ABSTRACT

PERSHING, MARY RENE. Evaluating the Flush of CO_2 as a Short-Term Biological Indicator of Soil Nitrogen Availability. (Under the direction of Dr. Alan Franzluebbers).

Determining the appropriate nitrogen (N) rate for crops is critical to farm economics and environmental protection. In North Carolina, N fertilizer recommendations do not consider residual inorganic N or biologically active N estimations, but only realistic yield expectations set for each soil type and crop. However, due to increasingly popular conservation management practices such as cover cropping and no-till farming, biologically active N can accumulate in soil, resulting in greater supply of N than expected. Measuring biologically active carbon (C) is strongly correlated to net N mineralization, and may be less complicated, expensive, and time consuming than measuring biologically active N, due to the rapidly changing nature of soil N. The purpose of this study was to analyze soil from different locations and soil types with a diversity of management practices and determine which soil properties were most related to plant N uptake and dry matter production. Soil samples from research stations and private farms representing three physiographic regions of North Carolina (coastal plain, piedmont, and mountains), as well as from cooperating locations in Virginia, Pennsylvania, Oklahoma, Nebraska, and Georgia were analyzed for various soil chemical and biological properties in the laboratory and utilized in greenhouse growth trials. Unfertilized *Sorghum bicolor* was grown for eight weeks in each soil sample to test for N availability. Shoot dry matter accumulation and N concentration of plants were measured, which allowed for determination of above ground plant N uptake. The flush of CO_2 following rewetting of dried soil was a key indicator of interest, as was net N mineralization during 24 days, soil microbial biomass C, particulate organic C and N, and

total organic C and N. In Greenhouse Trial 1, the flush of CO_2 was the second best indicator of greenhouse growth (R^2=0.78) behind net N mineralization (R^2= 0.81). In Greenhouse Trial 2, total soil N was the best indicator of greenhouse growth (R^2 = 0.82). In Greenhouse Trial 3, the flush of CO_2 was the best indicator of greenhouse growth (R^2 = 0.96). In Greenhouse Trial 4, the flush of CO_2 was the second best indicator of plant N uptake (R^2=0.83) behind net N mineralization (R^2= 0.88). Among all samples, the flush of CO_2 explained 69% of the variation in N uptake. The slope to predict greenhouse growth in soils from Oklahoma, and Pennsylvania, was significantly different from North Carolina, Georgia, and Virginia. The interaction effect of state on the flush of CO_2 improved prediction of dry matter production from 50 to 71% and of plant N uptake from 68 to 89%. Samples originating from NC and VA (n=516) were predicted by the flush of CO_2 with an R^2=0.81 for plant N uptake and R^2=0.66 for dry matter production. The biologically active fraction of organic matter was the most dominant and consistent way to determine plant N uptake in this study (i.e. through net N mineralization and potential C mineralization). Occasionally, other soil nutrient concentrations and residual inorganic N were additionally helpful. Results from this study clearly indicate that the flush of CO_2 has the potential to be a simple, rapid, and reliable predictor of potentially available N in the mid-Atlantic United States.

Evaluating the Flush of CO_2 as a Short-Term Biological Indicator of Soil Nitrogen Availability

by
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DEDICATION

To my family for their steadfast encouragement, and to my fellow graduate students for their wisdom, love, and guidance, without whom I could not have been successful.

BIOGRAPHY

Molly was born on November 2, 1991 in Webster Groves, Missouri, a suburb right outside of Saint Louis. She grew up hiking in Southern Missouri and swimming on the Black River on weekends. She attended the University of Missouri-Columbia and majored in Food Science, where she also minored in Sustainable Agriculture and found her passion for creating a better food and agriculture system to improve natural resources and ensure agricultural sustainability by working on the Bradford Research Farm in Columbia. Her senior year she developed an interest in soils at a soil judging competition in Springfield, Missouri. She then attended graduate school at NC State in August of 2014, where she pursued her master's degree in Soil Science.

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INTRODUCTION

Literature Review

Nitrogen (N) limits plant production, and therefore, is crucial to feed the world's population. An estimated 40% of the world's population relies on N fertilizer for dietary protein, which is fortunately accessible due to the invention of the Haber-Bosch process (Galloway, 2003; Keeney and Hatfield, 2008). Advancements in N fertilizer are largely responsible for tripling the global food supply in the last 50 years, proliferating the population from 1.6 billion to over 7 billion in the 20th century (Mosier, A.R., Syers, J.K. and Freney, 2004; Smil, 2011). Unfortunately, reactive forms of N cause detrimental effects to the environment, such as eutrophication, greenhouse gas emissions, and ozone depletion (Cassman et al., 2002; Galloway, 2003; Mosier et al., 2004; Howarth, 2008).

Agricultural nitrate (NO_3) leaching poses the greatest immediate risk to the economy and water resource protection. The United States Department of Agriculture (USDA) Economic Research Service estimates that $1.7 billion dollars are spent annually to remove agriculturally derived NO_3 from drinking water supplies. Studies have shown that groundwater NO_3 levels in North Carolina and other southeastern states often exceed the maximum allowable level of 10 mg/L NO_3-N (Hubbard et al., 2004). Reducing NO_3 levels by 1% in the US would decrease treatment costs by more than $120 million dollars each year (Ribaudo, 2011), and prevent the more than 50% of the population in the US that depend on groundwater as their primary drinking source from elevated blood NO_3 levels.

Algal blooms and fish kills caused by agricultural loss of nitrate damage many industries dependent on preservation of waterways and coasts. Fisheries from estuaries provide $1.9 billion annually, while millions of jobs and billions of dollars are created

through tourism and recreation along the coasts (CENR, 2003). In total, eutrophication from nutrient runoff into freshwater systems costs the United States $2.2 billion each year (Dodds et al., 2009). Given the large damaging effects of reactive N losses on ecosystems and the economy, and the prediction that worldwide use of N could double to 236 million metric tons by the year 2050 (Tilman et al., 2001), better prediction of plant-available N has never been more urgent.

In addition to the adverse economic and environmental impacts of excess N, farmers are more likely to attain maximum profit from optimum fertilizer rate decisions. In Wisconsin, adjusting the N application rate after using the presidedress soil nitrate test (PSNT) and considering manure and legume N credits resulted in an increase in profit by $34/ha (Andraski and Bundy, 2002). In the tidewater region of North Carolina, reducing fertilizer N by 39 kg ha^{-1} to wheat resulted in a decrease of 2.3 mg NO_3-N L^{-1} in groundwater and a 25% greater harvest N ratio (%N in grain or forage divided by total N applied), as compared to using a realistic yield expectation (RYE) database (Hong et al., 2006). In the same study by Hong et al., (2006) some sites saw a significant increase in yield with more fertilizer N applied than the RYE fertilizer recommendation.

Making better real-time N recommendations will potentially decrease N rates and environmental impacts, but the tools to do this are currently inadequate. Measuring plant available N from soil prior to fertilization in cereal crops has been researched for decades, but consensus has not been reached as to a soil N test that can make the best estimation of actual N availability relative to crop demands. Unpredictable climate coupled with variations in soil properties and other characteristics affecting N availability across regions and even fields make developing a single, robust soil N test an enormous challenge. Although many

scientific advances in N management have been made, much is still unknown about N cycling. The ability to predict N supplying capacity of soil has yet to be achieved, contributing to worldwide inefficient N use (Hong et al., 1990). Farmers need a robust soil N testing tool so that they can apply the right amount of N fertilizer to achieve high crop production while protecting the environment.

Current State of Soil Nitrogen Testing

Currently, state soil testing facilities in North Carolina and surrounding states do not routinely test for soil N. The rationale is that inorganic N is an unreliable predictor of plant available N because of its potential to be leached from the root zone prior to the growing season. Instead, farmers are instructed to utilize an online database (realistic yield expectation, RYE) that recommends N based on crop and soil type. Soil organic N is not explicitly accounted for in the database. The database was recently updated to reflect improved corn hybrid genetics and management, and shown to accurately predict N recommendations with increased yield using the same rate of N applications (Rajkovich et al., 2015). However, the database does not directly account for potentially available N prior to fertilization on a case-by-case basis.

Cover cropping, no-till, manure application and other increasingly common conservation management practices may stabilize soil aggregates, reduce erosion and subsequently protect microbial functioning, carrying over mineralization potential to the following growing season. In the United States, 36 Mha are farmed using no-till and adoption is growing (Horowitz et al., 2010). Cover cropping has increased every year for five consecutive years (Myers et al., 2014). A meta-analysis conducted on cover crops indicated

that over time, there is greater soil organic C sequestration, which also may increase organic N supply and subsequently plant available N (Poeplau and Don, 2015). A soil-testing tool for predicting soil N supplying capacity that is sensitive to management would allow farmers to adjust fertilizer application rates due to their particular management approach. Quantifying the change in the active fraction of organic matter, if resulting in better N supplying capacity, would also encourage farmers to adopt or continue to use these "sustainable" soil management methods.

Essential to soil N test adoption by farmers is reliability, minimal time and cost, and easy integration into current operations (Balkcom et al., 2000; Griffin, 2008; Kitchen et al., 2008). Decades have been spent researching a soil-testing tool that embodies these key components. This literature review assesses several options, both chemical and biological. The potential N indices are reviewed with three criteria in mind: i) summary of the methodology; ii) ability of test to predict soil N mineralization and/or plant N uptake across different soil types; and iii) review limitations and speculate on feasibility for widespread use. A biological test, the flush of CO_2 following rewetting of dried soil, will be described in more detail as a short-term biological indicator of soil N mineralization.

Nitrogen Availability Indices

Validation of a soil N test requires a statistical relationship between test results and crop yield response to N or crop N uptake (Griffin, 2008). Two tests to mention that are widely accepted measures of available N but are not often feasible for reasons of cost or time are the isotopic (^{15}N) tracer method that assess the fate of applied N sources (Griffin, 2008), and the long-term aerobic incubation method established by Stanford and Smith (1976)

whereby soil is incubated for 30 weeks. The isotopic tracer method uses expensive equipment and therefore may not be feasible as a routine soil test. The long-term incubation approach, however, is widely accepted as a standard for quantifying the potentially mineralizable N pool, and many studies often compare short-term indices against this method for validation (Griffin, 2008; Schomberg et al., 2009; Culman et al., 2013). Therefore, many tests, such as total soil N concentration are validated against aerobic incubation. It should be noted that there have been many modifications to the method first proposed by Stanford and Smith (1976), so variations in methodology exist, making it more difficult to compare methods against each other (Griffin, 2008).

Cropping systems and management affect the accumulation and loss of soil organic matter (Griffin, 2008). Therefore, total N and organic matter N have been evaluated as indicators of N availability. The relationship of plant available N, total N concentration, soil organic N, and fractions of soil N varied in different studies. The correlation coefficient between total organic N concentration and net mineralization in a study by Hassink (1994) was only 0.38 when both fine- and coarse-textured soils were used. Other various aerobic incubations against total N concentration had an R^2 of 0.57-0.94 depending on whether variations in soil types, textures, and geography were included. Larger areas of sampling weakened the correlation while similar areas and soil types strengthened it (Griffin, 2008; Schomberg et al., 2009). Few field experiments to assess N concentration as a measure of N availability have been conducted, although field studies are the best indicators of actual plant N uptake. Fox and Piekielek (1978) correlated total N concentration against actual N uptake in corn. The correlation coefficient was 0.68 from two years of field trials (Fox and

Piekielek, 1978)**.** The N uptake from the soil was determined from measuring corn grain and stover N content from zero N rate plots.

Extraction methods have been useful as a chemical index for soil N availability, because results are obtained rapidly. There are several extraction methods, including $CaCl_2$-extractable N, $NaHCO_3$-extractable N, and hot and cold KCl-extractable N. The N extracted by boiling $CaCl_2$ was highly correlated to the N taken up by corn in Pennsylvania soils (Fox and Piekielek, 1978). However, recent studies did not obtain similar results (Griffin, 2008). Field validations of the method by Fox and Piekielek (1978) found a lower correlation due to climatic factors, which reduced the reliability of the extraction tests. $NaHCO_3$-extractable N obtained mixed results similar to that of $CaCl_2$ (Griffin, 2008).The KCl extraction method was strongly correlated to N mineralization in an incubation experiment with a correlation coefficient of 0.95 by subtracting the initial soil NH_4 concentration (Hong et al., 1990). Subsequent evaluations indicated a much lower correspondence (Griffin, 2008). In field-testing, hot KCl extraction also had mixed results. In Scotland, 10 soils had a close correlation of KCl extractable NH_4 and N mineralization with barley N uptake (McTaggart and Smith, 1993), while Hong et al. (1990) found it not to be a strong predictor in Pennsylvania soils.

The pre-sidedress nitrate test (PSNT) (Magdoff, 1991) and leaf chlorophyll content (Scharf et al., 2006) are used by some farmers in the Midwest. The PSNT test is able to identify N-sufficient sites typically on manured soils. Limitations to this test are the inability to predict optimum N rates on medium potential soils, characterized by a shallow root zone, coarse-textured subsoil, and poor drainage (Andraski and Bundy, 2002). The PSNT is a time sensitive test, where the soil is tested for nitrate at the V4-V6 stage of plant growth. Less than

5% of farmers in Michigan use PSNT (Stuart et al., 2014). Farmers will either apply all of their N fertilizer at time of planting, or they are too busy to sample at the V4-V6 stage (Hong et al., 1990).

A limitation to chemical N indices is that they are only reflective of the inorganic N concentration at that time, and are not reflective of the active fraction of organic matter that may also contribute to N availability. One approach to measuring the biologically available N in soil organic matter is to partition the soil N into various pools, such as soil microbial biomass or particulate organic matter. Transformation of soil organic N is a biologically mediated process, so soil microbial biomass has been largely examined as a labile fraction of soil organic N for its ability to predict N mineralization (Griffin, 2008). Correlations in various studies range from 0.41-0.72 in aerobic incubations of different lengths. Franzluebbers et al. (2001) found that soil microbial biomass C accounted for 30% of the variation in N mineralized in soils from a wide range of moisture and temperature gradients in the US. Individual locations explained an additional 26% of the variation, totaling 56% of the variation accounted for by soil microbial biomass C.

Particulate organic matter is partially decomposed plant matter, and represents the "intermediate" fraction only partially available to microbes. It has been evaluated due to its sensitivity to management in agricultural systems (Griffin, 2008). There are no published field validations, but aerobic incubations support the relationship of particulate organic matter to mineralizable N. Particulate organic C was found to be strongly correlated (r=0.93) to N mineralized during a 140 day aerobic incubation (Chan, 1997). Schomberg et al. (2009) observed that particulate organic C or N were not as useful for predicting mineralizable N as other indices.

In New York and Illinois, the Illinois Soil Nitrogen Test (ISNT) has been useful. Field trials at Cornell State University determined 83% accuracy in N fertilizer recommendations (Lawrence et al., 2012). ISNT indicates the active fraction of organic matter by measuring the amino sugar content, which resides in soil microbial biomass (Griffin, 2008). It has been extremely useful in determining sufficiency of N in soils, as long as sampling did not occur directly after manure application. Williams et al. (2007) showed that the ISNT concentration and economically optimum N rate were strongly correlated in North Carolina (no threshold rate was established because all sites were insufficient in N) on well-drained and poorly drained soils with an r^2 of 0.87 and 0.78, respectively. Similar to all tests previously described, climate variability reduces the effectiveness for the ISNT to work.

Schomberg et al. (2009) reviewed many chemical and biological indices in relation to N mineralization from nine different sites in the southern US under different management systems. This study found that the highest correlation of N mineralization (a 41-week method by Wang et al., 2003) to laboratory indices were total C and N, anaerobic N mineralization, hot KCl extraction, and N mineralization during 24 days. They found N mineralization during 24 days to be a potentially useful short-term indicator of N availability in soils. The best combination of methods to estimate N mineralization in this study was total N and C mineralization during three days (i.e. flush of CO_2). The flush of CO_2 reflects microbial population dynamics and the steady-state rate of C mineralization, while total soil N is composed of active and recalcitrant fractions of soil organic N. Measurement of the flush of CO_2 is a promising tool, as it is relatively fast (three days) and more sensitive than N measurements, since 8 to 12 times more C is mineralized from soil organic matter (SOM)

than N (Schomberg et al., 2009). Measuring C mineralization is also inexpensive and feasible for laboratories to adopt.

Many N research papers confirm that biological tests are better indicators of N availability. Often times N and C mineralization are highly related due to soil microbial biomass decomposing SOM to obtain energy (i.e. C mineralization) and leaving behind N as inorganic NH_4^+ and NO_3 as by-products of decomposition (i.e. N mineralization). Franzluebbers et al. (2000) and Haney et al. (2001) have shown that C mineralization is highly correlated with N mineralization, as well as reflective of recent changes in management. Culman et al. (2013) evaluated current short-term and long-term tests to compare actual corn yield performance in a growing season in Michigan. The study found C mineralization to be the best indicator of N mineralization potential in six out of nine models tested.

The greatest impacts on soil biological activity are temperature and water content. The biological approach of C mineralization after re-wetting dried soil is useful in that it mimics the real-world occurrences in which rainfall and drought occur throughout a growing season (Franzluebbers et al., 2000). Incubating soil at optimal mineralization temperature (25°C) determines the amount of potential N mineralized. For these reasons, the flush of CO_2 has been proposed as a useful indicator of potential N mineralization for soils in the southeast United States (Franzluebbers et al., 1999a).

Relationship of Microbial Activity and Nitrogen Mineralization

In broad terms, total soil N is comprised of inorganic and organic N. Inorganic N is an important measurement in the soil because it contains NH_4^+ and NO_3^-, the only two plant

available forms of N. Inorganic N ranges from 1-300 kg N ha^{-1} in the top meter of soil, and its mobility and cycling depend on climatic conditions (Sylvia et al., 2005). Organic N from SOM is also important, for it can be mineralized for plant N availability. The three major pools of SOM are the active or labile fraction, the intermediate pool, and the slow or passive pool (Sylvia et al., 2005). Detection of differences in total organic matter due to changes in management practices can take several years. Total C is one measurement of soil organic matter, but is not often reflective of recent changes in management, such as with adoption of conservation tillage. The active or labile fraction of SOM with rapid turnover of C and N cycling is more indicative of available soil N within a growing season (Schomberg et al., 2009; Culman et al., 2013). Mineralization and immobilization of the labile fraction is mediated by microbes that are obtaining energy from carbon (C). The C comes from soil organic matter and decomposing plant and animal residues (Sylvia et al., 2005). The soil N cycle is thus a biologically mediated process. Soil microorganisms decompose organic substrates, supplying inorganic N as by-products for plant growth and development (Chen et al., 2003).

Rates of these processes are highly temperature and moisture dependent: they are optimal when soil temperatures are between 15-37°C and when moisture conditions are at field capacity. Climate is unpredictable and varies year to year. For this reason, measuring biological activity in the laboratory to predict soil microbial biomass C and respiration under ideal conditions is a better indicator of the potential N supplying capacity of the soil. While optimal conditions may not reflect what actually occurs in the field, it is prudent to measure the N supplying capacity from the field prior to a growing season to better predict the potential supplying capacity of the soil.

Conceptually, N mineralization is systematically related to microbial activity. Many studies have shown a significant positive relationship that verifies this concept. Franzluebbers et al. (2000) determined a standardized test using the flush of CO_2 following rewetting of dried soil that relates to the active pool of organic matter. They found that N mineralization was highly correlated with the flush of CO_2.

Gilmour et al. (1985) compared substrates with various C:N ratios (e.g. sewage sludge, alfalfa, clover, bermuda grass, and ryegrass) to evaluate the relationship of CO_2 evolution and N mineralization. Although N concentration of the substrate was not related to decomposition, a significant linear relationship between N mineralization and CO_2 evolution was determined for each substrate. Several studies on the relationship between soil microbial biomass N (or C) with N mineralization have produced mixed results (in various incubation lengths).

Several other studies have shown strong correlation of C and N mineralization (Franzluebbers et al., 2000; Haney et al., 2001). A study by Franzluebbers et al. (2000) found that the flush of CO_2 during 3 days accounted for 67% of N mineralization during 24 days of incubation, and 86% of the variability in soil microbial biomass in soils from Alberta-British Columbia, Maine, Texas, and Georgia. This study showed that the relationship of more resistant fractions of soil organic C (i.e. particulate organic C and total organic C) with the flush of CO_2 during three days was much lower than that of more active fractions of soil organic C (i.e. C mineralization during 24 days and soil microbial biomass C). Franzluebbers et al. (2000) also showed variation in relationships due to temperature and precipitation differences among regions. The results indicated that the flush of CO_2 during three days was

sensitive to differences in tillage management. This knowledge would allow farmers to make more accurate fertilizer decisions after changes in management practices.

In a field study in Michigan, yield and total biomass production were more strongly related to potential C mineralization during 0-1 day than any other indicator during early corn development stages (Culman et al., 2013). Other indicators strengthened their relationship as the season went on. Nitrogen mineralization ranked second, and soil nitrate was stronger by the third sampling. The results of this study suggest that multiple measurements might be needed to fully characterize the soil and to make optimal fertilizer N recommendations. Soil C mineralization is just one tool for farmers, as other measurements of N, such as inorganic N, will provide a fuller picture of N availability. Measurement of the flush of CO_2 is advantageous because it indicates to farmers and researchers the status of active C and N in the soil. This biologically active fraction indicates the N supplying capacity of the soil. In addition, this method is simple to set up with minimal equipment and rapid analysis (Franzluebbers, 2016).

In terms of methodology, the flush of CO_2 after re-wetting of dried soil has been determined with a standard approach for nearly 20 years. Validation of potential N mineralization has been with 24-day aerobic incubation at 25°C and 50% water-filled pore space (Franzluebbers, 1999b). Using field-moist, intact soil cores for determination of C and N mineralization and soil microbial biomass C may better estimate in-situ conditions, but using dried and sieved soil has yielded comparable results to undisturbed soil cores (Franzluebbers, 1999). Dried and sieved soil allows for routine sampling protocols to be used, including compositing of numerous cores within a field to better represent a field, and storage of samples without compromising sample integrity. Drying avoids seasonal

complications of obtaining in-situ cores from field soil that is too dry or too wet. Although breaking soil aggregates will cause a flush of C mineralization due to loss of physical protection of organic C (Franzluebbers and Arshad, 1997), sieving cores to <4.75 mm yielded C mineralization dynamics similar to the intact core method since many aggregates remained intact (Franzluebbers, 1999a). Standardization of the method is important, so that consistent results will be obtained. Although several research investigations have been conducted on N mineralization in laboratory incubations, these results may not be synonymous with plant N uptake.

The purpose of this study was to determine the actual amount of plant available N supplied in different locations and soil types, and to evaluate whether other properties might be associated with plant N uptake. Greenhouse growth trials would provide an intermediate step to understand plant N availability from soil organic N sources, between highly controlled laboratory conditions and uncontrollable field conditions. Few studies have evaluated the effectiveness of the flush of CO_2 to actual N uptake by plants across a wide range of soil types and climatic conditions, so a study of laboratory analyses followed by greenhouse trials with a wide range of soils was considered appropriate to evaluate the flush of CO_2 as an indicator of soil N availability.

Controlling for water, sunlight, and temperature is an important aspect, for these are inherent factors that affect soil N mineralization. A study by Stanford and Epstein (1974) held gravimetric soil water content at different levels, and concluded that soil N mineralization was a linear function of gravimetric soil water content for both coarse- and fine-textured soils. In addition to temperature and soil water content, texture is a major indicator of the magnitude of soil N availability, but it is difficult to measure its effects

directly because of the confounding effects of moisture content (Griffin, 2008). For this reason, determining soil texture along with C mineralization and dry matter production was considered important for this study. In general, SOM mineralizes to a greater extent in sandy soils than clayey soils, and sandy soils generally have less organic C and N overall (Griffin, 2008). There are mixed results on whether mineralization rate changes with soil texture, which is an additional question to be investigated in this analysis.

While some field studies have been done using a narrow range of soil types, greenhouse studies with a wide variety of un-amended soil have not yet been conducted for evaluating the flush of CO_2 as an indicator of actual plant N uptake. Sorghum-sudangrass (*Sorghum bicolor*) was considered an ideal test crop to grow in the greenhouse to test for N availability, because it is fast growing and has regrowth potential when clipped (Dial, 2012). It is also able to grow in limited soil volume and is easy to harvest, weigh, and measure the entire above ground biomass.

Objectives and Hypotheses

The objectives of this study were to identify soil properties associated with net N mineralization (laboratory incubations), plant dry matter growth, and plant N uptake (greenhouse growth trials) across various soil types in the southeastern and mid-Atlantic United States. It was hypothesized that soil biological activity is a major driving force controlling potential N mineralization and N availability, and subsequently that C mineralization is systematically related to potential N mineralization. It was also hypothesized that residual inorganic N and soil N mineralization are major sources of plant N

availability, so characterizing the various N fractions was critical to predicting soil N supplying capacity.

MATERIALS AND METHODS

Soil Sampling

Soil was collected from 51 different locations in North Carolina (NC), Virginia (VA), Georgia (GA), Pennsylvania (PA), Oklahoma (OK) and Nebraska (NE), for a total of 759 samples (Figure 1; Table 1). Sampling was generally obtained from topsoil to varying depths, but the majority of samples were collected as four field replicates from a composite of eight cores at depths of 0-10, 10-20, and 20-30 cm. Samples were mostly collected using a push probe with a core diameter of 4 cm.

Details of site management and sampling depth are presented in Table 1. Fields with different management practices, such as tillage, crop rotations, or manure applications rates on the same farm or research station were sampled to assess the sensitivity of soil biological quality indicators to management. Geographic location of sites, climate normals from nearby weather stations, soil taxonomy and textures are described in Appendix A and Appendix B.

The objective was to collect samples that represented a diversity of management, soil order, texture, and soil organic matter content. Replicate samples from farm fields were collected by subdividing fields based on landscape features (Petersen and Calvin, 1996). Laboratory analyses and greenhouse growth trials were conducted on all 759 samples (see section on laboratory analyses and greenhouse growth trials).

Soil Locations in Greenhouse Trial 1

The preliminary greenhouse trial contained 120 samples from five research stations in North Carolina and three livestock farms in Virginia. Soil was collected from the Tidewater Research Station in Plymouth, NC and the Peanut Belt Research Station in Lewiston-

Woodville, NC (courtesy of Carl Crozier as collaborator). Additional soil was collected from the Lower Coastal Plain Tobacco Research Station in Kinston, NC; the Piedmont Research Station in Salisbury, NC; and the Mountain Horticultural Crops Research and Extension Center in Mills River, NC (courtesy of Shelby Rajkovich and Deanna Osmond as collaborators). In VA, soil was collected from three tall fescue pasture farms near Blackstone (courtesy of Chris Teutsch as collaborator). Greenhouse Trial 1 was conducted from 28 Oct to 23 Dec 2014.

Soil Locations in Greenhouse Trial 2

A total of 184 samples were analyzed and used in Greenhouse Trial 2, which was conducted from 19 Jan 2015 to 16 Mar 2015. Soil was collected in June 2014 along the Dan River Valley shortly after the coal ash spill of 2014 (courtesy of Dean Hesterberg as collaborator). Soil from three different experiments evaluating two different winter cover crop mixtures in Pennsylvania was evaluated (courtesy of Charlie White as collaborator). Surface soil samples from a native prairie in Oklahoma were evaluated (courtesy of Pat Starks and Jean Steiner as collaborators). A half dozen samples from under various trees (Nebraska City NE) and grasses (Watkinsville GA) were also evaluated. In addition, 36 samples from Kinston, Lewiston, and Plymouth (12 each) were repeated from Greenhouse Trial 1 to test for similarity in greenhouse methodological approaches.

Soil Locations in Greenhouse Trial 3

A total of 101 samples were analyzed and subsequently used in Greenhouse Trial 3, which was conducted from 20 Mar 2015 to 15 May 2015. Soil from five farms in Cleveland

County, NC was collected in November-December 2014 prior to wheat fertilization evaluation (courtesy of Steve Gibson as collaborator). Soil samples from three different experiments that were previously analyzed for soil biological quality were selected. One set (n=20) was from an irrigated cropping study in Shellman, GA (courtesy of Marshall Lamb as collaborator). A second set (n=13) was from an integrated crop-livestock system study in Watkinsville GA (Franzluebbers and Stuedemann, 2008). A third set (n=23) was from a long-term pasture study in Watkinsville GA (Franzluebbers et al., 1999). These three sets created known gradients in soil biological activity that could be used to test the linkage between soil biological activity and plant growth.

Soil Locations in Greenhouse Trial 4

A total of 354 samples were analyzed in the laboratory and used in Greenhouse Trial 4, which was conducted from 15 Sep 2015 to 15 Nov 2015. Soil was sampled from the following research stations in association with 2015 field trials (courtesy of Shelby Rajkovich and Deanna Osmond as collaborators):

- Caswell Research Farm in Kinston, NC
- Piedmont Research Station in Salisbury, NC
- Mountain Horticultural Crops Research and Extension Center in Mills River, NC

Additional soil samples were collected from 2015 field trials without N application (courtesy of Carl Crozier and Gary Roberson as collaborators) from:

- Mountain Horticultural Crops Research and Extension Center in Mills River, NC

- Tidewater Research Station in Plymouth, NC from two fields (one dryland, one irrigated)
- Center for Environmental Farming Systems in Goldsboro, NC
- Private farm in Camden County NC

Additional soil samples were collected prior to corn planting in 2015 from:

- Piedmont Research Station in Salisbury, NC (two fields)
- Private farm in Stanly County NC (two fields)

Soil was collected in March 2015 from:

- Long-term pasture at the Tidewater Research Station in Plymouth, NC

Lastly, in collaboration with farmers and extension personnel, soil was collected in April-May 2015 from 14 farms in Augusta, Fauquier, and Rockingham Counties in Virginia (courtesy of Jeff Cline, Alec Lipscomb, Tim Mize, and Robert Shoemaker as collaborators).

Laboratory Analyses

Soil Sample Preparation

Each soil sample (n=759) was assigned a completely randomized lab ID to be used for all analyses. Soil cores were oven-dried (55 °C, 3 d), weighed and sieved through a 4.75 mm screen with stones removed to homogenize the sample without destroying all aggregates (Franzluebbers et al., 1999b). Bulk density was calculated from the dry weight of soil divided by the volume of soil cores. Soil was thoroughly mixed in its sampling bag prior to subsampling for biological, chemical, and physical analyses.

Carbon Mineralization after Re-wetting Dried Soil

To prepare samples for incubation, duplicate subsamples (50 g) were weighed from each composited sample into two 60-mL glass jars. Jars had markings in 5 mL increments, so soil was gently tamped to the nearest 2.5 mL to determine disturbed bulk density. Once the volume of soil was recorded, the amount of water needed to wet each subsample to 50% water-filled pore space (WFPS) was determined. Porosity was calculated as:

porosity = 1 - (bulk density / particle density)

assuming a particle density of 2.65 Mg m^{-3}

and milliliters (mL) of water needed to wet subsamples to 50% WFPS was calculated as:

$$H_2O\ (mL) = 0.5 * porosity * soil\ volume\ (mL)$$

At least 12 hours prior to Day 0 of incubation, canning jars were prepared with a 10 mL vial of water in each to maintain humidity. Duplicate subsamples were immediately placed in one canning jar after wetting, along with an alkali trap (30 mL plastic Nalgene bottle) containing 10 mL of 1 M NaOH to capture the evolved CO_2. The canning jars were capped and put into an incubator at 25 °C (± 1 °C) (Franzluebbers et al., 1999b). For every 11 samples in a box, one blank sample was added containing only the vial of water and an alkali trap without soil to subtract the background concentration of CO_2.

On Day 3 of the incubation, boxes were removed from the incubator and the alkali traps replaced with a new 10 mL bottle of 1 M NaOH. Canning jars were sealed and placed back into the incubator. Original alkali traps were capped and set aside to be titrated with

HCl to determine the amount of CO_2 evolved from the soil from 0-3 days, referred to as the "flush of CO_2."

For titrations, three items were added to each alkali trap, which would be uncapped one at a time: i) 2 mL of 1.5 M $BaCl_2$ to precipitate all sodium carbonate out of solution, ii) 2-3 drops of phenolphthalein color indicator, and iii) a magnetic stir bar. The alkali trap was then placed on a magnetic stir plate and slowly titrated with 1 M HCl until a color change from pink to white occurred. The HCl reacted with the leftover NaOH that was not neutralized by the evolved CO_2 from the soil, turning the solution white. The vial contained in the blank sample (with no soil) was also titrated and the blanks were averaged. The quantity of CO_2 evolved from each soil sample was calculated as follows (Franzluebbers, 2016):

CO_2-C (mg kg^{-1} soil) = (mL [blank] – mL [sample]) x N x M / S

where, N = normality of acid (mol L^{-1}), M = mass conversion from $cmol_c$ C to g C (6000), and S = soil weight (g)

Exceptions to titrations occurred with samples that had very high amounts of organic matter (e.g. from some shallow surface samples of pastures). Additional $BaCl_2$ was necessary to encourage a sharper turning point of the solution (4 mL). These samples were repeated using only 50 grams of soil total, but they still saturated the 10 mL of NaOH. In the future, it would be recommended to use 20 mL of NaOH with 30-50g soil and adjust the calculations accordingly (samples were repeated with this protocol).

Soil Microbial Biomass Carbon

Soil microbial biomass carbon (SMBC) was determined using the chloroform fumigation-incubation method (Jenkinson, 1977)**.** On Day 10 of the incubation, the vial of alkali was removed and immediately capped with an air-tight lid. The etched jar of subsample was removed and placed into a vacuum desiccator. A 50-mL beaker containing boiling chips and 30 mL of chloroform was placed into each desiccator containing ~26 samples. Desiccators were sealed and vacuumed for 30 seconds following rapid boiling of chloroform. A vacuum was retained and desiccators placed into a dark environment for 18-24 hours. On Day 11, desiccators were unsealed and chloroform was evacuated. Samples were placed into fresh canning jars with a 10 mL vial of water and an alkali trap. At Day 21, alkali traps with fumigated soil were titrated to determine SMBC, calculated using the equation:

$$\text{SMBC (mg kg}^{-1}\text{ soil)} = (\text{mL [blank]} - \text{mL [sample]}) \times \text{N HCl} \times 6 \times 1000 / ((\text{g soil}) / k_C)$$

where, 6 = equivalent weight of C and $k_C = 0.41$ (efficiency factor)

On Day 24, alkali traps were removed from the original mason jars and titrated for total C mineralized after 24 days with the remaining subsample.

Net Nitrogen Mineralization

Net N mineralization was determined by the difference in inorganic N (NH_4^+ and NO_3^-) concentration at 0 and 24 days of incubation (Hart et al., 1994). To determine initial inorganic N concentration (Day 0), 10 g (±0.02) of ball milled soil was weighed into 30 mL plastic Nalgene bottles, shaken with 20 mL of 2 M KCl for 30 min, and filtered through

Whatman #5 filter paper. Extracts were stored in a -20°C freezer until analysis using the Bran Luebbe Auto-Analyzer 3 (EPA Method 353.1). The process was repeated at 24 days for the final inorganic N concentration using a 10-g portion of oven-dried (55 °C, 3 d) and sieved (<2 mm) sample. Initial inorganic N (Day 0) and net N mineralized after 24 days were added together to calculate potentially available N for plant growth.

Soil Texture and Particulate Organic Matter

The previously fumigated 50-g subsample was dried in an oven (55 °C, 3 d), then scraped into 125-mL Nalgene plastic bottles with screw caps for textural analysis modified from Gee and Bauder (1986). Each bottle had 100 mL of 0.1M sodium pyrophosphate decahydrate added, and the bottles were placed on a reciprocation shaker for 12-16 hours. Soil solution was transferred to a 1-L cylinder and diluted to 1-L volume with deionized water. Solution was mixed with a plunger exactly 10 times and left to settle for exactly 5 hours. Soil clay concentration was determined by placing a hydrometer in the solution and reading the measurement at 5 hours (Gee and Bauder, 1986):

Clay (g/g) = ((((°C - 20) *0.36) + hydrometer reading (mg/L)) - blank) / soil weight (g)

The soil solution in the cylinder was poured over a 53 µm screen and washed with deionized water until only sand and particulate organic matter remained. Sand was calculated by the weight of the remaining material divided by the original soil weight. Silt concentration was calculated from difference between unity and the summation of clay and sand fractions.

Contents containing the sand and particulate organic matter on the sieve screen were transferred to a drying bottle with a stream of water, then dried in an oven (55 °C, 3 d). It was then ball milled to homogenize the sample. Particulate organic matter C and N concentration were determined from dry combustion (LECO, TruMac CN analyzer).

Soil Testing

Subsamples of 50 g were sent to the North Carolina Department of Agriculture and Consumer Services (NCDA & CS) state soil-testing lab for macro and micronutrient soil analysis. Soil was characterized for humic matter (HM), cation exchange capacity (CEC), pH, and base saturation. Mehlich-3 methodology was used (Mehlich, 1984).

Greenhouse Growth Trials

Preliminary Greenhouse Trial Protocol

Soil (dried and sieved to <4.75 mm) was thoroughly mixed in a bucket and scooped into six replicate 66 mL greenhouse growth tubes of 1.54-cm diameter, 16.1 cm height (Ray leach RLC4 cells “cone-tainers”). A cotton ball was positioned at the bottom of the tube to keep soil from falling out. Each replicate was assigned a random id number referred to as the Greenhouse id (GH id). Tubes were gently tapped 3 times to settle, and topped with additional sample to fill the tube to within 1 cm of headspace. Tubes were placed in racks in order of their GH id number, leaving a cell between tubes (Figure 2).

Two days prior to planting, sorghum-sudangrass (*Sorghum bicolor*) seeds were germinated by placing them on wet paper towels on trays in an incubator at 25°C. On the day of planting, three germinated seeds with $\leq$ 2mm of root growth were gently placed into every

tube on top of the soil. Seeds were covered with approximately 5 grams of sand to prevent accelerated rates of evapotranspiration and to protect seeds from drying out. Each tube was watered on top with a few mL of water to promote the growth of the germinated seedlings. Trays were then moved into a greenhouse kept at 20-30°C depending on the season and time of day. Average weekly temperatures for each greenhouse trial were calculated (Figure 4). Trays were randomly arranged on two rectangular tables under equal light and temperature conditions across greenhouse trials. Greenhouse lights remained on for equal length across all trials (12 hours), adjusted for differences in sunrise and sunset during the different seasons.

The six subsamples of a soil were divided into three different water levels. Water levels were managed by bringing them to full water-holding capacity after drying to approximately 80, 60, and 40% of water-holding capacity, in order to determine if variations in water content would affect plant growth. This initial experiment informed the process for the remaining greenhouse trials.

Tubes were placed in water trays to wet soil through capillary action. The amount of water at saturation was determined by weighing trays of soil and subtracting the weight of the soil, sand, tubes, and trays. Once saturated, water was released from trays and the soils were allowed to remain without water until approximately 80, 60, or 40% (depending on the treatment) water-holding capacity was reached. Water-holding capacity was calculated by determining the weight of the soil in each tube, adding the total dry weight of the tubes, and reweighing after initial saturation of tubes. The weight of the tray, empty tubes, and cotton balls were subtracted to determine the target weight of the rack for re-watering. The approximate time required to reach 80, 60, and 40% water-holding capacity was one, two,

and three days. Once target water levels (determined by weighing trays every day) were reached, trays were capillary wetted for 20 minutes, and the cycle repeated for a total of 8 weeks of growth. Watering was repeated 47, 25 and 14 times for 80, 60 and 40% water-holding capacity, respectively.

Plant dry matter was harvested twice: first at four weeks and again at eight weeks (regrowth). Plant dry matter at each harvest was placed into separate envelopes. Plant samples were dried in the oven at 55 °C for 3 days. Each plant sample was weighed and ground using a Udy Mill. The mg of dry matter per kg of soil was calculated from the weight of the plant sample divided by the dry soil weight of each tube.

At least 1 g of dry matter was needed to scan the plant samples using the Near Infrared Spectrometer (NIR) for N concentration. Most tubes did not produce enough dry matter, so plant samples from the same original soil sample were composited (across 3 water levels, 2 greenhouse replicates, and the four and eight-week harvest). Therefore, the 720 growth tubes were composited (after weighing each individual plant sample) into 120 envelopes for N content determination.

Statistical Analysis: Preliminary Trial 1

Data from Greenhouse Trial 1 were first analyzed for the effect of water stress levels on plant dry matter production and total N uptake. Total N uptake was the product of dry matter production and N concentration of plant material. Determining the effect of water stress level between 80, 60 and 40% water-holding capacity on dry matter production and total N uptake was the first step in setting a protocol for subsequent greenhouse trials.

A mixed model was determined to account for variation from management (crop or pasture), city (5 different cities), sites (9 different sites), stress (3), and greenhouse rep (2) using proc GLIMMIX in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). The Virginia samples were initially taken out of the data set because they were sampled only from 0-10 cm. Management (crop or pasture) and different sites were set as random effects because they were not evenly distributed under both managements. Least square means (LS Means) was used to determine the predicted weight of stress level and depth by management, and the adjustment for multiple comparison was made using Holm-Tukey. All possible interactions between variables were analyzed. The final model with the smallest amount of variance was chosen using the Akaike's Information Criterion (AICc) and the Bayesian Information Criterion (BIC). Using the square root of dry matter production (the dependent variable) improved the fit of the model in SAS. The significance level used for all data analysis in this study was $p < 0.05$.

To understand if there was significant difference in dry matter production from stress and depth at each site, an additional analysis of variance (ANOVA) using proc GLM for stress against dry matter production was used, and the nine different management x site sets were examined as blocks. If the dry matter production was in the same rank order for 80% water-holding capacity as with 60 or 40% water-holding capacity, then 60 or 40% water-holding capacity would be chosen, for reasons of time (watering every three days), better root distribution, and enhanced mineralization of nutrients.

Standardized Greenhouse Trial Protocol (Trial 2-4)

Subsequent greenhouse trials followed similar protocol to the preliminary greenhouse trial with some alterations. The growth tubes were larger (see Figure 3) than trial 1 (164 mL; 20.95 cm height; 3.81 cm diameter; SC10 cells), with three replicates for each sample. Five pre-germinated sorghum-sudangrass seeds were planted in each tube and covered with sand. Trays were saturated in the laboratory and covered with cloth to avoid evaporation of water. After seeds sprouted in the laboratory, trays were moved into the greenhouse and placed under equal light and temperature conditions.

There was no significant, practical difference in water treatment, so all trays were wetted in the same manner. Once approximately 40% water-holding capacity was reached, trays were capillary wetted for 20 minutes and the cycle repeated. Plants were harvested at four and eight weeks in the same manner as the preliminary greenhouse trial.

Plant Nitrogen Content

Nitrogen content was determined using a near infrared reflectance spectrophotometer (NIRS). Plant samples were ground to pass a 1 mm screen (Udy mill, model). All samples were scanned in a model 5000 NIRS with WinISI version 1.5 software (Foss North America, Inc., Eden Prairie, MN). Spectra were evaluated for outliers (‘H’ > 3.0) prior to sample selection for calibration resulting in seven samples removed. The ‘H’ statistic (0.6) was used to select samples with different spectra (n = 210) which were subsequently analyzed for N and C content using a LECO TruMac CN combustion analyzer. Calibration equations were developed for N using modified PLS regression with four cross validations. After equation

development, the equation was then applied to all samples to estimate N and C concentrations.

Plant N uptake (mg N kg^{-1} soil) was calculated as N concentration (g kg^{-1}) multiplied by dry matter production (g dry matter kg^{-1} soil). Plant N uptake and dry matter production were used in the statistical analysis as the response variables for this study, termed together as "greenhouse growth."

Statistical Analysis of All Data (Trials 1-4)

An initial correlation procedure (Proc CORR) was used for Greenhouse Trial 1 to understand the relationships between variables and to determine which would be significantly related or collinear to one another in order to determine the best variables to include in a model. A selection procedure (proc GLMSELECT) was used to determine the variables with the greatest potential to explain variability in greenhouse growth. A principal component analysis using the selected variables was conducted to determine which variables had the greatest proportion of variability explained by them, and to check for existence of collinearity among variables. Finally, multiple linear regression was conducted with the significant principal components. Adjusted R^2 is reported in the results to account for number of variables. Assumptions and residuals were tested with the Durbin-Watson test, Cook's Distance, Leverage, Residuals and Partial Residuals plots, and normal probability plots.

The following variables were used as potential predictors of greenhouse growth: sampling region, soil depth, C mineralization in 3, 10, and 24 days, available N, soil microbial biomass C, total organic C and N, particulate organic C and N, sand and clay content, humic matter, pH, base saturation, acidity, P, K, Ca, Mg, S, Na, Mn, Cu, and Zn.

Most important variables were considered from a combination of the lowest AIC, AICC, and SBC values with the highest R^2. After Greenhouse Trial 1 was analyzed, subsequent greenhouse trials with further evaluation among states and management practices were tested in a similar manner. All data were tested together to characterize the overall relationship between dry matter production and the flush of CO_2 as well as N mineralization across depths, soil types, and regions.

RESULTS AND DISCUSSION

Greenhouse Trial 1: Effect of Water Stress on Plant Dry Matter Production

An analysis of variance (ANOVA) using proc GLIMMIX was conducted to test how dry matter production varied as a function of water stress, soil depth, and field site for the 108 samples from North Carolina (648 experimental units total) in Greenhouse Trial 1. All main effects and the interaction of stress x depth x site ($p=0.04$) significantly impacted dry matter production.

The three-way interaction of stress x depth x site made the effect of stress on dry matter production complicated (Table 2). Field site was inherently different due to differences in soil types and management practices. Therefore, field site was removed from the model and the effect of water stress and depth on greenhouse growth was conducted separately for each field site.

The ANOVA (Table 3 and Table 4) determined for each field site individually resulted in depth as a significant main effect in all locations in NC ($p < 0.001$); stress as a significant main effect in pastured fields in McKinney, VA ($p =0.02$); and stress x depth as an interaction effect in the hayed forage site in Plymouth, NC ($p=0.04$). However, a trend for lower dry matter production occurred in almost all field sites as water stress increased (Figure 5).

Seven of the eight field sites had a significant main effect of depth on dry matter production. Dry matter production decreased significantly with each depth at all locations except Mills River, where depths 10-20 cm and 20-30 cm were not significantly different (mean dry matter production of 1.49 and 1.40 g DM kg^{-1} soil, at depths of 10-20 cm and 20-30 cm, respectively). The biological significance of depth was only interesting when

examining its interaction with stress on dry matter production. The significant interaction of water stress and depth was most likely due to increased plant growth from the surface depth, which is in turn more susceptible to water stress.

In McKinney, VA, stress additionally affected dry matter production at high stress compared to low stress ($p=0.01$). The hayed forage site in Plymouth, NC (shown separately in Table 4) was the only field site with a significant interaction of water stress x depth ($p=0.04$). At this site, dry matter production was significantly lower with increasing stress at the surface depth (0-10 cm). At lower depths at this site, dry matter production was not significantly altered by water stress, but a trend for lower dry matter production occurred with increasing water stress (Figure 5).

Conclusions of water stress level analysis

While stress was not significant at many field sites, it was significant at sites with elevated dry matter production. Hayed forage in Plymouth had elevated dry matter production compared with other sites, likely due to greater N availability, and therefore, greater water demands by larger plants. Water stress was likely more pronounced in surface samples from this site compared with other field sites due to greater water use by the elevated dry matter production.

A trend occurred where surface depths were generally more affected by stress than lower depths, and was significant at sites with greater plant dry matter production such as in Plymouth, NC. Greater total soil C and N at in the surface depths may have contributed to differences in dry matter production, although this may have been more an effect of plant

growth and water extraction than organic matter per se. The analysis in section 1 examines the drivers affecting dry matter production more closely.

Regardless of water stress level, the rank order of dry matter production among all 12 field sites was the same (this was important to note, because subsequent greenhouse trials were conducted at 40% water stress level). In addition, plant samples were combined across greenhouse growth replicates in order to determine plant N uptake (dry matter for NIR scan needed >1 g of dry plant material).

Statistical significance overall occurred in dry matter production, but among field sites, it only occurred at higher N supplying sites. Variation in dry matter production was due more to field site (49%) and soil depth (37%) than to water stress level (1%). While water stress level was significant overall in the data set, the magnitude of difference in dry matter production averaged only 0.2 mg g^{-1} soil.

For use as an assay, as long as water level was standardized, water stress level would not be a significant factor in studying greenhouse growth. In conclusion, while water stress level was a biologically interesting variable, it was not investigated further in subsequent greenhouse trials due to time and resource constraints. Subsequent greenhouse trials were conducted at one stress level (40%), in order to facilitate less frequent watering.

Soil Properties Associated with Greenhouse Growth

1. Greenhouse Trial 1

1.1. Selecting the key variables to predict greenhouse growth

To test the main factors that predicted greenhouse growth and N release by soils for plant uptake, both dry matter production and plant N uptake were analyzed against various

soil properties across the 120 soil samples from NC and VA. Dry matter production was highly related to plant N uptake ($R^2 = 0.96$; Figure 6). Interpretation of results was similar whether analyzing for dry matter production or plant N uptake, and therefore "greenhouse growth" described the general response across terms. The following analyses includes an initial correlation procedure of greenhouse growth with all 34 soil properties; a statistical selection procedure that chose the best variables to predict greenhouse growth and a principal component analysis using these selected variables, which was then run as a model to predict greenhouse growth.

1.2. Soil biological properties correlated to greenhouse growth

Table 5 summarizes the soil properties correlated with greenhouse growth. A number of soil biological properties were highly correlated to greenhouse growth (using an a priori threshold of $r > 0.70$), including C mineralization during 3, 10 and 24 days, soil microbial biomass C (SMBC), net N mineralization during 24 days, and particulate organic C and N. Some soil chemical properties were also highly correlated to greenhouse growth, including cation exchange capacity (CEC), initial inorganic N, and total available N (initial inorganic N + net N mineralization during 24 days). Total soil N was slightly less correlated to greenhouse growth ($r=0.68$). Total organic C (organic matter) was not highly correlated to greenhouse growth ($r=0.5$). This is most likely due to recalcitrant fractions in the organic matter that are not readily mineralizable by microorganisms.

1.3. Selection procedure to choose best variables to predict greenhouse growth

Proc GLMSELECT was used to select variables (total of 34 available) to predict dry matter production across the 120 samples in Greenhouse Trial 1. This analysis yielded variables of the flush of CO_2, SMBC, particulate organic C, available N, P, Mg, and Na as the most important. To predict plant N uptake, the selection procedure chose the same variables, excluding P and Na. Multiple linear regression using these variables yielded an adjusted R^2 of 0.91 and 0.92 for dry matter and plant N uptake, respectively. However, Na, P, net N mineralization, and SMBC were not significant when used in the Type III sum of squares regression analysis (Proc GLM), so they were removed from the model. A model with only the flush of CO_2, particulate organic C, available N, and Mg was then developed. This model accounted for 90 and 92% of the variation in dry matter and plant N uptake, respectively.

Two major concerns derived from this analysis were the absence of particulate organic N in the model (which was highly correlated with greenhouse growth), and the presence of Mg in the model, to explain greenhouse growth. Mg is an essential macronutrient for plant growth, but is not a major indicator of N uptake. In general, nutrient deficiencies may decrease dry matter production, but the relationship here showed that as greenhouse growth increased, Mg increased ($R^2 = 0.40$). An explanation for this is that the hay pasture was sprayed with swine lagoon sludge, which was high in Mg. Taking these 12 samples out of the model drastically decreased the relationship of Mg to greenhouse growth ($R^2 = 0.15$). Therefore, Mg was determined to be a covariate and not an actual indicator of greenhouse growth.

The selection procedure, after taking out the hayed pasture samples, proceeded to choose particulate organic N instead of Mg as an important variable to explain greenhouse growth. Particulate organic C and N were highly related ($R^2 = 0.91$; slope of 16.4), so it is clear why they would be chosen together in the model. With this information, a principal component analysis was conducted to account for the variance in the data using the variables selected by the GLMSELECT (flush of CO_2, available N, SMBC, and particulate organic C and N).

1.4. Principal component analysis of Greenhouse Trial 1 to test the explained variance

The first two principal components explained 94% of the variance in greenhouse growth using the flush of CO_2, available N, SMBC, and particulate organic C and N (Figure 7). The first principal component explained 89% of the variance, and the second principal component explained 5%. All of the variables had similar loading values of 0.43-0.46 in the first component, suggesting that these five measurements of organic matter were equally important (Table 6).

A model using simply the first principal component yielded an adjusted R^2 of 0.89 and 0.90 for plant dry matter and plant N uptake, respectively. This model would be sufficient, as it is simpler and very similar to the more complex model. While the model created by the principal component analysis is statistically sound, the variables measured are highly related, suggesting covariance was occurring (Table 7). Assessing these variables independently was the next step in order to understand which biological characteristics explained the most variability in greenhouse growth, and whether or not the flush of CO_2 would be a viable option as a predictor of soil N release for plant uptake.

1.5. Further testing the relationship of the biological properties with greenhouse growth

Available N, which is the sum of net N mineralization in 24 days plus initial inorganic N, was hypothesized to be the best indication of greenhouse growth. In a simple linear regression, available N explained 77% of the variation in dry matter production and 76% of the variation in plant N uptake (Table 8). As available N increased, greenhouse growth increased. A quadratic fit and transformation to the square root of dry matter and plant N uptake improved the model to explain 82 and 84% of dry matter and plant N uptake, respectively.

Net N mineralization, which excludes initial inorganic N, explained 81% of the variation in greenhouse growth in a simple linear regression (Table 8). Net N mineralization by itself was an excellent predictor of greenhouse growth, but the long evaluation period and multiple analyses needed (initial and ending NH_4 and NO_3 values) limit its suitability for soil testing facilities, which require rapid reporting of results to their clientele. The flush of CO_2, however, is a process that only takes three days of incubation and uses inexpensive equipment. How the flush of CO_2 related to net N mineralization and greenhouse growth was therefore preferentially explored as a potential rapid soil test of N availability.

In order to look at the possibility of the flush of CO_2 to predict greenhouse growth, the C:N ratio of various fractions was explored. Particulate organic C and N were highly related, with an R^2 of 91%. The more active fraction of C mineralization in 24 days was highly related to N mineralization in 24 days (R^2=0.71).

The flush of CO_2 (C mineralization in three days) was highly related to C mineralization in 24 days (R^2=0.97). The flush of CO_2 was thus a viable alternative for

predicting N mineralization in 24 days. Net N mineralization was highly related to plant N uptake and dry matter production (R^2=0.82). If net N mineralization was a good indicator of greenhouse growth, it would be possible to explore the flush of CO_2 instead as a predictor of greenhouse growth, since it was highly related to net N mineralization (R^2=0.77).

In a simple linear regression (proc GLM) the flush of CO_2 predicted 77% of the variation in net N mineralization, 78% of the variation in dry matter production, and 79% of the variation in plant N uptake. The equations to predict net N mineralization, dry matter production and plant N uptake in Greenhouse Trial 1 (n=120) were:

Net N mineralization = -0.41 + 0.35 (flush of CO_2) ; R^2= 0.77

Plant dry matter production = 1.5 + 0.013 (flush of CO_2) ; R^2= 0.78

Plant N uptake = 14.3 + 0.17 (flush of CO_2) ; R^2= 0.79

In conclusion, the flush of CO_2 was determined to be a viable surrogate for N mineralization to predict plant N uptake and dry matter production in Greenhouse Trial 1. Table 8 summarizes the strength of the relationship of the flush of CO_2 along with other soil properties to predict greenhouse growth. Subsequent analysis explored the flush of CO_2 with other soil properties to improve the model and strengthen prediction of greenhouse growth. Other soil factors such as soil texture, depth, and management practices were explored in the following sections.

1.6. Soil texture influence on the relationship between greenhouse growth and soil biological properties

An additional characteristic, soil texture, may influence the relationship of the biological indicators when predicting greenhouse growth (Pare and Gregorich, 1999). While the variable selection procedure (proc GLM) did not select texture as a variable, it was still necessary to conduct the analysis, as soil texture is proposed to affect the rate and magnitude of mineralization, especially for widely different soil textures such as sands and clays (Griffin, 2008). The key soil biological N indicators: available N, net N mineralization, and the flush of CO_2 were analyzed with texture in an ANOVA.

An ANOVA model to predict plant N uptake using the flush of CO_2 and texture in Greenhouse Trial 1 resulted in a significant main effect of the flush of CO_2 ($p<0.001$), as well as an interaction effect of the two terms ($p=0.053$) (Table 9). The model was improved by 7% when including texture as an additional variable to the flush of CO_2. Sandy loam was the reference variable with a slope of 0.15 for the flush of CO_2. Clay loam and loam had significantly greater slopes of 0.21 and 0.20, respectively (Table 10Table).

Predicting dry matter production also resulted in a main effect of the flush of CO_2 and the interaction of texture and the flush of CO_2. The slope of greenhouse growth as predicted by the flush of CO_2 was greater for clay, loam, and sandy clay loam classes than for sandy loam textural class (Figure 8; Table 10).

Predicting dry matter production with available N was also impacted by soil textural class (Table 9). The slope of greenhouse growth as predicted by available N was greater for clay and loam classes than for sandy loam and sandy clay loam textural class (Table 10). Predicting greenhouse growth using net N mineralization was not impacted by soil textural

class. For all biological indicators, the magnitude of difference in slope by soil texture was not large. The major differences noted were clayey versus sandy loam soil textures. shows the differences in slope for soil textures in Greenhouse Trial 1.

There are issues when interpreting texture (a categorical variable) in a model to predict greenhouse growth. Soil textures may be on the borderline of two different USDA classifications. In addition, soil texture in the greenhouse may have been confounded with soil water content, depth, or soil type, which ultimately affected the relationship of mineralization of C and N to greenhouse growth. Soil textural analysis would be better investigated with a balanced set of textural classes.

An additional way to analyze texture was to analyze the continuous variable of sand content and its effect on biological indicators to predict greenhouse growth. Sand content was used as an indirect method to study the effect of silt and clay on biological indicators. Therefore, an ANOVA with the flush of CO_2 and sand was analyzed. The main effect of sand was not significant ($p=0.24$). The interaction of the flush of CO_2 with sand was significant ($p<0.03$). The model indicated that as sand content increased (or clay and silt decreased), greenhouse growth decreased. However, the Type III sums of squares models placed significantly more emphasis on the F value of the flush of CO_2 (Table 11). Adding sand in the model only improved the model by 4%, indicating that sand content was not a major indicator of greenhouse growth.

In conclusion, while the magnitude of mineralization may be impacted by soil texture (Table 12) the impact on the rate of mineralization was not as clear in this analysis. A more controlled study of water content plus a balanced sample of soil textures with depth would be needed to investigate the effect of texture on the rate of mineralization.

1.7. Relationship between greenhouse growth and soil biological properties with soil depth

Available N in the surface depth (0-10 cm) had a significantly different relationship with dry matter production than in subsurface depths (10-20 cm and 20-30 cm) (Figure 9a). In contrast, soil depth did not significantly alter the relationship between greenhouse growth and net N mineralization in Greenhouse Trial 1 (Figure 9b). A similarly stable relationship of greenhouse growth with the flush of CO_2 occurred, independent of soil depth (Figure 9c).

All indicators had a similar trend of depth, but only available N was significantly different. Some soils sampled in Lewiston-Woodville, NC had a history of poultry litter application, resulting in high nitrate concentration, which may have been the driving factor in the disparate relationship of available N with depth. The relationship of biological indicators with management practices at Lewiston-Woodville was explored in greater detail in Section 1.7. In summary, soil depth did not significantly alter the relationship of either soil biological indicators (the flush of CO_2 and net N mineralization) with greenhouse growth in Greenhouse Trial 1.

1.8. Differences of zero, low, and high poultry litter applications on the flush of CO_2 in Lewiston-Woodville, NC

The flush of CO_2 in three days explained 82% of the variability in dry matter production (n=36) of soils from Lewiston-Woodville. Dry matter production in relationship with the flush of CO_2 was significantly affected by litter amendment (zero, low, and high poultry litter). High poultry litter application (n=12) had a slope of 0.19 while the zero and low poultry litter applications had a smaller slope of 0.11 and 0.12, respectively. High

poultry litter application had significantly more nitrate than the low and zero poultry litter applications (Table 13), and therefore, this additional N caused an increase in slope.

Available N explained 86% of the variation in dry matter production. The slopes for the three treatments were not significantly different to predict greenhouse growth with total available N. In essence, initial inorganic N helped explain the variability of the different treatments. Since the flush of CO_2 was highly correlated to N mineralization, it was speculated that the flush of CO_2 combined with inorganic N would be a good predictor of dry matter production in the greenhouse with these samples across treatments. Management practices such as manure application suggest that the need for initial inorganic N is an important addition to biological activity in predicting greenhouse growth production.

Zn was highly correlated (r=0.98) to greenhouse growth. Zn concentration predicted greenhouse growth in a simple linear regression model with an R^2 of 0.89. High Zn concentration was in the poultry litter, which increased as poultry litter increased, indicating that it was more of a coincidental variable than an actual predictor of N uptake. Some micronutrients can affect N uptake by plants, but this association here was likely only coincidental. Correlations to micronutrients and other variables will continue to be examined in order to determine whether they may be coincidence or a true explanatory variable of greenhouse growth.

1.9. Does land management affect the relationship between greenhouse growth and soil biological properties?

In Plymouth NC, soil was sampled from three different land management conditions: conventionally tilled cropland, no-tillage cropland, and hayed pasture. Available N accounted

for 88% of the variation in dry matter production and 92% of the variation in plant N uptake. The relationship between dry matter production and available N was significantly affected by management condition. The slope of dry matter production as a function of available N was greater with hayed pasture (0.032) than with no tillage (0.027) and conventional tillage (0.022). However, the relationship between plant N uptake and available N was not affected by management condition ($p=0.50$).

Net N mineralization was not as sensitive to land management as available N, resulting in no effect of management on the relationship between greenhouse growth and N mineralization. Net N mineralization explained 86 and 87% of the variation in dry matter production and plant N uptake, respectively, in soils from Plymouth NC.

The flush of CO_2 explained 86% of the variation in dry matter production and 89% of the variation in plant N uptake from soils in Plymouth NC. An additional 3% of variation was explained by management differences (i.e. conventional till, no till, and hay pasture) ($p=0.02$). Slopes of dry matter production as a function of the flush of CO_2 were 14, 16, and 22 mg dry matter mg^{-1} CO_2-C for no till, hay, and conventional till, respectively. The range of values in dry matter and the flush of CO_2 with conventional tillage was narrower than with hay or no tillage, so the relationship may not have been as robust in this situation.

The flush of CO_2 accounted for 86% of the variability in N uptake ($p < 0.001$). Land management explained an additional 5% of variation in plant N uptake. The relationship of plant N uptake with the flush of CO_2 was greater with conventional tillage than no tillage or hayed pasture ($p=0.02$). The slope of conventional tillage was 0.29 and the slope of no tillage and hay pasture was 0.21. Again, there was a smaller range of CO_2 and N uptake in the conventionally tilled samples, so the slope was influenced by these narrow range of samples.

The surface samples from the conventionally tilled soil potentially may have had lower biological activity due to soil disturbance with tillage prior to sampling.

While management differences did occur, overall, the flush of CO_2 explained the majority of the variation (86%) at this field site. By obtaining a larger range of values for conventionally tilled soil, a more robust conclusion could be drawn that would determine if the relationship in conventionally tilled soil is different than no till or hay pasture in predicting greenhouse growth as a function of CO_2 evolution. For these samples, the flush of CO_2 was highly effective by itself in predicting greenhouse growth across different management practices.

1.10. Conclusions from Greenhouse Trial 1

In conclusion, the biological soil properties such as soil microbial biomass C, the flush of CO_2 and total available N were all good predictors of greenhouse growth (both plant N uptake and dry matter production). High nitrate in soils managed with poultry litter (see section 1.7) altered relationships slightly. The flush of CO_2 was a viable surrogate as a predictor of greenhouse growth, and thus was a focus of tests in subsequent greenhouse trials.

2. Greenhouse Trial 2

2.1. Selecting the key variables to predict greenhouse growth

A total of 184 samples (excluding repeated samples from Greenhouse Trial 1) were analyzed in Greenhouse Trial 2 from multiple states, including: NC (n=73), OK (n=35), PA (n=70), GA (n=2), and NE (n=4). An additional 36 samples were repeated from Greenhouse Trial 1 for a total of 220 samples analyzed in total. Similar to Greenhouse Trial 1, the main objective was to determine the factors that predicted N release by soils for plant uptake and

dry matter production. An additional objective of this greenhouse trial was to determine if there were differences in the relationship of biological indicators to greenhouse growth due to state of origin (i.e. soil type) and management practices.

In Greenhouse Trial 2, several growth tubes had stunted sorghum-sudangrass plants. These samples were still harvested and analyzed, and were all found to be from soil along the Dan River. Something occurred in soil of the Dan River samples (n=73) that stunted plant growth. The flush of CO_2 of the Dan River samples were therefore only reported against N net mineralization in 24 days (see section 2.5). The following correlation and principal component analysis for greenhouse growth prediction included 111 soil samples, excluding those from Dan River, which were analyzed separately in section 2.5.

Dry matter production was relatively highly related to plant N uptake (R^2= 0.88) in Greenhouse Trial 2 (Figure 6). The slope of the relationship was 10.7 mg N g^{-1} dry matter (as compared with 12 mg N g^{-1} dry matter in Greenhouse Trial 1). The R^2 between plant N uptake and dry matter production in Greenhouse Trial 1 was 8% greater than that of Greenhouse Trial 2. Plant N uptake and dry matter production were thus reported separately in Greenhouse Trial 2.

In addition to an overall analysis of the 184 samples, states with different management practices were analyzed separately. Oklahoma samples (n=35) were analyzed in section 2.3 in order to evaluate the difference of grazing practices that were hypothesized to influence the relationship of the flush of CO_2 to greenhouse growth. Pennsylvania samples (n=70) were also analyzed separately (section 2.4) to evaluate potential differences from cover crop and location. Finally, Dan River, NC samples (n=70) were analyzed against net N mineralization to test for differences due to previous crop (section 2.6).

2.2. Soil biological properties correlated with greenhouse growth

Soil properties highly correlated to plant N uptake across the 111 samples (a priori r > 0.70) were: C mineralization after 3, 10 and 24 days, particulate organic N, initial NH_4, available N, net N mineralization, and particulate organic N. Relationships with dry matter production varied slightly: only the flush of CO_2, total soil N, potassium (K), initial NH_4 and available N were significantly correlated. Carbon mineralization in 24 days and particulate organic N were both slightly less correlated (r=0.68 and 0.69, respectively). Table 14 summarizes all variables highly correlated to greenhouse growth as well as net N mineralization to evaluate variables correlated with net N mineralization for Dan River samples.

The two samples from Georgia and four samples from Nebraska were outliers in the model (Figure 11). An explanation for why these samples did not mineralize at a rate that matched the CO_2 emissions was that the C substrate availability was greater than optimal, so that N was not readily available to the soil for plant N uptake. In these cases, from non-agricultural soil uses, the biological test may not be a good indicator of N availability. Looking at the C:N ratio of these samples, the C mineralization rate in 24 days was extremely high, but the N mineralization was extremely low. The C:N ratio of the mineralizable fraction ranged from 13-145 (Table 15). More samples would be needed to fully analyze and make conclusions about the high C availability effects on net N mineralization. These samples were taken out of the data set and only the samples from NC, OK, and PA were analyzed further.

2.3. Selection procedure to choose best variables to predict greenhouse growth

The variable selection procedure (proc GLMSELECT) across the 105 samples and 34 explanatory variables yielded the flush of CO_2, available N, particulate organic N, Zn, and K as the most important variables to predict plant N uptake in Greenhouse Trial 2. An ANOVA (proc GLM) was conducted to determine the significance of the selected variables. Particulate organic N, Zn, and K were not significant in the multiple linear regression (proc GLM). Using the flush of CO_2 and available N yielded an adjusted R^2 of 0.66 and 0.80 for dry matter and plant N uptake, respectively. Additional analysis was conducted to determine if other factors accounted for variability in greenhouse growth, due to the relatively low relationship of the flush of CO_2 and available N to dry matter production. This was conducted through a closer look at the sample groupings, such as in OK and PA in the following sections.

2.4. Key drivers of greenhouse growth in Oklahoma

In the pasture fields of Oklahoma, two grazing practices were used: continuous grazing and rotational grazing. All samples were taken at 0-6 cm depth (n=35). An ANOVA to predict greenhouse growth was conducted to test the relationship of the flush of CO_2 with grazing type to see if the relationship was altered by management. The analysis yielded only the flush of CO_2 as a significant main effect $F(1, 34) = 99$ ($p < 0.001$) to predict plant N uptake (Figure 12). The interaction of the flush of CO_2 and grazing type was not significant ($p=0.12$). Similar results were obtained for plant N uptake. In a simple linear regression model, the flush of CO_2 explained 73% of the variation in plant N uptake and 52% of the

variation in dry matter production. Continuous and rotational grazing types did not affect the relationship of the flush of CO_2 to predict greenhouse growth

To further explore the variation in greenhouse growth, a selection procedure (proc GLMSELECT) chose Zn and initial inorganic N as the best variables to predict plant N uptake (R^2=0.84). To predict dry matter production, the selection procedure chose clay, Cu, and Zn. This model predicted 87% of the variation in dry matter production.

Including clay in the model with the flush of CO_2 improved the fit of dry matter production from 52% to 76%. To predict plant N uptake, the model was improved by 11% (R^2=0.84). A simple correlation of clay and greenhouse growth was conducted, where clay content and greenhouse growth were highly negatively correlated (r= -0.81 and -0.74 for dry matter production and plant N uptake, respectively). Figure 13 shows the linear negative relationship of clay content and greenhouse growth, expressing that as clay content increased, the rate of the flush of CO_2 decreased. The range in clay from the OK samples was 14-30%. The high clay content in some surface samples was most likely due to historical soil erosion at this site. The organic rich, sandy topsoil was eroded over many years, leaving behind a substrate-poor clayey soil in its place. Therefore, the negative impact of clay in the model was potentially due to the erosion of the substrate-rich topsoil and exposure of low C substrate soil, rather than clay content per se.

The average Zn and Cu contents were not higher compared to other soils in the greenhouse trials. These variables were most likely collinear and not actual predictors of greenhouse growth. In conclusion, the flush of CO_2 by itself in this case was not the best indicator of greenhouse growth in the 35 samples from OK pasture. The interaction of the flush of CO_2 with clay was important in indicating greenhouse growth. Clay content

negatively impacted greenhouse growth, namely dry matter production. Understanding soil characteristics and management history were key in this greenhouse trial beyond using the flush of CO_2 alone.

2.5. Effect of cover crops in Pennsylvania

Across the 70 soil samples in Pennsylvania, the flush of CO_2 by itself explained 68% of the variance in plant N uptake and 62% of the variance in dry matter production. Adding cover crop management (simple versus complex) and location (Berks, Lancaster, and Montour) improved the model by 12% (R^2=0.80).

Available N by itself was the best predictor of plant N uptake (R^2= 0.89). The interaction of available N with location or management was not significant. This addition of initial inorganic N to help predict plant N uptake was seen before in other analyses suggesting that in some cases, initial N is important in explaining the variance of greenhouse growth and predicting potentially available N from soil. In high total organic C containing soils such as ones managed with cover crops or pasture, initial inorganic N may be an additional variable important to predict plant N uptake. For this reason, the explained variability by location and management to predict greenhouse growth with the flush of CO_2 may be due to initial inorganic N. Thus, initial inorganic N was added to the flush of CO_2 to determine if it improved the model without location or management.

Using initial N plus the flush of CO_2 improved the prediction of plant N uptake by 20% (R^2=0.88). This variability explained by the flush of CO_2 with initial N was almost as strong as using available N. Dry matter production was improved by 8%, with an R^2 of 0.70 when including initial N with the flush of CO_2. Due to the length of incubation for

mineralizable N (24 days), measuring the flush of CO_2 with initial inorganic N would be a more timely and effective indicator of greenhouse growth in the samples from PA.

2.6. Does previous crop affect the prediction of net N mineralization from soils along Dan River?

In the analysis of Dan River samples, only relationships among various soil biological properties were possible due to stunted growth of some sorghum sudangrass plants, namely in the tobacco fields along the Dan River. Soil biological properties highly correlated with net N mineralization in order from highest correlation to smallest were soil microbial biomass C, total organic C, the flush of CO_2, total soil N, particulate organic N, particulate organic C, C mineralization after 3, 10, and 24 days, bulk density, and available N (Table 14).

An ANOVA was conducted on simply the flush of CO_2 as a possible predictor of soil net N mineralization (Figure 14). The flush of CO_2 accounted for 77% of the variance in net N mineralization ($p<0.001$). The interaction of the flush of CO_2 and previous crop grown was not significant in these samples. Soil microbial biomass C and total organic C also predicted 77% of the variation in net N mineralization. Total soil N predicted 76% of the variation in net N mineralization. Clay and sand were not significant when added to the linear regressions of any of the biological predictors. In conclusion, the flush of CO_2 was a good indicator of net N mineralization in the Dan River samples in Greenhouse Trial 2, but was comparable to soil microbial biomass C, total organic C, and total soil N.

2.7. *Conclusions of Greenhouse Trial 2*

Relationship of the flush of CO_2 and net N mineralization to greenhouse growth was slightly altered among different states (Table 16). The best indicator of greenhouse growth across PA and OK was total soil N (R^2=0.82). The flush of CO_2 explained 64% of the variation in dry matter production and 70% of the variation in plant N uptake. A model including initial N with the flush of CO_2 improved the fit by 7% for dry matter production and 16% for plant N uptake. Including initial N with the flush of CO_2 would be the best indicators for plant N uptake.

3. Greenhouse Trial 3

3.1. Overall determinants of greenhouse growth

Greenhouse Trial 3 consisted of 101 soil samples from NC (n=45) and GA (n=56). Five farms in western NC were sampled from three depths following either corn, cotton, or soybean. The samples from Georgia were selected from three different studies for their ability to provide a gradient of low to high N supplying capacity. Soil samples were collected under conventional till, no till, and tall fescue pastures.

In Greenhouse Trial 3, dry matter production was related to plant N uptake (R^2 = 0.86) (Figure 15). Many soil biological properties were highly correlated to greenhouse growth (using an a priori threshold of $r > 0.70$), including the flush of CO_2. Some soil chemical properties were also highly correlated to greenhouse growth, including cation exchange capacity (CEC), initial inorganic N, and total available N. Total soil N was highly correlated to greenhouse growth in this trial (r=0.95). As opposed to Greenhouse Trial 1 and perhaps due to the limited number of soil types, total organic C was highly correlated to

greenhouse growth (r=0.94). Table 17 summarizes the variables highly correlated to dry matter production and plant N uptake.

3.2. Variable selection procedure

Proc GLMSELECT was used to select variables (total of 34 available) to greenhouse growth across the 101 samples in Greenhouse Trial 3. This analysis to predict plant N uptake yielded variables of the flush of CO_2, C mineralization after 10 and 24 days, available N, and initial inorganic N as the most important (R^2=0.98). An ANOVA model using multiple linear regression explained 97% of the variation. However, the Type III Sums of Squares determined that C mineralization after 10 and 24 days and initial inorganic N were not significant (p=0.74, p=0.21, and p=0.79), most likely because they were collinear to the flush of CO_2, so including them in the model did not explain additional variability. The flush of CO_2 and available N were significant (p<0.001). The Type I sum of squares analysis to explain plant N uptake using the flush of CO_2 yielded a much higher F Value (3478.5) than when using available N (42.6). This indicates that the flush of CO_2 by itself was a large contributor to predicting variation in plant N uptake. The flush of CO_2 by itself in a model explained 96% of the variation in plant N uptake (Table 8).

The selection procedure (proc GLMSELECT) chose the flush of CO_2, K, and initial inorganic N as the most important variables to predict plant dry matter production (R^2 =0.78). The ANOVA model with these three variables determined that K was not significant (p=0.38). Only the flush of CO_2 (p<0.001) and initial inorganic N (p=0.03) were significant. This model explained 79% of the variation in plant dry matter production. Samples were

analyzed more closely to determine additional variables that would improve the model to predict dry matter production in the following sections.

3.3. Previous crop effect in Cleveland County NC

Five farms near Shelby, NC (45 samples) were analyzed together to determine if there was an effect of previous crop for prediction of greenhouse growth. The best fitting model for plant N uptake was using the flush of CO_2, which explained 90% of the variance. Previous crop effects may have also been due to different farming styles, as there were few replications of management among different farmers. All farms operated under long term no tillage conditions. The interaction of the flush of CO_2 and previous crop was significant ($p<0.001$). One farm with corn as previous crop had a different relationship between the flush of CO_2 and plant N uptake. The slope of the flush of CO_2 to predict plant N uptake for four farms was between 0.010-0.012 mg N mg^{-1} CO_2-C, while the slope for the corn previous crop was 0.08 mg N mg^{-1} CO_2-C. Another farm that had corn as previous crop was not significantly different than farms with cotton or soybean as previous crop.

In conclusion, previous crop was not a major influence for determining the prediction of plant N uptake by the flush of CO_2. While one farm was different, the influence was possibly due to farm style or other inherent characteristics, and not necessarily the previous crop. The flush of CO_2 was a good overall indicator of plant N uptake for the farms in Cleveland County (R^2= 0.90).

3.4. Soil gradients from Georgia

Selected soil samples from three long-term studies in Georgia (Watkinsville and Shellman) were analyzed as a 'gradient' of soil biological activity. One set of samples from Watkinsville, GA was sampled in 2004 (n=13) and sampled under sorghum and wheat trials. Another set of soil from Watkinsville (n=23) was sampled in 1997 under grazed tall fescue pastures. In Shellman, GA 20 samples were collected from 0-15 cm depths in 2013 following peanut, cotton, and corn. The goal of the gradient studies was to relate the predetermined flush of CO_2 to greenhouse growth with the assumption that available N would be a direct process-level factor. The flush of CO_2 explained 76% of the variation in dry matter production and 96% of the variation in plant N uptake.

The discrepancy between the two parameters of greenhouse growth indicated that another soil property was preventing dry matter production as compared to the best fit in Greenhouse Trial 1 (Figure 15). The relationship between plant N uptake and dry matter production for all samples in Greenhouse Trial 3 was R^2=0.85. The 15% variation was thus explained by some other factor in the soil samples. Data points furthest from the regression line were primarily from samples in the tall fescue study, which had the highest level of greenhouse growth. In these samples, sorghum-sudangrass plants in the greenhouse had high N uptake but varied widely in dry matter production (Figure 15). Removing all samples from the tall fescue study (n=33) from the model improved the flush of CO_2 to explain 90% of the variation in dry matter production overall, up by 14%.

Other variables were scrutinized for possible contribution to the unique relationship in the tall fescue study. In the tall fescue pasture soils, S, K, Mg, and P were highly correlated to dry matter production. Other soil properties highly correlated were SMBC, the

flush of CO_2, CEC, initial nitrate, total soil N, total organic C, ending ammonium, total available N, and net N mineralization. These soil properties were further evaluated to determine underlying causes for unique dry matter production from this tall fescue study.

For the tall fescue soils, the flush of CO_2 predicted 95% of the variation in plant N uptake and 63% of the variation in dry matter production. Total soil N predicted 91% of the variation in plant N uptake and only 52% of the variation in dry matter production. Total organic C explained 91% of the variation in plant N uptake and 50% of the variation in dry matter production. Nutrient toxicities and deficiencies were explored to investigate the lower dry matter production in the tall fescue samples. Zn and Cu did not seem to cause toxicity to sorghum-sudangrass seedlings (Figure 16). The soil samples high in Zn (>30 meq/100cc) were lower in dry matter but also lower in plant N uptake. The samples with dry matter production much lower than plant N uptake did not have excess Zn or Cu. Dry matter production was always low when S was >61 meq/100cc. High S may have contributed to low dry matter production, but the relationship was not clear (Figure 16).

Another hypothesis is that robust sorghum-sudangrass plants with highest dry matter production became water stressed, thus limiting further growth despite availability of N (Figure 17). This was seen in the greenhouse stress analysis in the first section, where the only significant differences in stress occurred from plants with greater greenhouse growth. This variable was supposed to be controlled and thus not accounted for in this trial. In addition, Greenhouse Trial 3 had the highest average temperature among the four greenhouse trials (Figure 4), suggesting that higher temperature may have induced additional water stress.

Overall, the flush of CO_2 was an excellent indicator of plant N uptake. Understanding other soil properties that affect dry matter production was important for predicting dry matter production, such as nutrient toxicities, temperature, water holding capacity, and total C substrate availability. This trial demonstrated that in cases with sufficient N availability, crop yield may be limited by other factors in the soil. Thus, it is critical to characterize soil properties prior to fertilization to understand how dry matter production may be affected.

4. Greenhouse Trial 4

4.1. Selecting the key variables to predict greenhouse growth

A total of 354 soil samples from NC and VA farms were used in Greenhouse Trial 4. Dry matter production and plant N uptake were not as highly related in this data set (R^2=0.77), as in other trials. Three outliers were identified (Figure 18a) and removed. These outliers were sampling replicates of the same farm at the surface depth managed with poultry litter application. Zinc concentration in these samples was unusually high, potentially creating toxicity that limited dry matter production even with sufficient available N (Figure 18b). A model that analyzed dry matter production with outliers removed improved the fit by 9% (R^2=0.86) (Figure 19). Some results between dry matter production and plant N uptake were still not congruent in this trial, so these responses were reported separately.

The following analyses include an initial correlation procedure of greenhouse growth with all 34 soil properties; a statistical selection procedure that chose the best variables to predict greenhouse growth, and a principal component analysis using these selected variables, which was then run as a model to predict greenhouse growth.

4.2. Soil biological properties correlated to greenhouse growth

The flush of CO_2, net N mineralization, SMBC, particulate organic C and N, and other soil biological properties were highly correlated to greenhouse growth. Table 18 shows all variables that were highly correlated to both dry matter production and plant N uptake (using an a priori threshold of $r > 0.7$). Total organic C was not correlated to dry matter production (r=0.49) or plant N uptake (0.46). Total soil N was highly correlated with dry matter production (0.75) and plant N uptake (0.77). In conclusion, many biological indicators were highly correlated to greenhouse growth in this data set.

4.3. Selection procedure to choose best variables to predict greenhouse growth

Proc GLMSELECT was used to select variables (total of 34 available) to predict plant N uptake across 351 samples in Greenhouse Trial 4. The flush of CO_2, available N, total soil N, initial inorganic N, and P were chosen as the most important variables to predict plant N uptake in Greenhouse Trial 4. Variables chosen were congruent with variables chosen in other analyses, e.g. in Greenhouse Trial 1, the flush of CO_2, SMBC, particulate organic C, and available N were chosen. Multiple linear regression using the variables determined by the selection procedure for Greenhouse Trial 4 yielded an adjusted R^2 of 0.92 for plant N uptake.

Proc GLMSELECT chose the flush of CO_2, available N, clay, total soil N, and P as the best predictors of dry matter production. This model explained 79% of the variation in dry matter production. Additional variation was further investigated to predict dry matter production in the following sections.

4.4. Principal component analysis of Greenhouse Trial 4

The first three principal components predicted 94% of the variation in dry matter production using the flush of CO_2, total soil N, available N, and initial inorganic N (Table 19). The first principal component explained 73%, the second principal component explained 11%, and the third principle component explained 10%. The flush of CO_2 and available N had similar loading values of 0.48 and 0.49 in the first component, suggesting that these two biological indicators explained the most variability in plant N uptake (Table 20Table). The high correlation (r=0.92) between the flush of CO_2 and available N suggested strong collinearity. Running the first three principal components to predict plant N uptake in a multiple linear regression resulted in a model with an adjusted R^2 of 0.90.

In Greenhouse Trial 1, the model selected for the principal component analysis included the flush of CO_2, available N, and particulate organic C and N. This same model, when run in Greenhouse Trial 4 yielded similar results as Greenhouse Trial 1. The first principal component explained 90% of the variance, and the second component explained an additional 7%. A multiple linear regression of the first two components however only explained 71% of the variation in plant N uptake.

4.5. Further testing the relationship of soil biological properties with greenhouse growth

Similar to Greenhouse Trial 1, the biological indicators were explored individually to test the ability of these properties to predict greenhouse growth. While the principal components in this analysis were significant, the similar loading values suggest that covariance may have occurred among biological variables. Therefore, available N, net N

mineralization, the flush of CO_2, SMBC, total organic C, and total soil N were explored separately.

Table 8 summarizes the main effects of the biological predictors in Greenhouse Trial 4. The biological indicators predicted plant N uptake with greater strength than dry matter production. The best indicator of plant N uptake was available N (R^2 =0.83). This is another indication that in certain cases, knowing the initial inorganic N of a soil is helpful in predicting potentially available N. The best indicator of dry matter production was also available N followed by the flush of CO_2 and net N mineralization. To predict dry matter production, the R^2 of the regressions were weaker (R^2=0.70 and 0.67 for available N and the flush of CO_2, respectively).

4.6. Effect of texture on predicting plant N uptake

The interaction of texture with the flush of CO_2 to predict plant N uptake was not significant in the ANOVA model at the surface depth (p=0.32). The flush of CO_2 alone was significant in the model (p<0.001). The model explained 65% of the variance (n=118). Similar results were obtained for the subsurface depths as well.

Texture was not a significant factor in altering the rate of mineralization for Greenhouse Trial 4 (Figure 20). While the average plant N uptake for certain soil textures at the surface depth was different, the rate as affected by net N mineralization, or available N to predict plant N uptake was not different. To predict dry matter production, texture had a slight impact on the slope when using the flush of CO_2. Definitive statements on the impact of certain soil textures on the rate of N mineralization could not be made without a balanced design of soil texture in this experiment.

5. Overall analysis of the flush of CO_2

The flush of CO_2 was the parameter of interest in this study (see hypotheses and objectives p. 15), so it was analyzed for its ability to predict soil N mineralization in 24 days, plant N uptake, and dry matter production. Across 677 samples (excluding outliers from previous analyses), the flush of CO_2 explained 80% of the variation in net N mineralization, 69% of the variation in plant N uptake, and 50% of the variation in dry matter production (Table 21). Greenhouse growth (plant N uptake and dry matter production) were examined by state in order to improve the model due to largely different climate regimes and soil types (Appendix A and Appendix B).

The flush of CO_2 explained 89% of the variation in plant N uptake when the effect of state was included in the model (Table 21). The groupings of Oklahoma and Pennsylvania were significantly different from that of Virginia, North Carolina, and Georgia to predict plant N uptake using the flush of CO_2 (Figure 21; Table 22), indicating that the test may need to be calibrated for different regions with largely different climates regimes, soil types, and for cropping systems. To predict dry matter production, the interaction effect of state and the flush of CO_2 improved the model from 50% to 71% (Table 21). Dry matter had more variables associated with growth that improved the model than plant N uptake, but these variables changed depending on the soil samples and greenhouse trials. Specific instances of nutrient deficiencies or toxicities also likely occurred.

Table 22 summarizes and compares the ability of the flush of CO_2 to predict greenhouse growth by state using a linear equation versus a quadratic equation. For dry matter production, a quadratic equation markedly improved the R^2 values of GA, NC, OK,

and VA. Plant N uptake did not improve with a quadratic equation to the same magnitude as dry matter production.

Across all NC soil samples (n=336), 77% of the variation to predict plant N uptake was explained by the flush of CO_2 (slope = 0.14 mg N mg^{-1} CO_2-C). The flush of CO_2 explained 59% of the variation in dry matter production (slope = 8.6 mg DM mg^{-1} CO_2-C). Including initial inorganic N in the model to predict dry matter production improved the fit by 7% (R^2 = 0.66). The quadratic equation to predict dry matter production using only the flush of CO_2 improved the fit to predict 67% of the variation ($y= 0.01*CO_2 – 0.00002 * (CO_2)^2 + 1.09$) (Table 22).

In VA soil samples (n=180), 80% of the variation to predict plant N uptake was explained by the flush of CO_2 (slope=0.14 mg N mg^{-1} CO_2-C). The flush of CO_2 explained 70% of the variation in dry matter production (slope = 8.0 mg DM mg^{-1} CO_2-C). A quadratic transformation of the flush of CO_2 improved the prediction of dry matter production in VA by 7% (R^2=0.77). The slopes of the VA and NC samples were similar for greenhouse growth (Table 23).

Samples from NC and VA were the most closely related of all states, in terms of temperature and climate regimes. The flush of CO_2 predicted 81% of the variation in plant N uptake and 66% of the variation in dry matter production for the combined samples from NC and VA (n=516). The slope to predict dry matter was 8.3 mg dry matter mg^{-1} CO_2-C and the slope to predict plant N uptake was 0.15 mg N mg^{-1} CO_2-C (Table 24)

Available N, which was often the best indicator of greenhouse growth (especially plant N uptake), was examined to see its ability to predict greenhouse growth overall. Available N (n=677) predicted 62% of the variation in plant N uptake and 50% of the

variation in dry matter production. When including the effect of state, the model markedly improved to 90% of plant N uptake and 74% of the variation in dry matter (Table 21). Available N was not a good indicator of greenhouse growth in OK samples (Table 25). The flush of CO_2 was generally a better predictor of dry matter production than available N, but plant N uptake was best predicted by available N in most cases (see Table 26).

CONCLUSIONS

A large range in dry matter and plant N uptake occurred in Greenhouse Trials 1-4 from different agricultural sites. Table 21 summarizes the biological indicators' ability to predict greenhouse growth over all samples (n=677). The flush of CO_2 was an excellent predictor of plant N uptake with soil samples from the southeast (NC, VA, and GA). Other reliable predictors included available N, net N mineralization, and soil microbial biomass C. Soil organic C and total soil N were highly significant in some cases, but were less predictive across different soil types due to differences in stability of organic matter, where in many cases a high organic matter content did not release the N or have active C present.

Available N was the best indication of greenhouse growth in most cases. If resources and time were unlimited to measure any soil N parameter, available N would have been the best indicator of greenhouse growth in this study. However, given time restraints, the second best indicator would be the flush of CO_2, with initial inorganic N included when possible. The flush of CO_2 was highly related to net N mineralization, and adding initial inorganic N in certain cases helped predict more of the variation in greenhouse growth. Across all samples, initial inorganic N was not nearly as important as the flush of CO_2 in explaining variability.

Dry matter production was a more elusive response variable to predict for many reasons. Nutrient toxicities and deficiencies were potential problems for predicting dry matter production effectively. Water content and temperature are not controllable in the field, but should be controlled in a greenhouse experiment for use as a standard measure. Differences in water stress using the current study design may have been possible due to uniform tray watering across different soil textures. Management practices added some additional explanation for variability in dry matter production, but was usually due more to inorganic N

or the C:N ratio as a result of management. In NC, VA, GA, and OK, the R^2 improved by 7-10% when using a quadratic fit to predict both dry matter production and plant N uptake by the flush of CO_2 (Table 25).

Texture did not always significantly improve the model with the biological variables to predict greenhouse growth. However, a more balanced design of soil texture classes with a range of soil N supplying capacities could improve the understanding of soil texture and plant growth. Water content interacting with textural class may have been an uncontrolled factor in this study design.

Given the simple, cost effective, and rapid properties of this test, the flush of CO_2 has potential to be a routine test for predicting plant available N from soils in the mid-Atlantic United States, especially in North Carolina, Virginia, and Georgia. Additional field validation research needs to be conducted and would improve the understanding of the relationship of the flush of CO_2 to crop yield. Soil testing facilities and farmers could use the flush of CO_2 to make changes to N fertilizer applications to improve economic return and environmental protection. In terms of actual crop yield, it is crucial to examine all soil properties using a routine soil test for other factors that affect dry matter production. Knowing that conservation agricultural management approaches positively alter soil biological properties, farm advisors and producers have numerous options to help promote more biologically active soil.

The need for managing N effectively and efficiently is more pressing every day as the population increases and energy costs continue to rise. N fertilizer has the potential to be both a solution and a threat to food security, depending on how we choose to manage it. Application of the right N fertilizer rate, coupled with an understanding of each agricultural

soil on a case-by-case basis, are valid approaches to assist producers, satisfy consumers, and protect the environment for the long-term.

TABLES

Table 1. Description of soils used for laboratory analysis and greenhouse trials (grouped by state and greenhouse trial)

State, City	Source / Management §	Date Sampled	Sampling Depth (cm)	# Soil Samples	Soil pH
	GH Trial 1				
NC					
Lewiston-Woodville	Research station following corn in 3 fields with history of low poultry litter, high poultry litter, and no poultry litter or N applications	May 2014	0-10, 10-20, 20-30	36	5.9 ± 0.4
Plymouth	Research station in 3 fields with no till and conventional till following corn and with grass hay receiving livestock effluent	May 2014	0-10, 10-20, 20-30	36	5.4 ± 0.4
Kinston	Research station following soybean with conventional tillage	Apr 2014	0-10, 10-20, 20-30	12	6.0 ± 0.1
Mills River	Research station following wheat with conventional tillage	May 2014	0-10, 10-20, 20-30	12	5.9 ± 0.2
Salisbury	Research station following soybean with long-term no tillage	Apr 2014	0-10, 10-20, 20-30	12	6.4 ± 0.2
VA					
McKenney	3 farm fields with grazed tall fescue pasture	Jul-Aug 2014	0-10	12	6.4 ± 0.7
	GH Trial 2				
PA ¶					
Danville	Farm field with crop-pasture rotation; following barley and winter cover cropping with 4-species mix (canola, pea, rye, clover) and 3-species mix (triticale, pea, clover) and history of manure application	Jun 2014	0-10, 10-20, 20-30	24	6.9 ± 0.3

Table 1 continued

Reading	Farm field with crop-pasture rotation; following wheat and winter cover cropping with single species (rye) and 3-species mix (rye, radish, clover) and history of manure application	Jun 2014	0-10, 10-20, 20-30	22†	5.7 ± 0.3
Lancaster	Farm field with crop-pasture rotation; following spelt and winter cover cropping with single species (red clover) and 4-species mix (canola, pea, rye, clover) and history of manure application	Jun 2014	0-10, 10-20, 20-30	24	6.8 ± 0.1
NC					
Eden	Farm fields along Dan River with previous cropping of corn (n=27), soybean (n=9), tobacco (n=11), wheat (n=4) pasture (n=9), and fallow fields (n=12)	Jun 2014	0-15	73	5.5 ± 0.8
OK					
El Reno	Research station with native prairie grazed continuously and rotationally	Feb-Mar 2012	0-6	35	6.2 ± 0.2
NE					
Nebraska City	Arboretum under *Quercus rubra*, *Juglans nigra*, *Pinus strobus*, *Gingko biloba*	May 2014	0-10	4	6.1 ± 0.9
GA					
Watkinsville	Farm field with bamboo and turf	Oct 2014	0-10	2	5.7 ± 0.4
	GH Trial 3				
NC					
Shelby	5 farm fields with long-term no tillage following corn, cotton, and soybean	Nov-Dec 2014	0-10, 10-20, 20-30	45	6.2 ± 0.5
GA					
Shellman	Research station with conventional tillage and irrigation following peanut, cotton, and corn	Dec 2013	0-15	20	6.7 ± 0.4
Watkinsville	Research station following sorghum and wheat with cover crops under conventional and no till §	Dec 2004	3-6, 6-12, 12-20, 20-30	13	5.9 ± 0.2

Table 1 continued

Watkinsville	Research station in grazed tall fescue pastures with history of endophyte and fertilization differences ¶	Feb 1997	0-5, 5-7.5, 7.5-15	23	5.7 ± 0.2
	GH Trial 4				
NC					
Camden	Farm following corn with conventional tillage	Apr 2015	0-10, 10-20, 20-30	12	5.6 ± 0.2
Goldsboro	Research station following cotton with no till and irrigation	May 2015	0-10, 10-20, 20-30	12	6.2 ± 0.2
Kinston	Research station following soybean with no till	May 2015	0-10, 10-20, 20-30	12	5.3 ± 0.2
Mills River	Research station in 2 fields following corn with conventional till	May 2015	0-10, 10-20, 20-30	24	6.2 ± 0.2
Plymouth	Research station in 2 fields following soybean with conventional tillage	Apr 2015	0-10, 10-20, 20-30	24	5.9 ± 0.2
Plymouth	Research station in naturalized pasture over seeded annually with ryegrass	Mar 2015	0-10, 10-20, 20-30	45	5.7 ± 0.3
Salisbury	Research station in 2 fields following soybean with long-term no till and repeated poultry litter and dairy slurry application	Apr 2015	0-10, 10-20, 20-30	9	6.8 ± 0.2
Salisbury	Research station following soybean with long-term no till	Apr 2015	0-10, 10-20, 20-30	24	6.4 ± 0.4
Norwood	Farm with long-term no till and multi-species cover crops on upland (following cotton) and bottomland (following corn) fields	May 2015	0-10, 10-20, 20-30	24	6.2 ± 0.5
VA					
Calverton	3 farm fields following corn and soybean with long-term no till and history of manure application	Apr/May 2015	0-10, 10-20, 20-30	39	6.1 ± 0.5
Harrisonburg	11 farm fields following corn or soybean with long-term no till and history of manure application	Apr/May 2015	0-10, 10-20, 20-30	129	6.7 ± 0.5
Total				**759**	

† Sample from Depth 20-30 cm missing
§ Franzluebbers and Stuedemann, 2008
¶ Franzluebbers et al., 1999

Table 2. Analysis of variance for dry matter production as a function of field site, depth, and stress (n=108) Trial 1

Source of Variation	**Degrees of Freedom**	**F-Value**	**Pr > F**
Field Site	8	166.0	<0.001
Depth	2	167.5	<0.001
Field Site x Depth	16	5.2	0.024
Water Stress	2	3.2	<0.001
Depth x Water Stress	4	1.9	0.114
Field Site x Water Stress	16	1.8	0.025
Field Site x Depth x Water Stress	32	1.6	0.024

Table 3. Effect of soil depth (cm) and water stress level (% water at lower limit) on dry matter production (mg g^{-1} soil) at each field site in Greenhouse Trial 1

Location	Management	Depth (cm)	Dry Matter (g kg^{-1} soil)	Water Stress (%)	Dry Matter (g kg^{-1} soil)
Kinston	Conventional Tillage	**0-10**	1.79 a	**80**	1.56 a
		10-20	1.52 b	**60**	1.56 a
		20-30	1.33 c	**40**	1.52 a
Lewiston-Woodville	High Poultry Litter	**0-10**	2.20 a	**80**	1.80 a
		10-20	1.59 b	**60**	1.73 a
		20-30	1.42 c	**40**	1.42 a
Lewiston-Woodville	Low Poultry Litter	**0-10**	1.80 a	**80**	1.51 a
		10-20	1.37 b	**60**	1.46 a
		20-30	1.28 c	**40**	1.50 a
Lewiston-Woodville	Zero Poultry Litter	**0-10**	1.51 a	**80**	1.35 a
		10-20	1.33 b	**60**	1.37 a
		20-30	1.21 c	**40**	1.33 a
McKenney	Pasture 1	**0-10**	*	**80**	2.12 a
		10-20	*	**60**	2.13 a
		20-30	*	**40**	2.14 a
McKenney	Pasture 2	**0-10**	*	**80**	2.45 a
		10-20	*	**60**	2.31 a
		20-30	*	**40**	2.15 b
McKenney	Pasture 3	**0-10**	*	**80**	1.86 a
		10-20	*	**60**	1.84 a
		20-30	*	**40**	1.84 a
Mills River	Conventional Till	**0-10**	1.56 a	**80**	1.48 a
		10-20	1.49 b	**60**	1.46 a
		20-30	1.40 b	**40**	1.48 a

Table 3 continued

Plymouth	Conventional Till	**0-10**	1.93 a	**80**	1.64 a
		10-20	1.55 b	**60**	1.55 a
		20-30	1.38 c	**40**	1.38 b
Plymouth	No Till	**0-10**	1.86 a	**80**	1.66 a
		10-20	1.63 b	**60**	1.61 a
		20-30	1.40 c	**40**	1.62 a
Salisbury	No Till	**0-10**	1.89 a	**80**	1.65 a
		10-20	1.52 b	**60**	1.56 a
		20-30	1.40 c	**40**	1.60 a

* Sampling depths at 10-20cm and 20-30cm not taken

Table 4. Interaction effect of depth x water stress in Plymouth at hayed forage site in Greenhouse Trial 1

Location	**Stress (%)**	**Depth (cm)**		
		0-10	**10-20**	**20-30**
Plymouth, Hay	**80**	2.80a	2.13 a	1.91 a
	60	2.57b	2.03 a	1.84 a
	40	2.38c	2.10 a	1.88 a

Table 5. Pearson correlation coefficients of greenhouse growth versus various soil N properties in Greenhouse Trial 1 (n=120)

Dry Matter Production (g DM kg^{-1} soil)		**Plant N Uptake (mg N kg^{-1} soil)**	
Particulate Organic C	0.92	Particulate Organic C	0.92
Net N Mineralization	0.90	Net N Mineralization	0.90
Flush of CO_2	0.88	Soil Microbial Biomass C	0.90
Particulate Organic N	0.88	Flush of CO_2	0.89
Soil Microbial Biomass C	0.87	Particulate Organic N	0.88
Available N	0.87	Available N	0.88
C Mineralization (24d)	0.84	C Mineralization (3-10d)	0.85
C Mineralization (3-10d)	0.84	C Mineralization (24d)	0.85
C Mineralization (10-24d)	0.79	C Mineralization (10-24d)	0.80
CEC	0.76	Initial NH_4-N	0.78
Initial NH_4-N	0.75	CEC	0.78
Final NO_3-N	0.73	Final NO_3-N	0.73
Calcium	0.70	Total soil N	0.70
Total soil N	0.68	Calcium	0.69

Table 6. Weight (Eigenvectors) of each variable in principal components 1-4 in Greenhouse Trial 1

	Eigenvectors				
	Principal 1	**Principal 2**	**Principal 3**	**Principal 4**	**Principal 5**
Flush of CO_2	0.46	-0.03	-0.12	-0.84	-0.27
Particulate Organic C	0.46	0.09	-0.33	0.51	-0.64
Particulate Organic N	0.46	-0.24	-0.53	0.12	0.66
Soil Microbial Biomass C	0.43	0.77	0.37	0.06	0.27
Available N	0.43	-0.58	0.68	0.16	0.01

Table 7. Correlation matrix of variables used in principal component analysis of Greenhouse Trial 1

	Flush of CO_2	**Particulate Organic C**	**Particulate Organic N**	**Soil Microbial Biomass C**	**Available N**
Flush of CO_2	1.00	0.92	0.96	0.89	0.82
Particulate Organic C	0.92	1.00	0.96	0.90	0.84
Particulate Organic N	0.96	0.96	1.00	0.85	0.82
Soil Microbial Biomass C	0.89	0.90	0.85	1.00	0.76
Available N	0.82	0.84	0.82	0.76	1.00

Table 8. Slope and R^2 of biological predictors of greenhouse growth grouped in greenhouse trials 1-4 using simple linear regressions

Predictor	Predictor Units	Slope				R^2 (*100)			
		Trial 1 (n=120)	Trial 2 (n=105)	Trial 3 (n=101)	Trail 4 (n=351)	1	2	3	4
		Dry Matter (g DM kg^{-1} soil)							
Flush of CO_2	mg CO_2-C kg^{-1} soil 3 d^{-1}	0.013	0.003	0.006	0.008	78	64	78	67
N Mineralization	mg N kg^{-1} soil 24 d^{-1}	0.032	0.015	0.040	0.027	81	51	75	67
Available N*	mg N kg^{-1} soil	0.026	0.014	0.029	0.025	76	56	76	70
Soil Microbial Biomass C	mg C kg^{-1} soil	0.003	0.001	0.003	0.002	77	47	73	55
Total Organic C	g C kg^{-1} soil	0.036	0.063	0.150	0.045	24	74	72	23
Total Soil N	g N kg^{-1} soil	1.04	0.91	1.90	1.16	46	77	75	54
		Plant N Uptake (mg N kg^{-1} soil)							
Flush of CO_2	mg CO_2-C kg^{-1} soil 3 d^{-1}	0.167	0.039	0.148	0.141	79	70	96	83
N Mineralization	mg N kg^{-1} soil 24 d^{-1}	0.421	0.181	0.880	0.505	82	57	95	83
Available N*	mg N kg^{-1} soil	0.338	0.180	0.723	0.460	77	68	96	88
Soil Microbial Biomass C	mg C kg^{-1} soil	0.039	0.015	0.072	0.036	80	55	87	63
Total Organic C	g C kg^{-1} soil	0.484	0.762	3.68	0.698	27	79	89	21
Total Soil N	g N kg^{-1} soil	13.87	11.01	46.00	20.04	49	82	91	60

*Available N improved to 82 and 84% for dry matter and plant N uptake when using quadratic fit and square root of dependent variable

Table 9. Analysis of variance for dry matter and plant N uptake as affected soil biological parameters and their interaction with texture in Greenhouse Trial 1 (n=120)

Source	F Value	Pr > F	R^2
	Plant N Uptake (mg N kg^{-1} soil)		
Flush of CO_2	463	<0.001	0.86
Flush of CO_2 x Texture	13	<0.001	
Available N	422	<0.001	0.84
Available N x Texture	13	<0.001	
Net N Mineralization	403	<0.001	0.85
Net N Mineralization x Texture	6	0.004	
	Dry Matter (g DM kg^{-1} soil)		
Flush of CO_2	406	<0.001	0.84
Flush of CO_2 x Texture	12	<0.001	
Available N	315	<0.001	0.81
Available N x Texture	7.64	<0.001	
Net N Mineralization	343	<0.001	0.83
Net N Mineralization x Texture	3	0.011	

[1] Textural classes: clay (n=12) clay loam (n=22) loam (n=20) sandy clay loam (n=20) and sandy loam (n=46)

Table 10. Regression parameters (intercept and slope by textural class) to describe the effect of soil biological properties (available N, net N mineralization, and the flush of CO_2) on plant N uptake and dry matter production in Greenhouse Trial 1 (with sandy loam as reference variable) (n=120)

Source	Available N	Net N Mineralization	Flush of CO_2
	Plant N Uptake (mg N kg^{-1} soil)		
Intercept	13.9	16.5	13.8
Clay	0.38 b	0.47 a	0.19 a
Clay Loam	0.44 b	0.49 b	0.21 b
Loam	0.39 b	0.44 a	0.20 b
Sandy Clay Loam	0.35 b	0.41 a	0.14 a
Sandy Loam*	0.28 a	0.36 a	0.15 a
	Dry Matter (g DM kg^{-1} soil)		
Intercept	1.5	1.7	1.5
Clay	0.028 a	0.036 a	0.015 a
Clay Loam	0.033 b	0.037 b	0.016 c
Loam	0.028 b	0.033 a	0.015 a
Sandy Clay Loam	0.025 a	0.033 a	0.010 b
Sandy Loam*	0.022 a	0.30 a	0.12

* a, b, and c denote whether the slopes are significantly different ($p < 0.05$) for soil textures

Table 11. Analysis of variance for plant N uptake and dry matter production as affected by the flush of CO_2 and its interaction with sand content in Greenhouse Trial 1 (n=120)

Source	Degrees of Freedom	Type III Sums of Squares	F Value	Pr > F
		Plant N Uptake (mg N kg^{-1} soil)		
Flush of CO_2	1	9859.0	217	<0.001
Flush of CO_2 x Sand	1	1519.5	33	<0.001
		Dry Matter (g DM kg^{-1} soil)		
Flush of CO_2	1	56.2	186	<0.001
Flush of CO_2 x Sand	1	8.1	27	<0.001

Table 12. Mean and standard deviation of plant N uptake and dry matter production as affected by soil texture in Greenhouse Trial 1 (n=120)

Texture	# Observations	Mean	Standard Deviation
		Plant N Uptake (mg N kg^{-1} soil)	
Clay	12	30.4	7.1
Clay Loam	22	44.4	17.8
Loam	20	27.0	16.8
Sandy Clay Loam	20	31.7	16.4
Sandy Loam	46	31.0	15.7
		Dry Matter Production (g DM kg^{-1} soil)	
Clay	12	2.6	0.7
Clay Loam	22	3.8	1.4
Loam	20	2.4	1.4
Sandy Clay Loam	20	2.8	1.2
Sandy Loam	46	2.9	1.2

Table 13. Mean values for soil nitrate (NO_3-N) and available N in samples collected from Lewiston-Woodville, NC managed with three different poultry litter treatments

Treatment	Depth (cm)	Average NO_3-N (mg NO_3-N kg^{-1} soil)	Average Available N (mg N kg^{-1} soil)
No poultry litter	0-10	9 ± 7	37 ± 1
	10-30	1 ± 0.2	13 ± 4
Low poultry litter	0-10	21 ± 5	110 ± 19
	10-30	8 ± 4	20 ± 7
High poultry litter	0-10	53 ± 34	162 ± 80
	10-30	12 ± 3	31 ± 11

Table 14. Pearson correlation coefficients of various soil properties in relationship to dry matter production (n=111), plant N uptake (n=111), and net N mineralization (n=73) in Greenhouse Trial 2 for samples collected from Dan River Valley

Dry Matter Production (g DM kg^{-1} soil)		**Plant N Uptake (mg N kg^{-1} soil)**		**Net N Mineralization (mg N kg^{-1} soil 24 d^{-1})**	
Flush of CO_2	0.74	Flush of CO_2	0.80	Soil Microbial Biomass C	0.88
Total soil N	0.74	Initial NH_4-N	0.80	Total Organic C	0.88
Potassium	0.73	Available N	0.79	Flush of CO_2	0.88
Initial NH_4-N	0.72	C Mineralization (24d)	0.76	Total Soil N	0.87
Available N	0.71	Total soil N	0.75	Particulate Organic N	0.86
C Mineralization (24d)	0.69	Particulate Organic N	0.75	Particulate Organic C	0.85
Particulate Organic N	0.68	C Mineralization (3-10d)	0.73	C Mineralization (24d)	0.84
.	.	Net N Mineralization	0.71	C Mineralization (3-10d)	0.81
.	.	C Mineralization (10-24d)	0.70	C Mineralization (10-24d)	0.75
.	.	Potassium	0.69	Density	-0.71
.	.	.	.	Available N	0.70

Table 15. Plant N uptake and dry matter production in the greenhouse as related to total organic C and N of soil from samples collected under undisturbed, non-agricultural plant canopies in Greenhouse Trial 2

State	Plant Canopy	Plant N Uptake	Dry Matter Production	Total Organic C	Total Soil N	Ratio of C mineralized: N mineralized (24 days)
		mg N kg^{-1} soil	g DM kg^{-1} soil	g C kg^{-1} soil	g N kg^{-1} soil	
GA	Eremochloa ophiuroides	17.9	1.1	21.3	1.4	56.9
GA	Bambusoideae	19.6	1.4	31.2	1.7	144.7
NE	Pinus strobus	17.0	1.4	104.1	5.1	30.6
NE	Quercus rubra	32.9	2.9	56.1	3.5	48.0
NE	Juglans nigra	35.3	3.4	85.7	4.7	40.0
NE	Gingko biloba	33.7	3.7	44.0	3.1	13.2

Table 16. Regression parameters for predicting plant N uptake and dry matter production from the flush of CO_2 as affected by state of origin in Greenhouse Trial 2 (Dan River calculated against laboratory net N mineralization only)

State Origin		Plant N Uptake (mg N kg^{-1} soil)	Dry Matter (g DM kg^{-1} soil)
PA (n=70)	**Slope**	0.077	0.006
	R^2	0.68	0.62
OK (n=35)	**Slope**	0.040	0.003
	R^2	0.73	0.52
		Net N Mineralization (mg N kg^{-1} soil 24 d^{-1})	
NC (n=73)	**Slope**	0.27	
	R^2	0.77	

Table 17. Pearson correlation coefficients of various soil properties in relationship to dry matter production and plant N uptake in Greenhouse Trial 3 (n=111)

Dry Matter Production (g DM kg^{-1} soil)		**Plant N Uptake (g DM kg^{-1} soil)**	
Flush of CO_2	0.89	Flush of CO_2	0.98
Available N	0.87	Available N	0.98
Total soil N	0.87	C Mineralization (24d)	0.98
Net N Mineralization	0.86	Net N Mineralization	0.98
C Mineralization (24d)	0.86	C Mineralization (3-10d)	0.97
C Mineralization (3-10d)	0.86	C Mineralization (10-24d)	0.96
Initial Inorganic N	0.86	Total soil N	0.95
Soil Microbial Biomass C	0.85	Total Organic C	0.94
Total Organic C	0.85	Soil Microbial Biomass C	0.93
C Mineralization (10-24d)	0.83	Initial Inorganic N	0.92
Particulate Organic C	0.81	Ending NH_4	0.92
Initial NH4-N	0.81	Particulate Organic C	0.92
Particulate Organic N	0.79	Particulate Organic N	0.88
Ending NH_4	0.77	Initial NH_4-N	0.88
CEC	0.69	Plant N concentration	0.87
Plant N concentration	0.69	CEC	0.74
.	.	Potassium	0.71

Table 18. Pearson correlation coefficients of various soil properties in relationship to dry matter production and plant N uptake in Greenhouse Trial 4 (n=351)

Dry Matter Production (g DM kg^{-1} soil)		**Plant N Uptake (mg N kg^{-1} soil)**	
Available N	0.84	Available N	0.93
Final NO_3-N	0.83	Final NO_3-N	0.92
C Mineralization (24d)	0.82	Net N Mineralization	0.91
Net N Mineralization	0.82	Flush of CO_2	0.91
Flush of CO_2	0.82	C Mineralization (24d)	0.90
C Mineralization (3-10d)	0.81	C Mineralization (3-10d)	0.89
C Mineralization (10-24d)	0.79	C Mineralization (10-24d)	0.85
Soil Microbial Biomass C	0.74	Soil Microbial Biomass C	0.80
Total soil N	0.74	Particulate Organic C	0.79
Particulate Organic C	0.72	Total soil N	0.77
Particulate Organic N	0.69	Particulate Organic N	0.77
Zinc	0.65	Plant N Concentration	0.76
Phosphorus	0.64	Initial Inorganic N	0.72

Table 19. Eigenvalues and proportion of principal components accounting for variation in Greenhouse Trial 4 (n=351)

Component	Eigenvalue	Proportion	Cumulative
1	3.58	0.90	0.90
2	0.28	0.07	0.97
3	0.11	0.02	0.99
4	0.02	0.01	1.00

Table 20. Weight (Eigenvectors) of each variable in principal components 1-4

	Eigenvectors			
	Principal 1	**Principal 2**	**Principal 3**	**Principal 4**
Flush of CO_2	0.49	0.55	0.68	0.06
Available N	0.51	-0.44	0.06	-0.74
Particulate Organic N	0.50	-0.54	0.01	0.67
Particulate Organic C	0.50	0.46	-0.74	0.01

Table 21. Strength of relationship (R^2) in predicting dry matter production and plant N uptake from the flush of CO_2 and its interaction with state of origin for samples across all greenhouse trials (n=677)

Response Variable	**Dry Matter Production (g DM kg^{-1} soil)**	**Plant N Uptake (mg N kg^{-1} soil)**
Flush of CO_2	0.50	0.69
Flush of CO_2 x state	0.71	0.89
Available N	0.50	0.62
Available N x state	0.74	0.90
Net N mineralization	0.53	0.64
Net N Mineralization x state	0.72	0.90
Total Organic C	0.28	0.26
Total Organic C x state	0.61	0.74
Total soil N	0.48	0.52
Total soil N x state	0.67	0.81

Table 22. Regression fit (R^2) of the flush of CO_2 to predict greenhouse growth by state using linear and quadratic components across all greenhouse trials

State	Plant N Uptake (mg N kg^{-1} soil)		Dry Matter Production (g DM kg^{-1} soil)	
	Linear	**Quadratic**	**Linear**	**Quadratic**
GA (n=56)	0.96	0.96	0.76	0.86
NC (n=336)	0.77	0.79	0.59	0.67
OK (n=35)	0.73	0.80	0.52	0.62
PA (n=70)	0.68	0.72	0.62	0.65
VA (n=180)	0.80	0.82	0.70	0.77

Table 23. Regression parameters (intercept and slope by state of origin) for estimating plant N uptake, dry matter production, and net N mineralization from the flush of CO_2 across all greenhouse trials (n=677)

State	Plant N Uptake (mg N kg^{-1} soil)		Dry Matter Production (g DM kg^{-1} soil)		Net N Mineralization (mg N kg^{-1} soil 24 d^{-1})	
	Estimate	Pr > F	Estimate	Pr > F	Estimate	Pr > F
GA (n=56)	0.141	<0.001	0.006	<0.001	0.157	<0.001
NC (n=336)	0.140	<0.001	0.009	<0.001	0.260	<0.001
OK (n=35)	0.038	<0.001	0.002	<0.001	0.185	<0.001
PA (n=70)	0.050	<0.001	0.001	0.04	0.268	<0.001
VA (n=180)	0.149	<0.001	0.008	<0.001	0.261	<0.001

Table 24. Separate and combined analyses of NC and VA samples to predict greenhouse growth by biological parameters

Predictor		**Slope**			**R^2 (*100)**		
		NC (n=336)	VA (n=180)	NC and VA (n=516)	NC	VA	NC and VA
		Dry Matter Production (g DM kg^{-1} soil)					
Flush of CO_2	mg CO_2-C kg^{-1} soil 3 d^{-1}	0.009	0.008	0.008	59	70	66
N Mineralization	mg N kg^{-1} soil 24 d^{-1}	0.030	0.027	0.028	70	51	65
Available N	mg N kg^{-1} soil	0.026	0.024	0.025	71	68	71
Total Organic C	g C kg^{-1} soil	0.038	0.137	0.042	31	72	25
Total Soil N	g N kg^{-1} soil	1.00	1.40	1.15	50	65	54
		Plant N Uptake (mg N kg^{-1} soil)					
Flush of CO_2	mg CO_2-C kg^{-1} soil 3 d^{-1}	0.140	0.143	0.144	77	80	81
N Mineralization	mg N kg^{-1} soil 24 d^{-1}	0.465	0.490	0.486	83	80	83
Available N	mg N kg^{-1} soil	0.404	0.441	0.430	82	85	84
Total Organic C	g C kg^{-1} soil	0.549	2.36	0.624	31	78	22
Total Soil N	g N kg^{-1} soil	14.98	25.15	18.71	55	77	58

Table 25. Regression parameters (slope and R^2) for predicting plant N uptake and dry matter production from available N (mg N kg^{-1} soil) by state using linear and quadratic components

State	Plant N Uptake (mg N kg^{-1} soil)			Dry Matter Production (g DM kg^{-1} soil)		
	Linear R^2	Slope of Linear Regression	Quadratic R^2	Linear R^2	Slope of Linear Regression	Quadratic R^2
GA (n=56)	0.95	0.74	0.95	0.72	0.028	0.85
NC (n=336)	0.82	0.71	0.87	0.40	0.026	0.76
OK (n=35)	0.30	0.19	0.50	0.13	0.008	0.39
PA (n=70)	0.87	0.70	0.89	0.19	0.014	0.71
VA (n=180)	0.85	0.68	0.86	0.44	0.024	0.73

Table 26. Significance of regression ($R^2 < 0.5$ = --; $R^2 > 0.5$ = *; $R^2 > 0.7$ = **; $R^2 > 0.9$ = ***) for predicting dry matter production and plant N uptake from various soil properties within each of the four greenhouse trials

Predictor	**Predictor Units**	**Dry Matter (g DM kg^{-1} soil)**				**Plant N Uptake (mg N kg^{-1} soil)**			
		Greenhouse Trial				**Greenhouse Trial**			
		1 (n=120)	2 (n=105)	3 (n=101)	4 (n=351)	1 (n=120)	2 (n=105)	3 (n=101)	4 (n=351)
Flush of CO_2	mg CO_2-C kg^{-1} soil 3 d^{-1}	**	*	**	*	**	*	***	**
N Mineralization	mg N kg^{-1} soil 24 d^{-1}	**	--	**	*	**	*	***	**
Available N	mg N kg^{-1} soil	**	*	**	**	**	*	***	**
Soil Microbial Biomass C	mg C kg^{-1} soil	*	--	**	*	**	--	**	*
Total Organic C	g C kg^{-1} soil	--	--	**	--	--	--	***	--
Total Soil N	g N kg^{-1} soil	--	*	**	*	--	*	***	*
Particulate Organic C	g C kg^{-1} soil	**	--	*	*	**	--	**	*
Particulate Organic N	g N kg^{-1} soil	**	--	*	--	**	*	**	*
C Mineralization (24 days)	mg CO_2-C kg^{-1} soil 24 d^{-1}	**	--	**	*	**	*	***	**
Initial Inorganic N	mg N kg^{-1} soil	--	--	**	--	--	--	**	*
Humic Matter	g/100cc	--	--	--	--	--	--	--	--

FIGURES

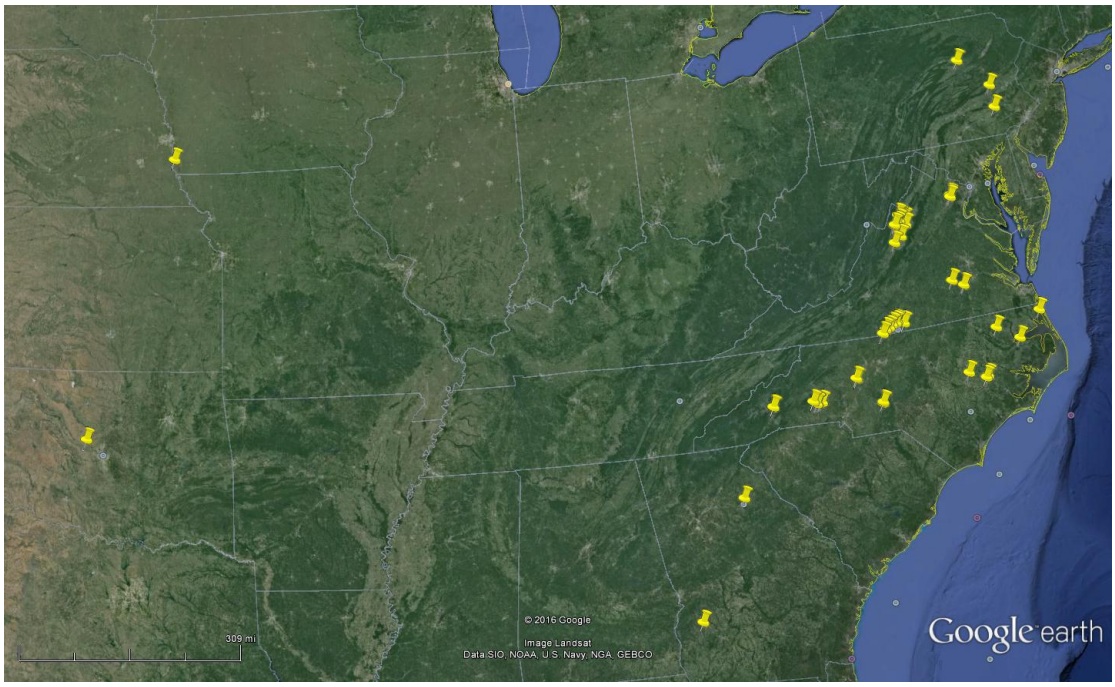

Figure 1. Soil sampling locations

Figure 2. Layout of growth tube rack in Greenhouse Trial 1 with randomly selected samples

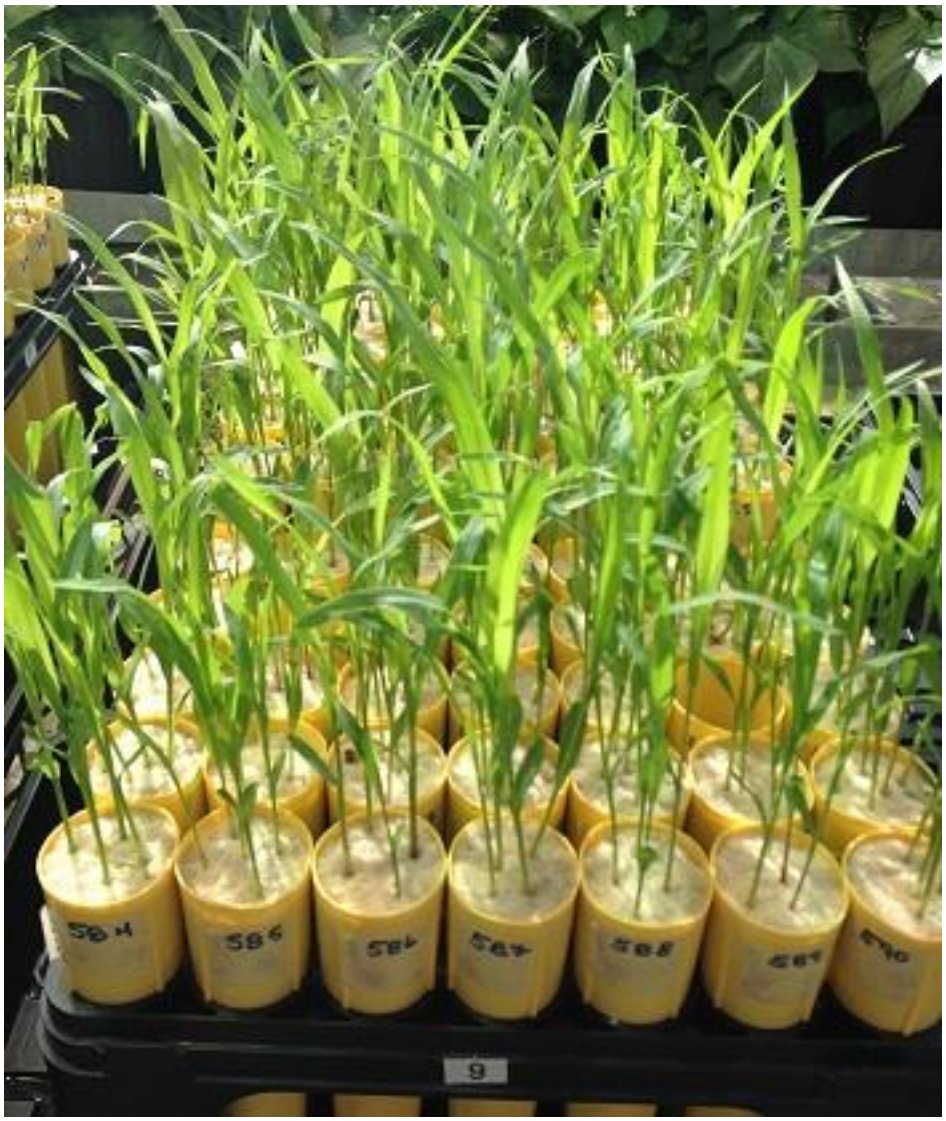

Figure 3. Layout of growth tube rack in Greenhouse Trials 2-4

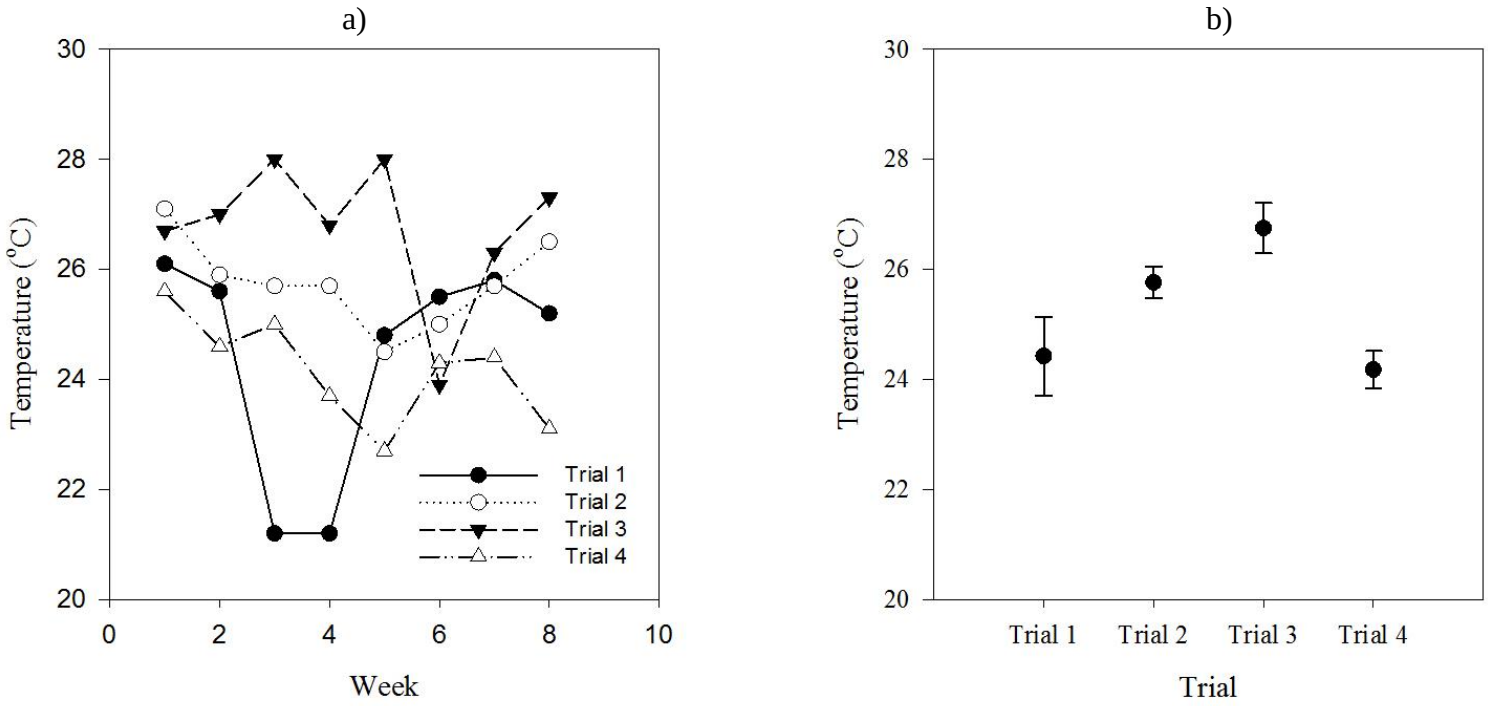

Figure 4. Mean air temperature by week for each greenhouse trial (a) and mean and standard deviation by week for each greenhouse trial (b)

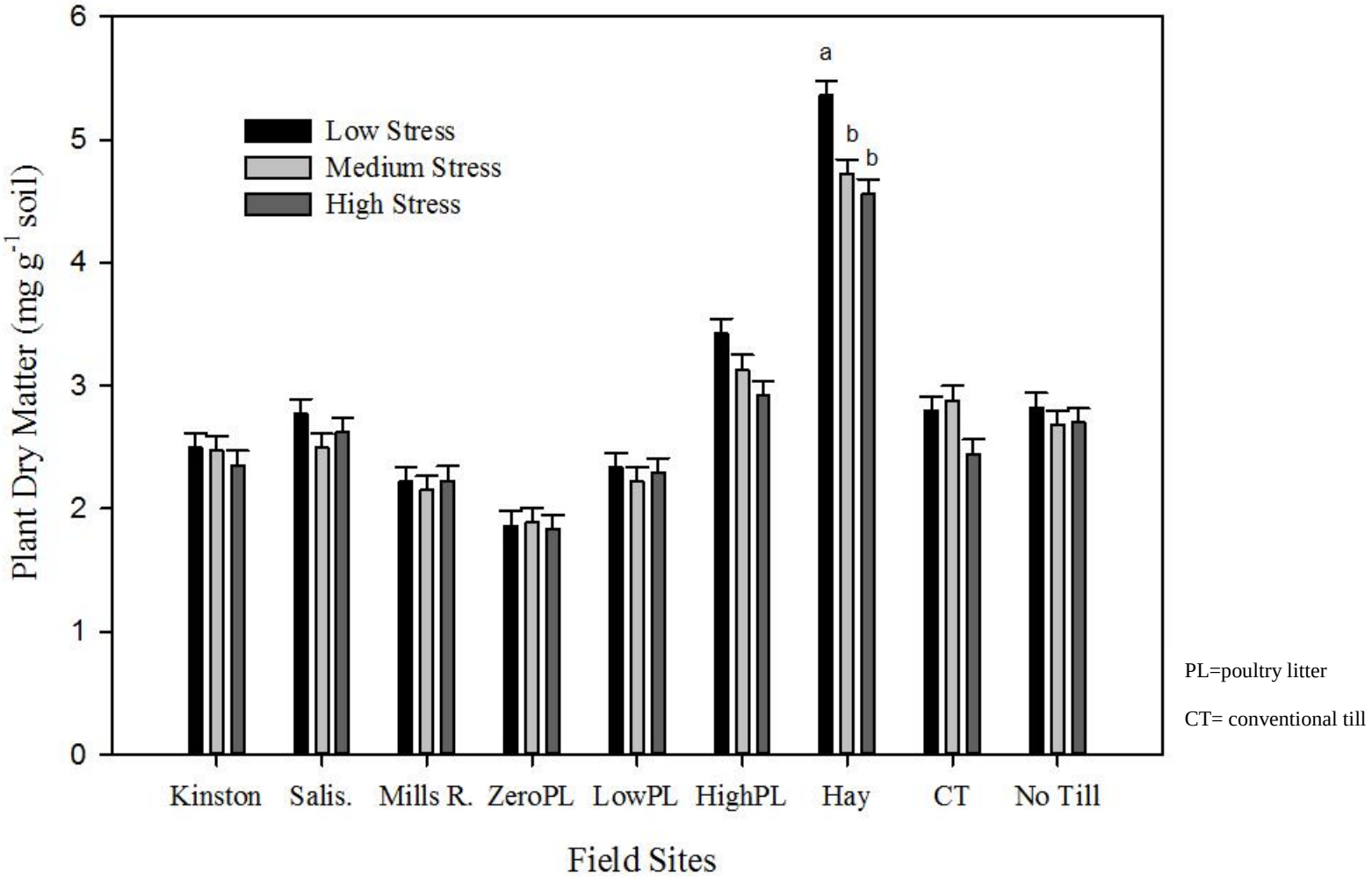

Figure 5. Mean and standard error of dry matter production (g DM kg^{-1} soil) by site as affected by water stress level (means not sharing the same letter are significant at $p<0.05$)

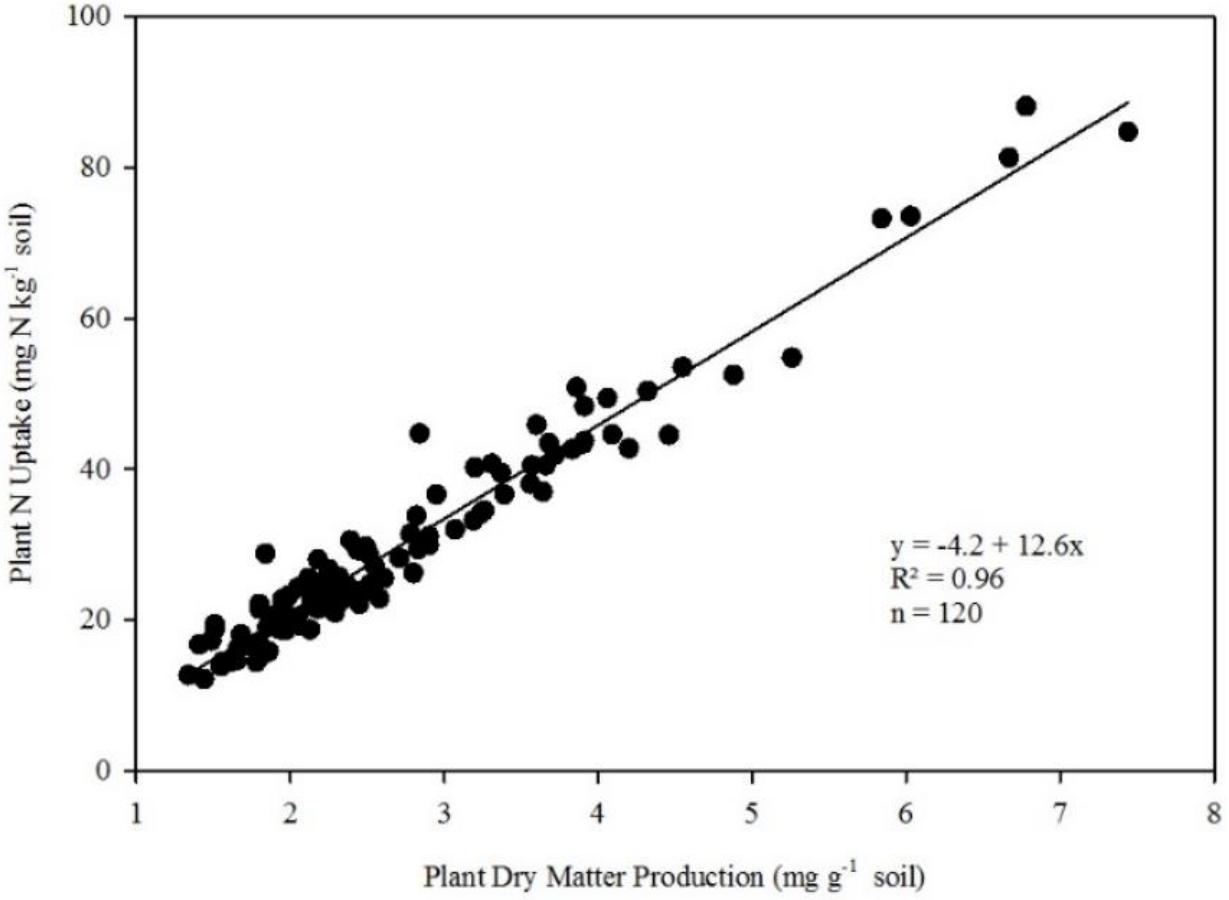

Figure 6. Plant N uptake in relationship with dry matter production in Greenhouse Trial 1

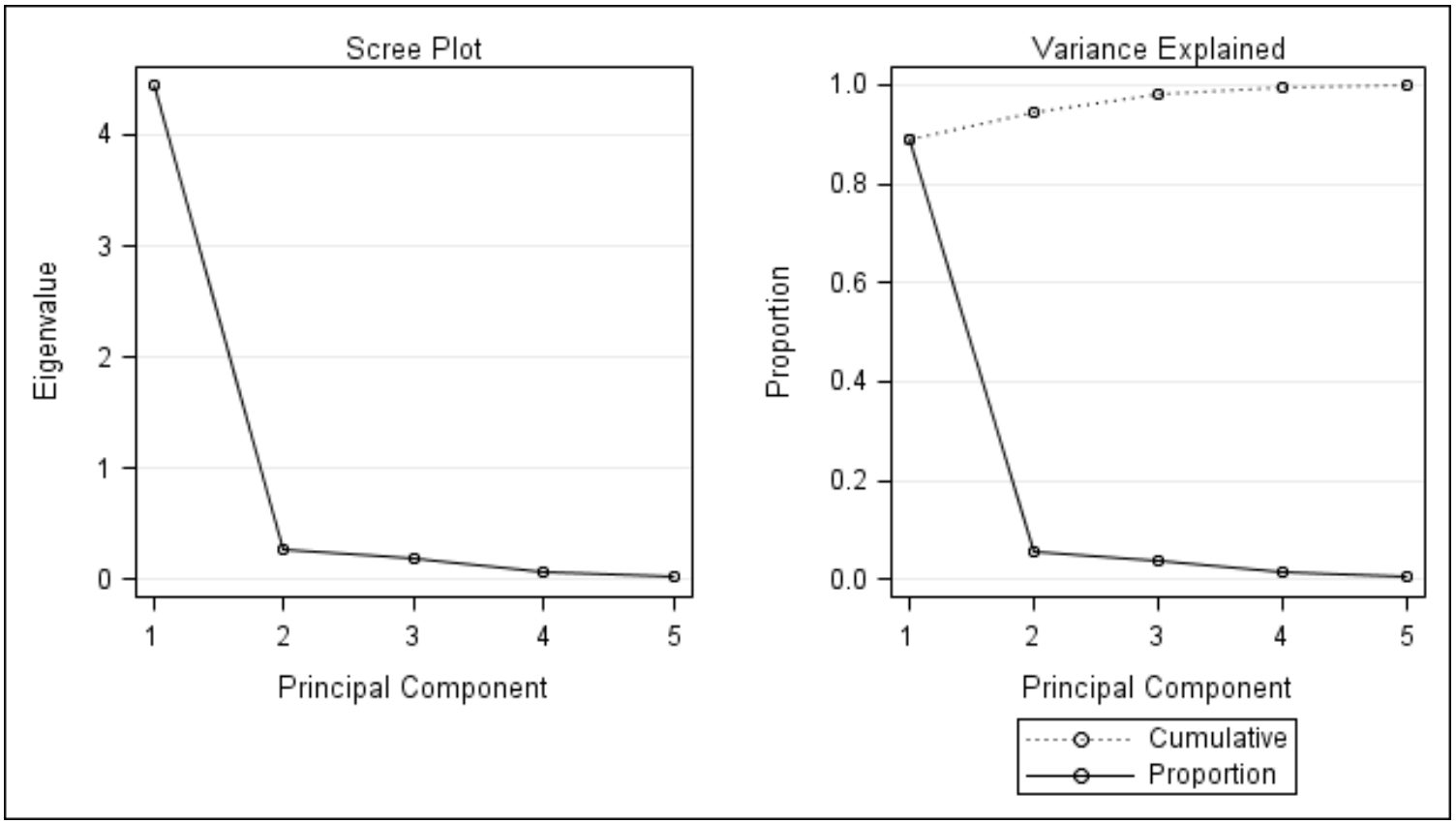

Figure 7. Principal component analysis of plant N uptake from Greenhouse Trial 1 using the flush of CO¬2, soil microbial biomass C, particulate organic C and N, and available N as independent variables (chosen by proc GLM selection procedure)

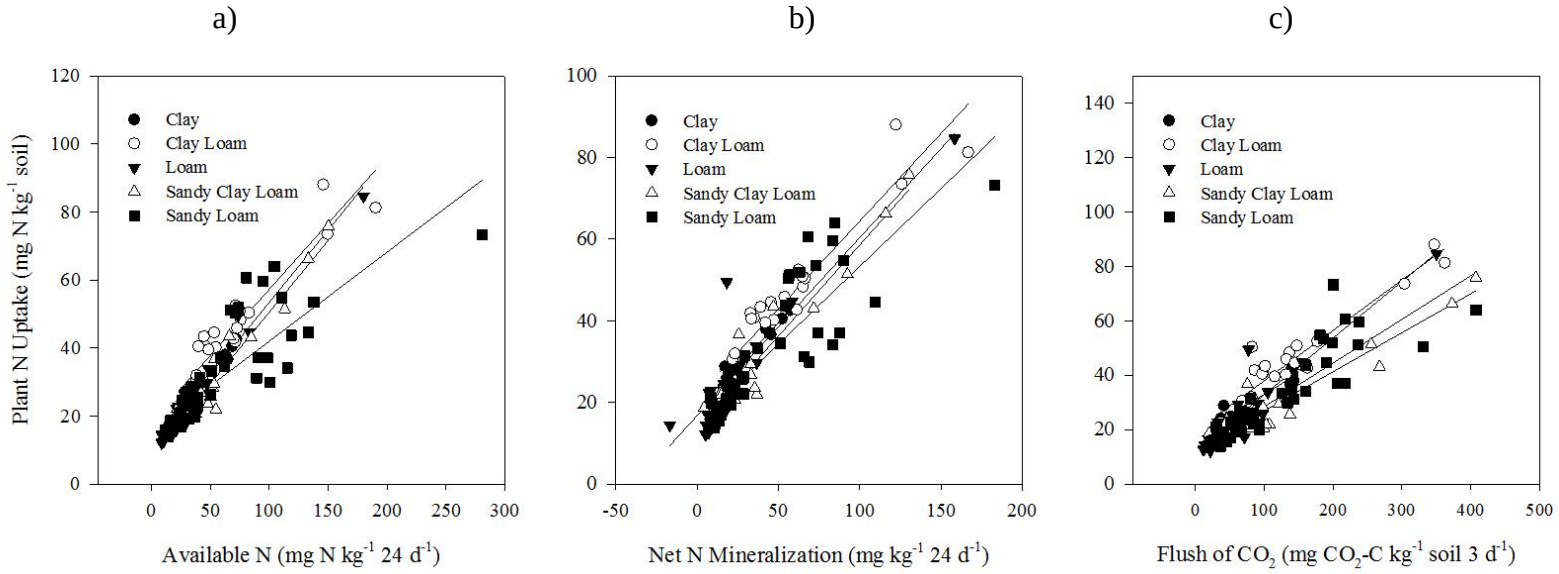

Figure 8. Plant N uptake in relationship with available N (a), net N mineralization (b), and the flush of CO_2 (c) as affected by soil textural class in Greenhouse Trial 1 (n=108)

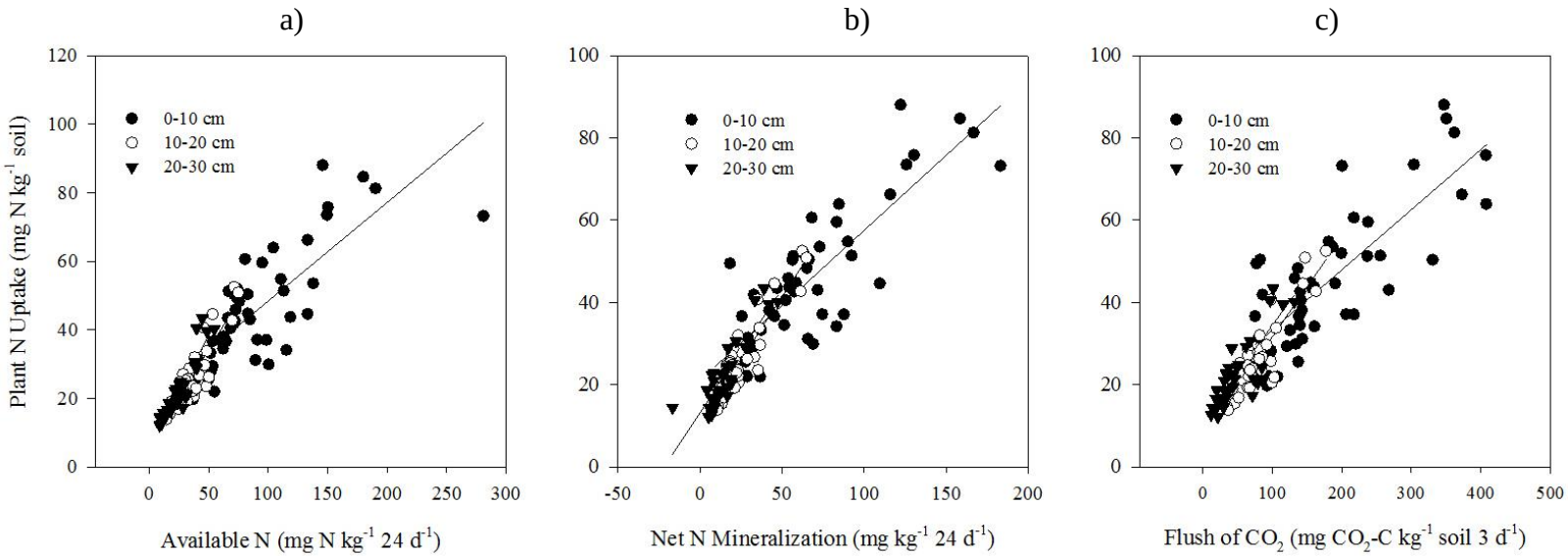

Figure 9. Plant N uptake in relationship with available N (a), net N mineralization (b), and the flush of CO_2 (c) as affected by soil depth in Greenhouse Trial 1 (n=108)

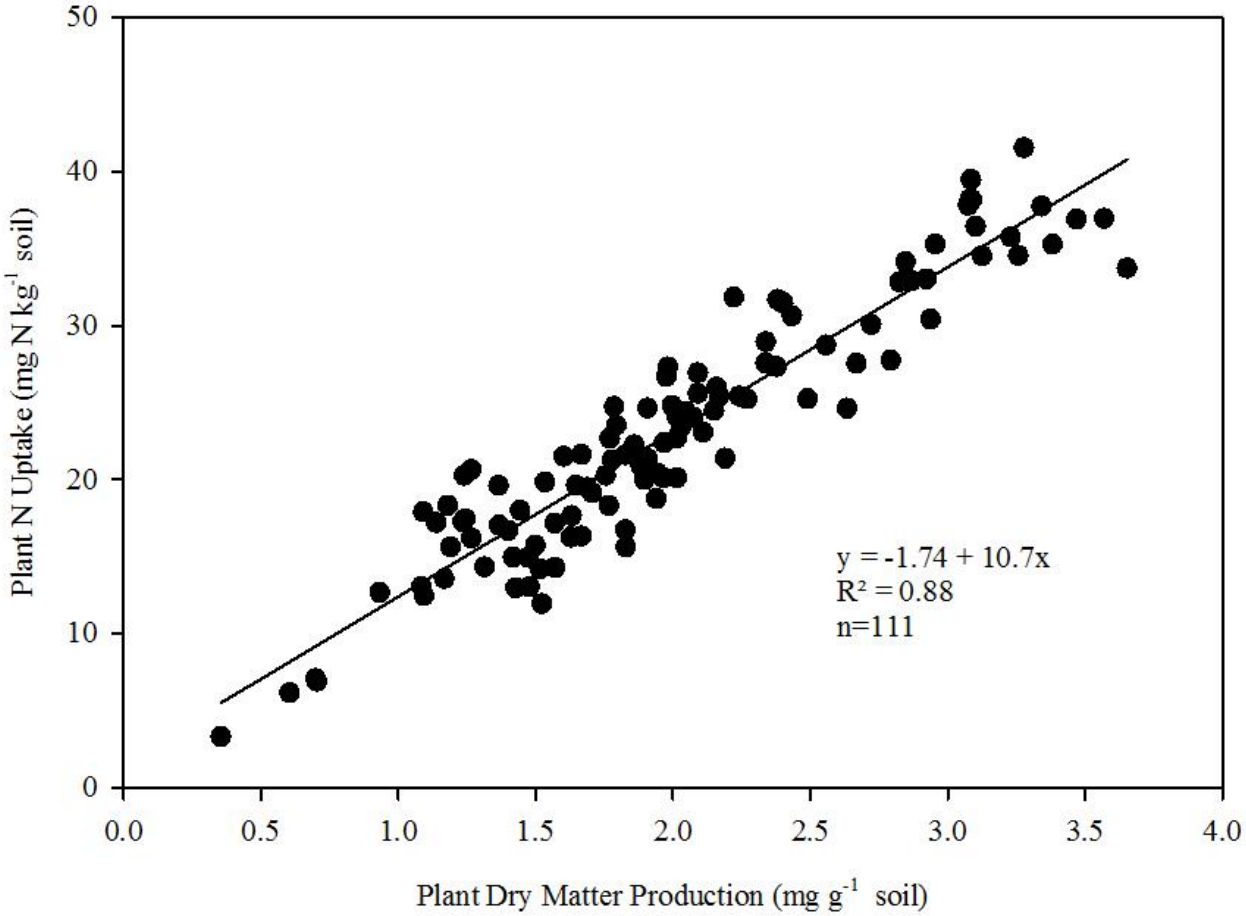

Figure 10. Relationship of plant N uptake with dry matter production in Greenhouse Trial 2

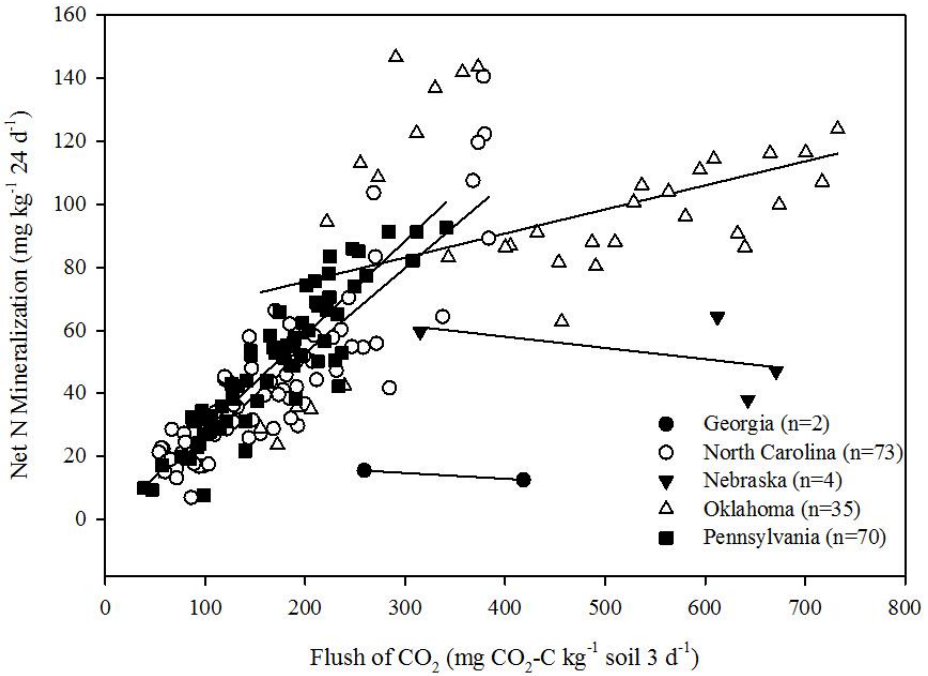

Figure 11. Net N mineralization in relationship with the flush of CO_2 as affected by state of origin in Greenhouse Trial 2

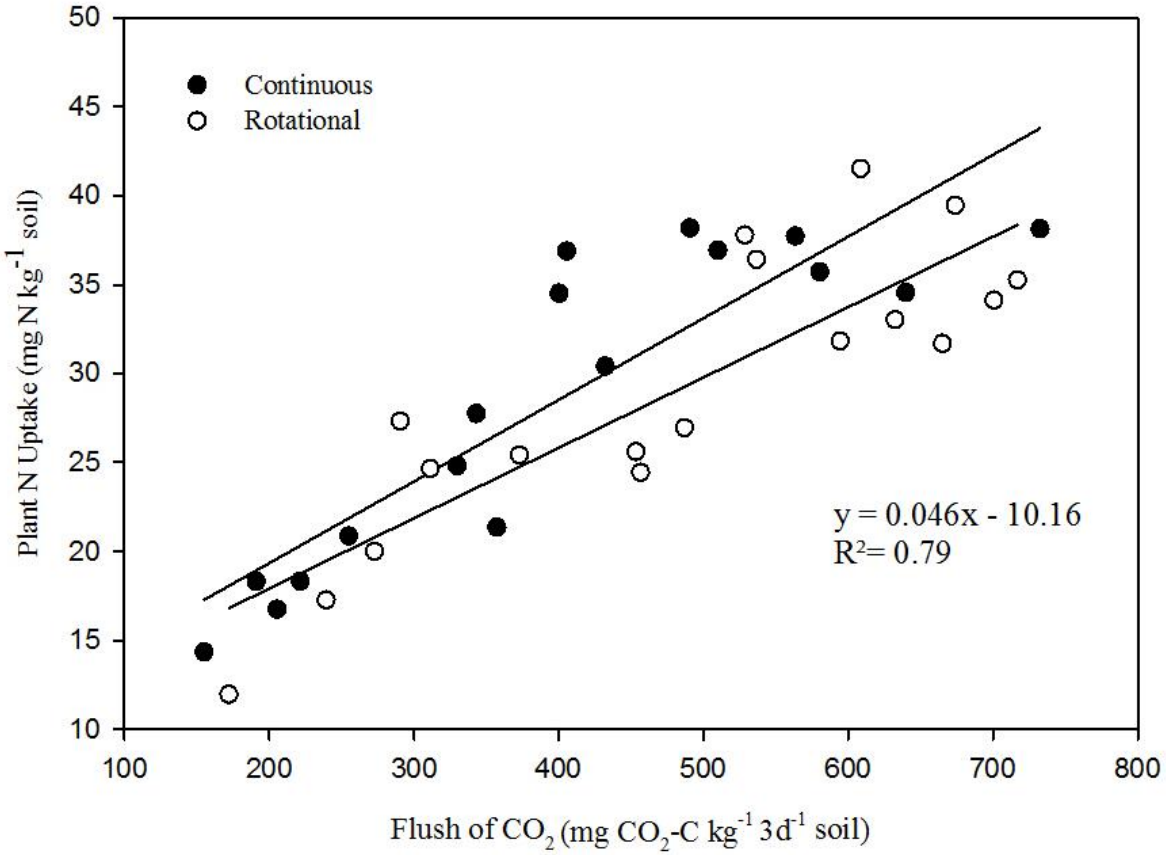

Figure 12. Plant N uptake in relationship with the flush of CO_2 as affected by grazing management (continuous or rotational) in Oklahoma in Greenhouse Trial 2 (n=35)

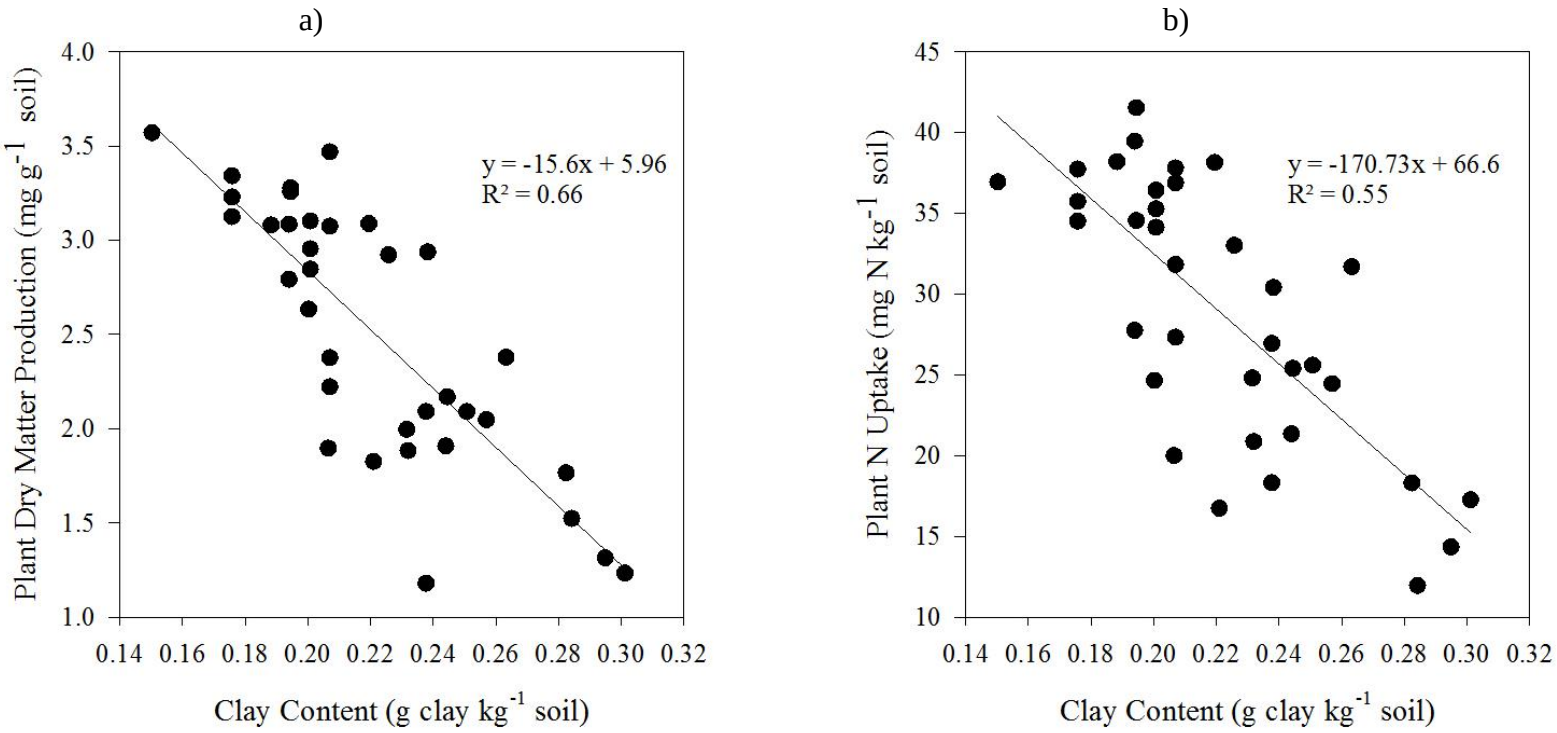

Figure 13. Plant dry matter production (a) and plant N uptake (b) in relationship with clay content from samples in OK in Greenhouse Trial 2 (n=35)

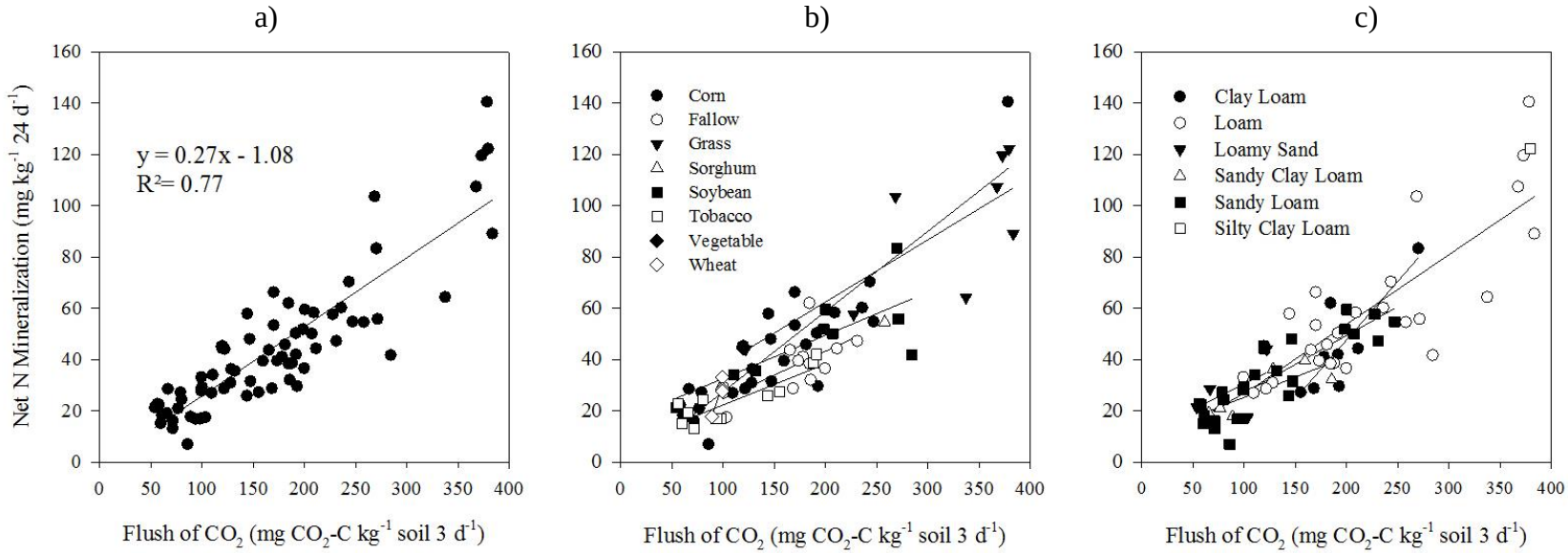

Figure 14. Net N mineralization in relationship with the flush of CO_2 in Dan River samples (n=73) overall (a), as affected by previous crop (b), and as affected by soil textural class (c)

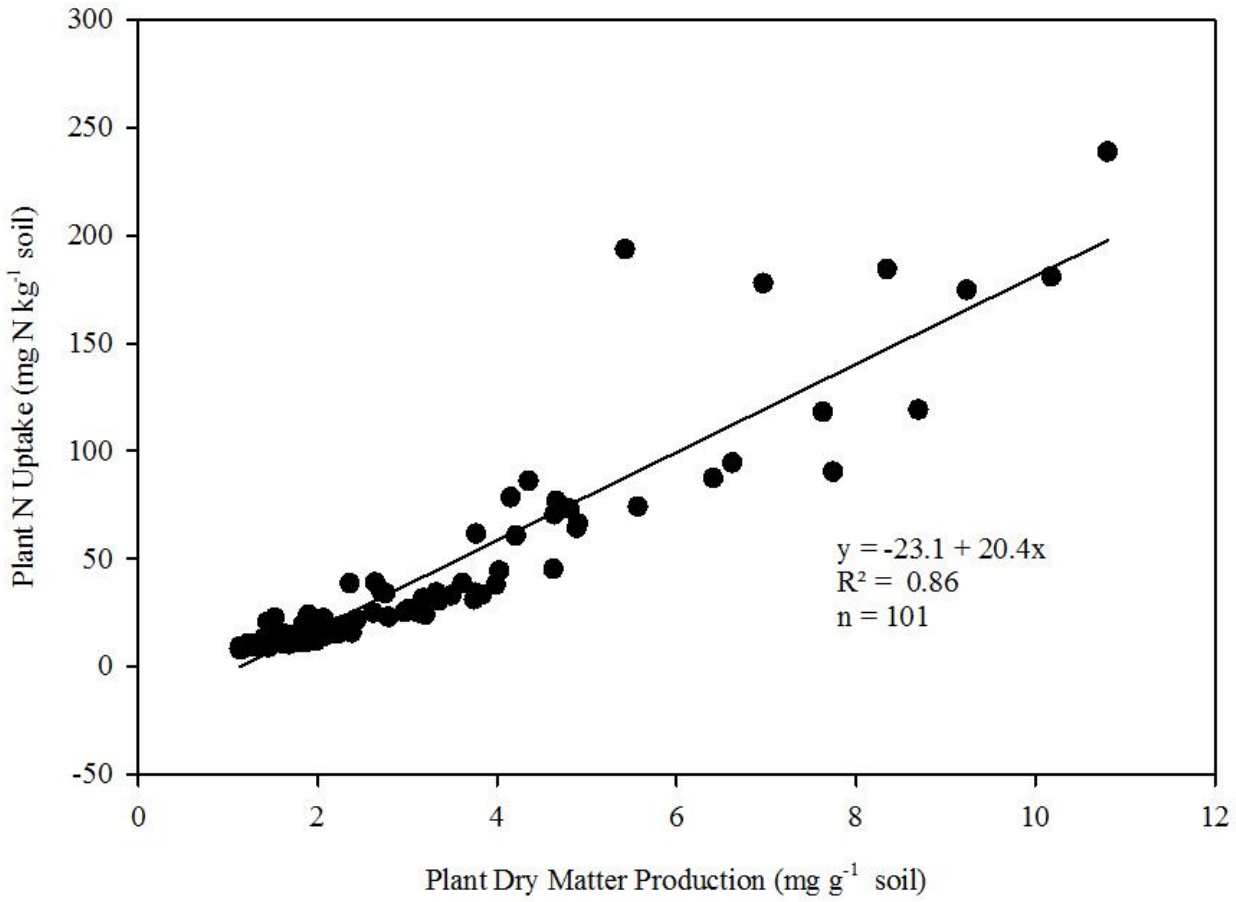

Figure 15. Plant N uptake in relationship with dry matter production in Greenhouse Trial 3 (n=101)

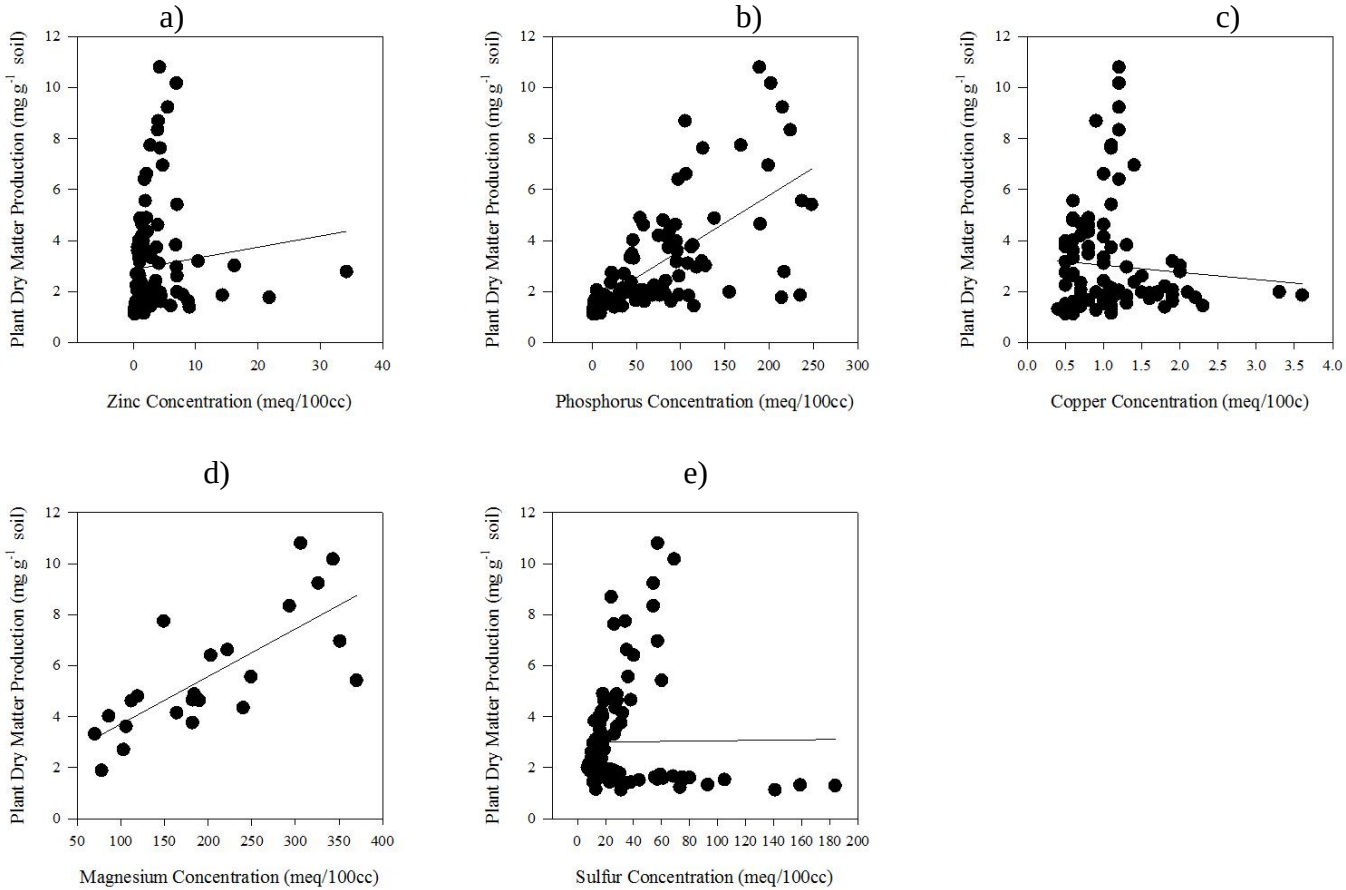

Figure 16. Plant dry matter production in relationship with soil-test Zn (a), P (b), Cu (c), Mg (d), and S (e) in Greenhouse Trial 3 (n=354)

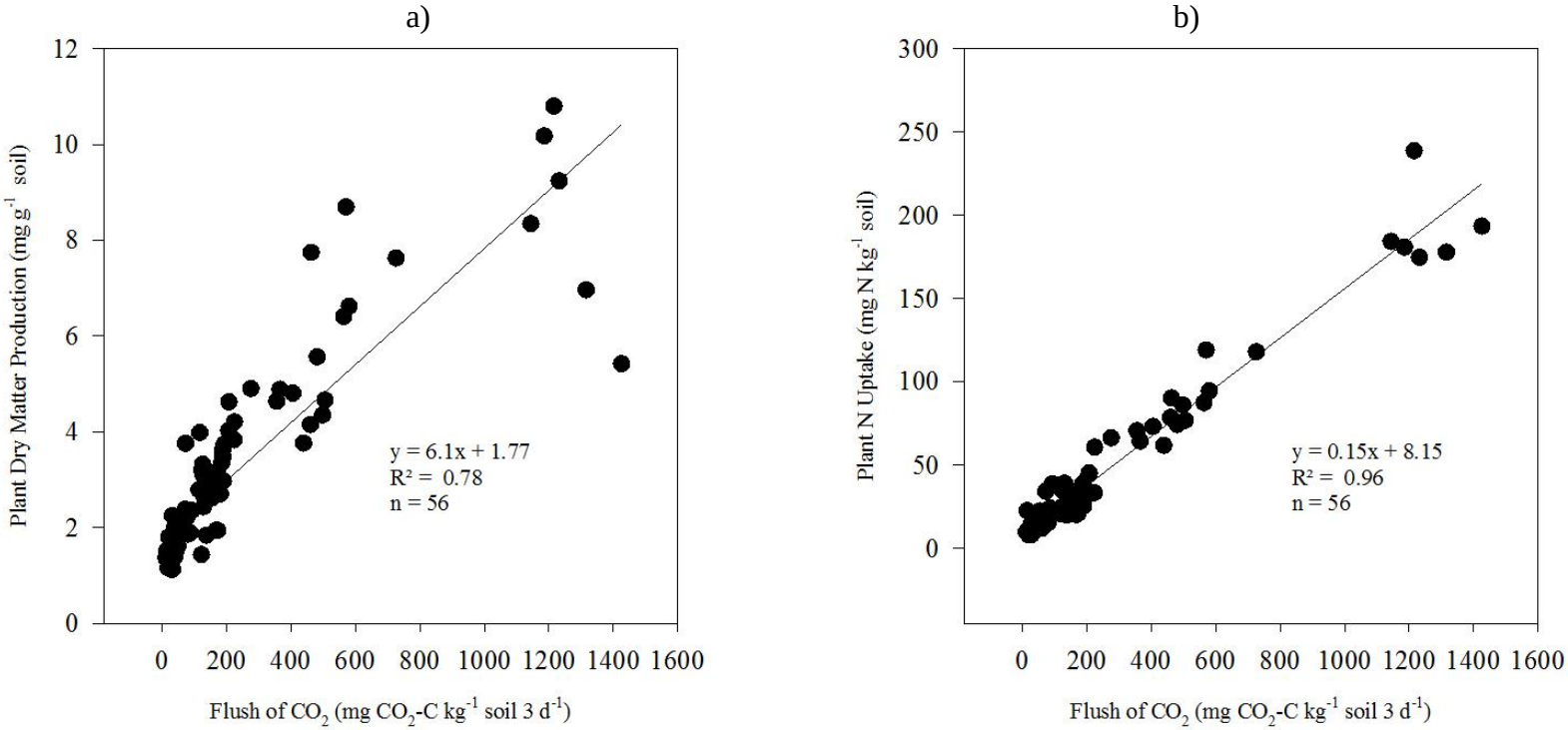

Figure 17. Plant dry matter production (a) and plant N uptake (b) in relationship with the flush of CO_2 from GA samples in Greenhouse Trial 3 (n=56) (Note: highest values represent tall fescue pastures)

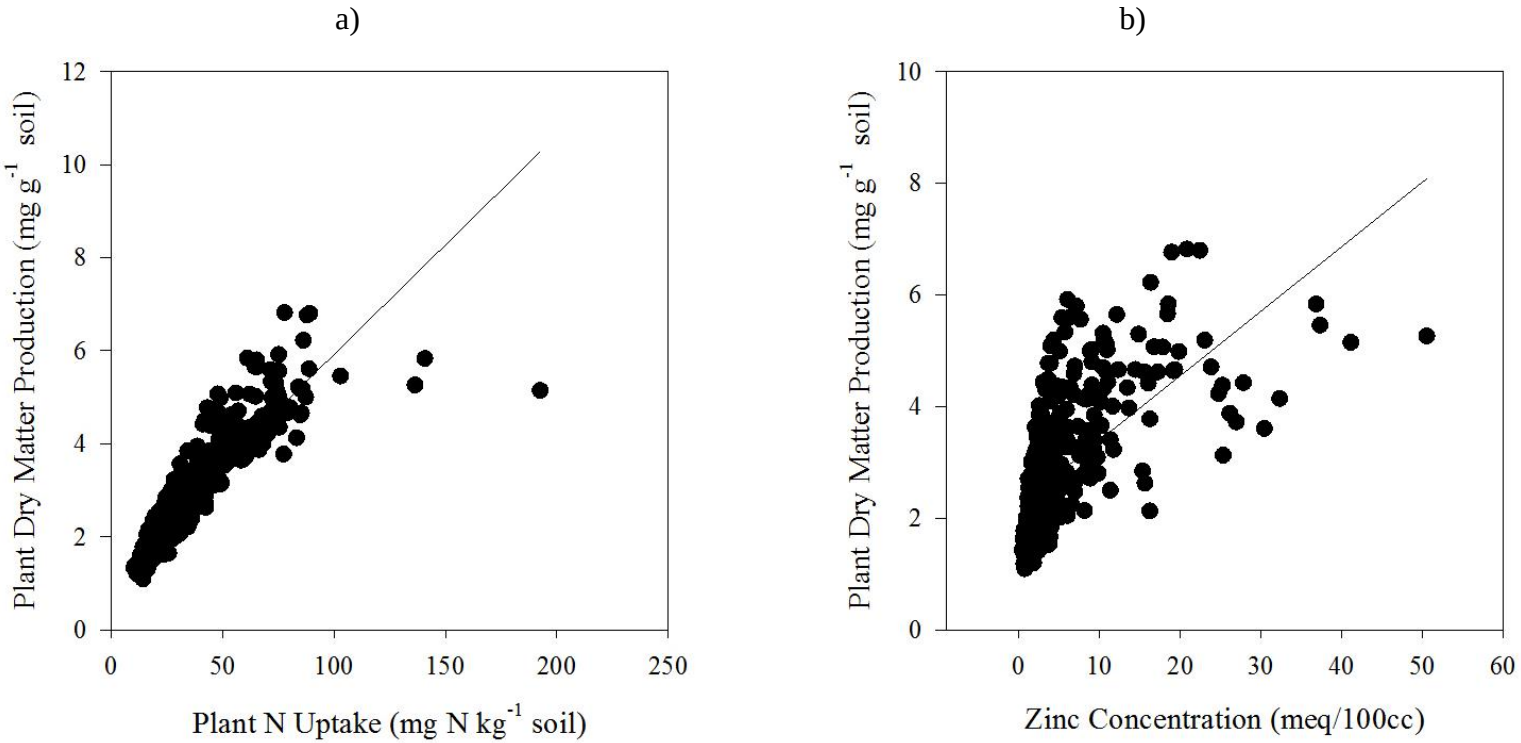

Figure 18. Plant dry matter production in relationship with plant N uptake (a) and soil Zn concentration (b) in Greenhouse Trial 4 (n=354)

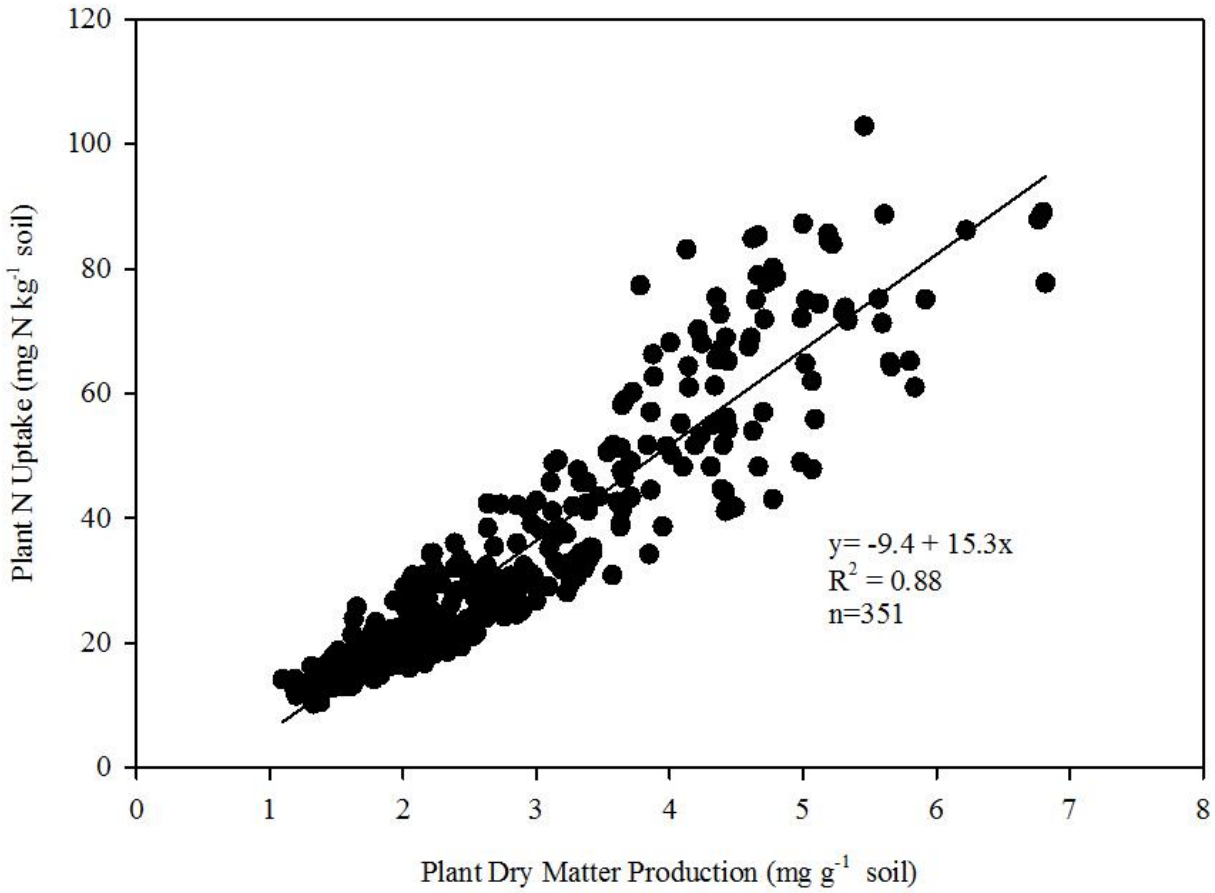

Figure 19. Plant N uptake in relationship with dry matter production in Greenhouse Trial 4 (n=351 with three outliers having high soil Zn removed – Salisbury B15 samples)

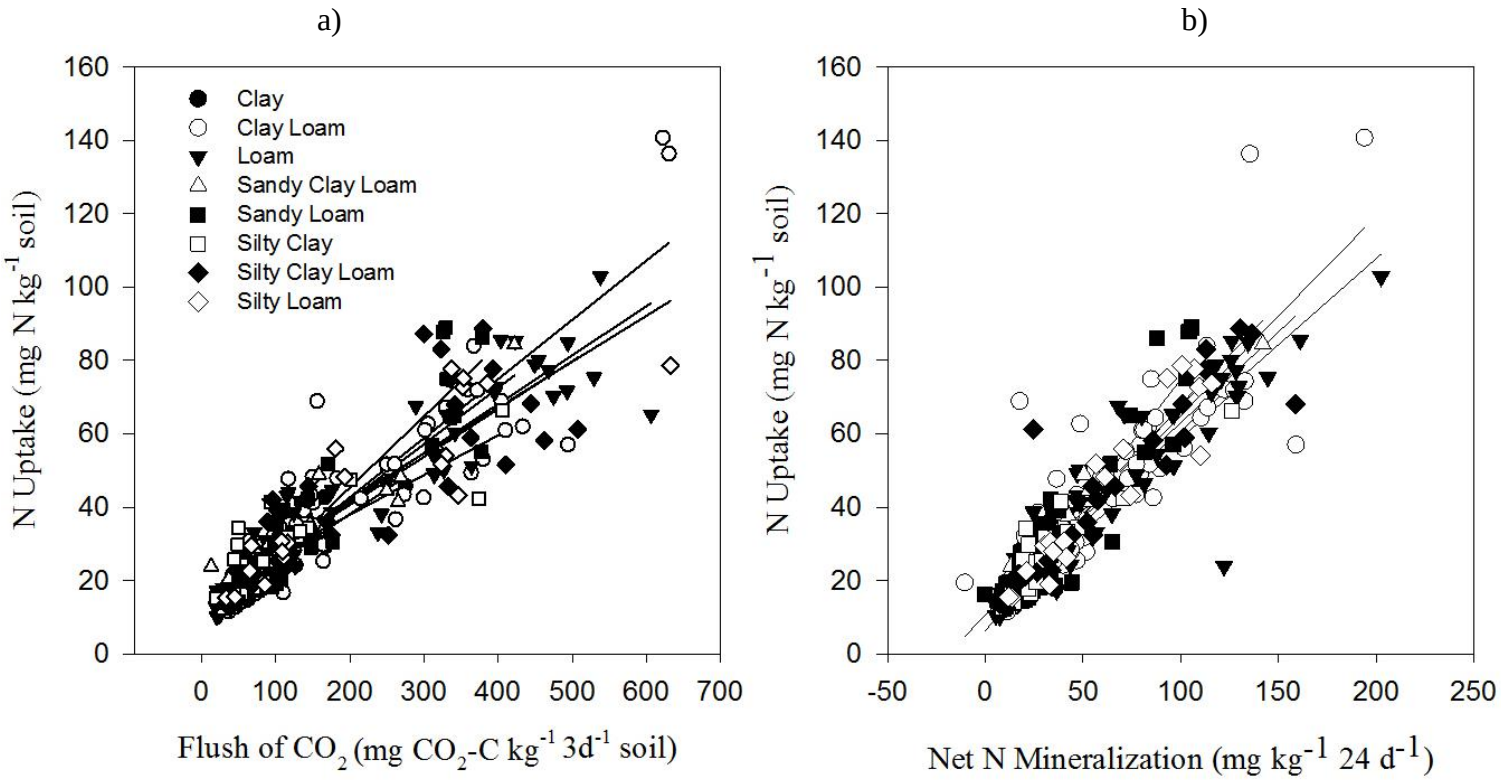

Figure 20. Plant N uptake in relationship with the flush of CO_2 (a) and net N mineralization (b) as affected by soil textural class in Greenhouse Trial 4 (n=354)

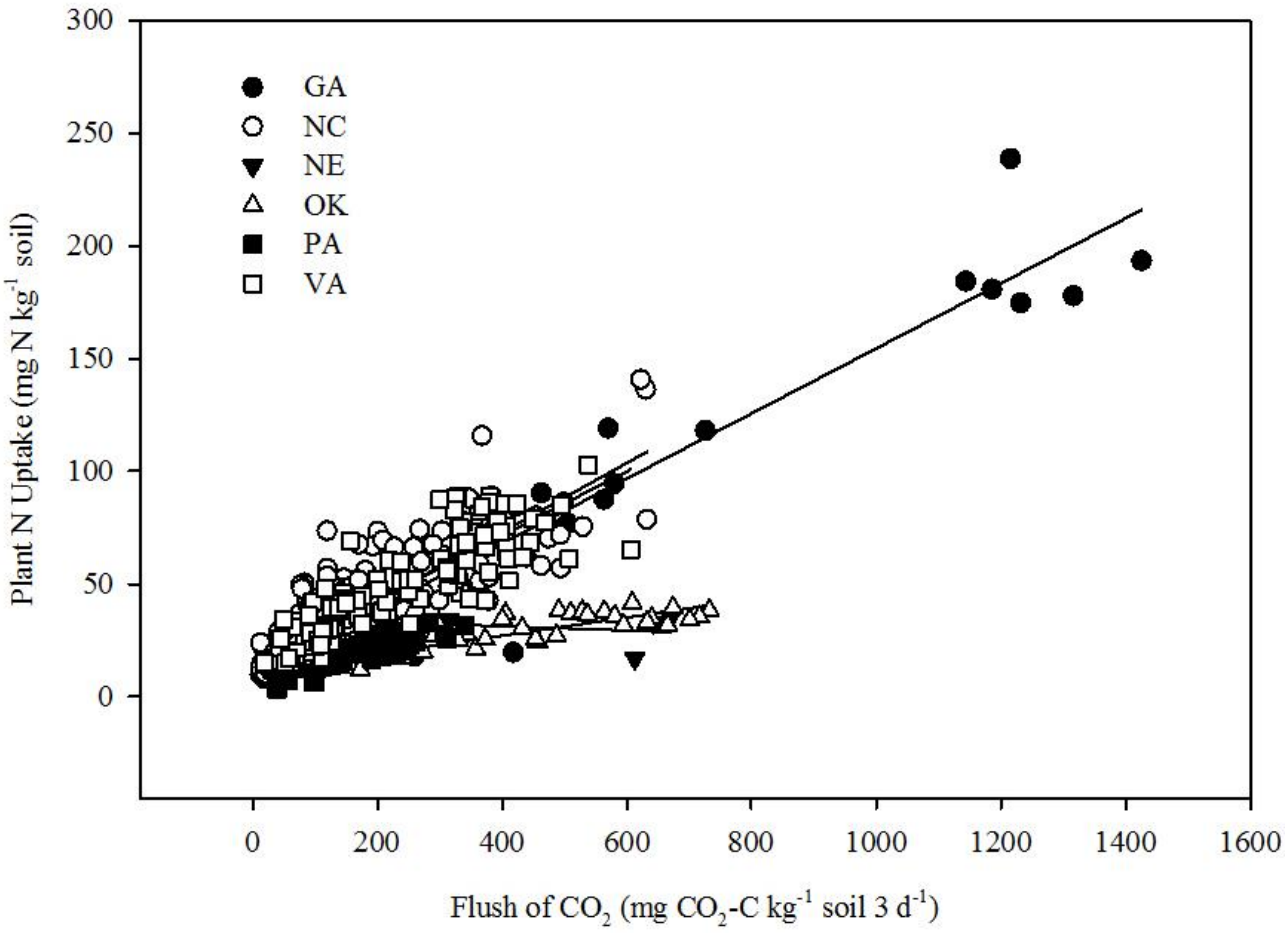

Figure 21. Plant N uptake in relationship with the flush of CO_2 as affected by state of origin across all greenhouse trials (n=745)

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APPENDICES

Appendix A Soil textural class, series, and family descriptions of samples used in laboratory and greenhouse analyses

Location	Soil Textures	Map Unit Name	Family
Blackstone, VA	SL, SCL	Appling sandy loam	Fine, kaolinitic, thermic Typic Kanhapludults
Blackstone, VA	SCL	Cecil fine sandy loam	Fine, kaolinitic, thermic Typic Kanhapludults
Camden County, NC	L, SiL, CL	Perquimans silt loam	Fine-silty, mixed, semiactive, thermic Typic Endoaquults
Eden, NC	L, SL, SCL, LS, CL	Dan River loam	Fine-loamy, mixed, active, mesic Oxyaquic Dystrudepts
Eden, NC	CL	Riverview silt loam	Fine-loamy, mixed, active, thermic Fluventic Dystrudepts
Eden, NC	SL, L, SCL, LS	Toccoa fine sandy loam	Coarse-loamy, mixed, active, nonacid, thermic Typic Udifluvents
Eden, NC	SCL	Clover sandy loam	Fine, mixed, semiactive, mesic Typic Hapludults
Eden, NC	L, CL	Bolling fine sandy loam	Fine-loamy, mixed, active, thermic Aquic Hapludalfs
Eden, NC	CL	Wehadkee silt loam	Fine-loamy, mixed, active, nonacid, thermic Fluvaquentic Endoaquepts
Eden, NC	L, CL	State sandy loam	Fine-loamy, mixed, semiactive, thermic Typic Hapludults
Eden, NC	L, SL, SiCL	Chenneby loam	Fine-silty, mixed, active, thermic Fluvaquentic Dystrudepts
Eden, NC	L, SL	Chenneby loam and Toccoa fine sandy loam	Fine-silty, mixed, active, thermic Fluvaquentic Dystrudepts Coarse-loamy, mixed, active, nonacid, thermic Typic Udifluvents
Eden, NC	L	Riverview silt loam and Toccoa fine sandy loam	Fine-loamy, mixed, active, thermic Fluventic Dystrudepts/Coarse-loamy, mixed, active, nonacid, thermic Typic Udifluvents
Eden, NC	L, CL	No digital data available	No digital data available
Eden, NC	L	Dan River loam and Codorus loam	Fine-loamy, mixed, active, mesic Oxyaquic Dystrudepts/Fine-loamy, mixed, active, mesic Fluvaquentic Dystrudepts
Eden, NC	SL, L, SCL	Codorus loam	Fine-loamy, mixed, active, mesic Fluvaquentic Dystrudepts

Appendix A continued

El Reno, OK	L, CL, SiL, SiCL	Kirkland-Pawhuska complex, 1 to 3 percent slopes	Fine, mixed, superactive, thermic Udertic Paleustolls
El Reno, OK	SiL	Norge silt loam, 1 to 3 percent slopes	Fine-silty, mixed, active, thermic Udic Paleustolls
El Reno, OK	SiL	Pond Creek silt loam, 0 to 1 percent slopes	Fine-silty, mixed, superactive, thermic Pachic Argiustolls
El_Reno, OK	SiCL, SiL, CL	Bethany silt loam, 0 to 1 percent slopes	Fine, mixed, superactive, thermic Pachic Paleustolls
Fauquier County, VA	L, SiL, SiCL	Ashburn silt loam, 2 to 7 percent slopes	Fine-silty, mixed, active, mesic Oxyaquic Hapludalfs
Goldsboro, NC	SL, SiL, L, CL, SiCL	Wickham sandy loam, 2 to 6 percent slopes, eroded	Fine-loamy, mixed, semiactive, thermic Typic Hapludults
Kinston, NC	SL	Lynchburg sandy loam/Rains sandy loam	Fine-loamy, siliceous, semiactive, thermic Aeric Paleaquults/Fine-loamy, siliceous, semiactive, thermic Typic Paleaquults
Kinston, NC	CL, SCL, L	Portsmouth loam	Fine-loamy over sandy or sandy-skeletal, mixed, semiactive, thermic Typic Umbraquults
Lewiston, NC	L, SCL, SL	Goldsboro, NC sandy loam	Fine-loamy, siliceous, subactive, thermic Aquic Paleudults
Mills River, NC	CL, SCL, L	Comus (colvard) fine sandy loam	Coarse-loamy, mixed, active, nonacid, mesic Typic Udifluvents
Mills River, NC	SCL, SL	Codorus loam (arkaqua)	Fine-loamy, mixed, active, mesic Fluvaquentic Dystrudepts
Nebraska City, NE	SiL	Cecil sandy loam	Fine-silty, mixed, mesic Typic Eutrochrepts
Plymouth, NC, NC	SL, L, SCL	Portsmouth fine sandy loam	Fine-loamy over sandy or sandy-skeletal, mixed, semiactive, thermic Typic Umbraquults
Plymouth, NC	CL, SCL, L	Cape Fear loam	Fine, mixed, semiactive, thermic Typic Umbraquults
Plymouth, NC	L, CL, SCL	Cape Fear Loam/Roanoke loam	Fine, mixed, semiactive, thermic Typic Umbraquults/Fine, mixed, semiactive, thermic Typic Endoaquults
Salisbury, NC	C, CL	Lloyd clay loam	Fine, kaolinitic, thermic Rhodic Kanhapludults
Salisbury, NC	CL, C	Mecklenburg clay loam, 2 to 8 and 8 to 15 percent slopes, moderately eroded	Fine, mixed, active, thermic Ultic Hapludalfs/
Salisbury, NC	CL, C	Lloyd clay loam	Fine, kaolinitic, thermic Rhodic Kanhapludults

Appendix A continued

Shelby, NC	SCL, SC, C	Cecil sandy clay loam, 2 to 8 percent slopes, moderately eroded/Appling sandy loam	Fine, kaolinitic, thermic Typic Kanhapludults
Shelby, NC	SL, SCL, SL, SC	Cecil sandy clay loam, 2 to 8 percent slopes, moderately eroded	Fine, kaolinitic, thermic Typic Kanhapludults
Shelby, NC	SCL, SL	Appling sandy loam, 1 to 6 percent slopes	Fine, kaolinitic, thermic Typic Kanhapludults
Shellman, GA	SCL	Greenville sandy clay loam, 2 to 5 percent slopes	Fine, kaolinitic, thermic Rhodic Kandiudults
Watkinsville, GA	SL, SCL	Cecil sandy loam	Fine, kaolinitic, thermic Typic Kanhapludults
Shenandoah Valley, VA	SiC, SICl, C	Edom silt loam, 2 to 7 percent slopes, eroded	Fine, illitic, mesic Typic Hapludalfs
Shenandoah Valley, VA	L, SiL, SiCL, SiC, CL, C	Frederick and Lodi silt loams, 2 to 15 percent slopes, eroded	Fine, mixed, semiactive, mesic Typic Paleudults/Fine, mixed, mesic Typic Hapludults
Shenandoah Valley, VA	L, CL, SiL	Ashburn silt loam, 2 to 7 percent slopes	Fine-silty, mixed, active, mesic Oxyaquic Hapludalfs
Shenandoah Valley, VA	SL	Craigsville cobbly fine sandy loam, 0 to 4 percent slopes, frequently flooded	Loamy-skeletal, mixed, superactive, mesic Fluventic Dystrudepts
Shenandoah Valley, VA	CL, C, SiCL	Sequoia-Berks silt loams, 2 to 7 percent slopes, eroded/Berks-Weikert channery silt loam, 7 to 15 percent slopes, eroded	Fine, mixed, semiactive, mesic Typic Hapludults/Loamy-skeletal, mixed, active, mesic Typic Dystrudepts
Shenandoah Valley, VA	L, CL	Chagrin loam	Fine-loamy, mixed, active, mesic Dystric Fluventic Eutrudepts
Shenandoah Valley, VA	L, SL, SCL	Chavies fine sandy loam, 0 to 4 percent slopes, rarely flooded	Coarse-loamy, mixed, active, mesic Ultic Hapludalfs
Stanly County, NC	SiL, SiCL, SiC	Tarrus channery silty clay loam, 2 to 8 percent slopes, moderately eroded	Fine, kaolinitic, thermic Typic Kanhapludults
Stanly County, NC	L, SL	Chewacla loam, 0 to 2 percent slopes, frequently flooded	Fine-loamy, mixed, active, thermic Fluvaquentic Dystrudepts
University Park, PA	SiCL, SiL	Washington silt loam and Edom complex	Fine-loamy, mixed, mesic Ultic Hapludalfs/Fine, illitic, mesic Typic Hapludalfs
University Park, PA	L, CL, SCL	Berks-Weikert complex	Loamy-skeletal, mixed, active, mesic Typic Dystrudepts

Appendix A continued

University Park, PA	SL, SCL, L	Berks-Weikert complex	Loamy-skeletal, mixed, active, mesic Typic Dystrudepts
University Park, PA	CL, L, SiL	Hagerstown silt loam	Fine, mixed, semiactive, mesic Typic Hapludalfs

[i] C=clay; S=sand; Si=silt; L=loam

Appendix B. Mean annual temperature and precipitation from closest NOAA weather station to field sites by city (1980-2010 climate normals)

Location	Mean Annual Temp (C)	Mean Annual Precipitation (cm)	Latitude/Longitude
Shellman, GA	19.0	130.6	31.5°, -84.1°
Watkinsville, GA	16.3	121.9	33.8°, -83.4°
Camden, NC	16.2	123.2	36.3°, -76.2°
Goldsboro, NC	16.2	124.0	35.3°, -77.9°
Kinston, NC	17.6	123.4	35.3°, -77.6°
Mills River, NC	12.8	128.5	35.4°, -82.6°
Shelby, NC	14.8	121.7	35.3°, -81.5°
Eden, NC	14.4	114.3	36.5°, -79.7°
Plymouth, NC	16.6	108.7	35.9°, -76.7°
Salisbury, NC	14.4	108.7	35.7°, -80.6°
Lewiston, NC	15.6	118.9	36.1°, -77.2°
Norwood, NC	15.5	124.0	35.4°, -80.2°
Nebraska City, NE	10.8	85.6	40.7°, -95.9°
El Reno, OK	16.4	92.7	35.4°, -97.6°
University Park, PA	10.2	109.7	40.9°, -76.9°
Harrisonburg, VA	12.7	102.9	37.8°, -79.4°
Harrisonburg, VA	12.8	113.0	38.7°, -77.8°
Harrisonburg, VA	11.8	112.8	37.8°, -79.4°
Harrisonburg, VA	11.2	92.71	38.5°, -78.9°
Calverton, VA	12.8	113.0	38.7°, -77.8°
Blackstone, VA	14.5	116.1	36.8°, -77.8°