all the piedmont and most of the coastal plain sites (Table 3.1). The tidewater site-years were on organic or mineral-organic soils that generally have shallow water tables. For example it is not unusual to have winter water table depths of 1 m or less at these sites. This was not the case in the 2007-2008 winter wheat cropping season, which was characterized by severe drought in late 2007 that continued into spring 2008 (Fig. 3.3) causing water table levels to drop below 3 m. Consequently, when dividing site-years into well- and poorly-drained sites, the 2008 tidewater experiments (Table 3.1) were included with the well-drained data. Poorly-drained sites were defined as those with soils classified as poorly drained and that did not have adequate artificial drainage. As described above the one exception to this poorly-drained rule was the 2008 drought tidewater sites that were classified as well drained. Only the data from the well-drained site-years was considered for further analysis as there were too few poorly-drained sites from which to draw conclusions.

One piedmont site (site 36, Table 3.1) which had high NUP_{Zero} but low mineral N and medium ASNT and SOM remained an outlier during data analysis. This was especially noticeable in Fig. 3.2B where this site was indicated with an "x". A fertilizer spreader calibration error at this site resulted in an exceptionally high rate of fertilizer N (500 kg ha^{-1}) applied pre-plant for corn the spring 2005 prior to winter wheat planting. Considerable N may have been present deep in the soil profile that went undetected with our 0- to 30-cm soil sampling. However, since such high rates of N fertilizer are not practiced in agricultural cropping systems this site was deemed an anomaly and excluded from further analysis.

Amino Sugar Nitrogen Test as a Measure of Mineralizable Soil Nitrogen In Well-Drained Soils

Fig. 3.4A shows the relationship between NUP_{Zero} and ASN for the well-drained site-years. The overall relationship continued to be poor. However the data aggregated into two distinct groups based on previous crop management (solid and open symbols in Fig. 3.4A). Site-years represented with solid symbols were fields that had cover-crop soybean [*Glycine max* (L.) Merr.] (beans plus stover turned under before wheat planting), that had manure applied in the previous 12 months, or that were preceded by a corn crop that received both the full recommended 165 kg N ha^{-1} (60 kg ha^{-1} at planting and 105 kg ha^{-1} at sidedress time, growth stage V7) and then an additional 112 kg N ha^{-1} after tasseling (VT-R1) (Table 3.1). Site-years represented with open symbols were fields that had no manure application history, no additional N applied beyond normal recommended rates, and that were preceded by either harvested corn or harvested soybean. Because of the management practices used at the experimental sites represented with solid symbols, we anticipated that these sites would have higher carry-over soil N available to the winter wheat crop. This is supported by the higher values of NUP_{Zero} in these sites (Fig. 3.4A). If these higher NUP_{Zero} values were indeed due to carry-over soil N pools, the ASNT was clearly not adequate in predicting them. When used as a source of N for cereal crops, broiler litter N efficiency has been shown to range from 10 to 49% (Nicholson et al., 1999; Nyakatawa and Reddy, 2000) resulting in a large percentage of manure derived N potentially becoming available in subsequent years. Klapwyk and Ketterings (2006) raised possible concerns when using the ASNT following manure applications and suggested that predicted crop responses may be inaccurate. In other studies (Mulvaney et al. 2005; Laboski et al 2008) the failure of the ASNT to correctly predict non-responsive corn sites was deemed to be the result of a combination of factors that would affect crop N availability (e.g., crop rotation, corn plant population density, manure applications or the occurrence of a fertility limitation). These studies suggested that where there were relatively large amounts of residual mineral N derived from fertilizer, recent manure, or incorporated soybean, the ASNT may not be accurate in predicting crop N availability. Our data from sites with manure history or that were preceded with tilled-under soybeans is consistent with these previous reports (Fig. 3.4A).

This contrasts sharply with the data from sites without manure, additional fertilizer N, or tilled-under soybean. In these sites (open symbols Fig. 3.4A) NUP_{Zero} was linearly related to ASN (r^2 = 0.92). At these sites where low levels of carry-over N were expected the ASNT was a strong indicator of soil N available for winter wheat uptake. This linear relationship between ASNT and NUP_{Zero} indicated that the ASNT was apparently measuring a fraction of mineralizable and crop available N. Another way of interpreting Fig. 3.4A is that across all the well-drained site-years (both the solid and open symbols in Fig. 3.4A) the ASNT could be used to predict the minimum amount of N-uptake expected from soil N supplies.

Across the well-drained sites NUP_{Zero} and YLD_{Zero} were highly correlated (r = 0.88, P < 0.001, not shown). Fig. 3.4B shows the linear relationship between YLD_{Zero} and ASN (r^2 = 0.83) for the sites where low carry-over N was expected (open symbols). The strong relationship between YLD_{Zero} and ASNT showed that the ASNT could be used to predict minimum winter wheat yield across all the well-drained sites also.

There was also a strong linear relationship between NUP_{Zero} and SOM for the low residual N sites (Fig.3.4C). Across these well-drained soils ASNT and SOM were highly correlated ($r = 0.91$, $P < 0.001$, not shown) so the similarity between Figs. 3.4A and 3.4C was not surprising. Other researchers have found a strong correlation between ASNT and SOM also (Klapwyk and Ketterings, 2006; Laboski et al., 2008). In similar studies ASNT has also been shown to be highly correlated with total N (Khan et al., 2001; Klapwyk and Ketterings 2005; Laboski et al., 2008). These authors suggested that the ASNT is apparently measuring a constant fraction of total soil N (Laboski et al., 2008) and is just a crude measure of SOM. Figure 3.5A shows the linear relationships between ASN and SOM for the different site classes based on expected carry-over N. The slopes of these two relationships were statistically different. In fields with relatively low SOM levels (< 2%) ASN was similar for both the "high" and "low carry-over fields". However at higher SOM levels, soil ASN was higher in the "high" compared to "low carry-over fields". It appeared that the expected high carry-over management did increase ASN levels somewhat but this was only evident when using SOM as a covariable. Apparently ASNT is not just estimating a constant fraction of total soil N but is dependent on the quality and forms of nitrogenous compounds contained in the SOM fraction at the time of sampling. Further evidence that ASNT is measuring a pool of potentially available N that is somewhat independent of SOM can be seen in Fig. 3.4A to D. While SOM was a good predictor of NUP_{Zero} and YLD_{Zero} at the site-years where carry-over N was expected to be low (open symbols Fig. 3.4C and D, $r^2 = 0.86$ and 0.79 respectively) it was not as strong a predictor as ASNT (open symbols Fig. 3.4A and B, r^2 = 0.92 and 0.83 respectively). These results suggest that the ASNT predicted somewhat more accurately than SOM the readily mineralizable fraction of soil organic N as would be required to assess the soil contribution to available N supply. This conclusion is consistent with the results reported in Chapter II, where I showed that ASNT and SOM were highly correlated across sites, but that temporal changes in ASNT in any given field were independent from SOM.

Tests of Soil Mineral Nitrogen In Well-Drained Soils

The NUP_{Zero} had a strong linear-plateau relationship with NH_4-N in these well-drained soils (Fig. 3.6A). As in Fig. 3.4A, the data tended to aggregate into the same two groups based on previous crop management. The group indicated with open symbols had mean soil NH_4-N and NUP_{Zero} values of 3.09 mg kg^{-1} and 79 kg ha^{-1} respectively. The group represented by solid symbols (from fields that we anticipated would have higher carry-over N pools) had mean soil NH_4-N and NUP_{Zero} values of 6.28 mg kg^{-1} and 165 kg ha^{-1} respectively. Based on the soil NH_4-N test, higher carry-over N was indeed present in these fields and may have contributed to the higher NUP_{Zero} values. Figure 3.5B shows the relationship between NH_4-N and SOM for the different site classes based on expected carry-over N. The slopes of these two relationships were statistically different. As SOM levels increased, NH_4-N increased and more so in the high compared to the low carry-over fields. The “high carry-over management” appeared to increase NH_4-N more than it did ASNT (Fig. 3.5, A vs. B) across the range of SOM levels in this study.

Across both management groups NUP_{Zero} was well modeled using a linear plateau response function (r^2 = 0.73, Fig. 3.6A). A NUP_{Zero} plateau was reached at 6.1 mg NH_4-N kg^{-1}. This model showed that at that critical NH_4-N level of 6.1 mg kg^{-1} the crop would have taken up 179 kg N ha^{-1}. As this model was able to predict NUP_{Zero} across all the well-drained sites, it indicated that a soil test for residual NH_4-N might have promise for improving winter wheat N-fertilizer recommendations in this region. The potential use of the soil NH_4-N test is further supported by Fig. 3.6B that shows the relationship between the YLD_{Zero} and soil NH_4-N. The YLD_{Zero} increased as soil NH_4-N levels increased up to approximately the critical level of 6.1 mg kg^{-1} found in the NUP_{Zero} model. At that soil NH_4-N level grain yields ranged from about 5 to 7 Mg ha^{-1}. These yields are representative of the maximum levels that can be expected from North Carolina winter wheat production.

However, the data points in Figs. 3.4A to D and 3.6A and B represented with solid symbols and the eight open symbols with the highest NUP_{Zero} or YLD_{Zero} were all from the 2007-2008 wheat season that started with a serious drought. These were also the data that spanned the greatest range in NH_4-N levels and that defined the relationships of soil NH_4-N with NUP_{Zero} and YLD_{Zero}. Other studies have shown that soil NH_4-N concentrations tended to increase and remain relatively high in dry years or during summer drought (Salazar Sosa et al., 1998; Soon et al., 2001). As the elevated NH_4-N detected in this study can be attributed to some extent to drought conditions in the 2007-2008 wheat season, it is possible that the NH_4 model (Fig. 3.6A) may not be a good predictor of NUP_{Zero} in years with more normal rainfall. Clearly further evaluation is needed to increase confidence in the predicted critical NH_4-N level.

NUP_{Zero} was not correlated with NO_3-N (Fig. 3.7). Over all the well-drained sites NO_3-N remained relatively low (<4.5 mg kg^{-1}). NO_3-N levels were not elevated at sites where N carryover was expected (solid symbols) and had a similar range to low N carryover sites (open symbols). This shows that soil NO_3 tests are not useful for predicting soil N supply in North Carolina. This finding is consistent with previous studies in this region that reported NO_3-N to be too transient to be used to predict crop response to N fertilization (Gilliam and Boswell, 1984; Scharf and Alley 1994).

Combining Tests of Soil Mineral Nitrogen, Soil Organic Matter, and the Amino Sugar Nitrogen Test, to Predict Soil Nitrogen Availability In Well-Drained Soils

Multiple regression using a stepwise regression procedure was used to evaluate multiple parameter models including the soil mineral N tests, the ASNT, and SOM. The best-fit model for predicting NUP_{Zero} in the well-drained soils was as follows;

$$NUP_{Zero} = -11.55 + 37.24*NH_4\text{-}N - 1.65*(NH_4\text{-}N)^2 + 0.0079*(ASNT)^2 - 4.76*(SOM)^2 \quad [3.1]$$

$$R^2 = 0.80 \qquad P < 0.001 \qquad n = 58$$

In this model, NH_4-N and $(NH_4\text{-}N)^2$ alone explained 72% of the variability in NUP_{Zero}, suggesting that soil NH_4-N levels contributed greatly to the differences in NUP_{Zero} among sites. If either ASNT or SOM were added individually to the model, the resultant parameters were non-significant. The addition of ASNT to the model was only significant when SOM was also included, and together they explained an additional 8% of the variability in NUP_{Zero}. The NUP_{Zero} as a function of ASNT and SOM is shown in Fig. 3.8A. At a constant level of

ASNT, NUP_{Zero} decreased as SOM increased. For example at an ASN value of 80 mg kg^{-1} (vertical dotted line in Figs. 3.8A and B), SOM values from the well-drained sites ranged from 1.5 to 3.8% with a corresponding decrease in NUP_{Zero} from >175 to 100 kg ha^{-1}. In contrast at a constant SOM value of 1.5% (horizontal dotted line in Fig. 3.8A), ASNT ranged from 48 to 100 mg kg^{-1} with a corresponding increase in NUP_{Zero} from <50 to >175 kg ha^{-1}. This shows the importance of including both ASNT and SOM together when modeled. In New York, Klapwyk and Ketterings (2006) also found that SOM was needed in conjunction with the ASNT to predict the need for additional N for corn beyond starter fertilizer.

In our study, ASNT was correlated with NH_4-N (r = 0.53, $P < 0.001$). According to Khan et al. (2001) and Osterhaus et al. (2008), the ASNT measures NH_4 + ASN released during digestion. They also reported that this method gives quantitative recovery of NH_4-N and glucosamine-N added to soil. Barker et al. (2006b) showed that the ASNT is a good estimate of hydrozyzable NH_4-N. Consequently, the correlation between ASNT and NH_4 was not surprising. The NUP_{Zero} as a function of ASNT and NH_4-N is shown in Fig. 3.8B. Consistent with Eq. [3.1], as either of these two measures of soil N increased, NUP_{Zero} increased. For example at an ASN value of 80 mg kg^{-1}, NH_4-N ranged from 1.5 to 12.7 mg kg^{-1} with a corresponding increase in NUP_{Zero} from 100 to >175 kg ha^{-1}. There was also a wide range in ASN values for a given NH_4-N level, however the corresponding response in NUP_{Zero} was much lower with increasing ASN than with increasing NH_4-N levels. This graphically illustrates the fact that NH_4-N explained much more of the variability in NUP_{Zero} than did ASNT.

CONCLUSIONS

The ASNT values decreased as the soil sampling interval expanded to deeper depths, and the mean values for all intervals were much lower than those reported from more northern regions of the USA. The lower values reported in this study may have been a result of using an incubator for the ASNT digestion as well as the differences in SOM types and levels in the soil. The ASNT was strongly related between different sampling depths. However, prediction of ASNT levels in the 0- to 30-cm profile using either 0- to 10-cm or 0- to 20-cm samples were less accurate at higher soil ASNT levels. These prediction errors lead to the conclusion that sampling for the ASNT in the southeastern USA should probably be done from 0 to 30 cm.

NUP_{Zero} had relatively poor relationships with ASNT, NO_3-N, NH_4-N, and SOM when all sites were considered together. Using only the well-drained sites, NUP_{Zero}, still had a poor relationships with ASNT, NO_3-N and SOM. However, NH_4-N was strongly related to NUP_{Zero} for the well-drained sites. A critical NH_4-N value of 6.1 mg kg^{-1} was determined at which the highest yields were reached and above which NUP_{Zero} leveled off. This relationship between NUP_{Zero} and NH_4-N needs to be further explored, as the model developed was heavily influenced by data from a single dry year.

For well-drained sites with no manure application history, or where no additional fertilizer N was applied beyond recommended rates, or in which soybean (beans and stover) were not tilled under prior to wheat planting, the ASNT was strongly related to NUP_{Zero} and YLD_{Zero}. At these sites, ASNT was able to accurately predict NUP_{Zero} and may be a good measure of available soil N made available through mineralization. Based on these results, the ASNT would be of limited use to predict NUP_{Zero} or YLD_{Zero} under a wide range of crop management circumstances in the southeastern USA, however it may be useful for predicting baseline NUP_{Zero} for winter wheat N management in well-drained soils. Winter wheat growers may also be able to use the ASNT to predict minimum grain yield for their production fields which is important when assessing their financial risk and managing their crop nutrient inputs.

When modeled together, the ASNT, NH_4-N and SOM tests were able to predict NUP_{Zero} for all well-drained soils with a range of different crop management histories, but it was NH_4-N that explained the overwhelming majority of the variance. While further research is needed to validate this NUP_{Zero} prediction model, the results of this study do show the potential of these soil N tests to improve N management in North Carolina.

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FIGURE CAPTIONS

Fig. 3.1. A: Linear regression of soil amino sugar-N test (ASNT) sampled at the 0- to 30-cm depth and ASNT sampled at 0 to 10 cm for all sites (n = 69). B: Model residuals from the linear regression of ASNT sampled at the 0- to 30- and 0- to 10-cm depths. C: Linear regression of ASNT sampled at the 0- to 30-cm and 0- to 20-cm depths for all sites (n = 69) and B: Model residuals.

Fig. 3.2. Wheat N uptake with no spring-N fertilizer applied (NUP_{Zero}) (kg ha^{-1}) vs. (A) soil amino sugar-N test (ASNT) (mg kg^{-1}), (B) soil NH_4-N (mg kg^{-1}), (C) soil organic matter (SOM) (%), and (D) soil NO_3-N (mg kg^{-1}) for all sites (n=69). The data from site-year 36 (see Table 1) is indicated with an "x".

Fig. 3.3. Total annual precipitation (mm) for 2006, 2007, 2008, and the 30-yr average for the Cunningham and Caswell Research Stations (CRS) in the Coastal Plain, the Piedmont Research Station (PRS), and Tidewater Research Station (TRS).

Fig. 3.4. A: Wheat N uptake (NUP_{Zero}) (kg ha^{-1}) and B: grain yield (YLD_{Zero}) (Mg ha^{-1}) with no spring-N fertilizer applied vs. soil amino sugar-N (ASNT) (mg kg^{-1}), C: NUP_{Zero} and D: YLD_{Zero} vs. soil organic matter (SOM) (%), for all well-drained sites (n=58). Open symbols represent sites that had no manure applied in the previous 12 mo, no additional N fertilizer applied beyond the recommended rates for the previous crop, or no soybean plus stover turned under (i.e., sites with low expected residual N). The solid symbols represent sites that had one or more of these management practices (i.e., sites with high expected residual N). Linear regression models represent the relationships for the open symbols only.

Fig. 3.5. Relationships of (A) soil amino sugar-N test (ASNT) (mg kg^{-1}) and (B) NH_4-N (mg kg^{-1}) with SOM (%) for all well-drained sites. Open symbols: sites with low expected residual N due either to no manure applied in the previous 12 mo, no additional N fertilizer applied beyond the recommended rates for the previous crop, or no soybean plus stover turned under. Solid symbols: sites with high expected residual N due to one or more of these management practices.

Fig. 3.6. A: Wheat N uptake (NUP_{Zero}) (kg ha^{-1}) and B: grain yield (YLD_{Zero}) (Mg ha^{-1}) with no spring-N fertilizer applied vs. soil NH_4-N (mg kg^{-1}), for all well-drained sites (n=58). Open symbols: sites with low expected residual N due either to no manure

applied in the previous 12 mo, no additional N fertilizer applied beyond the recommended rates for the previous crop, or no soybean plus stover turned under. Solid symbols: sites with high expected residual N due to one or more of these management practices. The regression model represents the relationships over all sites.

Fig. 3.7. Wheat N uptake with no spring-N fertilizer applied (NUP_{Zero}) (kg ha^{-1}) vs. soil NO_3-N (mg kg^{-1}) for all well-drained sites (n=58). Open symbols: sites with low expected residual N due to either, no manure applied in the previous 12 mo, no additional N fertilizer applied beyond the recommended rates for the previous crop, or no soybean plus stover turned under. Solid symbols: sites with high expected residual N due to one or more of these management practices.

Fig. 3.8. A: Contour plot of wheat N uptake with no spring-N fertilizer applied (NUP_{Zero}) (kg ha^{-1}) vs. soil organic matter (SOM) (%) on the Y-axis, and soil amino sugar-N test (ASNT) (mg kg^{-1}) on the X-axis. B: Contour plot of NUP_{Zero}, vs. soil NH_4-N (mg kg^{-1}) on the Y-axis and soil ASNT on the X-axis for all well-drained sites (n=58).

Table 3.1. Study site, year, soil series map unit, region and soil taxonomic classification, site drainage, previous crop, tillage used, whether manure had been applied in the previous 12 months, and winter wheat cultivar for the 48 trial sites used in this study of the relationships between soil N tests and winter wheat N uptake and yield with no spring topdress N.

Site	Year	Soil series map unit	Region and soil taxonomic classification	Site Drainage†	Previous Crop‡	Tillage§	Manure	Cultivar¶
			Tidewater					
1	2006	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	P	C	CT	No	U
2	2006	Cape Fear loam	Clayey, mixed, thermic Typic Umbraquults	P	C	CT	No	U
3	2007	Cape Fear loam	Clayey, mixed, thermic Typic Umbraquults	P	C	CT	No	U
4	2007	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	P	C	CT	No	U
5	2008	Cape Fear loam	Clayey, mixed, thermic Typic Umbraquults	W	C	CT	No	P, U
6	2008	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	W	C	CT	No	P, U
7	2008	Cape Fear loam	Clayey, mixed, thermic Typic Umbraquults	W	C	CT	No	P, U
8	2008	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	W	C	CT	No	P, U
			Coastal Plain					
9	2006	Johns sandy loam	Sandy, siliceous, thermic Aquic Hapludults	W	S	CT	No	U
10	2006	Torhunta loam	Coarse-loamy, siliceous, thermic Typic Humaquepts	P	S	CT	No	U
11	2006	Bayboro loam	Fine, mixed, thermic Umbric Paleaquults	P	C	CT	No	U
12	2006	Pantego fine sandy loam	Fine-loamy, silicious, thermic Umbric Paleaquults	P	C	CT	No	U
13	2006	Rains fine sandy loam	Fine-loamy, siliceous, thermic Typic Paleaquults	P	C	CT	No	U
14	2006	Goldsboro loamy sand	Fine-loamy, siliceous, thermic Aquic Paleudults	W	S	CT	No	U
15	2006	Lynchburg sandy loam	Fine-loamy, siliceous, thermic Aeric Paleaquults	W	S	CT	No	U
16	2006	Norfolk loamy sand	Fine-loamy, siliceous, thermic Typic Paleudults	W	S	CT	No	U
17	2006	Rains fine sandy loam	Fine-loamy, siliceous, thermic Typic Paleaquults	P	S	CT	No	U
18	2006	Lynchburg sandy loam	Fine-loamy, siliceous, thermic Aeric Paleaquults	W	C	CT	No	U
19	2006	Norfolk loamy sand	Fine-loamy, siliceous, thermic Typic Paleudults	W	C	CT	No	U
20	2006	Goldsboro loamy sand	Fine-loamy, siliceous, thermic Aquic Paleudults	W	C	CT	No	U

Table 3.1 continued

21	2007	Pocalla loamy sand	Loamy, siliceous, thermic Arenic Plinthic Paleudults	W	C	CT	No	U
22	2007	Johns sandy loam	Sandy, siliceous, thermic Aquic Hapludults	W	S	CT	No	U
23	2007	Duplin sandy loam	Fine, kaolinitic, thermic Aquic Paleudults	W	C	CT	No	U
24	2007	Marvyn sandy loam	Fine-Loamy, kaloinitic, thermic Typic Kanhapludults	W	C	CT	No	U
25	2007	Lynchburg sandy loam	Fine-loamy, siliceous, thermic Aeric Paleaquults	W	C	CT	No	U
26	2007	Pantego fine sandy loam	Fine-loamy, silicious, thermic Umbric Paleaquults	P	C	CT	No	U
27	2008	Portsmouth loam	Sandy, mixed, thermic Typic Umbraquults	W	CN	CT	No	P, U
28	2008	Kenansville loamy sand	Loamy, siliceous, thermic Arenic Hapludults	W	CS	CT	No	P, U
29	2008	Norfolk loamy sand	Fine-loamy, siliceous, thermic Typic Paleudults	W	CS	CT	No	P, U
30	2008	Pocalla loamy sand	Loamy, siliceous, thermic Arenic Plinthic Paleudults	W	CS	CT	No	P, U
31	2008	Duplin sandy loam	Fine, kaolinitic, thermic Aquic Paleudults	W	CS	CT	No	P, U
32	2008	Goldsboro loamy sand	Fine-loamy, siliceous, thermic Aquic Paleudults	W	CS	CT	No	P, U
33	2008	Lynchburg sandy loam	Fine-loamy, siliceous, thermic Aeric Paleaquults	W	C	CT	No	P, U
34	2008	Rains fine sandy loam	Fine-loamy, siliceous, thermic Typic Paleaquults	W	C	CT	No	P, U
			Piedmont					
35	2006	Hiwassee clay loam	Fine, kaolinitic, thermic Typic Rhodudults	W	S	NT	No	U
36	2006	Hiwassee clay	Very fine, kaolinitic, thermic Rhodic Kanhapludults	W	C	NT	No	U
37	2006	Davidson clay loam	Fine, kaolinitic, thermic Rhodic Kanhapludults	W	S	NT	No	U
38	2006	Cecil loam	Fine, kaolinitic, thermic Typic Kanhapludults	W	C	NT	No	U
39	2007	Davidson clay loam	Fine, kaolinitic, thermic Rhodic Kanhapludults	W	S	NT	No	U
40	2008	Mecklenburg clay loam	Fine, mixed, thermic Ultic Hapludalfs	W	C	NT	YES	P, U
41	2008	Mecklenburg loam	Fine, mixed, thermic Ultic Hapludalfs	W	C	NT	YES	P, U
42	2008	Chewacla soils	Fine-Loamy, mixed, thermic Fluvaquentic Dystrudepts	W	C	NT	YES	P, U
43	2008	Cecil clay loam	Fine, kaolinitic, thermic Typic Kanhapludults	W	C	NT	YES	P, U
44	2008	Pacolet clay	Fine, kaolinitic, thermic Typic Kanhapludults	W	CS	NT	NO	P, U
45	2008	Hiwassee clay	Very fine, kaolinitic, thermic Rhodic Kanhapludults	W	CS	NT	NO	P, U
46	2008	Congaree soils	Fine-Loamy, mixed, thermic Oxyaquic Udifluvents	W	C	CT	YES	P, U

Table 3.1 continued

47	2008	Hiwassee clay loam	Fine, kaolinitic, thermic Typic Rhodudults	W	C	NT	NO	P, U
48	2008	Altavista fine sandy loam	Fine-Loamy, mixed, thermic Aquic Hapludults	W	CS	NT	NO	P, U

† P: Poorly drained sites (i.e., those with soils classified as poorly drained and without adequate artificial drainage), W: Well drained sites (i.e., those with soils that are naturally well drained, or with functional tile drainage, or sites assayed during the 2008 drought).

‡ C, corn; CN, corn with an additional 112 kg N ha^{-1} applied late season above recommended N rates; S, soybean; CS, cover-crop soybean bushoged and incorporated before wheat planting

§ CT, conventional tillage; NT, no-till

¶ P, Pioneer 26R12; U, UniSouth Genetics 3209

Table 3.2. Correlations (Pearson's *r*) of winter wheat N uptake and grain yield (both with no spring N fertilizer), with four soil tests: amino sugar-N test (ASNT), NO_3-N, NH_4-N, and soil organic matter (SOM) (n = 69). Soil samples were taken from 0 to 30 cm.

Soil Test Value	Wheat N Uptake	Wheat Grain Yield
ASNT	0.25*	0.26*
NO_3-N	NS†	NS
NH_4-N	0.55***	0.47***
SOM	NS	NS

† NS, not statistically significant at the 0.05 probability level
* Significant at the 0.05 probability level
*** Significant at the 0.001 probability level

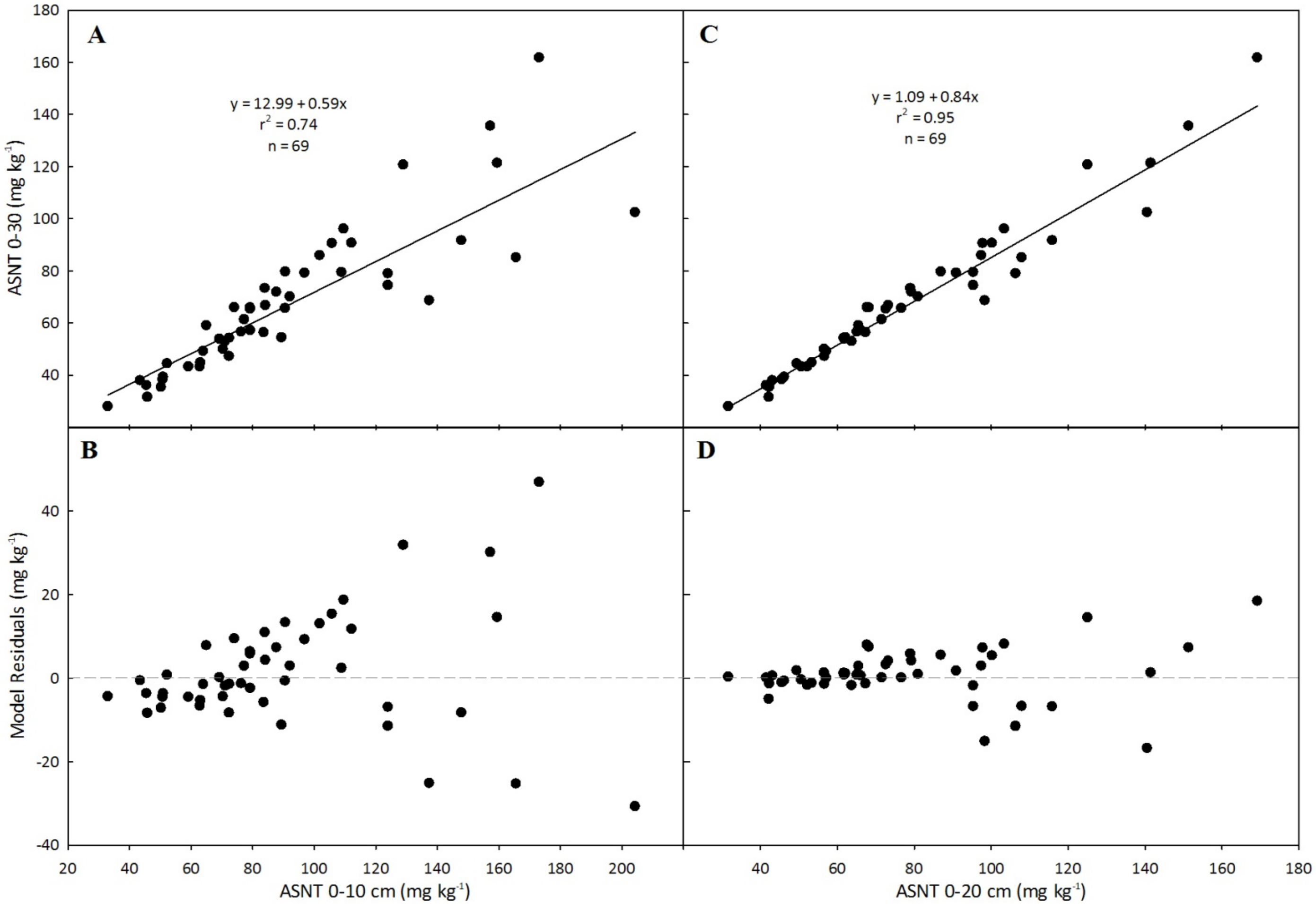

Fig. 3.1. A: Linear regression of soil amino sugar-N test (ASNT) sampled at the 0- to 30-cm depth and ASNT sampled at 0 to 10 cm for all sites (n = 69). B: Model residuals from the linear regression of ASNT sampled at the 0- to 30- and 0- to 10-cm depths. C: Linear regression of ASNT sampled at the 0- to 30-cm and 0- to 20-cm depths for all sites (n = 69) and B: Model residuals.

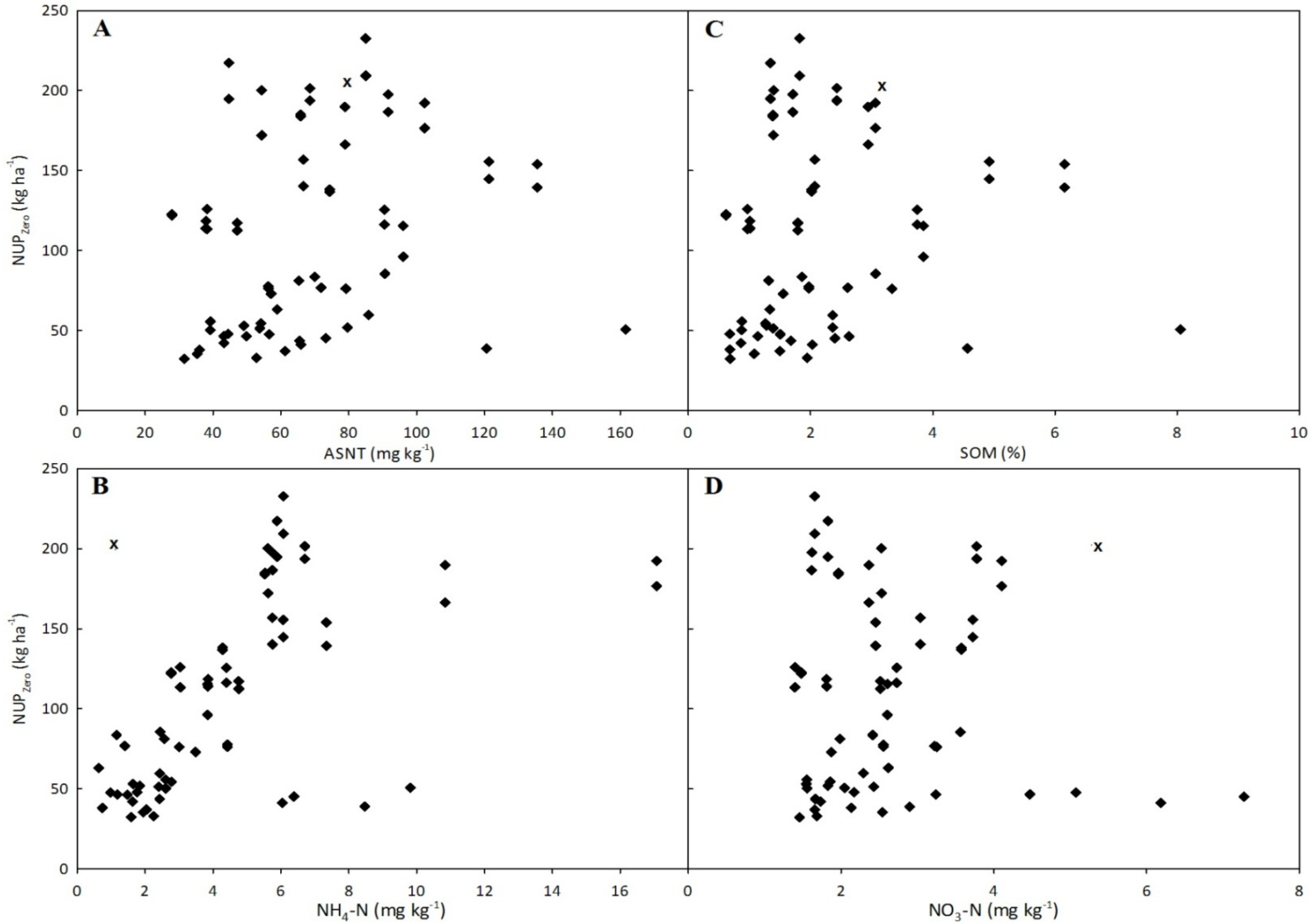

Fig. 3.2. Wheat N uptake with no spring-N fertilizer applied (NUP_{Zero}) (kg ha^{-1}) vs. (A) soil amino sugar-N test (ASNT) (mg kg^{-1}), (B) soil NH_4-N (mg kg^{-1}), (C) soil organic matter (SOM) (%), and (D) soil NO_3-N (mg kg^{-1}) for all sites (n=69). The data from site-year 36 (see Table 1) is indicated with an "x".

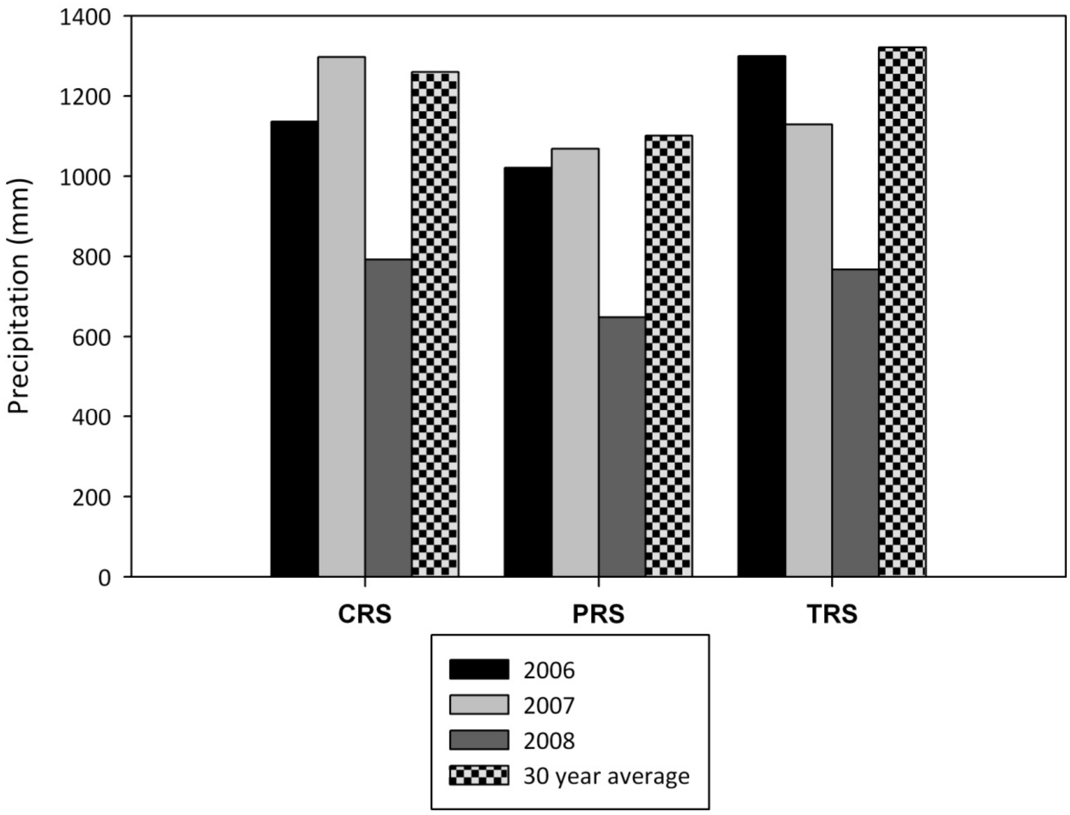

Fig. 3.3. Total annual precipitation (mm) for 2006, 2007, 2008, and the 30-yr average for the Cunningham and Caswell Research Stations (CRS) in the Coastal Plain, the Piedmont Research Station (PRS), and Tidewater Research Station (TRS).

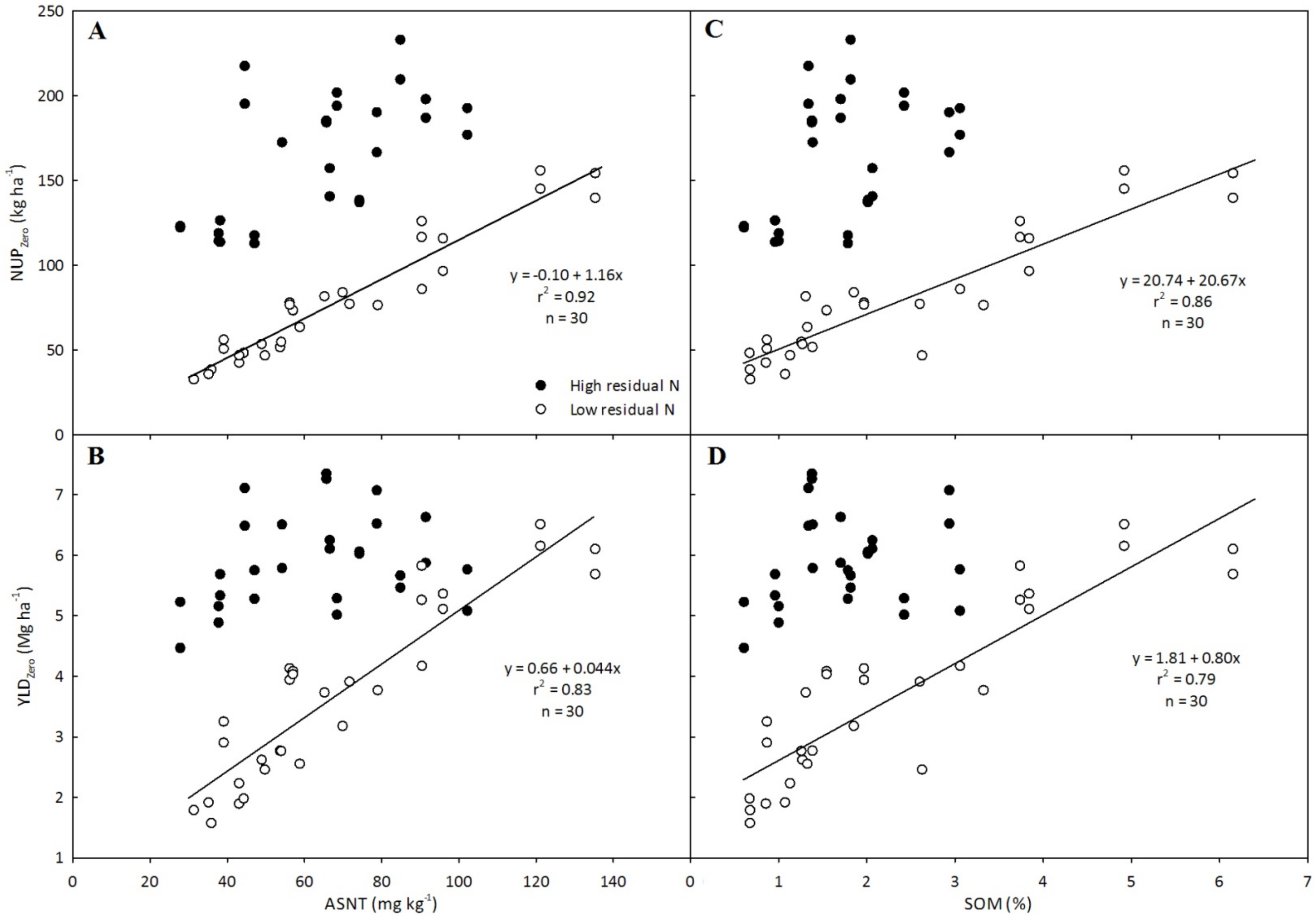

Fig. 3.4. A: Wheat N uptake (NUP$_{Zero}$) (kg ha^{-1}) and B: grain yield (YLD$_{Zero}$) (Mg ha^{-1}) with no spring-N fertilizer applied vs. soil amino sugar-N test (ASNT) (mg kg^{-1}), C: NUP$_{Zero}$ and D: YLD$_{Zero}$ vs. soil organic matter (SOM) (%), for all well-drained sites (n=58). Open symbols represent sites that had no manure applied in the previous 12 mo, no additional N fertilizer applied beyond the recommended rates for the previous crop, or no soybean plus stover turned under (i.e., sites with low expected residual N). The solid symbols represent sites that had one or more of these management practices (i.e., sites with high expected residual N). Linear regression models represent the relationships for the open symbols only.

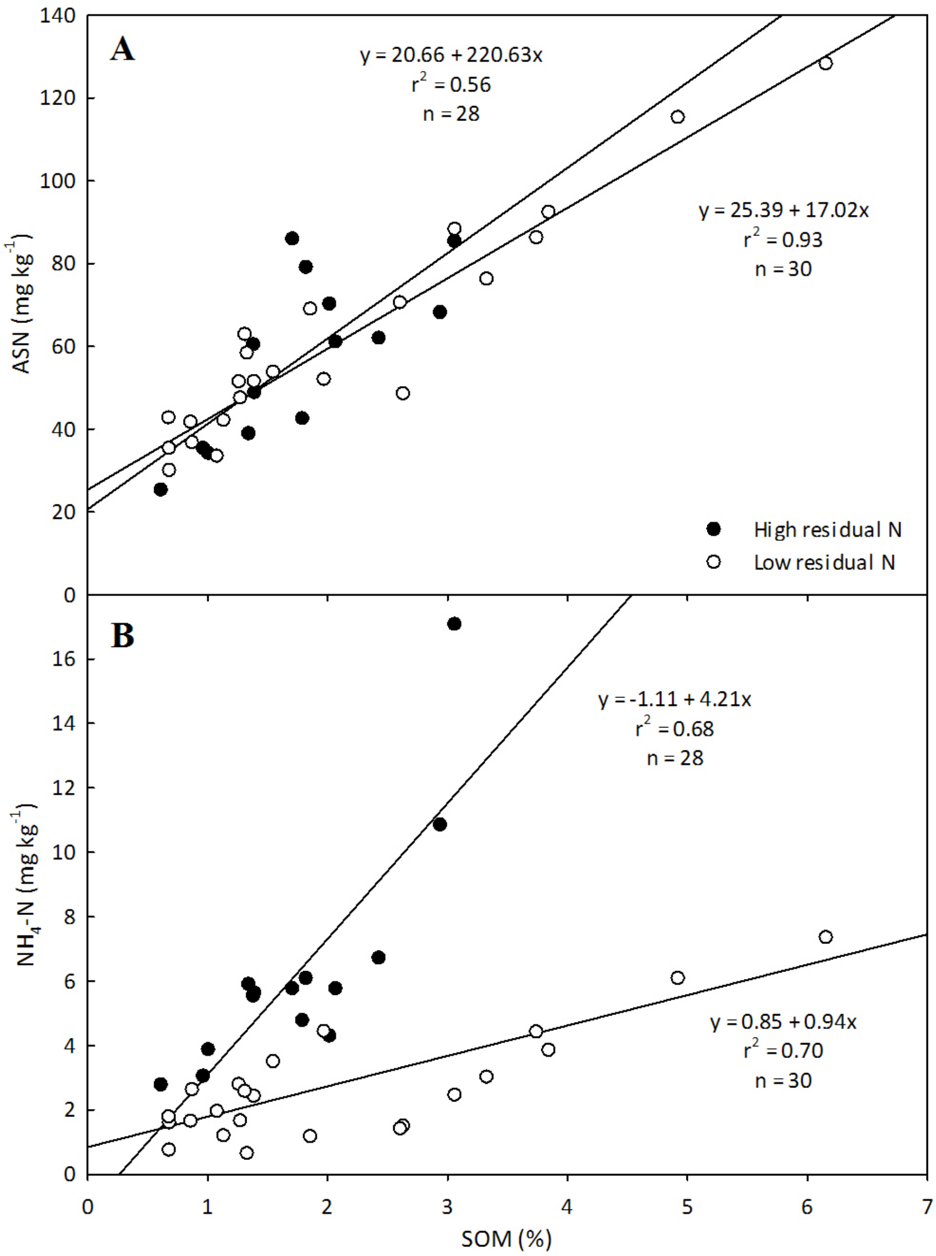

Fig. 3.5. Relationships of (A) soil amino sugar-N (ASN) (mg kg^{-1}) and (B) NH_4-N (mg kg^{-1}) with SOM (%) for all well-drained sites. Open symbols: sites with low expected residual N due to either to no manure applied in the previous 12 months, no additional N fertilizer applied beyond the recommended rates for the previous crop, or no soybean plus stover turned under. Solid symbols: sites with high expected residual N due to one or more of these management practices.

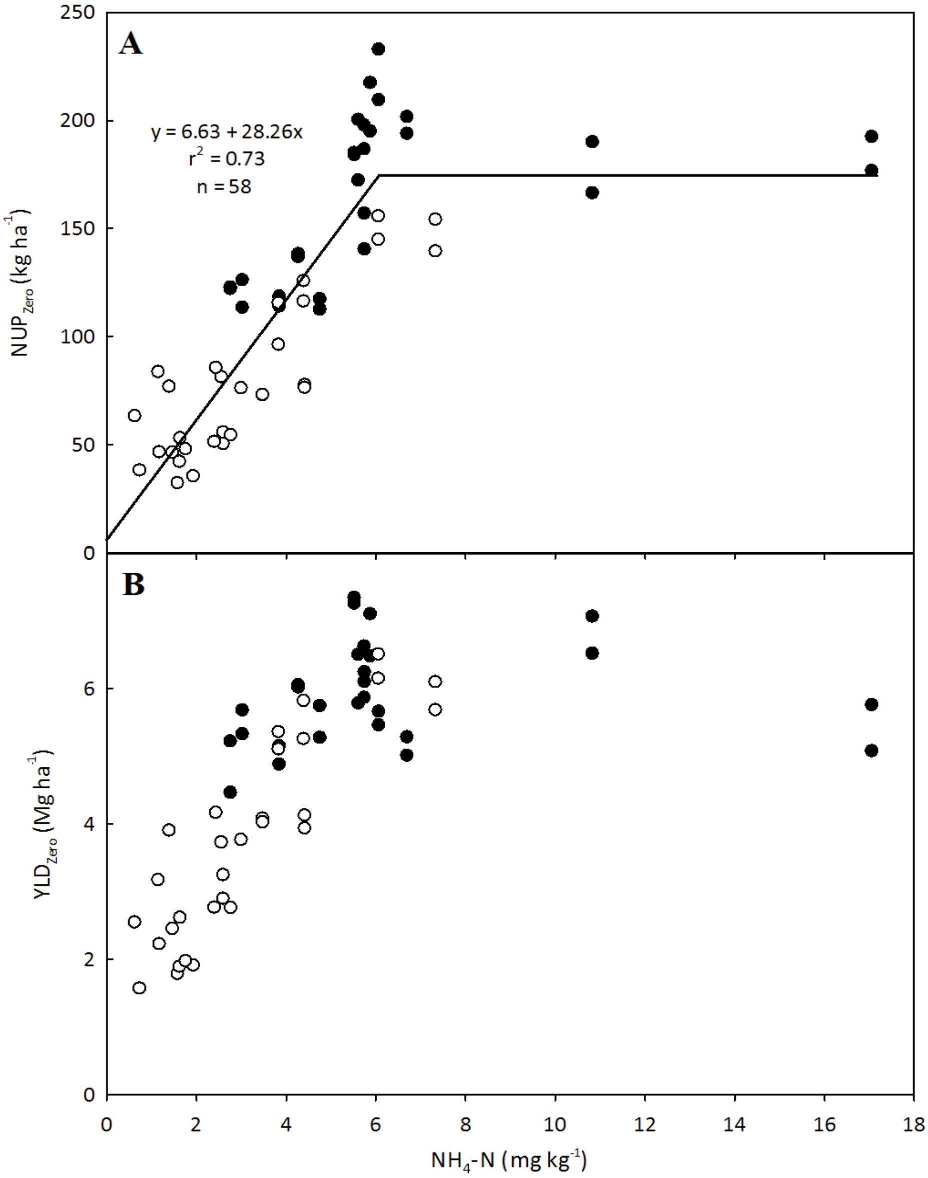

Fig. 3.6. A: Wheat N uptake (NUP_{Zero}) (kg ha^{-1}) and B: grain yield (YLD_{Zero}) (Mg ha^{-1}) with no spring-N fertilizer applied vs. soil NH_4-N (mg kg^{-1}), for all well-drained sites (n=58). Open symbols: sites with low expected residual N due either to no manure applied in the previous 12 mo, no additional N fertilizer applied beyond the recommended rates for the previous crop, or no soybean plus stover turned under. Solid symbols: sites with high expected residual N due to one or more of these management practices. The regression model represents the relationships over all sites.

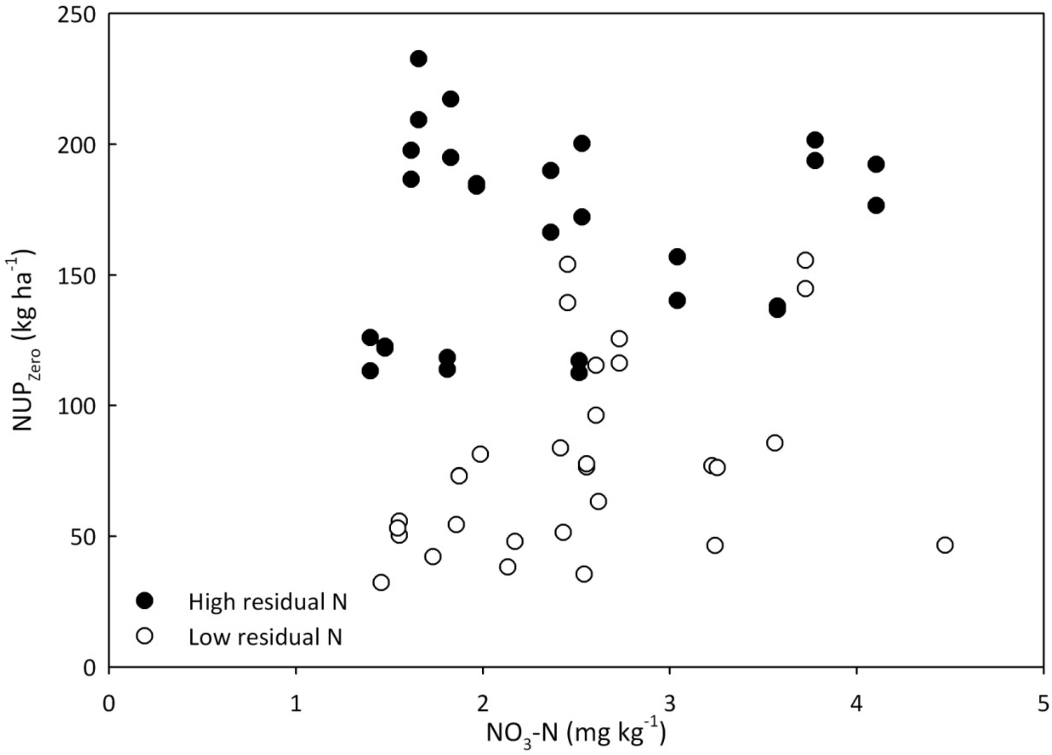

Fig. 3.7. Wheat N uptake with no spring-N fertilizer applied (NUP_{Zero}) (kg ha^{-1}) vs. soil NO_3-N (mg kg^{-1}) for all well-drained sites (n=58). Open symbols: sites with low expected residual N due to either no manure applied in the previous 12 mo, no additional N fertilizer applied beyond the recommended rates for the previous crop, or no soybean plus stover turned under. Solid symbols: sites with high expected residual N due to one or more of these management practices.

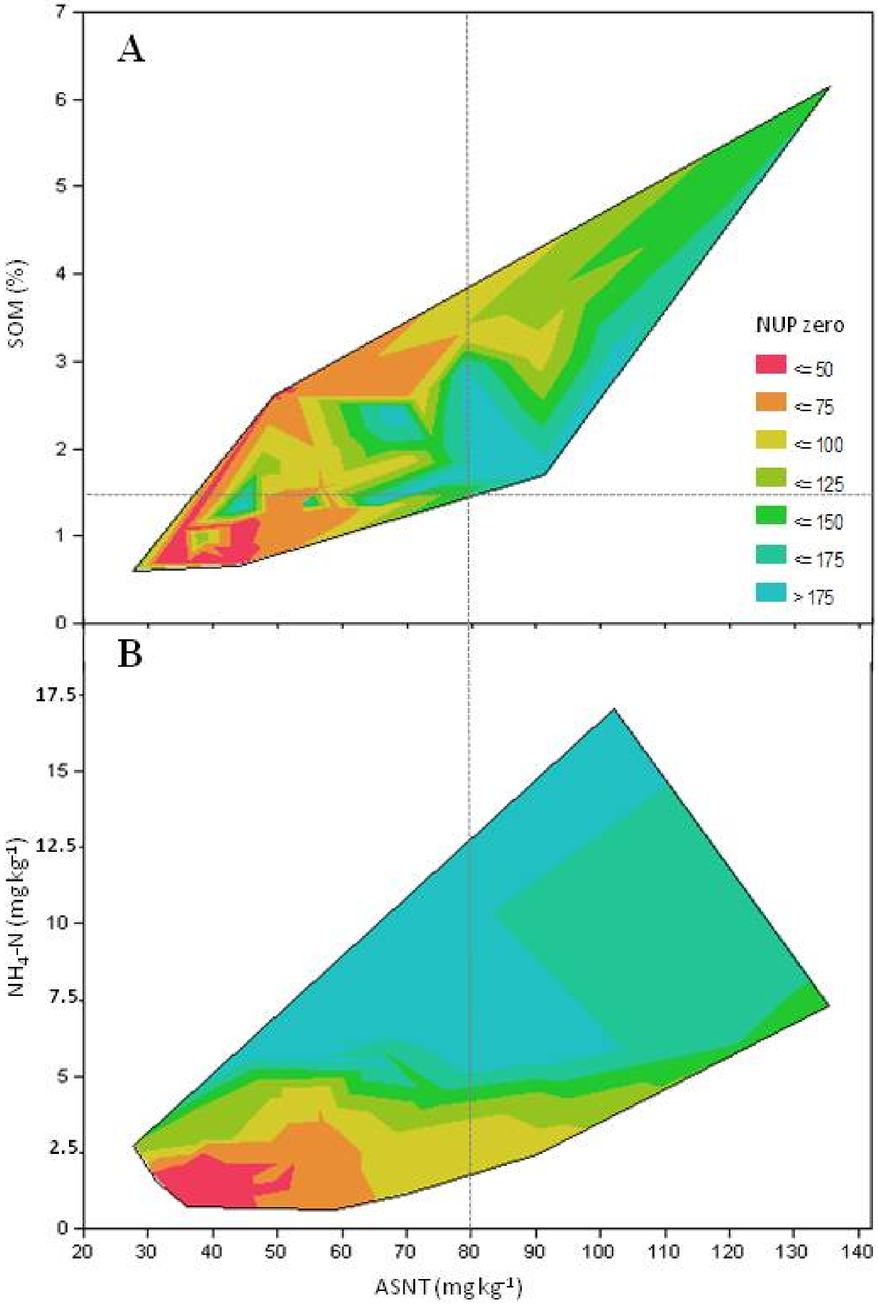

Fig. 3.8. A: Contour plot of wheat N uptake with no spring-N fertilizer applied (NUP_{Zero}) (kg ha^{-1}) vs. soil organic matter (SOM) (%) on the Y-axis, and soil amino sugar-N test (ASNT) (mg kg^{-1}) on the X-axis. B: Contour plot of NUP_{Zero}, vs. soil NH_4-N (mg kg^{-1}) on the Y-axis, and soil ASNT on the X-axis for all well-drained sites (n=58).

CHAPTER IV

ASSESSMENT OF THE AMINO SUGAR-N TEST AND SOIL MINERAL NITROGEN TESTS FOR WINTER WHEAT NITROGEN MANAGEMENT: CALIBRATION TO PREDICT ECOMOMIC OPTIMUM FERTILIZER NITROGEN RATES

ABSTRACT

In the southeastern USA, the amount of available soil N is generally not taken into account when deciding winter wheat (*Triticum aestivum* L.) spring N fertilizer application rates. Soil mineral N is prone to loss from the crop rooting zone in this warm humid region, therefore soil mineral N tests are regarded as poor predictors of season-long crop N fertilizer needs. The amino sugar-N test (ASNT) estimates a potentially mineralizable pool of organic N which is relatively stable compared to soil mineral N. The ability of the ASNT to accurately predict mineralizable N likely to become available to a crop during the growing season could have important implications for in-season N fertilizer recommendations. This study was conducted to examine the relationships between soil N tests (i.e. the ASNT, KCl-extractable soil NH_4-N and NO_3-N, and soil organic matter [SOM] tests) and economic optimum spring N fertilizer rate (OptN) for winter wheat over a range of soils and crop management practices and to evaluate if the ASNT, and/or soil mineral N and SOM tests can be used to predict OptN for winter wheat in North Carolina. Winter wheat N response trials (n=58) were conducted during the 2006 to 2008 growing seasons over a wide range of soils and previous crop management practices across North Carolina. Soil samples were taken in February each season and analyzed using these soil N tests. The data aggregated into two distinct groups: 1) sites with low residual N and 2) sites with high residual N. The ASNT by itself, was a poor predictor of winter wheat OptN for all well-drained sites, however the ASNT was able to predict N uptake at the optimum spring N fertilizer rate (NUP_{Opt}) and N uptake at wheat growth stage (GS) 30 (NUP_{GS30}) for sites with low residual N. The ASNT

could not satisfactorily predict OptN for these sites with low residual N, but could predict the minimum NUP_{Opt} and wheat yield at the optimum GS 30 N fertilizer rate (YLD_{Opt}) for well-drained sites. To predict OptN for all well-drained sites a combination of soil tests was required (i.e., NH_4-N and SOM with resultant $R^2 = 0.59$). The ability of soil NH_4-N to predict OptN for well-drained sites was improved by segregating sites by SOM levels (> 1.5 and < 1.5 %). Soil-test NO_3-N showed no benefit for winter wheat N management. The results of this study indicate the potential of the other soil N tests to account for mineralizable and residual soil N and improve N management for winter wheat in southeastern USA.

Abbreviations: ASNT, amino sugar-N test; ASN, amino sugar-N; EONR, economic optimum nitrogen rate; GS, growth stage; NUP_{Opt}, wheat N uptake at optimum growth stage 30 N fertilizer rates; NUE, apparent N fertilizer use efficiency; NUP_{Zero}, wheat N uptake with no growth stage 30 N fertilizer applied, OptN, wheat optimum growth stage 30 N fertilizer rates. PSNT, pre-sidedress N test; SOM, soil organic matter; YLD_{Zero}, winter wheat yield with no growth stage 30 N fertilizer applied; YLD_{Opt}, grain yield at optimum growth stage 30 N fertilizer rates;

INTRODUCTION

With rapidly increasing worldwide demand for N fertilizer, more prudent use of this valuable resource is critical. Nitrogen fertilizers are relied upon to maintain winter wheat yields all over the world. The appropriate amount of N fertilizer varies depending ,among other things, on yield potential, residual soil N, and the N supplying capacity of the soil. Even with good management practices, fertilizer N-use efficiency is usually less than 50% for annual cultivated crops (Gilliam et al., 1985). This may result in large amounts of N being released into the environment and NO_3-N contamination of groundwater under agricultural land (Spruill et al., 1996; Hong et al., 2006, 2007). Matching fertilizer N rates and application timing with crop N needs can reduce N losses and minimize a potential source of surface and groundwater pollution (Ferguson et al. 2002; Hong et al. 2007). Additionally, there is growing concern among the farming community with regard to increasing N fertilizer prices and the potential financial risks of applying N fertilizers to their crops. Therefore growers need to be able to accurately predict N rates to meet potential crop requirements.

Predicting N fertilizer rates for winter wheat may be improved by using soil tests that determine plant-available N. One soil property that can effect crop requirements for N fertilizer is SOM. Rasmussen et al. (1994) found that long-term decline in SOM, and specifically organic N, was tracked closely by a decline in wheat yields. Johnson (1986) reported that crops grown in soils with higher SOM may benefit from higher water holding capacity, better soil physical structure and availability of N in ways that cannot be mimicked by fertilizer N applications. Agricultural soils that differ in organic matter inputs would be expected to differ in rates of soil N transformations, competition for NH_4-N by immobilizers and nitrifiers, and fates of NO_3-N. Consequently, SOM could be an important parameter for predicting crop N fertilizer rates.

Another factor that can influence N fertilizer requirements is residual soil inorganic N (NO_3- and NH_4-N). Residual inorganic N can remain in the soil where fertilizer N was in excess of the crop need. Scharf and Alley (1994) observed substantial amounts of NO_3-N in the soil profile in the Virginia Coastal Plain during the winter wheat growing season. This situation is more pronounced where yields may have been lower than expected due to drought and where dry and cold weather conditions aid winter carryover of this inorganic N. If weather conditions permit, this remaining inorganic N will be available for crop uptake the following year (Bundy et al., 1992; Vonotti and Bundy, 1994; Halvorson et al. 2001). Vaughan et al. (1990) found that soil NH_4-N levels explained more wheat yield variation than did NO_3-N. They reported a significant relationship between spring soil NH_4-N and grain yield. However, they cautioned against using soil NH_4-N alone in assessing N needs for winter wheat, as low NH_4-N may coexist with high NO_3-N. Residual soil mineral-N can contribute greatly to crop N uptake (Scharf and Alley, 1994; Alley et al., 1996), and N fertilizer recommendations need to be adjusted where there is residual N carryover. However, in the humid southeastern USA, it is generally assumed that profile mineral-N is too transient to permit reliable use of soil tests like the pre-sidedress nitrate test (Magdoff et al., 1984) for prediction of crop response to N fertilization (Gilliam and Boswell, 1984; Bundy and Malone, 1988; and Scharf and Alley, 1994).

The Illinois Soil N Test (referred to in this paper as the amino sugar-N test, ASNT) estimates a potentially mineralizable fraction of soil organic N (Khan et al 2001; Mulvaney et al, 2001). A number of researchers have attempted to make N recommendations for corn using the ASNT (Mulvaney et al., 2001, 2005; Klapwyk and Ketterings, 2006; Barker et al., 2006; Williams et al., 2007; Laboski et al., 2008). Their success was been quiet variable. Initial studies in Illinois using the ASNT (Khan et al., 2001) show that N fertilizer response in corn was related to amino sugar-N test (ASNT) values, whereby as ASNT increased corn N fertilizer response decreased to zero once a threshold ASNT was reached. Further studies in the north central region of the USA showed no relationship between ASNT and economic optimum nitrogen rate (ENOR) for corn (Barker et al. 2006; Osterhaus, 2005; Laboski et al. 2008). Laboski et al. (2008) concluded that the ASNT should not be used for adjusting corn N rates. In a study conducted in Saskatchewan, Canada, Torrie et al. (2004) showed that there was a poor correlation between ASNT and N response in wheat. Klapwyk and Ketterings (2006) found that the ASNT estimates a potentially available pool of mineralizable N, and that when SOM is also considered, responsive and non-responsive sites can be distinguished for corn in New York. Williams et al. (2007b) found strong correlations between ASN and responses to fertilizer N in corn in North Carolina and successfully calibrated the ASNT to predict EONR on mineral soils (Williams et al., 2007a).

In Chapter 3, I showed that the ASNT could be used to predict winter wheat N uptake in plots that had not received spring N-fertilizer (NUP_{Zero}) at trial sites with low residual mineral N. Across all trial sites with a wide range in residual mineral N, combination of soil NH_4-N, ASNT, and SOM could be used to predict NUP_{Zero} ($R^2 = 0.80$). Clearly, winter wheat nitrogen uptake was dependent on a combination of both residual mineral- and potentially mineralizable-N. In light of these results, the objectives of this study were to: 1) examine the relationships between soil N tests (i.e., the ASNT, mineral N, and SOM tests) and winter wheat N uptake when optimum N fertilizer rates are applied, and 2) determine if these soil tests could be used to predict economic optimum N fertilizer rates for winter wheat in North Carolina.

MATERIALS AND METHODS

Field Selection

Research sites used in these experiments have been previously described in detail (Chapter 3). In brief, sites were selected so as to represent the three main wheat growing regions in North Carolina: Piedmont, Coastal Plain, and Tidewater. Experiments were conducted at 47 sites during the 2006, 2007, and 2008 winter wheat growing seasons. The sites were located at the Piedmont Research Station in Rowan County (PRS), at the Cunningham and Caswell Research Stations in Lenoir County (CRS), and at the Tidewater Research Station in Washington County (TRS). At each research station trial sites were selected based on soil series and mapping unit so as to represent the main soils used for crop production in these regions of North Carolina. As reported previously (Chapter 3), we found that wheat N uptake behaved differently between the poorly- and well-drained sites. As there were too few poorly-drained sites for meaningful data analysis (n = 10), these sites were discarded and results from only the 37 remaining well-drained sites are reported here (Table 4.1).

Pre-plant N applications (34 kg N ha^{-1}) was applied at all sites in the fall, based on North Carolina standard recommendations (Weisz et al., 2001) for early planted wheat. Phosphorus, K, S, lime and micronutrients were applied as needed at each site according to standard North Carolina recommendations (Hardy et al., 2002; Tucker et al., 1997) based on pre-season soil test results. Appropriate crop pesticides were applied as needed (Weisz et al., 2000) and weed, insect, and disease pest control was excellent at all sites.

Experimental Design and Soil Sampling

At each site, a RCBD N-response experiment with five replications and seven N-rates was initiated at winter wheat planting in the fall of each year. In the 2008 season at all sites, a split-plot treatment consisting of two winter wheat cultivars (Table 4.1) was incorporated into the experimental design. This resulted in 37 site-years, and because two cultivars were tested in 21 of those site-years, there were a total of 58 N-rate experiments on well-drained soils. The main plot treatment was growth stage 30 (GS30, Zadocks, 1974) N. The GS30 N rates were 0, 28, 56, 84, 112, 140, and 168 kg N ha^{-1} surface applied as urea-ammonium nitrate solution ($CO[NH_2]_2$-NH_4NO_3: 30%N) using a custom-built spray applicator.

At each site, within each experimental block, 1.7-cm diameter soil cores were taken to three depths (0 to 10, 0 to 20 and 0 to 30 cm) in the first week of February when the wheat was near GS25. For each depth, a minimum of seven soil cores were taken per block and bulked to give a total of 5 composite samples for each sampling depth and site-year. Previously, I reported (Chapters 2 and 3) that sampling from 0 to 30 cm gave the most reliable results. Consequently only soil test data from the 0- to 30-cm samples are reported here. These soil samples were analyzed for ASNT, NH_4- and NO_3-N, and SOM as described below. In 2008, additional 2.5-cm-diameter soil cores were extracted from 0- to 90-cm depth using a tractor mounted hydraulic probe (Giddings Machine Company Inc. Windsor, CO) and similar sampling protocol. These 90-cm cores were analyzed for NO_3-N as described below.

Wheat Biomass and Whole-Plant Nitrogen Concentration at Growth Stage 30

At wheat GS30 at each site within each experimental block, a 1-m section of row was randomly selected. All plant tissue above the soil surface within the selected row length was collected. The plant tissue from each of the 5 blocks was then composited, dried, and weighed to determine average GS30 dry matter biomass (kg ha^{-1}) for each cultivar-by-site-year. A wheat tissue subsample was analyzed for N concentration using a CHN analyzer (McGeehan and Naylor, 1988). The GS30 N uptake (NUP_{GS30}, kg ha^{-1}) was computed as the product of GS30 biomass and N concentration.

Grain Yield and Nitrogen Concentration

All wheat plots were harvested with a Wintersteiger Delta combine (Wintersteiger, Salt Lake City, UT) and grain yields were measured with a HarvestMaster grain guage (Juniper Systems, Inc., Logan, UT). Grain yield was adjusted to 135 g kg^{-1} moisture for analysis. At all sites in 2006 and 2007 grain yields (kg ha^{-1}) was determined by harvesting the center

1.75-m-wide strip from each plot; at all sites in 2008, grain yield was determined by harvesting each 1.52-m-wide sub plot. A 2.0-kg grain sample was collected from each plot at all sites and subsampled for grain N concentration analysis using a CHN analyzer (McGeehan and Naylor, 1988). Grain N uptake (kg N ha^{-1}) was computed as the product of grain yield and N concentration.

Straw Yield and Nitrogen Concentration

After separation from the grain by the combine the straw was collected from each harvested strip within the plot and weighed in the field. A 100-g straw subsample was also weighed in the field, dried and then reweighed to determine moisture content. Average straw dry weight was then determined for each plot. For all plots and sites, a straw sample was analyzed for N concentration using a CHN analyzer (McGeehan and Naylor, 1988). Straw N uptake (kg N ha^{-1}) was computed as the product of straw biomass and N concentration.

Amino Sugar-Nitrogen Test

Soil samples were air dried, and ground to pass through a 2-mm mesh. The sieved soil samples were analyzed in triplicate for alkali hydrolysable amino sugar-N plus NH_4-N (ASNT) using the procedure described by Khan et al. (2001) and modified by Williams et al. (2007a). The modification consisted of using 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 the ensemble heated at 48 to 50^o C in an incubator for 5 hr. Thermometers were positioned randomly inside the incubator to verify the temperature was within ±1 °C of 49 °C. After the incubation, samples were allowed to cool to room temperature, the petri dishes were removed from the jars, and the indicator solution was diluted with 5 mL of deionized H_2O. 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* was milliliters of H_2SO_4 used in titrating and *T* was 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).

Other Soil Analyses

Soil samples were also extracted with 2 *M* KCl (Keeney and Nelson, 1982) and analyzed for soil mineral N (NO_3- and NH_4- N). Extract NO_3-N was determined colorimetrically with a QuikChem Automated Ion Analyzer (Lachat Instruments, Milwaukee, WI) according to QuikChem Method No. 12-107-04-1-B, a Griess-Ilosvay method (Keeney and Nelson, 1982). Extract NH_4-N was also measured colorimetrically with the same instrument and QuikChem Method No. 12-107-06-2-A. Soil organic matter content was determined by the loss on ignition (360 ^{o}C) (Schulte et al., 1991).

Data Analysis

Statistical analyses for these data were conducted using SAS Version 9 and JMP 7.0 (SAS Institute, Cary, NC). Initially, winter wheat grain yield response to N rate and site-year was analyzed with ANOVA for either RCBD repeated at multiple sites (2006 and 2007 sites) or for a split-plot design repeated at multiple sites (2008 sites). Then, for each site-year by cultivar combination the GS30-N rate resulting in the highest economic return (OptN) was determined using the procedure described by Sripada et al. (2007). The steps of this procedure were as follows:

1) The yield response to GS30 N was determined by ANOVA in PROC GLM. If the response to GS30 N was not significant, OptN was assumed to be zero. This was the case in 17 of the N-response trials.

2) For site-year-by-cultivar combinations for which the response was significant, Fishers Protected LSD ($\alpha = 0.05$) was used for means separation. If the highest mean yield occurred at 0 kg N ha^{-1}, then OptN was determined to be zero. This occurred in 6 of the N-response trials.

3) If there was a significant and positive yield response to GS30 N, this was analyzed as follows:

   a. A linear-plateau function was fit to the data using PROC NLIN. In every case (n=35), the 95% confidence interval for the estimated slope of the linear portion of the model and for the breakpoint (the GS30-N rate beyond which the yield response was flat) did not contain zero, so the lowest N rate resulting in the maximum yield (MaxN) was determined to be equal to the breakpoint. In two trials, the yield response to GS30 N was initially positive, reached a plateau, and then decreased at higher N rates. When this occurred, the GS30 N rates that had significantly decreased yield compared to the GS30 N rate with the highest yield as determined by the Fisher's protected LSD (alpha=0.05) mean separation were deleted before fitting the linear-plateau model.

b. When the yield response to GS30-N is modeled with a linear-plateau function, MaxN (estimated at the breakpoint) is equal to OptN if the linear slope (in units of Mg grain per kg N) is greater than or equal to the N-cost-to-grain-price ratio. We define this ratio as the cost of 1kg N divided by the price of 1 Mg wheat and for this analysis used a critical N to grain price ratio of 0.011. If the linear-slope is lower than the N to grain price ratio, then the linear-plateau model predicts that OptN will be equal to zero. This approach was used for the 35 data sets that conformed to rule 3a above. In 4 of these trials, the linear slope was less than the N-cost-to-grain-price ratio of 0.011 and OptN was set to zero. In the other 31 trials OptN was equal to MaxN.

Once OptN was calculated for a given site-year-by-cultivar combination, the optimum yield (YLD_{Opt}) was defined as the modeled yield at OptN. Wheat N uptake (the sum of straw plus grain N uptake) response to GS30 N was also modeled using a linear-plateau function for each site-year-by-cultivar combination. Using this model, the N uptake at OptN (NUP_{Opt}) was determined. Relationships between OptN and NUP_{Opt} and the different soil tests were further explored using correlation, and linear and stepwise regression.

RESULTS AND DISCUSSION

There was a yield response to N fertilizer (i.e., OptN > 0) in 31 of the 58 trials conducted on well-drained sites between 2006 and 2008 (Table 4.2). For the yield response to GS30-N for the 2006 and 2007 sites, the interaction of site X GS30 N, and the main effects of site, and GS30 N were significant ($P < 0.001$), indicating that the response to N rate differed across sites. For the 2008 sites, the interactions of site X GS30 N X cultivar ($P < 0.05$), and site X GS30 N, and the main effects of site and GS30 N, and cultivar were significant ($P < 0.001$), while the interaction of cultivar X GS30 N was not. The significance of the three-way interaction suggested that the yield response to GS30-N rate differed across sites, and that these differences were cultivar specific. To meet the objectives of this research we were primarily interested in three parameters: NUP_{Opt}, OptN, and the YLD_{Opt} for each site-year by cultivar combination. To determine if these parameters could be pooled across cultivars, a t-test was used to test for differences between the two cultivars in 2008. There was no significant difference between wheat cultivars for NUP_{Opt}, OptN, or $YLD_{Opt,}$ which allowed the data to for each variety to be pooled and analyzed as one data set (n = 58).

All 27 of the non-responsive trials occurred in 2008. The 2008 wheat season was characterized by drought conditions in late 2007 that continued into spring 2008 (Fig. 4.1). Previous analysis of these data (Chapter 3) for wheat N uptake and yield for the 0 kg ha^{-1} GS30-N plots (NUP_{Zero} and $YLD_{Zero,}$ respectively) showed that the trials segregated into two groups based on past management. In one group that was expected to have high carry-over soil N (n = 28), either the previous crop had been cover-cropped soybeans [*Glycine max* (L.) Merr.] that were turned under prior to wheat planting, manure had been applied to the field in the previous 12 mo, or the previous crop had been corn that received both the full recommended N-fertilizer rate plus an additional 112 kg N ha^{-1} after tasseling. These fields were also shown to have elevated levels of soil NH_4-N and ASNT. All these high carry-over trials were from 2008 and it is likely that the drought conditions in that season were at least in part responsible for this carry-over N being detected as NH_4-N. Given the past management of these 28 trials and the detection of carry-over soil N, it is not surprising that 23 were found to be non-responsive to GS30 N. The second group of trials consisted of those that were preceded by either corn or soybeans harvested for grain and had not received any manure or fertilizer N beyond recommended rates. This low carry-over group consisted of the remaining 30 N-response trials of which only 4 were non-responsive to GS30 N.

The Amino Sugar Nitrogen Test for Predicting Nitrogen Fertilizer Response at Low Carry-Over Sites

If the ASNT truly measures a fraction of potentially mineralizable N, its relationships with NUP_{Opt}, YLD_{Opt}, and OptN should be strongest when considering only the low carry-over N sites. For these low carry-over sites, ASNT was correlated with NUP_{Opt} (r = 0.79, P < 0.001,) and YLD_{Opt} (r = 0.55, P < 0.001, Table 4.3). Figure 4.2 shows relationships between NUP_{Zero} (data from Fig. 3.4A in Chapter 3), and NUP_{Opt} with ASNT for the low carry-over trial sites. There was a linear-plateau relationship between ASN and NUP_{Opt} for these low residual N sites ($r^2 = 0.73$, $P < 0.001$). For NUP_{Opt} the model predicted that a winter wheat crop will assimilate increasing quantities of N to achieve NUP_{Opt} as soil ASN levels increase up to 74 mg kg^{-1}. A plateau was reached at this level, above which NUP_{Opt} leveled off at approximately ~149 kg N ha^{-1}. The YLD_{Opt} and NUP_{Opt} were linearly related (Fig. 4.3, $r^2 = 0.68$, $P < 0.001$). Consequently, at ASNT values below 74 mg kg^{-1}, site-year yield potential was decreasing and that probably resulted in the reduction in NUP_{Opt}. The trail sites with these lower ASNT values were on sandy coastal plain soils or piedmont soils with lower SOM and/or potential water holding capacity, which might explain the lower yield potential compared to the trials at sites with higher ASN levels (above 74 mg kg^{-1}) that were consistently higher yielding. These data make it clear that as ASN decreased, there was a corresponding decrease in yield potential that could not be overcome with the addition of fertilizer N. In other words, ASN and yield potential are confounded (as both are linked to SOM and soil texture) making it unlikely that a simple relationship between OptN and ASN could be found. This is consistent with the findings of Klapwyk et al. (2006) for corn who reported that a combination of ASN plus SOM was needed to separate responsive from non-responsive sites in New York.

In Fig. 4.2 NUP_{Opt} minus NUP_{Zero} likely represents predominantly N that was supplied from fertilizer N sources. This difference increased as soil ASNT increased up to 74 mg kg^{-1}. For soils with ASNT > 74 mg kg^{-1} the proportion of NUP_{Opt} supplied by N fertilizer decreased again as soil ASN levels continued to increase. Four trials with ASNT values between 91 and 135 mg kg^{-1} appeared to have their NUP_{Opt} requirement fully supplied by mineralized soil N. Figure. 4.4 shows the quadratic relationship between OptN and ASN for these low carry-over sites. This relationship was similar to the difference between NUP_{Zero} and NUP_{Opt} for these sites illustrated in Fig. 4.2, however the relationship was not strong enough for ASNT to be used to predict OptN (r^2 = 0.42). Given that OptN at these low N carry-over sites was determined not only by potentially mineralizable soil N levels reflected in the ASNT, but by a declining yield potential of the soils with lower SOM, the weakness of this relationship was not surprising. Additionally, the apparent fertilizer N-use efficiencies (NUE, NUP_{Opt} minus NUP_{Zero}, divided by OptN) for these trial sites ranged from 29 to 100%. This range in NUE highlights the difficulty in using a soil test to predict N fertilizer rates for winter wheat over a number of different soil types and years. In Illinois, ASNT critical levels have been used to identify corn sites that were responsive and non-responsive to N fertilizer (Khan et al. 2001). They determined a critical ASN range of 225 to 235 mg kg^{-1}. above which corn was not responsive to fertilizer N. Kalpwyk et al. (2006) also developed critical levels for corn in New York, however, they concluded that SOM must also be considered. In our study, a winter wheat critical ASNT level could not be reliably established, however the data suggests that a critical level for winter wheat may lie in the range of 120 to 150 mg ASNT kg^{-1} for well-drained soils in North Carolina (Fig. 4.2). Spargo et al. (2008) reported that the average recovery of ASNT using the incubator method (Williams et al., 2007a) was only 58% of that recovered by the original laboratory protocol where a commercial griddle is used to heat the samples (Khan et al., 2001). Given the differences in methods used to determine ASNT, 120 to 150 mg ASNT kg^{-1} is probably very similar to the critical levels for corn reported by Khan et al., (2001).

Combining the Soil N Tests to Predict Optimum N at All Sites

A correlation matrix was initially used to explore whether any soil test (NO_3-N, NH_4-N, ASNT, SOM) was related to OptN, YLD_{Opt}, or NUP_{Opt} for all the N response trials conducted between 2006 and 2008 (n=58, Table 4.3). These relationships are graphically illustrated in Fig. 4.5A-D. Soil SOM, ASNT, and NH_4-N were correlated with NUP_{Opt} with *r* values ranging from 0.28 to 0.58, however NO_3-N was not correlated with NUP_{Opt}. These correlations suggested that it might be possible to better predict NUP_{Opt} using a combination of these soil N tests. Multiple regression using a stepwise regression procedure was used to evaluate multiple parameter models including the soil mineral N tests, the ASNT, and SOM. The best-fit model for predicting NUP_{Opt} was:

$$NUP_{Opt} = 40.05 + 20.48*NH_4\text{-N} - 0.95*(NH_4\text{-N})^2 + 0.92*ASNT - 2.86*(SOM)^2 \quad [4.1]$$

$$R^2 = 0.62 \qquad P < 0.001 \qquad n = 58$$

Ammonium-N and its quadratic term $(NH_4\text{-N})^2$ were major components of this model explaining approximately 52% of the variability in NUP_{Opt} (consistent with Fig. 4.5B). The addition of either ASNT or SOM individually to the model was not significant. Only when the two were entered together was there a statistically significant improvement to the model with a further explanation of 10% of the variability in NUP_{Opt}. When combined, these soil N tests (NH_4-N, ASNT, and SOM) explained 62% of the variability in NUP_{Opt}. While the model predicted that increasing soil NH_4-N had a positive linear and negative quadratic influence on NUP_{Opt} the relationship between NUP_{Opt} and the combination of ASNT and SOM was more complex (Fig 4.6). As reported previously (Chapters 2 and 3) ASNT and SOM were highly correlated (r = 0.91, see Fig. 4.6), indicating that an experimental site with elevated SOM generally also had higher ASNT levels. Figure 4.6 makes it clear that there was a positive response of NUP_{Opt} to ASNT at a given level of SOM, and that this response was greater at low SOM. For example, at a low SOM value of ˜2.5%, increasing ASNT by 10 mg kg^{-1} resulted in an increase of approximately 40 kg N ha^{-1} in NUP_{Opt}, but at a higher SOM of ˜5% a 10 mg kg^{-1} increase in ASNT had little to no effect on NUP_{Opt} (see Fig. 4.6). This model showed that there was potential for these soil N tests to predict NUP_{Opt} and to aid winter wheat N management decisions for well-drained sites in North Carolina.

Figure 4.7A-D shows the relationships between OptN and the four soil N tests. Soil NH_4-N was the only soil test significantly correlated with OptN (r = -0.57, $P < 0.001$, Table 4.3). Both ASNT and SOM were weakly correlated with YLD_{Opt} (r = 0.33 and 0.30 respectively, $P < 0.05$). Multiple regression using a stepwise regression procedure was used to evaluate multiple parameter models including the soil mineral N tests, the ASNT, and SOM. The best-fit model for predicting OptN was:

$$\text{OptN} = 96.79 - 31.95 * NH_4\text{-N} + 1.26 * (NH_4\text{-N})^2 + 46.50 * \text{SOM} - 6.05 * (\text{SOM})^2 \qquad [4.2]$$

$$R^2 = 0.59 \qquad P < 0.001 \qquad n = 58$$

Using this model, soil NH_4-N and SOM explained 59% of the variability in OptN. These soil tests show potential for predicting OptN for well-drained sites over a range of prior management histories in North Carolina. Soil NH_4-N and its quadratic term $(NH_4\text{-N})^2$ were major components of this model explaining approximately 50% of the variability in OptN. Adding SOM and its quadratic term $(SOM)^2$ improved the relationship between OptN and NH_4-N by accounting for differences in OptN possibly due to differences in NUP_{Opt} between sites as SOM levels increased. One way of interpreting Eq. 4.2 is that the relationship between OptN and NH_4-N changed depending on SOM. To illustrate this, we divided the data into sites with SOM levels above or below 1.5% and fitted these two groups of data with linear-plateau models (Fig. 4.8). Sites with SOM > 1.5% (solid symbols) required more N fertilizer than sites with SOM < 1.5% (open symbols) for $NH_4\text{-N} \leq 6.29$ mg kg^{-1}. Critical soil NH_4-N levels above which no additional spring N fertilizer was required were 4.95 and 6.29 mg NH_4-N kg^{-1} for sites with SOM < 1.5% and > 1.5% respectively. These relationships demonstrated that there is a need to adjust N fertilizer rates among fields due to SOM variation.

Soil Nitrate Nitrogen Levels and Winter Wheat N Management

Soil NO_3-N is generally assumed to be too transient to be used to predict crop response to N fertilizer in the southeastern USA (Gilliam and Boswell, 1984; Bundy and Malone, 1988; Scharf and Alley, 1994). However some studies in humid regions have shown benefits of deep soil sampling (0 to 90 cm) to account for NO_3-N movement deeper in the soil profile which may be available to crops later in the growing season as their root system develops (Wehrmann and Scharpf, 1979; Scharf and Alley, 1993). Figures 4.5D and 4.7D showed that there was no relationship between NO_3-N (sampled from 0 to 30 cm) and

NUP_{Opt} or OptN over all sites. During the stepwise procedure reported above soil test NO_3-N was rejected for inclusion in the best-fit regression models for NUP_{Opt} and OptN. Clearly, NO_3-N in soil sampled from 0 to 30 cm was of no benefit in determining optimum fertilizer rates.

We were concerned that we may have missed soil NO_3-N pools that were below our sampling depth but still available for crop uptake. To test for this possibility, in 2008 we took additional soil cores to a depth of 90 cm and tested these for NO_3-N. This gave us one year of data containing two estimates of soil NO_3-N for each trial. Multiple regression analysis using a stepwise regression procedure was used to evaluate multi-parameter models using this 2008 data set. This analysis was conducted using soil tests for NH_4-N, ASNT, and SOM (sampled from the 0 to 30 cm) and included soil NO_3-N measured from both this and the deeper (0 to 90 cm) depths. The best-fit model for predicting OptN for the 2008 sites was:

$$\text{OptN} = 119.96 - 22.94 \cdot NH_4\text{-N} + 0.94 \cdot (NH_4\text{-N})^2 \qquad [4.3]$$

$$R^2 = 0.24 \qquad P < 0.01 \qquad n = 42$$

Although this model was poor, it shows that NH_4-N explained more variability in OptN for the 2008 sites than any other soil test, as it did in Eq. 4.2 (the model for all the 2006, 2007, and 2008 data using only the 0- to 30-cm-d samples). Again soil test NO_3-N at either sampling depth was rejected during the stepwise procedure. The OptN prediction was not improved by NO_3-N sampled from the deeper depths based on the 2008 season. These results showed that soil NO_3-N, including that sampled in February to 90-cm depth, was not a good predictor of crop-available soil N and had no benefit for winter wheat N management in North Carolina.

Differences Between Expected High and Low Carry-Over Trial Sites

Twenty-seven of our 58 trials were not responsive to fertilizer N. Of these unresponsive trials, ten had manure applied in the 12 mo prior to these experiments and 11 had cover-cropped soybean tilled under just prior to wheat planting. Both these practices should have increased the organic N pools in these fields, but there was only a slight increase in ASNT values (Chapter 3). In these fields and in two additional trials where high levels of N fertilizer had been applied late in the previous corn season we also expected high levels of mineral N as NO_3- and/or NH_4-N. Soil NO_3-N levels were relatively low for all trials (< 7.4 mg kg^{-1}) and did not come close to approaching the PSNT NO_3-N critical range of 20 to 25 mg kg^{-1} for corn (Bundy and Meisinger,1994; Evanylo and Alley, 1998) or the 20 mg kg^{-1} level reported for wheat (Guillaumes et al., 2006). Furthermore, there were no differences in mean NO_3-N levels between the fields where we expected high carry-over N and in those where we did not. The mean NH_4-N values from these two groups of fields, did however, differ statistically. In the fields that had manure applied in the previous 12 mo, cover-crop soybean turned under, or excess fertilizer applied the mean soil NH_4-N level was 6.28 mg kg^{-1} compared to 3.09 mg kg^{-1} in the low carry-over fields. These differences in soil NH_4-N can also be visualized in Figs. 4.5B and 4.7B.

In the mid-Atlantic USA, most N is taken up by winter wheat in the 30 to 50 d after GS30 (Baethgen and Alley, 1989). As NH_4-N is usually highly dynamic, it seemed unlikely to us that elevated levels of NH_4-N in early February could supply enough N in late March and April to account for the differences in NUP_{Zero} (see Fig. 3.6A Chapter 3) and NUP_{Opt} (Figs. 4.5A-D) between the high and low carry-over fields. Fig. 4.9A shows the relationships between NUP_{GS30} and ASNT for the 28 trials where we expected high N carry-over (solid symbols) and for the 30 trials where we expected low carry-over N (open symbols). The slopes of these linear regressions were not statistically different so a common slope was fitted to the data. As ASNT increased, NUP_{GS30} also increased, but that increase could not account for the differences in NUP_{GS30} between the two groups. Differences in the intercepts indicated that the high carry-over fields had average NUP_{GS30} values that were 89 kg ha^{-1} higher than those in the low carry-over fields. At harvest the mean NUP_{Opt} was 130 and 47 kg ha^{-1} for the high and low carry-over fields, respectively, representing a difference of 83 kg N ha^{-1}. In other words, by the time these winter wheat trials had reached GS30, the difference in wheat N uptake in the low and high carry-over fields was sufficient to account for the differences observed in N uptake at harvest. Figure 4.9B shows the quadratic relationships between NUP_{GS30} and soil NH_4-N (r^2 = 0.67), demonstrating that a single relationship could account for differences in NUP_{GS30} across the two residual N groups. The elevated levels of soil N in the high carry-over fields were apparent in early February as NH_4-N and this mineral N likely resulted in increased crop N uptake very early in the season.

CONCLUSIONS

Predicting OptN for a winter wheat crop using soil tests was complicated, as relationships between OptN and the soil test values were dependent on many factors including soil moisture variability between normal and drought-stressed years, crop yield potential, and previous field management history.

At sites with manure application history, or where additional fertilizer was applied beyond recommended rates, or where cover-crop soybean (beans and stover) were tilled under prior to wheat planting the usefulness of the ASNT to predict OptN was poor. The ASNT was able to predict NUP_{GS30} and NUP_{Opt} for well-drained sites where none of these management practices had occurred. Consistent with our previous findings (Chapter 3) and contrary to the conclusions of Laboski et al. (2008) these results demonstrated that the ASNT was estimating a pool of potentially mineralizable N plus NH_4-N that did become available for crop uptake. However, the relationship between ASNT and crop N uptake was masked in fields where the crop N requirements were being supplied from a previously mineralized or fertilizer derived soil mineral N pool. At best, the ASNT could be used to predict minimum wheat N uptake or minimum grain yield for a field assuming that optimum N rates were applied (Fig. 4.5A and 4.3). This may help farmers assess the financial risks of applying that optimal rate of N fertilizer. However, the ASNT was not able to satisfactorily predict that optimum N fertilizer rate. This was due, at least in part due to the fact that wheat yield potential and ASNT were both correlated to SOM. Soils with lower SOM had lower ASNT suggesting, the need for higher rates of N-fertilizer to compensate for a reduction in mineralizable N. However, this was not the case in our tests because the low SOM soils in our tests also had reduced yield potentials and conversely needed less fertilizer N. Based on our results, the ASNT appears to have, limited potential use to predict OptN for wheat under a wide range of crop management circumstances in the southeastern USA.

When modeled together, ASNT, NH_4-N, and SOM tests were able to predict NUP_{Opt} for all well-drained soils with a range of different crop management histories. Ammonium-N explained most of the variability in NUP_{Opt} in this model. Ammonium-N and SOM modeled together were able to predict OptN for all well-drained soils with a range of crop management histories, and as found with NUP_{Opt}, NH_4-N accounted for the majority of the variability in OptN. This indicates the potential of an early-spring test of soil NH4-N to guide spring top-dress N-fertilizer recommendations. The ability of soil NH_4-N to predict OptN for the well-drained sites was improved somewhat when the data were segregated into two groups by SOM: less than or greater than 1.5%. Therefore, southeastern USA winter wheat growers may be able to use NH_4-N and SOM tests in February prior to GS30 to predict OptN for their well-drained production fields.

Sampling for KCl-extractable NO_3-N in February (even to 90 cm) was not useful for predicting OptN, as NO_3-N levels in the soil profile had depleted by that time, possibly due to crop N uptake during the fall and winter and/or NO_3-N losses prior to sampling. Consistent with previous research, our results indicate that soil NO_3-N has little or no potential to guide winter wheat N management in North Carolina (and probably elsewhere in the southeastern USA), likely due to the effects of our warm and humid weather.

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FIGURE CAPTIONS

Fig. 4.1. Total annual precipitation (mm) for 2006, 2007, 2008, and the 30-yr average for the Cunningham and Caswell Research Stations (CRS) in the Coastal Plain, the Piedmont Research Station (PRS), and Tidewater Research Station (TRS).

Fig. 4.2. The relationship between wheat N uptake with no growth stage 30 N fertilizer applied (NUP_{Zero}) (kg ha^{-1}) and soil amino sugar-N test (ASNT) (mg kg^{-1}) (open symbols, solid regression line) and the relationship between wheat N uptake at optimum growth stage 30 N fertilizer rate (NUP_{Opt}) (kg ha^{-1}) and ASNT (closed symbols, dashed regression line) for well-drained sites that had no manure applied in the previous 12 mo, no additional N fertilizer applied beyond the recommended rates for the previous crop, or no soybean plus stover turned under (i.e., sites with low expected residual N, n=30).

Fig. 4.3. Relationship between wheat grain yield at optimum growth stage 30 N fertilizer rate (YLD_{Opt}) (Mg ha^{-1}) and N uptake at optimum growth stage 30 N fertilizer rate (NUP_{Opt}) (kg ha^{-1}) for the well-drained sites that had no manure applied in the previous 12 mo, no additional N fertilizer applied beyond the recommended rates for the previous crop, or no soybean plus stover turned under (i.e., sites with low expected residual N, n=30).

Fig. 4.4. Relationship between wheat growth stage 30 optimum N fertilizer rate (OptN) (kg ha^{-1}) and soil amino sugar-N test (ASNT) (mg kg^{-1}) for the well-drained sites that had no manure applied in the previous 12 mo, no additional N fertilizer applied beyond the recommended rates for the previous crop, or no soybean plus stover turned under (i.e. sites with low expected residual N, n=30).

Fig. 4.5. Wheat N uptake at optimum growth stage 30 N fertilizer rate (NUP_{Opt}) (kg ha^{-1}) vs. A: amino sugar-N test (ASNT) (mg kg^{-1}), B: KCl-extractable soil NH_4-N (kg ha^{-1}), C: soil organic matter (SOM) (%), and D: KCl-extractable soil NO_3-N (kg ha^{-1}) for all well-drained sites (n=58). Open symbols represent sites that had no manure applied in the previous 12 mo, no additional N fertilizer applied beyond the recommended rates for the previous crop, or no soybean plus stover turned under (i.e., sites with low

expected residual N). The solid symbols represent sites that had one or more of these management practices (i.e., sites with high expected residual N).

Fig. 4.6. Contour plot of wheat nitrogen uptake (NUP_{Opt}) (kg ha^{-1}) vs. soil organic matter (SOM) (%) on the Y-axis and soil amino sugar-N test (ASNT) (mg kg^{-1}) on the X-axis.

Fig. 4.7. Wheat growth stage 30 optimum N fertilizer rate (OptN) (kg ha^{-1}) vs. A: amino sugar-N test (ASNT) (mg kg^{-1}), B: KCl-extractable soil NH_4-N (kg ha^{-1}), C: soil organic matter (SOM) (%), and D: KCl-extractable soil NO_3-N (kg ha^{-1}) for all well-drained sites (n=58). Open symbols represent sites that had no manure applied in the previous 12 mo, no additional N fertilizer applied beyond the recommended rates for the previous crop, or no soybean plus stover turned under (i.e., sites with low expected residual N). The solid symbols represent sites that had one or more of these management practices (i.e., sites with high expected residual N).

Fig. 4.8. The relationships between wheat growth stage 30 optimum N fertilizer rate (OptN) (kg ha^{-1}) and KCl-extractable NH_4-N (mg kg^{-1}) for sites with soil organic matter (SOM) levels > 1.5% (soild symbols, solid regression line) and SOM < 1.5% (open symbols, dashed regression line) for well-drained sites.

Fig. 4.9. The relationships between winter wheat N uptake at growth stage 30 (NUP_{GS30}), and A: soil amino sugar-nitrogen test (ASNT) and B: KCl-extractable soil NH_4-N. Open symbols and solid regression line represent sites that had no manure applied in the previous 12 mo, no additional N fertilizer applied beyond the recommended rates for the previous crop, or no soybean plus stover turned under (i.e., sites with low expected residual N). The solid symbols and dashed regression line represent sites that had one or more of these management practices (i.e., sites with high expected residual N).

Table 4.1. Study site, year, soil series map unit, soil taxonomic classification, previous crop, tillage used, whether manure had been applied in the previous 12 mo, and winter wheat cultivar for the 38 trial sites in this study of the relationships between soil N tests and winter wheat N uptake, and economic optimum spring top-dress N rate. All sites were well drained (i.e., either had soils that were naturally well drained, functional tile drainage, or were assayed during the 2008 drought).

Site	Year	Soil series map unit	Region and soil taxonomic classification	Previous Crop†	Tillage‡	Manure	Cultivar§
			Tidewater				
1	2008	Cape Fear loam	Clayey, mixed, thermic Typic Umbraquults	C	CT	No	P, U
2	2008	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	C	CT	No	P, U
3	2008	Cape Fear loam	Clayey, mixed, thermic Typic Umbraquults	C	CT	No	P, U
4	2008	Portsmouth fine sandy loam	Fine-loamy, mixed, thermic Typic Umbraquults	C	CT	No	P, U
			Coastal Plain				
5	2006	Johns sandy loam	Sandy, siliceous, thermic Aquic Hapludults	S	CT	No	U
6	2006	Goldsboro loamy sand	Fine-loamy, siliceous, thermic Aquic Paleudults	S	CT	No	U
7	2006	Lynchburg sandy loam	Fine-loamy, siliceous, thermic Aeric Paleaquults	S	CT	No	U
8	2006	Norfolk loamy sand	Fine-loamy, siliceous, thermic Typic Paleudults	S	CT	No	U
9	2006	Lynchburg sandy loam	Fine-loamy, siliceous, thermic Aeric Paleaquults	C	CT	No	U
10	2006	Norfolk loamy sand	Fine-loamy, siliceous, thermic Typic Paleudults	C	CT	No	U
11	2006	Goldsboro loamy sand	Fine-loamy, siliceous, thermic Aquic Paleudults	C	CT	No	U
12	2007	Pocalla loamy sand	Loamy, siliceous, thermic Arenic Plinthic Paleudults	C	CT	No	U
13	2007	Johns sandy loam	Sandy, siliceous, thermic Aquic Hapludults	S	CT	No	U
14	2007	Duplin sandy loam	Fine, kaolinitic, thermic Aquic Paleudults	C	CT	No	U
15	2007	Marvyn sandy loam	Fine-Loamy, kaloinitic, thermic Typic Kanhapludults	C	CT	No	U
16	2007	Lynchburg sandy loam	Fine-loamy, siliceous, thermic Aeric Paleaquults	C	CT	No	U
17	2008	Portsmouth loam	Sandy, mixed, thermic Typic Umbraquults	CN	CT	No	P, U
18	2008	Kenansville loamy sand	Loamy, siliceous, thermic Arenic Hapludults	CS	CT	No	P, U
19	2008	Norfolk loamy sand	Fine-loamy, siliceous, thermic Typic Paleudults	CS	CT	No	P, U

Table 4.1 continued.

20	2008	Pocalla loamy sand	Loamy, siliceous, thermic Arenic Plinthic Paleudults	CS	CT	No	P, U
21	2008	Duplin sandy loam	Fine, kaolinitic, thermic Aquic Paleudults	CS	CT	No	P, U
22	2008	Goldsboro loamy sand	Fine-loamy, siliceous, thermic Aquic Paleudults	CS	CT	No	P, U
23	2008	Lynchburg sandy loam	Fine-loamy, siliceous, thermic Aeric Paleaquults	C	CT	No	P, U
24	2008	Rains fine sandy loam	Fine-loamy, siliceous, thermic Typic Paleaquults	C	CT	No	P, U
			Piedmont				
25	2006	Hiwassee clay loam	Fine, kaolinitic, thermic Typic Rhodudults	S	NT	No	U
26	2006	Davidson clay loam	Fine, kaolinitic, thermic Rhodic Kanhapludults	S	NT	No	U
27	2006	Cecil loam	Fine, kaolinitic, thermic Typic Kanhapludults	C	NT	No	U
28	2007	Davidson clay loam	Fine, kaolinitic, thermic Rhodic Kanhapludults	S	NT	No	U
29	2008	Mecklenburg clay loam	Fine, mixed, thermic Ultic Hapludalfs	C	NT	YES	P, U
30	2008	Mecklenburg loam	Fine, mixed, thermic Ultic Hapludalfs	C	NT	YES	P, U
31	2008	Chewacla soils	Fine-Loamy, mixed, thermic Fluvaquentic Dystrudepts	C	NT	YES	P, U
32	2008	Cecil clay loam	Fine, kaolinitic, thermic Typic Kanhapludults	C	NT	YES	P, U
33	2008	Pacolet clay	Fine, kaolinitic, thermic Typic Kanhapludults	CS	NT	No	P, U
34	2008	Hiwassee clay	Very fine, kaolinitic, thermic Rhodic Kanhapludults	CS	NT	No	P, U
35	2008	Congaree soils	Fine-Loamy, mixed, thermic Oxyaquic Udifluvents	C	CT	YES	P, U
36	2008	Hiwassee clay loam	Fine, kaolinitic, thermic Typic Rhodudults	C	NT	No	P, U
37	2008	Altavista fine sandy loam	Fine-Loamy, mixed, thermic Aquic Hapludults	CS	NT	No	P, U

† C, corn; CN, corn with an additional 112 kg N ha^{-1} above recommended N rates applied late season; S, soybeans; CS, cover-crop soybeans bushoged and incorporated before wheat planting.

‡CT, conventional tillage; NT, no-till.

§ P, Pioneer 26R12; U, UniSouth Genetics 3209.

Table 4.2. Study site; wheat cultivar; amino sugar-N test (ASNT), KCl-extractable NO_3-N and NH_4-N, and soil organic matter (SOM) from the 0- to 30-cm samples; N uptake with no growth stage 30 N fertilizer applied ($NUP_{Zero,}$from Chapter 3); N uptake at optimum growth stage 30 N rate (NUP_{Opt}); optimum growth stage 30 N rate (OptN); and yield at OptN (YLD_{Opt}). Data are ranked by ASNT.

Site	Cultivar	ASNT	NO_3-N	NH_4-N	SOM	NUP_{Zero}	NUP_{Opt}	OptN	YLD_{Opt}
		mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	%	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	Mg ha^{-1}
21	Pioneer 26R12	28	1.5	2.8	0.6	122	122	0	5.4
21	USG 3209	28	1.5	2.8	0.6	123	123	0	4.5
11	USG 3209	32	1.5	1.6	0.7	32	73	89	4.6
15	USG 3209	35	2.5	1.9	1.1	35	85	66	4.1
14	USG 3209	36	2.1	0.7	0.7	38	77	79	3.2
22	Pioneer 26R12	38	1.8	3.9	1.0	118	118	0	5.1
22	USG 3209	38	1.8	3.9	1.0	114	114	0	4.9
18	Pioneer 26R12	38	1.4	3.0	1.0	126	183	128	7.2
18	USG 3209	38	1.4	3.0	1.0	113	113	0	6.4
23	Pioneer 26R12	39	1.6	2.6	0.9	56	106	93	6.1
23	USG 3209	39	1.6	2.6	0.9	50	103	94	5.8
16	USG 3209	43	4.5	1.2	1.1	47	96	91	4.8
6	USG 3209	43	1.7	1.6	0.9	42	84	68	4.5
8	USG 3209	44	2.2	1.8	0.7	48	106	78	4.4
35	Pioneer 26R12	45	1.8	5.9	1.3	217	217	0	6.5
35	USG 3209	45	1.8	5.9	1.3	195	195	0	7.1
34	Pioneer 26R12	47	2.5	4.8	1.8	112	176	116	7.5
34	USG 3209	47	2.5	4.8	1.8	117	169	103	7.1
28	USG 3209	49	1.5	1.6	1.3	53	101	101	5.4
10	USG 3209	50	3.2	1.5	2.6	46	90	127	4.2
5	USG 3209	54	2.4	2.4	1.4	51	104	101	4.8
9	USG 3209	54	1.9	2.8	1.3	54	86	91	4.1
37	Pioneer 26R12	54	2.5	5.6	1.4	200	200	0	6.5
37	USG 3209	54	2.5	5.6	1.4	172	172	0	5.8
36	Pioneer 26R12	56	2.6	4.4	2.0	76	135	70	6.3
36	USG 3209	56	2.6	4.4	2.0	76	111	59	5.9
24	Pioneer 26R12	57	1.9	3.5	1.6	73	110	62	6.3

Table 4.2. Continued

		mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	%	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	Mg ha^{-1}
24	USG 3209	57	1.9	3.5	1.6	73	114	85	6.2
12	USG 3209	59	2.6	0.6	1.3	63	125	102	4.4
7	USG 3209	65	2.0	2.6	1.3	81	126	103	5.9
17	Pioneer 26R12	66	2.0	5.5	1.4	185	185	0	7.3
17	USG 3209	66	2.0	5.5	1.4	184	184	0	7.3
31	Pioneer 26R12	67	3.0	5.8	2.1	157	157	0	6.1
31	USG 3209	67	3.0	5.8	2.1	140	140	0	6.2
30	Pioneer 26R12	69	3.8	6.7	2.4	202	202	0	5.3
30	USG 3209	69	3.8	6.7	2.4	194	194	0	5.0
13	USG 3209	70	2.4	1.2	1.9	84	143	140	5.2
26	USG 3209	72	3.2	1.4	2.6	77	171	153	7.8
33	Pioneer 26R12	74	3.6	4.3	2.0	138	182	148	8.1
33	USG 3209	74	3.6	4.3	2.0	137	165	58	7.4
32	Pioneer 26R12	79	2.4	10.8	2.9	190	190	0	7.1
32	USG 3209	79	2.4	10.8	2.9	166	166	0	6.5
25	USG 3209	79	3.3	3.0	3.3	76	165	137	7.1
20	Pioneer 26R12	85	1.7	6.1	1.8	233	233	0	5.7
20	USG 3209	85	1.7	6.1	1.8	209	209	0	5.5
27	USG 3209	91	3.6	2.4	3.1	86	175	113	7.6
19	Pioneer 26R12	92	1.6	5.8	1.7	187	187	0	5.9
19	USG 3209	92	1.6	5.8	1.7	198	198	0	6.6
29	Pioneer 26R12	102	4.1	17.1	3.1	177	177	0	5.1
29	USG 3209	102	4.1	17.1	3.1	192	192	0	5.8
1	Pioneer 26R12	91	2.7	4.4	3.7	126	142	50	6.5
1	USG 3209	91	2.7	4.4	3.7	116	116	0	6.3
3	Pioneer 26R12	96	2.6	3.8	3.8	115	115	0	6.7
3	USG 3209	96	2.6	3.8	3.8	96	141	98	6.2
4	Pioneer 26R12	121	3.7	6.1	4.9	156	156	0	6.5
4	USG 3209	121	3.7	6.1	4.9	145	152	31	6.7
2	Pioneer 26R12	135	2.5	7.3	6.2	139	173	22	6.1
2	USG 3209	135	2.5	7.3	6.2	154	154	0	6.1

Table 4.3. Correlation matrix of soil test values (ASNT, amino sugar-N test; KCl-extractable NO_3- and NH_4-N; SOM, soil organic matter), winter wheat economic optimum spring top-dress N rate (OptN), winter wheat yield at OptN (YLD_{Opt}), and winter wheat nitrogen uptake at OptN (NUP_{Opt}) for N-response trials at all sites and only those at sites with low residual soil nitrogen.

	Trials at all sites (n=58)			Trials at low residual N sites (n=30)		
Variable	**OptN**	**YLD_{Opt}**	**NUP_{Opt}**	**OptN**	**YLD_{Opt}**	**NUP_{Opt}**
ASNT	NS	0.33**	0.44***	-0.55**	0.55**	0.79***
NO_3-N	NS	NS	NS	NS	NS	0.49**
NH_4-N	-0.57***	NS	0.58***	-0.75***	0.48**	0.54**
SOM	NS	0.30*	0.28*	-0.55**	0.52**	0.74***

* Significant at the ≤ 0.05 probability level.
** Significant at the ≤ 0.01 probability level.
*** Significant at the ≤ 0.001 probability level.

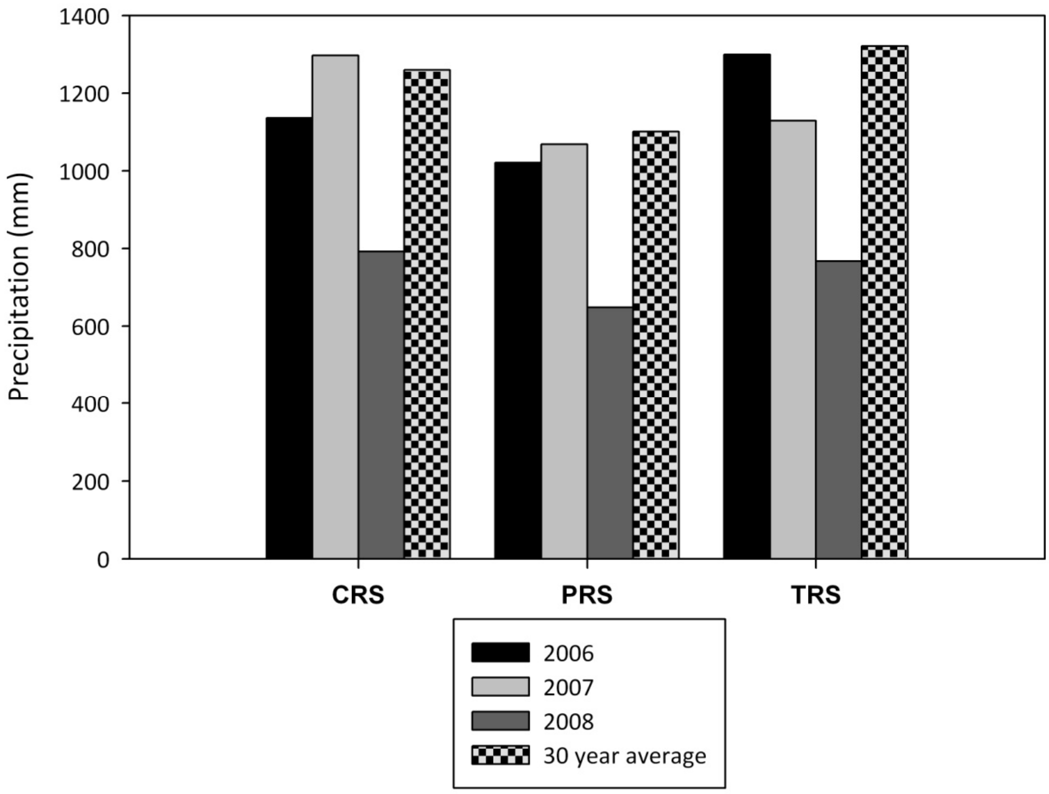

Fig. 4.1. Total annual precipitation (mm) for 2006, 2007, 2008, and the 30-yr average for the Cunningham and Caswell Research Stations (CRS) in the Coastal Plain, the Piedmont Research Station (PRS), and Tidewater Research Station (TRS).

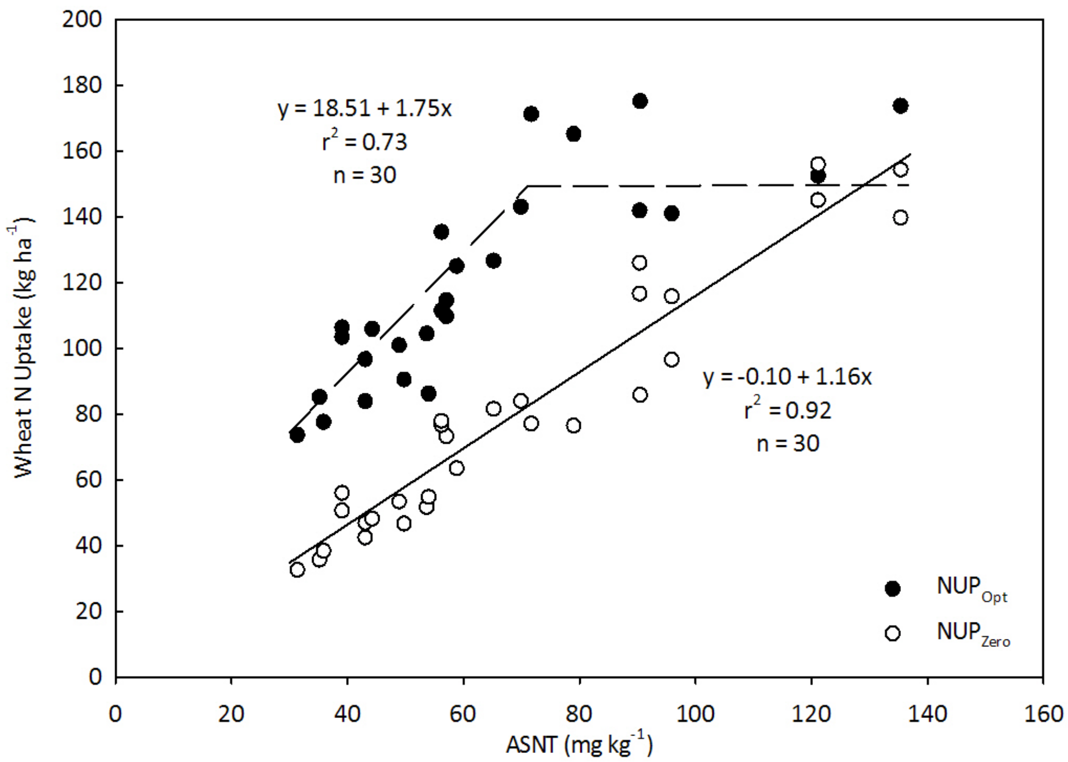

Fig. 4.2. The relationship between wheat N uptake with no growth stage 30 N fertilizer applied (NUP_{Zero}) (kg ha^{-1}) and soil amino sugar-N test (ASNT) (mg kg^{-1}) (open symbols, solid regression line) and the relationship between wheat N uptake at optimum growth stage 30 N fertilizer rate (NUP_{Opt}) (kg ha^{-1}) and ASNT (closed symbols, dashed regression line) for well-drained sites that had no manure applied in the previous 12 mo, no additional N fertilizer applied beyond the recommended rates for the previous crop, or no soybean plus stover turned under (i.e., sites with low expected residual N, n=30).

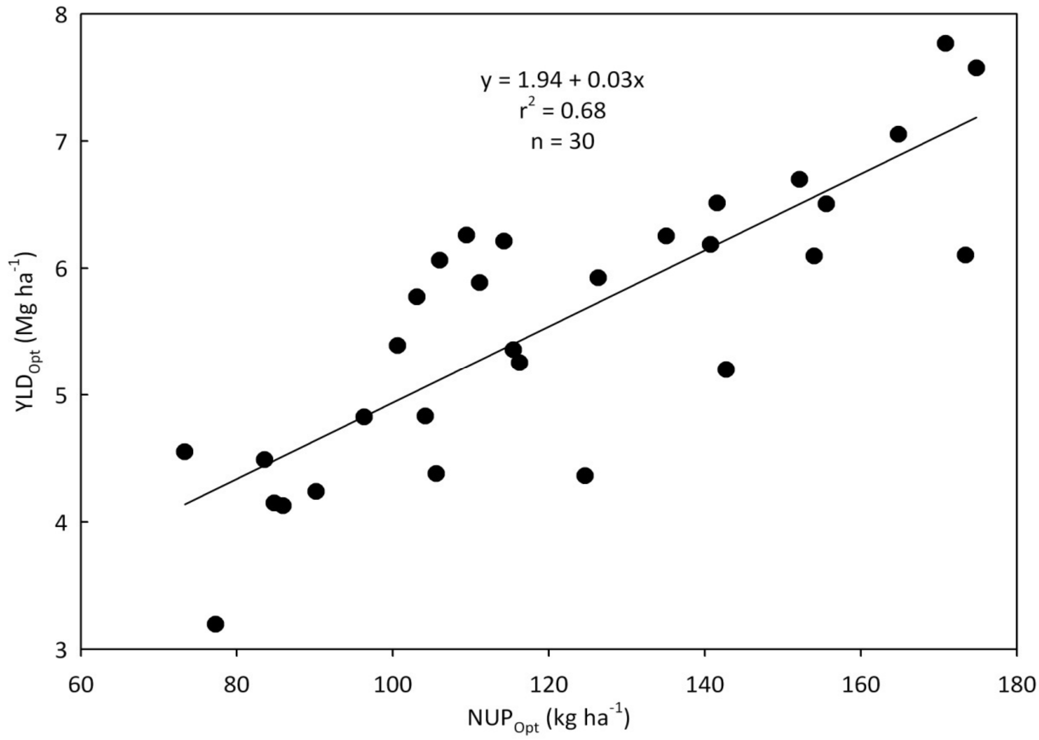

Fig. 4.3. Relationship between wheat grain yield at optimum growth stage 30 N fertilizer rate (YLD_{Opt}) (Mg ha^{-1}) and N uptake at optimum growth stage 30 N fertilizer rate (NUP_{Opt}) (kg ha^{-1}) for the well-drained sites that had no manure applied in the previous 12 mo, no additional N fertilizer applied beyond the recommended rates for the previous crop, or no soybean plus stover turned under (i.e. sites with low expected residual N, n=30).

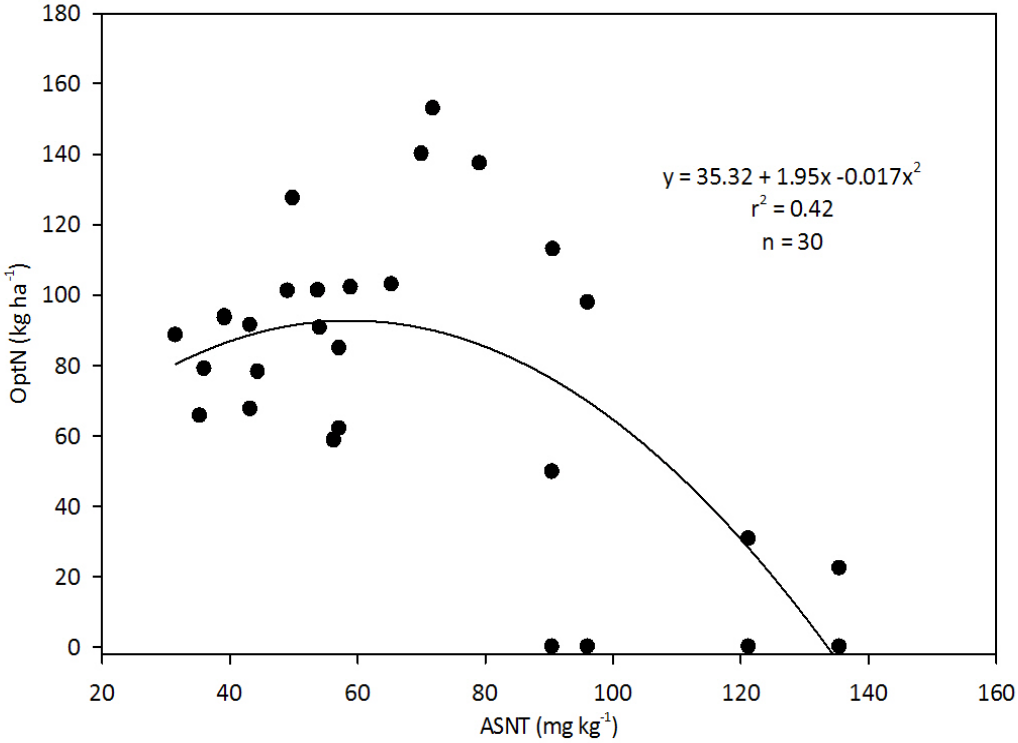

Fig. 4.4. Relationship between wheat growth stage 30 optimum N fertilizer rate (OptN) (kg ha^{-1}) and soil amino sugar-N test (ASNT) (mg kg^{-1}) for the well-drained sites that had no manure applied in the previous 12 mo, no additional N fertilizer applied beyond the recommended rates for the previous crop, or no soybean plus stover turned under (i.e., sites with low expected residual N, n=30).

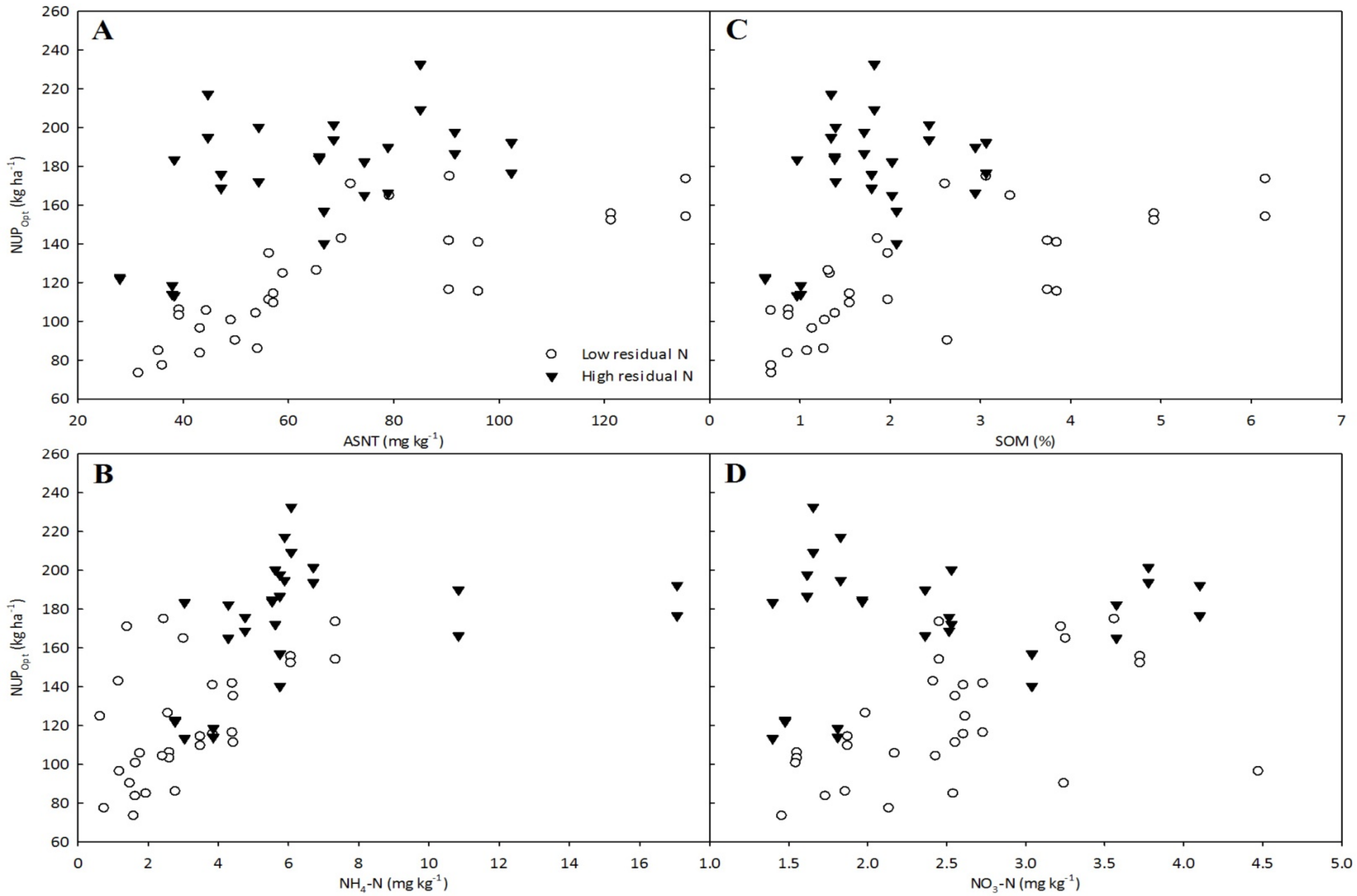

Fig. 4.5. Wheat N uptake at optimum growth stage 30 N fertilizer rate (NUP_{Opt}) (kg ha^{-1}) vs. A: amino sugar-N test (ASNT) (mg kg^{-1}), B: KCl-extractable soil NH_4-N (kg ha^{-1}), C: soil organic matter (SOM) (%), and D: KCl-extractable soil NO_3-N (kg ha^{-1}) for all well-drained sites (n=58). Open symbols represent sites that had no manure applied in the previous 12 mo, no additional N fertilizer applied beyond the recommended rates for the previous crop, or no soybean plus stover turned under (i.e., sites with low expected residual N). The solid symbols represent sites that had one or more of these management practices (i.e., sites with high expected residual N).

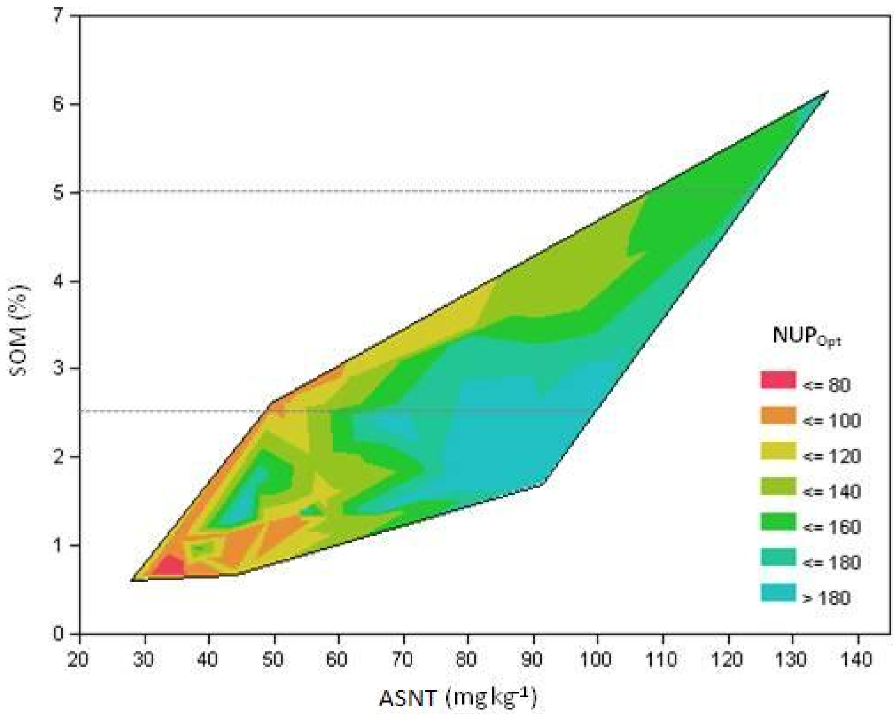

Fig. 4.6. Contour plot of wheat nitrogen uptake (NUP_{Opt}) (kg ha^{-1}) vs. soil organic matter (SOM) (%) on the Y-axis and soil amino sugar-N test (ASNT) (mg kg^{-1}) on the X-axis.

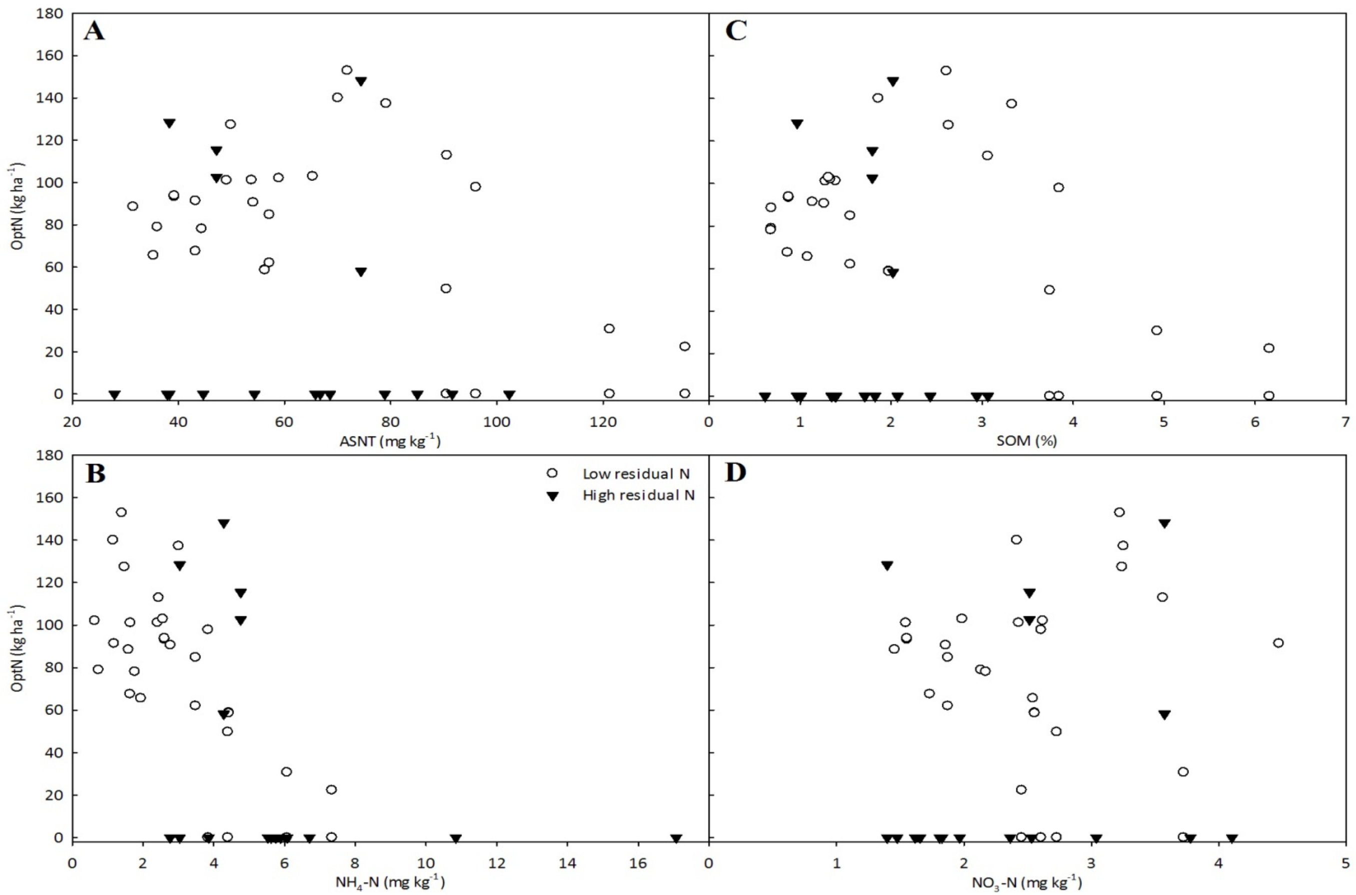

Fig. 4.7. Wheat growth stage 30 optimum N fertilizer rate (OptN) (kg ha^{-1}) vs. A: amino sugar-N test (ASNT) (mg kg^{-1}), B: KCl-extractable soil NH_4-N (kg ha^{-1}), C: soil organic matter (SOM) (%), and D: KCl-extractable soil NO_3-N (kg ha^{-1}) for all well-drained sites (n=58). Open symbols represent sites that had no manure applied in the previous 12 mo, no additional N fertilizer applied beyond the recommended rates for the previous crop, or no soybean plus stover turned under (i.e., sites with low expected residual N). The solid symbols represent sites that had one or more of these management practices (i.e., sites with high expected residual N).

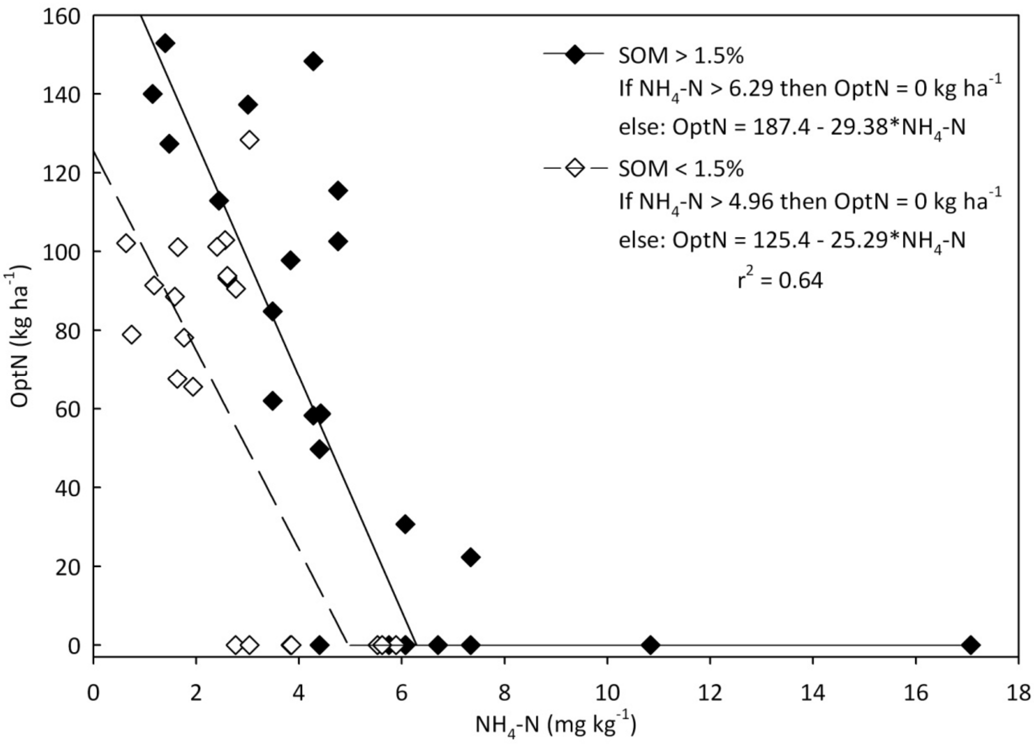

Fig. 4.8. The relationships between wheat growth stage 30 optimum N fertilizer rate (OptN) (kg ha^{-1}) and KCl-extractable soil NH_4-N (mg kg^{-1}) for sites with soil organic matter (SOM) levels >1.5% (solid symbols, solid regression line) and SOM <1.5% (open symbols, dashed regression line) for well-drained sites.

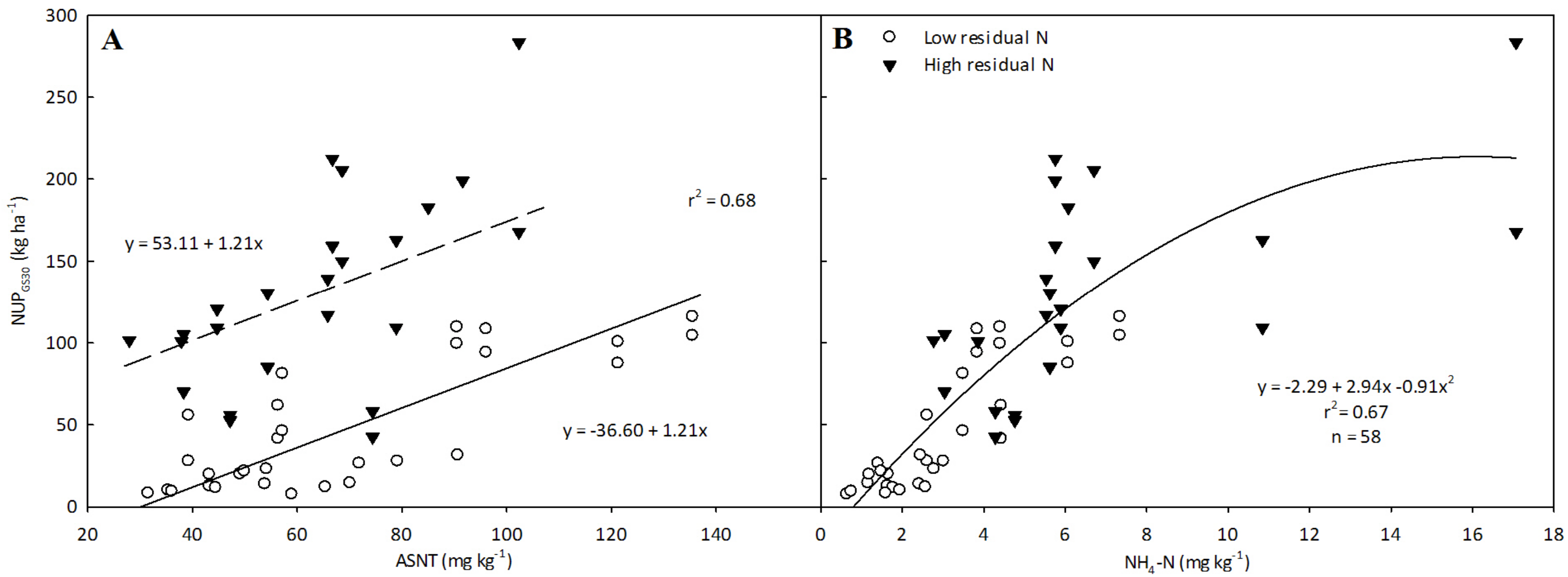

Fig. 4.9. The relationships between winter wheat N uptake at growth stage 30 (NUP_{GS30}), and A: soil amino sugar-nitrogen test (ASNT) and B: KCl-extractable soil NH_4-N. Open symbols and solid regression line represent sites that had no manure applied in the previous 12 mo, no additional N fertilizer applied beyond the recommended rates for the previous crop, or no soybean plus stover turned under (i.e., sites with low expected residual N). The solid symbols and dashed regression line represent sites that had one or more of these management practices (i.e., sites with high expected residual N).