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SOIL MANAGEMENT AND NITROGEN DYNAMICS IN BURLEY TOBACCO ROTATIONS

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SOIL MANAGEMENT AND
NITROGEN DYNAMICS IN BURLEY TOBACCO ROTATIONS

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture, Food and Environment
at the University of Kentucky

By
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Lexington, Kentucky

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and  Dr. John H. Grove, Professor of Plant and Soil Sciences

Lexington, Kentucky

2015

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ABSTRACT OF DISSERTATION

SOIL MANAGEMENT AND NITROGEN DYNAMICS IN BURLEY TOBACCO ROTATIONS

Agronomic practices, including tillage, crop rotation and N fertilization, have been developed to efficiently manage soil N dynamics and crop N nutrition. These practices can affect soil organic carbon (SOC) and soil total nitrogen (STN) sequestration, and consequently influence soil nitrogen mineralization (SNM) and crop N nutrition. However, little research has been systematically and simultaneously conducted to examine the effect of agronomic management on (1) SOC and STN stocks; (2) SNM; and (3) crop N nutrition. Burley tobacco (Nicotiana tobacum L.) is a N demanding crop and subject to inefficiency in N fertilization. Moreover, conservation tillage and rotation have been integrated into traditionally tillage intensive tobacco cropping systems. Thus, a tobacco tillage and rotation study was used to test how agronomic practices can affect N dynamics and crop N status in a series of sequential experiments.

Firstly, different tobacco production systems were utilized to investigate the effects of tillage and rotation on soil aggregate stabilization and associated SOM sequestration. No-tillage and rotation management enhanced SOC and STN stocks, mainly by increasing the proportion of macroaggregates and SOC and STN concentrations.

Secondly, a series of studies were conducted on SNM, including: (1) comparison of laboratory and \textit{in situ} resin-core methods in estimating SNM; (2) evaluation of the influence of N fertilizer application on SNM; and (3) comparison of chemical indices for predicting SNM across management treatments over time. Laboratory method had different results relative to \textit{in situ} method due to sample pretreatments. Fertilizer N application had a priming effect on SNM, but priming depended on both the N fertilizer rate and the background SOM level. The effect of rotation/tillage treatments on SNM was stable across years and SOC appeared to be the best indicator of SNM among other soil carbon and N estimates.

Thirdly, a N fertilizer study for different tillage systems was conducted in 2012 and 2013. Crop parameters and plant available N (PAN) were collected to investigate the impact of tillage on tobacco production. Crop parameters showed that no-tillage can result in N deficiency in dry years. Similar PAN for both tillage methods suggested N deficiency in
no-till tobacco was due to the crop’s lower N uptake capacity. In 2014, tobacco root analysis confirmed that no-tillage can result in less root exploration of the soil volume than conventional tillage.

KEYWORDS: Nitrogen Nutrition, No-tillage, In situ Resin-Core Method, Net Soil N Mineralization, Tobacco

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DEDICATION

This dissertation is dedicated to my family; my loving wife, Jinglin Xiang, and my growing son, David Congming; for all of their support up to and during this process.
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Chapter 1: A Literature Review of Agronomic Practices and Soil N Dynamics

1.1 Introduction

The Development of Agronomic Practices Reflects the History of Managing Soil N Dynamics

Nitrogen (N) is the most important plant mineral nutrient (Epstein, 1972). Nitrogen was first discovered in the late 18th century, and N’s role in improving crop production was widely recognized by the mid-19th century (Galloway and Cowling, 2002). Long before these discoveries, ancient farmers often unknowingly employed agronomic practices that resulted in the management of soil N availability, helping to ensure the human food supply and nutrition. Before synthetic N fertilizers, there were two major sources of N in agro-ecosystems - soil N and legume based biological N fixation. Ancient farmers constructively developed tillage schemes and rotated non-legume and legume crops to manage both N sources for millennia. However, the appearance of commercial synthetic N fertilizers in the early 20th century brought significant changes to those traditional agronomic practices. The history of agronomic practices from the perspective of managing soil and biologically fixed N dynamics would seem to be a fruitful review.

Plow tillage is a form of soil N management. Much of the soil N is in complex organic forms, such as decomposing plant and animal residues (Marschner and Marschner, 2012). Most plants can only take up inorganic N (NH₄⁺ and NO₃⁻) forms (Keeney, 1982), although the basic amino acids are rarely absorbed by some plant species (e.g. Picea abies.) (Boukcim and Plassard, 2003). Inorganic N and basic amino acids in the soil are mainly derived from N mineralization processes. Mineralization can be promoted by
tillage practices because disturbance can expose naturally-protected (i.e. aggregate-protected) soil organic matter (SOM) to microbes, enhancing microbial activity and N mineralization (Tisdall and Oades, 1982). Therefore, plow tillage was considered a great agricultural advance and, from the archeological evidence, has a very long history. There were foot plows (Curwen, 1953), also called “digging sticks”, shown in Egyptian tomb paintings (Burke and Connections, 1978). Also, a wooden model of oxen and plow found in an Egyptian tomb was dated to 2000 BCE (Burke and Connections, 1978). In Asia, one of the oldest existing Chinese books titled “Lü Shi Chun Qiu” (compiled in 239 BCE) or “The Annals of Lu Buwei” (Lü et al., 2000), demonstrated the details of when and how to till according to soil and weather conditions and served as an early example of a practical farming guide.

Rotation can also be a tool to manage soil N through legume bio-fixation of N, depending on the chosen crop species. Mono-cropping, especially with non-legumes and heavy nutrient using crops (e.g. tobacco and corn), can deplete soil N (Bationo, 2004). Rotation practices, even simple fallow, helps to restore soil N (Giller et al., 1997). This practice was evident in early Roman times. One of Rome’s greatest poets, Virgil (70-19 BCE), wrote in his poem Georgics (from the Greek, “On Working the Earth”) “For the field is drained by flax-harvest and wheat-harvest, drained by the slumber-steeped poppy of Lethe, but yet rotation lightens the labour”. This emphasizes that fallow was necessary to rotate with those crops that required more nutrients. On the other hand, rotations which include a legume crop can bring biological N fixation into agricultural production systems. Although ancient farmers knew nothing of the biological N fixation process and nothing about the importance of mineral N to plant growth, they intentionally included
legume crops into crop sequences. This was evidenced in the book of natural history where Pliny the Elder (23-79 CE) mentioned several legume-successions as alternatives to conditions that forbade fallowing (White, 1970).

Synthetic fertilizer N application in agriculture production has a relatively short history compared to tillage and rotation practices, because knowledge regarding N in plant nutrition and N synthesis techniques are recent. In 1836, Jean-Baptiste Boussingault (1801-1887) did experiments on manure, crop rotation, and N sources and for the first time concluded that N was a major component of plants and that the nutritional value of fertilizer was proportional to its N content (Smil, 2004). However, ammonia could not be easily synthesized from constituent elements until 1908, when the Haber-Bosch process was developed. After that, synthetic fertilizer N started to play a greater role in agricultural production, helping to improve global food security (Erisman et al., 2008).

The Influence of Synthetic Fertilizer N on Traditional Agronomic Practices

The appearance of synthetic fertilizer N brought a huge increase in the global food supply and Erisman et al. (2008) estimated that around 50% of the world population’s food requirements are currently met by the use of synthetic fertilizer N. However, synthetic fertilizer N fundamentally disturbed the soil N cycling balance in agro-ecosystems, and brought significant changes in traditional agronomic practices.

Synthetic fertilizer N played a role in the development of modern no-tillage farming. While few people recognized the fertilizer N contribution to no-tillage, early Kentucky no-tillage by N fertility trials unveiled its importance (Rice et al., 1986). No-tillage without N fertilizer significantly lowered yield compared to conventional tillage without
N fertilizer. However no-tillage with N fertilizer reached yields comparable to those of
conventional tillage with fertilizer N. From this perspective, one can speculate that added
fertilizer N compensated for the reduction in soil N mineralization with the lack of
tillage. Other factors, including herbicide and equipment development, also made
Kentucky no-tillage farming feasible, beginning in the 1960s (Phillips and Young Jr,
1973). At the time, the move away from tillage was viewed with much skepticism, but
eventually no-tillage was accepted as a revolution in farming. By 2009 approximately 36
% of U.S. cropland, planted to eight major crops, was under no-tillage soil management
(Horowitz et al., 2010). Agriculture derives numerous benefits from no-tillage, including
fuel and labor savings, increased soil C stocks and erosion resistance.

Although ancient farmers knew nothing of biological N fixation, legume crops had been
an important cropping systems component, worldwide, before synthetic N became
available (White, 1970). However, crop rotation was discouraged during the Green
Revolution, partially because pest control benefits from crop rotation could be replaced
by chemical crop protectants (Bruns, 2012). Also, the N credits from biological N
fixation could be easily replaced by synthetic fertilizer N. However, soon after the height
of the Green Revolution, many studies reported that no amount of chemical fertilizer or
pesticide could fully compensate for crop rotation benefits (Karlen et al., 1994; Roth,
1996). Rotation systems then came back into fashion. Currently, 80 percent of all corn,
soybean, and wheat planted acres in the United States are under rotation.
Systematic Understanding of Agronomic Practices and N Dynamics

This brief review of agricultural history establishes that management of N dynamics is one of the central reasons farmers developed and implemented specific agronomic practices. Furthermore, in the last few decades, new knowledge indicates how transient N can have negative impacts on global environments and human health (Townsend et al., 2003). A systematic understanding of “How does soil and crop sequence management influence nitrogen dynamics?” will have a significant impact on agronomic practice development, but also has global meaning for the quality of human life. The aim of optimal agricultural N management is to enhance net N mineralization at times when crops need N, to synchronize soil N mineralization with crop N uptake, and to minimize N loss. To systematically understand this topic, three sequential steps need clarification:

i. **How do agronomic practices affect soil organic matter pools?**

ii. **How do soil organic matter pools contribute to soil N availability?**

iii. **How do agronomic practices influence crop N uptake capacity?**

Soil organic and crop residue N pools provide the organic N for N mineralization. This microbial process, primarily heterotrophic, also requires soil organic C (SOC) as an energy source (Sollins et al., 1984; Chen et al., 2014). Thus, to understand how soil and crop management affect mineralized soil N, it is critical to first evaluate whether and how tillage, rotation, and fertilizer N application affect SOC and N sequestration. Soil organic matter sequestration has been reported to be linked with soil aggregate formation, thus the dominant concept that explains SOC and N sequestration is based on the aggregation-SOM model (Six et al., 2004). The basic idea is that soil organic matter can function as a
nucleus/binding agent for aggregate formation. Aggregates are considered important reservoirs of SOC and N that are protected from microbial access and less subject to physical, chemical, microbial, and enzymatic degradation (Six et al., 2000).

Proper and precise estimation of soil N mineralization has been a challenge since the early 1900s (Bundy and Meisinger, 1994). Temporal and spatial variability are large because this process is determined by both internal soil factors (e.g. SOM level, labile C and N pools, soil microbial community) and external environment factors (e.g. temperature, precipitation, and aeration) (Goncalves and Carlyle, 1994; Sierra, 1997; Zech et al., 1997). Agronomic management, such as plant species and N fertilizer application, may also affect N mineralization (Gill et al., 1995; Van Der Krift and Berendse, 2001). With current technology it is impossible to predict N mineralization by taking these factors into consideration at once. Instead of being a measure of available N supply, N mineralization estimates by current methods should be considered an index of N availability (Binkley and Hart, 1989). Isotopic tracers and incubation methods are the two main approaches used to estimate N mineralization. The isotopic tracer method can measure gross N mineralization, but isotope methods are most expensive and can also have methodological problems with mineralization rate estimates and other assumption violations (Hart et al., 1994). Although incubation methods can only measure net soil N mineralization (net soil N mineralization = gross N mineralization – N immobilization), incubation can fairly estimate the available N pool, which has a practical value for efficient N management in agro-ecosystems. Therefore, long-term biological mineralization has been considered the most suitable soil N availability index, and is often used to validate other indices derived from more rapid chemical or biological
essays (Keeney, 1982; Griffin et al., 2007). There are, however, many variations to incubation methods, including environment, sample pretreatment, and incubation time, and each variation has advantages and disadvantages. To use incubation to meet research objectives, assumptions, pros and cons of each variation should be considered.

An experimentally derived N availability index might not necessarily reflect total crop N uptake. Besides the amount of available soil N, crop N accumulation also depends on N uptake capacity. Crop N uptake capacity might be determined by either/both genetic and environmental controls. Genetics can control crop growth rate and biomass accumulation, which would result in different N demands at different growth stages (Gastal and Lemaire, 2002). Crop species have different root architectures, mostly controlled by genetics (Clark et al., 2011). However, roots, the dominant nutrient uptake organ directly exposed to the soil, interact with a wide array of soil physical, chemical, and biological factors that vary in time and space (Lynch, 1995). To understand the impact of agronomic management practices on crop N uptake or yield, both soil N availability and root architecture need to be taken into consideration.

Literature concerning the impact of agronomic practices on crop N uptake or yield is reviewed in three sequential steps. First, the mechanism and effect of agronomic practices on SOC and STN sequestration are described. Second, the pros and cons of long-term incubation methodologies for estimating N mineralization are described. Finally, the potential effects of soil and crop management on root architecture are discussed.
1.2 Mechanisms and Effect of Agronomic Practices on Soil C and N Sequestration

The link between SOC and total soil N (STN) decomposition and stabilization and soil aggregate dynamics has been developed, recognized and intensively studied since the 1900’s (Six et al., 2004). Soil organic C and N dynamics are important to agricultural production because these affect soil nutrient cycling and plant productivity (Bauer and Black, 1994). The C and N dynamics are also important to the environment because they can affect greenhouse gas emissions and water quality (Spalding and Exner, 1993; Cole et al., 1997). However, these processes happen in a heterogeneous soil matrix and have multiple interactions with soil biota (Six et al., 2004). The task of elucidation is complex. Aggregate-SOM models have the potential to explain some of these complexities. Aggregates not only physically protect SOC and SON, but also influence soil microbe community structure (Lupwayi et al., 2001), limit oxygen diffusion (Sullivan, 1990), regulate water flow (Prove et al., 1990), determine nutrient adsorption and desorption (Linquist et al., 1997; Zhang et al., 2011), and reduce surface runoff and erosion (Bissonnais, 1996). All these processes have fundamental effects on soil C and N sequestration and stabilization. Most current studies to understand the impact of agronomic practices on soil C and N sequestration have been based on the aggregate hierarchy concept, proposed and developed by (Tisdall and Oades, 1980).

To apply the theoretical aggregate-SOM models, the first consideration is the physical separation of soil into different aggregates size classes. Two main methods to separate soil aggregates are widely used by researchers: dry sieving and wet sieving (Kemper and Rosenau, 1986). The disruption of aggregates is due mainly to slaking and micro-cracking when the soil is initially dry. Therefore, dry sieving of air-dried samples is used
to characterize the aggregate size distribution with minimum destruction. Wet sieving is used to simulate micro-cracking and slaking (Puget et al., 1995). However, water-stable aggregate stability from wet sieving procedures was reported to be closely correlated with SOM stabilization because SOM can act as a transient binding agent (Tisdall and Oades, 1982) and has served as an effective early indicator of soil C change in numerous studies (Veum et al., 2012). Therefore, the wet-sieving procedure has been frequently used to evaluate the agronomic practice effects on both SOM sequestration and soil structural stability (Beare et al., 1994a; Angers and Giroux, 1996). In the wet-sieving procedure, there have been some modifications in sample pretreatment (Bissonnais, 1996). The rewetting pretreatments for soils can cause different results when comparing soils and management history treatments (Le Bissonnais, 1996). Cambardella and Elliott (1993a) showed that capillary-wetted soils retained larger amounts of macroaggregates (> 250 µm) than slaked soils. (Le Bissonnais, 1996) demonstrated that the different aggregate breakdown methods and frequency of crusting soil samples can dramatically affect soil aggregate stability within the same soil management system. Therefore, adopting minimum breakdown aggregates in the sieving procedure would keep comparisons between treatments relative to the natural field conditions.

The effect of agronomic practices; including tillage, rotation, and fertilizer N application; on SOC and STN according to aggregate-SOM models have been studied intensively in grass and grain crop production systems (Haynes and Naidu, 1998; Six et al., 1998; Kong et al., 2005; Sainju et al., 2009; Brye et al., 2012), but not in tobacco production systems. In these studies, no-tillage was reported to increase or maintain SOC and STN compared to conventional tillage. With aggregate separation, results have found conventional tillage
can increase large aggregate turnover rate, resulting in the loss of macro-aggregate proportion and SOC and STN concentrations (Cambardella and Elliott, 1993a). In contrast, no-tillage increases macro-aggregates and SOC and STN accumulation.

Most studies have shown that rotation increased SOC and STN sequestration, compared to mono-cropping (West and Post, 2002; Kong et al., 2005). Crops in rotation schemes have different impacts on SOM stabilization, depending on the quantity and quality of crop residues. (Wright and Hons, 2005a) found that crop residue production was similar among wheat, sorghum and soybean fields, but the wheat field had significant higher SOC and STN in surface soil than the other two fields, which indicates that the higher C:N ratio in wheat residue can play a role in SOM stabilization. Kong et al. (2005) reported that the quantity of crop residue/carbon production had a linear relationship with SOC sequestration in sustainable cropping systems. Therefore, when evaluating crop rotation schemes on SOM sequestration, an examination of crop residue quantity and quality is important.

Studies of the effect of fertilizer N application on SOM sequestration have produced the most controversial results. Some studies report fertilizer N application to increase SOM because higher fertilizer N input can cause more crop residue to be returned to soil (Haynes and Naidu, 1998). Mulvaney et al. (2009) reported that fertilizer N application decreased soil N in the long-term Morrow plot study and argued that synthetic N application enhanced soil microbial decomposition due to the decreasing C:N ratio. However, others found no effect of fertilizer N application on SOM sequestration (Su et al., 2006; Brown et al., 2014).
1.3 Methodologies of Soil N Mineralization Measurement

There are many different methods available for long term aerobic incubation, in both laboratory and field, depending on soil sample pretreatment and other incubation conditions (Beauchamp et al., 1986).

1.3.1 Laboratory Incubation Methods

Most aerobic laboratory incubation methods have common features, including maintenance of optimal soil water status (typically 60 % water filled pore space), constant temperature (commonly 25, 30, or 35 C), and periodic sampling with time so as to estimate N mineralization rates (Griffin et al., 2007). Although there have been several standardized protocols (Bundy and Meisinger, 1994; Honeycutt et al., 2005), there is significant variation in aerobic incubation details.

Leaching versus Non-Leaching Processes

In early studies with long-term N mineralization incubation, samples were usually incubated continuously in a container without periodic leaching of the accumulated inorganic N. The merit of this method was convenience, but there could be cumulative inhibitory effects, such as pH decline, on mineralization during the incubation (Allison and Sterling, 1949). Thus, non-leaching approaches were not recommended for long incubation periods. Stanford and Hanway, (1955) proposed a periodic leaching approach during incubation. Briefly, 0.01 M CaCl$_2$ was used to leach mineralized N out of the sample at the end of each incubation period (Stanford and Smith, 1972). The merit of leaching would be avoidance of accumulation of unspecified toxins. While a time-consuming and apparatus requiring process, there was also a technical concern with
potential leaching of soluble organic N during the incubation (Smith et al., 1980; Beauchamp et al., 1986).

**Excluded Crop Residue versus Included Crop Residue**

Crop residue can contribute to the soil inorganic N pool either by N mineralization or immobilization, depending on the residue C:N ratio. However, most laboratory incubation methods exclude such contributions by discarding visible pieces of residue in the pretreatment sieving process (Hart et al., 1994). Some laboratory methods cut entrained residues into pieces that are mixed with soil for incubation (Heumann et al., 2002). Certainly, discarding big portions of residue might cause inaccurate estimates of the N credit from the previous crop because soil fertility guidelines usually recommend a different fertilizer N rate for the current crop that depends on the previous crop.

**Field-Moist Soil Sample versus Dried and/or Ground Soil Sample**

Using dried and/or ground soil is convenient for a large amount of soil samples that require time to process or for cooperative projects where soil samples come from multiple locations at different times. However, several days are needed to rewet soil for pre-incubation, which also causes an N mineralization flush during the first weeks of incubation. Numerous studies have reported that sample sieving and drying-rewetting can cause rapid microbial death and enhance microbial respiration and activity, producing an N mineralization bloom (Mikha et al., 2005; Miller et al., 2005; Wu and Brookes, 2005; Xiang et al., 2008). Using field-moist samples might cause less physical damage during pre-incubation protocols and cause a better transition from field to lab conditions than dried and/or ground soil samples. However, field-moist soil samples intended for
incubation need to be gently crushed through the sieve (usually 2 to 4 mm) immediately after sample collection.

**Homogenized Soil versus Undisturbed Soil Cores**

Most laboratory incubation methods utilize a homogenized sample created by sieving. However, there are reports that homogenized samples do not well represent the effects of field soil tillage. Laboratory soil should have a physical structure similar to that of the field environment the sample represents, but sieving artificially “tills” soil from undisturbed/no-tillage environments. This can expose aggregate-protected SOM and enhance microbial activity, resulting in an over-estimate of N mineralization. Therefore, undisturbed cores may be a better option for laboratory incubations intended to differentiate the impact of tillage on N mineralization (Rice et al., 1987).

**Constant Temperature versus Variable Temperature**

Most laboratory incubation methods use a constant temperature, which does not reflect temperature fluctuation under field conditions. Carpenter-Boggs et al. (2000) proposed a variable-temperature method for laboratory incubation where soil samples are incubated in a variable temperature incubator (VTI) that mimicked field soil temperatures under a growing corn canopy. They reported that the VTI technique provided lower sample variance and a smaller initial flush of N mineralization than constant temperature (35 C) incubation.
1.3.2 Field (In-situ) Incubation Methods

Due to the uncertainty regarding the extrapolation of laboratory N mineralization values to the field, estimating N mineralization from SOM and crop residues under field conditions would be a compelling research topic for investigators because more efficient N fertilization practices could be hastened if a reliable in-situ N mineralization method was developed. So far, there have been three dominant in-situ research techniques, using buried polyethylene bags, covered cylinders, or resin-trap core methods.

**Buried Polyethylene Bag Method**

The buried polyethylene bag method for in situ N mineralization was proposed by Eno (1960). The main driving force behind this technical development was the realization that soil temperature variance would result in considerable changes in the rate of soil nitrate production. In that preliminary laboratory study, soil in sealed polyethylene bags had an equal rate of nitrification compared to that contained in ventilated bottles. Polyethylene is permeable to oxygen and carbon dioxide, but no nitrate diffused through the polyethylene bag during the 24 week incubation period. The preliminary results and polyethylene characteristics caused this technique to have the potential to estimate aerobic in-situ soil N mineralization.

Advantages to this technique include mimicking field temperature conditions at a low cost. However, the technique does not reflect transient field moisture conditions (Hanselman et al., 2004). Elevated concentrations of nitrate and carbon dioxide inside the bags may promote denitrification (Subler et al., 1995). Physical damage to the bags by insects or plant roots may result in losses of mineralized N into field soil via diffusion...
and mass flow (Eno, 1960; Hanselman et al., 2004). Another major limitation of this technique was the inevitable disturbance of soil, which does not allow a valid comparison of tillage effects on N mineralization under field conditions (Rice et al., 1987).

**Covered Cylinder Method**

The covered cylinder method was developed as a more durable alternative to the buried bag and this technique allows incubation of intact soil cores (Raison et al., 1987b). Covered cylinders are usually constructed from PVC or metal pipes that are capped to exclude rainfall, which is also assumed to stop inorganic N leaching (Adams and Attiwill, 1986). Although the tubes are open at the bottom, aeration is less than that found in field soil, which might result in higher denitrification potential. Therefore, modifications such as use of less than air-tight caps or perforations in the tube sidewall were often added to promote air exchange and reduce denitrification potential (Rapp et al., 1979; Dou et al., 1997). However, those sidewall aeration holes could potentially cause mineralized N loss. Water might enter the soil tubes through aeration holes, causing N leaching at the bottom of the soil column. Furthermore, plants roots might potentially grow into the soil column via aeration holes or the open bottom, absorbing mineralized soil from the tubes. Another major limitation of this technique is that the soil in the tube usually has a lower soil moisture than that in the field (Hanselman et al., 2004).

The basic principle of the covered cylinder method was the limiting of N leaching by sheltering incubating soil from precipitation. Based on the same principle, there was another *in-situ* method called the “rain shelter” (Powelson, 1980; Rice et al., 1987), which simply used a shelter over the sampled area to prevent leaching. However, except for
considerations regarding the quality and durability of the rain shelter and surface water run-on during intense rainfall, the major drawback to this technique was that a lack of ability to reflect field soil moisture fluctuations.

**Resin-Trap Soil Core Method**

Buried polyethylene bags and covered cylinder methods can capture variation in field temperature, while failing to reflect moisture and aeration conditions, which are reported to play a large role in soil N mineralization (Sierra, 1997). Therefore, an alternative *in-situ* method was proposed that employed ion exchange resins to capture mineralized N leaching from undisturbed soil cylinders (DiStefano and Gholz, 1986; Kolberg et al., 1997). The major modification of this technique were an open cylinder top, which allowed the precipitation and air to freely enter the intact soil column, and a resin trap at the bottom to capture inorganic N that might otherwise leach from the tube. There were some concerns about whether the soil tube caused abiotic differences between soil in the tube and the surrounding field soil. Wienhold et al. (2009) reported that soil inside the cylinders was slightly wetter and warmer than adjacent soil, which would likely increase soil N mineralization. However, they further pointed out that the magnitude was likely much less than the normally observed field core-to-core variation. Therefore, this method was found to better track true field conditions (Hanselman et al., 2004) and has potential to become a standard procedure (Khanna and Raison, 2013).

The drawback with the method is a large resource demand. This technique requires preliminary studies to ensure leached ions are efficiently trapped under field conditions. Resin duality, adsorption capacity and bypass flow are all factors that can potentially
influence resin effectiveness in capturing leached N. Also, the extraction of adsorbed N from the resin is time consuming. Kolberg et al. (1997) reported that five extractions with KCl were required to completely release adsorbed N.

**Other Modifications to In-Situ Incubation Methods**

Except for the major design developments mentioned above, some minor modifications to *in-situ* incubation methods have been suggested. Hatch et al. (1990) proposed a method to combine the soil core with acetylene inhibition, which would limit N loss by denitrification due to uncontrolled soil *in-situ* incubation conditions. The big concern with this modification would be that the tube must be sealed at the top, causing a loss in practical application to the field environment if rainfall were a concern. Given consideration of different drainage characteristics in resin-trap soil cores, relative to the surrounding soil, Hanselman et al. (2004) developed a “new” type of resin-trap soil core method in which resin is mixed with soil to create an artificial uniform soil column. This method is impractical when undisturbed soil structure is a research concern, as in a comparison of conventional and conservation tillage (Rice et al., 1987).

**1.3.3 Method Selection**

As discussed above, each method, including laboratory and *in-situ* methods, has unique assumptions, advantages, and disadvantages. There is no a standard method that will work for every situation. The selection of method would depend on the nature of the study, available resources, and site-specific factors. Although laboratory methods might not reflect natural field conditions, these can provide reasonable relative values to estimate differences due to soil type and certain management practices. The primary
merit to field incubation is a more practical estimation of N mineralization, which might be more useful in management decision making. However, the substantial time and apparatus requirement for the in-situ incubation methods needs to be taken into consideration. The principle is that both biotic and abiotic factors control the soil N mineralization process. Knowing the advantage and disadvantage to each method can help the investigator choose the best method while avoiding misinterpretation of results.

1.4 The Influence of Agronomic Practices on Root Architecture

Plant roots are a fundamental component of terrestrial ecosystems and function to maintain nutrient and water supply to the plant (Russell, 1977). Although root system architecture is controlled mainly by genetic factors (de Dorlodot et al., 2007), plant root systems exhibit highly plastic development. This plasticity is possible because root development results from continuous propagation of new meristems. In a heterogeneous soil matrix, a wide array of physical, chemical, and biological factors can affect the initiation and activity of root meristems (Lynch, 1995). Previous studies have reported that certain crop root traits can enhance productivity in resource-limited environments due to improved nutrient and water scavenging abilities (Liao et al., 2001; Ribaut et al., 2009; Lynch, 2011). Therefore, agronomic practices have the potential to influence crop nutrient uptake capacity by affecting the root growth environment.

Tillage can affect root growth mainly by changing soil structure, strength and penetration resistance. Any particular root increases its length through primary growth when cells of the meristem divide, elongate, and push the root tip forward through the surrounding materials. Turgor pressure in the elongating cells is the driving force and must be
sufficient to overcome cell wall constraints and other additional constraints imposed by the surrounding environment (Foy and Carson, 1974). Compared to conventional plow tillage, numerous studies on grain crops report that no-tillage can increase mechanical impedance, which can result in reduced root length density, root surface density, and lower biomass production (Gajri et al., 1992; Mosaddeghi et al., 2009; Guan et al., 2014). Similar results were found in a no-tillage burley tobacco study (Zartman et al., 1976). Furthermore, greater mechanical impedance with no-tillage not only restricts root growth but also changes root morphology, restricting main root axis elongation, stimulating lateral root branching, and root thickening (Griffith et al., 1977; Cook et al., 1996).

Nutrient supply and distribution (or fertilizer application) can affect root system architecture mainly by signaling (ROBINSON, 1996; López-Bucio et al., 2003). Typically, roots proliferate in volumes where nutrients are most concentrated (Robinson, 1994). However, the mechanisms of plant root response to the different nutrient elements might be controlled by different pathways and signals (Zhang and Forde, 1998; Zhang et al., 1999; Williamson et al., 2001; Mantelin and Touraine, 2004).

There have been few studies of the effect of crop rotation on plant root architecture. However, given the basic factors controlling root development, the hypothesis that crop rotation might have different effects on root architecture than mono-cropping systems. If rotated with residue-rich or deep rooted crops which can increase SOM levels and soil structure. In this case, rotation can affect root proliferation by changing soil structure in a manner similar to that observed with no-tillage. If rotation involved legumes, more N nutrition is provided than that found with mono-cropping. In this case, rotation could
affect root architecture by changing soil nutrient supply in a manner similar to that found with fertilizer application.

From the discussion above, the effects of agronomic practices on crop N uptake cannot only affect SOM sequestration and soil N mineralization, but can also cause a soil environment for plant root proliferation. Similarly, in the paper titled “A New Worldview of Soils” (Lin, 2014), soil productivity is broadly defined as the soil’s unique ability to supply water, nutrients, air, and heat, among other life-sustaining resources, adjusting that supply to the demands of plants and microbes. Soil resources fall into two main components; a) nutrients and moisture; and b) an environment suited for root growth and microbial activity.

1.5 Conclusions and Dissertation Overview

Agronomic practices reflect agriculture’s N management history. Currently, agronomic practices have two major responsibilities: a) to promote global food production; and b) to maintain the agro-ecosystem environment. This review demonstrates that soil N dynamics have the potential to provide a framework for understanding how agronomic practices can connect these two responsibilities. Systematically understanding N cycling in the context of a suite of soil and crop management practices provides a foundation to understanding, developing, evaluating and reshaping those agronomic practices.

In this dissertation, laboratory and in-situ studies were conducted at two long-term study sites at the University of Kentucky’s Spindletop Research Farm. In Chapter 2, the effect of tillage, rotation, and fertilizer N application on aggregate distribution and associated C and N storage in burley tobacco production systems is evaluated. In Chapter 3, there are
three topics, including a comparison study of laboratory and *in-situ* incubation methods for estimating the relative impact of tillage and rotation management on soil N mineralization, a study evaluating fertilizer N management on *in-situ* net N mineralization, and a study evaluating soil indicators of laboratory soil N mineralization. In Chapter 4, no-tillage culture and N fertilizer management for burley tobacco production is discussed. Finally, in Chapter 5, a summary of the findings obtained in this series of studies is presented.
2.1 Introduction

Burley tobacco has been produced in Kentucky since the 1860s (Kleber, 1992). While still a leading producer, Kentucky burley production has fluctuated because of the Tobacco Transition Payment (Tobacco Buy Out) Program (Womach, 2004), stricter production requirements, and global market demand. Although Kentucky was a pioneer in no-till crop production (Phillips and Young Jr, 1973), most burley tobacco production is still tillage intensive due to grower tradition, the expense of no-till tobacco transplanters, and the limited number of herbicides labeled for tobacco production in the USA (Zou, 2013). One result of the tobacco buy out has been that field production units have become larger, resulting in less crop rotation and, in some cases, no rotation at all. Intensive tillage and monoculture tend to accelerate the depletion of soil organic matter, degradation of water stable macroaggregates, and weakening of soil physical structure, leading to increased production cost and soil erodibility (Zotarelli et al., 2007; Kasper et al., 2009). No-tillage and rotation with grass sod or row crops are alternative management strategies for more sustainable tobacco production. Information is available to demonstrate the impact of no-tillage practices on tobacco productivity (Phillips and Zeleznik, 1989; Pearce and Zeleznik, 1996b), but little is known about how soil aggregates and associated SOC and STN stocks in tobacco production fields respond to these conservation practices.
No-tillage management has been shown to be effective at increasing SOC and STN stocks in agricultural ecosystems (Six et al., 1998; Wright and Hons, 2005a). Under no-tillage, more shoot and root residues at the surface soil, combined with less large aggregate disruption, results in higher carbon (C) and nitrogen (N) sequestration and a greater macroaggregate fraction (Sainju et al., 2009). In contrast, tillage mixes crop residues into the surface soil and disrupts aggregates, especially macroaggregates, which enhances residue decomposition and humus oxidation (Beare et al., 1994a). Similarly, increases in SOC and STN have been observed in diverse and intensive crop rotation systems, relative to monoculture, in numerous studies (Wright and Hons, 2005a; Sainju and Singh, 2008; Veum et al., 2012). The SOC and STN accumulation is regulated by the quantity and quality of crop residue returned to the soil (Wright and Hons, 2005a). Compared to other row or sod crops, burley tobacco production returns fewer residues to the soil because burley tobacco is a short-season, shallow rooted crop and the entire above ground portion of the plant is harvested.

Burley tobacco’s high cash value can lead to ineffective excess N fertilization of the crop (MacKown and Sutton, 1997; MacKown and Sutton, 1998). However, there might be two impacts of excess fertilizer N application on aggregation and SOC and STN sequestration, with contrary results. One is that fertilizer N can have a positive effect on SOM accumulation and macroaggregate formation due to increased residue biomass input (Lugato et al., 2010; Yu et al., 2012). The other is that fertilizer N can have a negative effect on SOM accumulation via increased SOC mineralization primed by N fertilizer addition (El-Haris et al., 1983a; Jordan et al., 2004; Khan et al., 2007). Several studies found no difference between N fertilized and unfertilized treatments with regard
to aggregate size fractions and associated SOC and STN stocks (Plaza-Bonilla et al., 2013; Tripathi et al., 2014). Given these results, fertilizer N application effects on soil aggregation, C and N might vary with different crop rotation and tillage management practices.

SOC and STN sequestration have been closely linked to aggregate stability (Six et al., 2004). Soil organic matter can be a nucleus for aggregate formation and act as a binding agent (Tisdall and Oades, 1982). Aggregates are considered an important reservoir of SOC and STN that is protected from microbial access and less subject to physical, chemical, microbial, and enzymatic degradation (Bajracharya et al., 1997; Trujillo et al., 1997; Six et al., 2000b). Therefore, aggregate-SOM models are an important and classical way to investigate SOC and STN stabilization (Six et al., 2004). This stabilization offers physicochemical protection in hierarchical soil aggregates and is critical to building and maintaining SOC and STN stocks (O'Brien and Jastrow, 2013). Among the several aggregate sizes, the proportion of the macroaggregate fraction (> 250 µm) reportedly serves as an early effective indicator of the SOM dynamics influenced by agronomic practices (Six et al., 1999; Veum et al., 2012).

Soil aggregate size distribution and stability are also considered important physical indicators of soil quality, reflecting the impact of land use and management (Kemper and Rosenau, 1986; Castro Filho et al., 2002) on aggregation or degradation (Boix-Fayos et al., 2001; Barthes and Roose, 2002), nutrient supply potential (Bronick and Lal, 2005) and soil health (Seybold and Herrick, 2001). Thus, physically separating aggregates and determining the associated SOC and STN stocks is a systematic way to evaluate the
influence of tillage, rotation and N fertilization management on structure, SOC and STN sequestration in burley tobacco production systems.

Conventional burley tobacco production would be considered non-sustainable due to the negative impacts on aggregation, SOC and STN stocks. The primary objective of this experiment was to investigate whether tillage, rotation and recent N fertilization practices influenced aggregate size distributions and aggregate-associated SOC and STN stocks in soils under burley tobacco production. The hypotheses were: i) no-tillage, rotation with sod and high N fertilization rates would result in a greater proportion of macroaggregates and improved soil structure when compared with conventional tillage, monoculture, and low N fertilization rates; and ii) no-tillage, rotation with sod and high mineral N input would increase bulk SOC and STN stocks by increasing macroaggregate-associated SOC and STN concentrations and stocks.

2.2 Materials and Methods

Site description

This research was conducted at the University of Kentucky Spindletop Research Farm near Lexington, Kentucky, USA (38°07'36.8"N 84°29'26.4"W). The soil was a deep well-drained Bluegrass-Maury silt loam (fine, mixed, active, mesic Typic Paleudalf), formed in phosphatic limestone residuum, with a 2 to 4 percent slope. When the study was established in 2007, the baseline soil water pH (1:1, w/v) was 6.3, and Mehlich III (Mehlich, 1984) extractable nutrient levels were 85, 178, 1800, 110 and 3 mg kg⁻¹ for phosphorus, potassium, calcium, magnesium, and zinc, respectively. The soil texture was 12 % sand, 68 % silt, and 20 % clay. The location is characterized by wide variation in
mean monthly air temperatures, from 0°C in January to 24°C in July and August, but a relatively uniform distribution in mean monthly precipitation, with an annual average rainfall of 1160 mm.

**Field experiment design**

The experiment was laid out in randomized complete blocks with four replications. The four tillage-crop sequence treatments: i) no-tillage continuous tobacco (TTT-NT); ii) conventional tillage continuous tobacco (TTT-CT); iii) 2-yr fescue sod (*Festuca arundinacea* Schreb.) and 1-yr no-tillage tobacco (SST-NT); and iv) 2-yr fescue sod and 1-yr conventional tillage tobacco (SST-CT); were randomly assigned plots (6.4 m wide by 24.4 m long) in each block. From 2007 to 2012, continuous burley tobacco was grown for 6 seasons and in the 2-yr sod plus 1-yr burley tobacco rotation 2 cycles were completed with 4 years of fescue and 2 years of tobacco. Primary conventional tillage consisted of moldboard plowing, with secondary tillage consisting of disk ing (twice), followed by a rotary tillage operation. Other management practices were as recommended by the University of Kentucky Cooperative Extension Service (Seebold and Pearce, 2011). In 2012, when all plots were in tobacco, broadcast N rates of 0, 140 or 280 kg N/ha, as NH₄NO₃ (34 % N), were randomly assigned to split plots on 22 May (6.4 m wide by 8.2 m long).

**Soil sampling and sample preparation**

Soil samples were taken on 12 February 2013. For this study, the sub-plots that had received 0 and 280 kg N/ha fertilizer N rates the previous spring were sampled. Soil bulk density was measured in each plot using an aluminum cutting ring 5.4 cm high by 6 cm in
diameter in the midst of the 0 to 10 cm depth increment. The soil cores were oven-dried at 105 °C for 24 hours. For determination of wet aggregate size distribution and both SOC and STN concentrations, soil cores (15 cores) were randomly collected from the 0 to 10 cm depth increment in each sub-plot using a 2.5 cm diameter probe and then compositied into one representative field moist sample. The field moist soil was passed through an 8 mm sieve. In this study, aggregates from all treatments were smaller than 8 mm in diameter. Visible pieces of root residue and rock were removed. The field moist samples were brought to the laboratory and kept at 4 °C in sealed plastic bags until wet sieving. Wet sieving fractionation was performed within one week of sampling.

**Wet aggregate sieving procedure**

Aggregate size classes were physically separated with wet sieving (Cambardella and Elliott, 1993b; Cambardella and Elliott, 1993a), but, to avoid potential breakage during rewetting of air-dried soil (Kemper and Rosenau, 1986), field moist soil was used. Subsamples of field moist soil were taken to determine gravimetric water content after oven drying at 105 °C for 24 hr. Another subsample of field moist soil, equal to 50 g of oven dried soil after moisture content correction, was slowly wetted via capillary action for 10 min in deionized water to reduce slaking, and then samples were wet sieved through a series of three sieves to obtain four aggregate size fractions: i) > 2000 µm (large macroaggregates); ii) 250 to 2000 µm (small macroaggregates); iii) 53 to 250 µm (microaggregates); and iv) < 53 µm (silt plus clay-sized particles). For the rewetting process, aggregate disruption was manually accomplished by moving the sieve 3 cm vertically 50 times during a period of 2 minutes. Materials remaining on the sieves were quantitatively transferred to glass jars and dried at 50° C in a forced-air oven. The
suspension containing the < 53 μm particles was brought to 5 L, well stirred, and a 500 mL sub-sample of that suspension was transferred to a glass jar and dried at 50° C in a forced-air oven. Aggregate fractions were weighed at room temperature. A subsample of each 50 °C dried aggregate fraction was dried at 105 °C for 24 hours for final aggregate moisture correction.

In this soil, both sand and iron-manganese nodules could be present in the aggregates, without actually being part of any aggregate (Elliott et al., 1991; Rhoton et al., 1993). A dry mass correction for the presence of sand and other coarse primary particles in intact aggregates was accomplished by dispersing 2 g of 50 °C–dried aggregates in 15 mL of a 5 % (w/v) solution of sodium hexametaphosphate and shaking the suspension overnight in a reciprocal shaker (180 min⁻¹). The dispersed particles were then sieved through a 53 μm sieve, washed using ionized water, and oven-dried at 105° C. The mass of coarse primary particles was subtracted from the previously obtained mass for that aggregate fraction.

**Soil C and N determination**

Bulk soil and aggregate subsamples were analyzed for SOC and STN concentrations on an Elementar Vario MAX CNS Analyzer (Elementar Americas Inc., Mount Laurel, NJ). Prior to chemical analysis, visible shoot and root residue was hand removed with a forceps and the subsamples were then ground in a mortar and pestle.
Calculations

To assess changes in soil structure due to the tillage, rotation and fertilizer N treatments, parameters expressing the aggregate size distribution were calculated. Aggregate mean weight diameter (MWD) was calculated with Equation 1 (Kemper and Rosenau, 1986):

\[
MWD = \sum_{i=1}^{n} X_i \times W_i
\]

Equation 1;

where \(X_i\) is the weight fraction (percentage) of each sand/coarse particle corrected aggregate size fraction and \(W_i\) is the mean diameter for each size fraction. The aggregate geometric mean diameter (GMD) was calculated with Equation 2 (Kemper and Rosenau, 1986):

\[
GMD = \exp\left[\frac{\sum_{i=1}^{n} W_i \times \ln X_i}{\sum_{i=1}^{n} W_i}\right]
\]

Equation 2;

Sand/coarse particle corrected aggregate SOC or STN concentration (g/kg) was expressed as: (aggregate STN or SOC concentration (g/kg)) \times 100/ (100 – sand/coarse particle %)

Stocks of SOC (SOCs) or STN (STNs) in whole soil (g/m²) were calculated with Equation 3:

\[
SOCs \text{ (or STNs)} = D \times BD \times SOC \text{ (or STN)} \times 10
\]

Equation 3;

where \(D\) is the thickness (cm) of the soil layer, \(BD\) is the bulk density (g/cm³), and SOC (or STN) is the SOC (or STN) concentration (g/kg) of the 0 to 10 cm soil layer.

Stocks of SOC (SOCsi) or STN (STNsi) in each aggregate size fraction (g/m²) found in the 0 to 10 cm soil layer were calculated with Equation 4:
SOCsi (or STNi) = \frac{D \times BD \times SOCi(\text{or STNi}) \times Wi}{10} \quad \text{Equation 4;}

where SOCi (or STNi) is the OC (or TN) concentration of the $i$th size fraction (g/kg aggregate), and $Wi$ is the weight proportion of the total soil in the $i$th size fraction (%).

**Statistical analyses**

Data were analyzed with the General Linear Model (GLM) procedure of the SAS 9.3 computer package (SAS Institute Inc., Cary, NC). Fertilizer N rate was considered a discrete variable and was not analyzed as a quantitative variable because no interpolation of fertilizer N effects over the 0 to 280 kg N/ha rate range was intended. Rather, we wanted to understand fertilizer N impacts at the chosen rates. Replicate measurements on composite soil samples were averaged for statistical analysis of treatment effects. Treatment effects were declared significant when the probability ($p$) of a greater $F$ statistic was $\leq 0.05$. Means separation was done by using the Tukey's HSD (honest significant difference) test at the 95 % level of confidence.

**2.3 Results**

**Soil Structure and Water Stable Aggregate Distribution**

Bulk density was significantly affected by tillage, but not by rotation or previous N fertilizer rate (Table 2.1 and Figure 2.1). The MWD and GMD were significant affected by tillage and the rotation by N rate interaction (Figure 2.2). In the rotation by N rate interaction, rotation with fertilizer N resulted in significantly higher MWD than rotation without fertilizer N, while monoculture tobacco with fertilizer N resulted in a
significantly lower MWD than monoculture without fertilizer N. The same trend was observed in GMD values.

Aggregate size fractions were significantly affected by two interactions, tillage by rotation and rotation by N rate (Table 2.1). In the tillage by rotation interaction, no-tillage significantly increased large and small macroaggregates and rotation with sod was synergistic to no-tillage. In contrast, conventional tillage and monoculture tobacco degraded soil structure, reducing the proportion of large and small macroaggregates and raising the fraction in silt-clay sized particles (Figure 2.3A). In the rotation by N rate interaction, rotation with fertilizer N resulted in significantly more large macroaggregates than rotation without fertilizer N, while monoculture tobacco with fertilizer N resulted in significantly less large macroaggregates than monoculture without fertilizer N. However, the results with small macroaggregates was reversed (Figure 2.3B).
Table 2.1 Analysis of variance for the effects of tillage, rotation, fertilizer N rate, and their interactions, on bulk density, large macroaggregates, small macroaggregates, microaggregates, silt-clay particles, and aggregate mean weight diameter (MWD), and geometric mean diameter (GMD).

<table>
<thead>
<tr>
<th>Effect/contrast</th>
<th>Bulk density</th>
<th>Large macroaggregates</th>
<th>Small Macroaggregates</th>
<th>Microaggregates</th>
<th>Silt-clay particles</th>
<th>MWD</th>
<th>GMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tillage (T)</td>
<td>0.0351</td>
<td>0.0007</td>
<td>0.1383</td>
<td>0.2801</td>
<td>0.0035</td>
<td>0.0005</td>
<td>0.0002</td>
</tr>
<tr>
<td>Rotation (R)</td>
<td>0.9597</td>
<td>0.0261</td>
<td>0.0060</td>
<td>0.8727</td>
<td>0.0095</td>
<td>0.0158</td>
<td>0.0038</td>
</tr>
<tr>
<td>Fertilizer N rate (N)</td>
<td>0.1784</td>
<td>0.5014</td>
<td>0.7477</td>
<td>0.2746</td>
<td>0.4931</td>
<td>0.4252</td>
<td>0.9963</td>
</tr>
<tr>
<td>T*N</td>
<td>0.2740</td>
<td>0.7009</td>
<td>0.3730</td>
<td>0.4350</td>
<td>0.2109</td>
<td>0.5208</td>
<td>0.2055</td>
</tr>
<tr>
<td>R*N</td>
<td>0.4484</td>
<td>0.0001</td>
<td>0.0015</td>
<td>0.0007</td>
<td>0.8115</td>
<td>0.0004</td>
<td>0.0351</td>
</tr>
<tr>
<td>T*R</td>
<td>0.5267</td>
<td>0.0505</td>
<td>0.0004</td>
<td>0.0011</td>
<td>0.0085</td>
<td>0.2959</td>
<td>0.1110</td>
</tr>
<tr>
<td>T<em>R</em>N</td>
<td>0.7124</td>
<td>0.1370</td>
<td>0.1075</td>
<td>0.6069</td>
<td>0.7476</td>
<td>0.2303</td>
<td>0.2756</td>
</tr>
</tbody>
</table>
Figure 2.1 The effect of tillage on bulk density and aggregate mean weight diameter (MWD), and geometric mean diameter (GMD). For any one measured variate, vertical bars with different letter at the top are significantly different at the $P \leq 0.05$ level.
Figure 2.2 The rotation by N rate interaction on aggregate mean weight diameter (MWD), and geometric mean diameter (GMD). For any one measured variate, vertical bars with different letters at the top are significantly different at the P ≤ 0.05 level.
Figure 2.3 The proportion of large macroaggregates, small macroaggregates, microaggregates and silt-clay particles due to the tillage by rotation (A) and rotation by N rate (B) interactions. For any one aggregate size class, vertical bars with different letters at the top are significantly different at the $P \leq 0.05$ level.
Aggregate C and N Concentrations

Tillage significantly affected whole-soil and aggregate-associated SOC concentrations, except for silt-clay particle associated SOC. Rotation significantly affected whole-soil and silt-clay particle associated SOC, while fertilizer N application had no effect on any aggregate size associated SOC (Table 2.2). Compared to conventional tillage, no-tillage burley tobacco production increased whole-soil SOC and, when rotated with a grass sod crop, there was a synergistic effect. Also, the NT-Rotation treatment exhibited the highest aggregate-associated SOC for any aggregate size class (Figure 2.4A). There were significant interaction effects; tillage by rotation on small macroaggregate and microaggregate associated SOC (Figure 2.4A), and rotation by fertilizer N application on microaggregate associated SOC (Figure 2.4B).

Tillage significantly affected whole-soil and all aggregate associated STN concentrations. Rotation significantly affected bulk soil and small macroaggregate associated STN, while fertilizer N application only significantly affected microaggregate associated STN (Table 2.2). Compared to conventional tillage, no-tillage burley tobacco production increased bulk soil STN and rotation with grass sod also synergized this soil parameter. The CT-Rotation treatment exhibited the highest aggregate associated STN for any aggregate size class (Figure 2.5A). There was a significant tillage by rotation interaction on small macroaggregate and microaggregate associated STN (Figure 2.5A), and there was a significant rotation by fertilizer N application interaction on large macroaggregate associated STN (Figure 2.5B).
Table 2.2 Analysis of variance for the effects of tillage, rotation, fertilizer N rate, and their interactions, on organic carbon (SOC) and total nitrogen (STN) concentrations for 0 to 10 cm whole-soil and aggregate fractions.

<table>
<thead>
<tr>
<th>Effect/contrast</th>
<th>Whole-soil SOC</th>
<th>Whole-soil STN</th>
<th>Large macroaggregates SOC</th>
<th>Large macroaggregates STN</th>
<th>Small macroaggregates SOC</th>
<th>Small macroaggregates STN</th>
<th>Microaggregates SOC</th>
<th>Microaggregates STN</th>
<th>Silt-clay particles SOC</th>
<th>Silt-clay particles STN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tillage (T)</td>
<td>0.0071</td>
<td>0.0096</td>
<td>0.0004</td>
<td>0.0015</td>
<td>0.0129</td>
<td>0.0025</td>
<td>0.0129</td>
<td>0.0098</td>
<td>0.0569</td>
<td>0.0066</td>
</tr>
<tr>
<td>Rotation (R)</td>
<td>0.0020</td>
<td>0.0080</td>
<td>0.1955</td>
<td>0.8481</td>
<td>0.3212</td>
<td>0.0225</td>
<td>0.2288</td>
<td>0.6378</td>
<td>0.0105</td>
<td>0.1413</td>
</tr>
<tr>
<td>Fertilizer N rate (N)</td>
<td>0.9711</td>
<td>0.6117</td>
<td>0.8657</td>
<td>0.4366</td>
<td>0.3812</td>
<td>0.0949</td>
<td>0.9937</td>
<td>0.0209</td>
<td>0.0733</td>
<td>0.0853</td>
</tr>
<tr>
<td>T*N</td>
<td>0.7817</td>
<td>0.6117</td>
<td>0.9856</td>
<td>0.5358</td>
<td>0.9793</td>
<td>0.4562</td>
<td>0.0917</td>
<td>0.0140</td>
<td>0.5143</td>
<td>0.9727</td>
</tr>
<tr>
<td>R*N</td>
<td>0.8040</td>
<td>0.3932</td>
<td>0.1548</td>
<td>0.0071</td>
<td>0.0748</td>
<td>0.8144</td>
<td>0.0201</td>
<td>0.7248</td>
<td>0.8979</td>
<td>0.8111</td>
</tr>
<tr>
<td>T*R</td>
<td>0.4323</td>
<td>0.7399</td>
<td>0.8056</td>
<td>0.8535</td>
<td>0.0298</td>
<td>0.0352</td>
<td>&lt;.0001</td>
<td>0.0003</td>
<td>0.8415</td>
<td>0.3295</td>
</tr>
<tr>
<td>T<em>R</em>N</td>
<td>0.5317</td>
<td>0.9470</td>
<td>0.2764</td>
<td>0.2137</td>
<td>0.3595</td>
<td>0.5910</td>
<td>0.9076</td>
<td>0.8538</td>
<td>0.4825</td>
<td>0.5634</td>
</tr>
</tbody>
</table>
Figure 2.4 The SOC concentrations of whole-soil, large macroaggregates, small macroaggregates, microaggregates and silt-clay particles for the tillage by rotation (A) and rotation by fertilizer N rate (B) interactions. Vertical bars within an aggregate size class, with different letters at the top, are significantly different at the P < 0.05 level. Note: * < 8000 indicates whole soil. The SOC of whole soil is without sand correction, while the other aggregate associated SOC concentrations were sand-corrected.
Figure 2.5 The STN concentrations for whole-soil, large macroaggregates, small macroaggregates, microaggregates and silt-clay particles for the tillage by rotation (A) and rotation by fertilizer N rate (B) interactions. Vertical bars within an aggregate size class, with different letters at the top, are significantly different at the P ≤ 0.05 level. Note: * < 8000 indicates whole soil. The STN of whole-soil is without sand correction, while the other aggregate associated STN concentrations were sand-corrected.
Soil Aggregate Organic Carbon and Total Nitrogen Stock

Whole-soil and all aggregate associated SOC stocks were significantly influenced by tillage, but not by fertilizer N application. Rotation significantly affected whole-soil and aggregate associated SOC stocks, except for microaggregate associated SOC stocks (Table 2.3). In the tillage by rotation interaction (Figure 2.6A), the order of whole-soil SOC stock is NT-Rotation > NT-Tobacco > CT-Rotation > CT-Tobacco. Generally, no-tillage and rotation tend to maintain whole-soil SOC stocks by maintaining large and small macroaggregate associated SOC stocks, while conventional tillage and tobacco monoculture can deplete whole-soil SOC stocks by depleting large and small macroaggregate associated SOC stocks. In the rotation by fertilizer N interaction (Figure 2.6B), rotation with fertilizer N application resulted in significantly higher large macroaggregate associated SOC stocks than rotation without fertilizer N, while tobacco monoculture with fertilizer N resulted in significantly lower large macroaggregate associated SOC stocks than tobacco monoculture without fertilizer N.

Whole-soil and all aggregate associated STN stocks were significantly influenced by tillage, while rotation significantly affected whole-soil, small macroaggregate and silt-clay particle associated STN stocks. Fertilizer N application affected only microaggregate associated STN stocks (Table 2.3). In the tillage by rotation interaction (Figure 2.7A), the whole-soil STN stock order was NT-Rotation > NT-Tobacco > CT-Rotation > CT-Tobacco. As with SOC stocks, no-tillage and rotation maintained whole-soil STN stocks by maintaining large and small macroaggregate associated STN, while conventional tillage and tobacco monoculture depleted whole-soil STN by depleting large and small macroaggregate associated STN stocks. In the rotation by fertilizer N interaction (Figure
2.7B), rotation with fertilizer N application resulted in significantly higher large macroaggregate associated STN stocks than rotation without fertilizer N application, while tobacco monoculture with fertilizer N application resulted in significantly lower large macroaggregate associated STN stocks than tobacco monoculture without fertilizer N application.

Table 2.4 shows the quality of the correlations between whole-soil SOC stocks (SOCS) or STN stocks (STNS) and the SOCS or STNS for the different aggregate sizes. These results showed that whole-soil SOCS and STNS have greater correlation with large and small macroaggregate SOCS and STNS than with the SOCS and STNS found in other aggregate size fractions. Aggregate SOCS, STNS, size of the aggregate fraction (%), bulk density and aggregate SOC and STN are shown in Table 2.5. These results demonstrated that large and small macroaggregate SOCS and STNS contributed more to whole-soil SOCS and STNS via their fraction size (%) than did bulk density or aggregate SOC and STN.
Table 2.3 Analysis of variance for the effects of tillage, rotation, and N fertilizer rate on whole-soil and aggregate-associated SOC stocks (SOCS) and STN stocks (STNS), for 0 to 10 cm whole-soil and aggregate fractions.

<table>
<thead>
<tr>
<th>Effect/contrast</th>
<th>Whole-soil SOCS</th>
<th>Whole-soil STNS</th>
<th>Large macro aggregates SOCS</th>
<th>Large macro aggregates STNS</th>
<th>Small macro aggregates SOCS</th>
<th>Small macro aggregates STNS</th>
<th>Microaggregates SOCS</th>
<th>Microaggregates STNS</th>
<th>Silt-clay particles SOCS</th>
<th>Silt-clay particles STNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tillage (T)</td>
<td>0.0040</td>
<td>0.0034</td>
<td>0.0007</td>
<td>0.0006</td>
<td>0.0008</td>
<td>0.0017</td>
<td>0.0006</td>
<td>0.0017</td>
<td>0.0310</td>
<td>0.0445</td>
</tr>
<tr>
<td>Rotation (R)</td>
<td>0.0419</td>
<td>0.0286</td>
<td>0.0276</td>
<td>0.0622</td>
<td>0.0010</td>
<td>0.0013</td>
<td>0.5385</td>
<td>0.8524</td>
<td>0.0297</td>
<td>0.0179</td>
</tr>
<tr>
<td>Fertilizer N rate (N)</td>
<td>0.5765</td>
<td>0.8241</td>
<td>0.7762</td>
<td>0.2871</td>
<td>0.8645</td>
<td>0.5034</td>
<td>0.1250</td>
<td>0.0162</td>
<td>0.9989</td>
<td>0.9796</td>
</tr>
<tr>
<td>T*N</td>
<td>0.8020</td>
<td>0.3685</td>
<td>0.9898</td>
<td>0.5942</td>
<td>0.8705</td>
<td>0.9414</td>
<td>0.6948</td>
<td>0.5767</td>
<td>0.2890</td>
<td>0.1815</td>
</tr>
<tr>
<td>R*N</td>
<td>0.5841</td>
<td>0.7442</td>
<td>0.0001</td>
<td>0.0004</td>
<td>0.0275</td>
<td>0.0082</td>
<td>0.0199</td>
<td>0.0027</td>
<td>0.9789</td>
<td>0.8830</td>
</tr>
<tr>
<td>T*R</td>
<td>0.6047</td>
<td>0.6742</td>
<td>0.0141</td>
<td>0.0207</td>
<td>0.0206</td>
<td>0.0247</td>
<td>0.0860</td>
<td>0.0364</td>
<td>0.1257</td>
<td>0.2463</td>
</tr>
<tr>
<td>T<em>R</em>N</td>
<td>0.7312</td>
<td>0.8218</td>
<td>0.4978</td>
<td>0.2958</td>
<td>0.4122</td>
<td>0.3722</td>
<td>0.8604</td>
<td>0.8600</td>
<td>0.8985</td>
<td>0.9666</td>
</tr>
</tbody>
</table>
**Figure 2.6** The SOC stocks (SOCs) of whole-soil, large macroaggregates, small macroaggregates, microaggregates and silt-clay particles for the tillage by rotation (A) and rotation by fertilizer N rate (B) interactions. Vertical bars within an aggregate size class, with different letters at the top, are significantly different at the $P < 0.05$ level. Note: * < 8000 indicates whole soil.
Figure 2.7 The STN stocks (STNS) of whole-soil, large macroaggregates, small macroaggregates, microaggregates and silt-clay particles for the tillage by rotation (A) and rotation by fertilizer N rate (B) interactions. Vertical bars within an aggregate size class, with different letters at the top, are significantly different at the P < 0.05 level. Note: * < 8000 indicates whole soil.
Table 2.4 The relationships between whole-soil SOC stocks (SOCS) or STN stocks (STNS) and the SOCS and STNS stocks found in different aggregate size fractions (n= 32).

<table>
<thead>
<tr>
<th></th>
<th>Large macroaggregates</th>
<th>Small macroaggregates</th>
<th>Microaggregates</th>
<th>Silt-clay particles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SOCS</td>
<td>STNS</td>
<td>SOCS</td>
<td>STNS</td>
</tr>
<tr>
<td>Whole-soil SOCS</td>
<td>0.865†</td>
<td>0.786</td>
<td>0.651</td>
<td>-0.437</td>
</tr>
<tr>
<td>Whole-soil STNS</td>
<td>0.807</td>
<td>0.748</td>
<td>0.562</td>
<td>-0.397</td>
</tr>
</tbody>
</table>

†values in the table are correlation coefficients, all of which are significant (p ≤ 0.05).

Table 2.5 The relationships between aggregate SOC stocks (SOCS) or STN stocks (STNS) with aggregates size fraction (%), bulk density and aggregate SOC (or STN) concentrations (n= 32).

<table>
<thead>
<tr>
<th></th>
<th>Large macroaggregates</th>
<th>Small macroaggregates</th>
<th>Microaggregates</th>
<th>Silt-clay particles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SOCS</td>
<td>STNS</td>
<td>SOCS</td>
<td>STNS</td>
</tr>
<tr>
<td>Size of Aggregate Fraction (%)</td>
<td>0.912†</td>
<td>0.908</td>
<td>0.839</td>
<td>0.873</td>
</tr>
<tr>
<td>Bulk Density</td>
<td>0.689</td>
<td>0.707</td>
<td>0.476</td>
<td>0.424</td>
</tr>
<tr>
<td>Aggregate SOC (or TSN) Concentration</td>
<td>0.479</td>
<td>0.317*</td>
<td>0.358</td>
<td>0.459</td>
</tr>
</tbody>
</table>

†values in the table are correlation coefficients. Coefficients not followed by a * sign are significantly different (p ≤ 0.05).
2.4 Discussion

Soil Structure and Water Stable Aggregate Size Distribution

No-tillage for burley tobacco production significantly increased soil aggregate stability (MWD and GMD) when compared to conventional tillage, which was consistent with earlier reports (Whalen et al., 2003; Wright and Hons, 2005a; Anders et al., 2012). In this study, no-tillage soil bulk density was significantly higher than that found with conventional tillage, which was not consistent with some reports. Tisdall and Oades (1980) argued that conventional tillage destroys the original soil structure, breaking up the macroaggregates into microaggregates, resulting in increased micro-porosity and bulk density, though this might depend on soil type. Six et al. (2002) reported that bulk density values under NT and CT were rarely different in tropical and subtropical soils, while NT bulk density was generally higher than CT in temperate soils. Previous research on Maury-Bluegrass soil found CT had significantly higher penetrometer resistance than NT in wheel trafficked areas, due to weakened CT soil structure and lower bearing capacity (Ritchey, 2010). In areas without significant traffic, compaction in CT and NT was similar, even lower in CT. In the present study, plot traffic was limited due to the small size of the plots and the presence of sod borders around each plot.

Water stable macroaggregate content was reported to be an easily measured early indicator of improved aggregation due to soil conservation management (Kemper and Rosenau, 1986; Beare et al., 1994a), which mostly increases mean weight diameter (MWD) values (Kemper and Rosenau, 1986). The macroaggregate proportion and both GMD and MWD values found here were consistent with earlier studies and supported
initial hypotheses that no-tillage and rotation in burley tobacco production would improve soil structure. Compared to conventional tillage, no-tillage can significantly reduce macroaggregate turnover rate and increase fungal hyphae and microbial byproducts that promote agglomeration of small aggregate factions into macroaggregates (Chan and Heenan, 1999; Whalen et al., 2003; Spaccini and Piccolo, 2013). Whether rotation can increase soil aggregation mainly depends on increased amounts/quality of crop residue returned to soil and its impacts on SOC, STN and the microbial biomass/activity that contributes to increased aggregation (Mendes et al., 1999). Burley tobacco is typically harvested as whole plants, thus much less residue is left compared to residue-rich crops like corn and wheat. Rotating perennial grass sod with tobacco adds extensive shoot and root residues. The sod’s dense, fibrous root system promotes soil structure and binds small aggregates into larger ones. Furthermore, the CT-Rotation treatment in this study was tilled only once per three year cycle because no tillage was necessary during the sod growth period, causing the CT-Rotation treatment to include two years of no-tillage as well as rotation. Therefore, only two tillage events within the two rotation cycles could dramatically deplete water stable large macroaggregates – as compared to the NT-Rotation treatment.

The N fertilizer application to tobacco had no main effect on water stable aggregate size distribution. This finding is similar to that of Plaza-Bonilla et al. (2013). They found similar water stable macroaggregate size distributions among different N fertilizer rates within grain production systems. However, there was a significant rotation by N interaction. Rotation with 280 kg N/ha had a higher macroaggregate content than rotation without fertilizer N, which can be explained by N immobilization with sufficient
available carbon. Before planting tobacco, sod was chemically killed, resulting in significant amounts of decaying above and belowground biomass, which can cause microbial immobilization of added inorganic N (Hart et al., 1994), increasing microbial biomass, fostering large macroaggregate formation. However, there was no clear mechanism to explain why tobacco monoculture without fertilizer N resulted in greater levels of large macroaggregates than monoculture with fertilizer N. There is less carbon input with tobacco monoculture than tobacco rotation. The addition of N fertilizer during tobacco production might decrease the soil C:N ratio, which could promote SOM decomposition and enhance large macroaggregate turnover.

In general, soil aggregate stability (MWD and GMD) and water stable aggregate distribution data suggested that traditional burley tobacco production (tobacco monoculture plus conventional tillage) was not sustainable with regards to soil structure. Tobacco monoculture with conventional tillage can enhance macroaggregate turnover and there was a corresponding increase in the proportion of silt-clay particles, resulting in lower MWD and GMD values. Conversation practices, both no-tillage and rotation, have the potential to slow soil degradation and maintain soil quality.

**Soil and Aggregate SOC and STN Concentrations**

No-tillage and rotation with sod significantly increased whole-soil SOC and STN concentrations. Some reports have suggested that water stable aggregate SOC is concomitant with STN (Jastrow et al., 1996; Six et al., 2000a; Zibilske and Bradford, 2007). This study’s findings are comparable to those reports. The SOC and STN exhibited similar patterns across all aggregate size classes. These results indicate that
SOC and STN concentration in > 53µm aggregates were higher than those in < 53µm aggregates and the trend was similar to that reported by Cambardella and Elliott (1993a). This result confirmed the hierarchical soil aggregate-SOM model - that SOC and STN in large aggregates were less susceptible to the physical, chemical and biological decomposition (O'Brien and Jastrow, 2013).

Whole-Soil and Aggregate SOCS and STNS

The SOCS and STNS play a central role in soil functions that produce a wide range of vital environmental goods and services (Banwart et al., 2014). In the evaluation of every conversation plan for agricultural production, SOCS and STNS become important parameters (Amundson, 2001; Dersch and Böhm, 2001; Hobbs et al., 2008a). The SOCS and STNS were distributed among different aggregate size classes and various size of aggregate have different formation and turnover responses to agronomic management (Six et al., 2000a). This caused interest as regards how no-tillage and rotation practices in burley tobacco production affected SOCS and STNS by physically separating the different size aggregates from the whole soil.

The SOCS and STNS for each aggregate size depended on the fraction of whole soil found in that aggregate size, bulk density and aggregate SOC and STN. The study showed no-tillage and rotation contributed to greater levels of SOC and STN and greater fraction of larger aggregates, which was consistent with other reports where increased aggregation, concomitant with increased SOC, have been observed (Paustian et al., 2000; Six et al., 2000b). The result from this study also suggest that maintaining SOCS and STNS with no-tillage and rotation practice in burley tobacco production is due to the
preserving of SOCS and STNS in macroaggregates (Tables 2.4 and 2.5), which was consistent with Wei et al. (2013). This study further showed that changes in SOCS or STNS as a result of agronomic practices were mainly influenced by the different aggregate size proportions, rather than SOC or STN (Tables 2.4 and 2.5). Whole-soil SOCS and STNS were found largely in large and small macroaggregates and silt-clay particles, but only a small percentage of SOCS and STNS were contained in microaggregates. The main reason for the small amount of microaggregate SOCS or STNS was not due to low SOC or STN, but to the relatively small proportion of microaggregates (Figure 2.3). However, this phenomenon can be explained by the pretreatment procedure before wet-sieving. Cambardella and Elliott (1993a) compared two re-wetting processes, including slaking and capillary wetting, and found that the more disruptive slaking procedure can convert macroaggregates into microaggregates, compared to capillary wetting. A field-moist (average 26 % gravimetric water content) or capillary wetted soil sample was used in this study, thereby conserving macroaggregates.

There are numerous studies showed that a greater fertilizer N rate could increase SOM and structure by increasing plant residue inputs in grain production systems (Graham et al., 2002; Halvorson et al., 2002). However, fertilizer N application in burley tobacco production did not affect SOCS and STNS in whole soil, probably because the entire plant is taken during tobacco harvest, resulting in similar plant residue levels at the different fertilizer N rates. Although N application had no effect on SOCS and STNS in the whole soil, a significant rotation by fertilizer N interaction on > 53 µm aggregate SOCS and STNS was observed and attributed mainly to the significant impact of that same interaction on the proportion of aggregates in that size fraction.
In general, whole soil SOCS and STNS data also suggested that the traditional burley tobacco production, tobacco monoculture with conventional tillage, was not sustainable. Tobacco monoculture with conventional tillage can deplete macroaggregate SOCS and TSNS and there was a corresponding increase in silt-clay particle SOCS and STNS.

2.5 Conclusion

No-tillage and rotation with sod in burley tobacco production resulted in significantly greater whole soil SOCS and STNS and improved soil structure relative to conventional tillage and tobacco monoculture, respectively. A majority of the burley tobacco grown in Kentucky is still produced using intensive conventional tillage, often with limited rotation. The results of this study suggested that these production practices not only reduced water stable macroaggregate content, but also decreased macroaggregate SOC and associated SOCS and STNS. While the degradation of water stable macroaggregates may be slowed with no-tillage soil management, potentially rebuilding structure and SOCS and STNS, the inclusion of other high biomass residue crops and sods in rotation with burley tobacco may be necessary to maintain or enhance soil productivity and quality. Although no-tillage management for tobacco might be difficult due to compaction impacts on root development and limitations in equipment and herbicides, one viable alternative is strip tillage. Rotating tobacco with grain crops or grass sod and inclusion of a winter cereal cover crop has been gradually accepted by growers. Over fertilization with N in tobacco production did not affect soil structure and SOCS and STNS. However, there is a risk that excess N could cause groundwater pollution by leaching. Production of burley tobacco with good agricultural practices, including proper
tillage, rotation and N management practices, could synergistically increase SOC and STN, enhance soil structure, and promote soil quality.
Chapter 3 Part I: Laboratory or *In Situ* Resin-Core Methods to Estimate Net Nitrogen Mineralization for Different Rotation and Tillage Practices

3.1.1 Introduction

Obtaining an accurate and realistic estimate of soil N mineralization in agriculture settings using current techniques is challenging because N mineralization processes can be affected by numerous dynamic, complex, and site-specific factors. These include biotic factors (e.g. soil organic N fractions, soil fauna activity, soil microbial community structure) and abiotic factors (e.g. soil temperature, moisture, aeration, field soil structure) and their interactions (Nadelhoffer et al., 1991; Knoepp and Vose, 2007). Soil N fertility would be managed more efficiently if the relative effect of crop sequences and tillage management on net soil N mineralization could be reasonably estimated. Soil N mineralization is mainly derived from biological decomposition of soil organic matter (SOM) and the plant residues (Hart et al., 1994), which are affected by tillage and crop rotation management (Six et al., 1998; Wright and Hons, 2005a). Conservation tillage (reduced or no-tillage) can increase the soil N pool as compared to traditional intensive tillage; simultaneously increasing the potentially mineralizable N pool (Sainju et al., 2009). Rotation could also affect the SOM (Kong et al., 2005). However, the quality of crop residue can have different effects on soil N mineralization. Rotation with high C:N ratio residue crops will result in soil N immobilization, while rotation with legume crop species that have a low C:N ratio residue will promote soil N mineralization for the following crop (Smith and Sharples, 1990). This is one of the key principles supporting adjustment of N fertilizer recommendations according to the preceding crop (Franzen,
Therefore, understanding the relative effect of agronomic practices on soil N mineralization would result in better N fertility management decisions.

Long-term aerobic biological incubation to predict N mineralization has been reported to be an acceptable N availability index (Stanford, 1982; Bundy and Meisinger, 1994). It has been used to validate other more rapid chemical or biological indices (Curtin and McCallum, 2004; Griffin et al., 2007). However, numerous long-term incubation methods have been proposed, depending on incubation environment and pretreatment. Two general incubation environment categories have been used are well-controlled laboratory incubations and in situ incubations (Khanna and Raison, 2013). Each might provide an N mineralization value that could be extrapolated to the field application with various levels of confidence (Hanselman et al., 2004). Most laboratory incubation methods were based the procedure described by Stanford and Smith (1972), where soil was incubated in the well-controlled conditions. Thus, the biggest concern for laboratory methods is that these do not reflect the fluctuations of soil moisture and temperature occurring in field. Some in situ incubation methods, using buried polyethylene bags or covered-cylinder, can capture the field temperature variation, but fail to reflect moisture and aeration change (Sierra, 1997). The in situ resin-core method, proposed by DiStefano and Gholz (1986) and developed by Kolberg et al. (1997), was shown to cause contained soil to most closely mimic field soil temperature, moisture, and aeration fluctuations (Khanna and Raison, 2013).

Soil sample handling and pretreatment can also affect soil N mineralization (Stanford, 1982). Most laboratory incubation methods utilize a pretreatment procedure where soil is sieved and large pieces of plant residue are discarded to create homogenous samples.
(Stanford and Smith, 1972; Hart et al., 1994). Some researchers argue that sieved soil
does not reflect field soil structure, especially for no-tillage fields (Rice et al., 1987),
therefore, they suggested the intact soil core for laboratory incubation. Furthermore,
Smith and Sharpley (1990) reported that previous crop residue could affect net soil N
mineralization. Some researchers cut crop residue into pieces, mixing these with soil to
retain residue during incubation (Honeycutt, 1999; Heumann et al., 2002). Given
concerns with different pretreatment methods for determination of soil N mineralization,
agricultural scientists have often appealed for standardization of the biological soil N
mineralization incubation procedure (Bremmer, 1965; Honeycutt et al., 2005). However,
a methods choice mostly depends on resource availability, the nature of the study and site
specific factors. In most cases, biological incubation methods are used to compare
agronomic practices; from which N management recommendations could be made.
However, as mentioned above, the soil pretreatment procedure can introduce error in the
estimate of agronomic practice effects on N mineralization due to poor simulation of the
“real-world” field conditions. Therefore, N management recommendations could be
somewhat risky if one neglects artifacts created by soil sample pretreatments.

As important sustainability strategies, conservation tillage and crop rotation will play
more and more important roles in future agriculture production (Tilman et al., 2002;
Hobbs et al., 2008b). Properly estimating soil N mineralization as a consequence of
agronomic practices would result in better N management. Therefore, the main objective
of the present work was to determine the relative effect of tillage and rotation practices
on soil net N mineralization using current common aerobic laboratory and in situ resin-
core incubation methods. The hypotheses were: (1) laboratory incubation method with
sieving pretreatment can overestimate soil net N mineralization in no-tillage soil, compared to the in situ resin-core method; and (2) laboratory incubation with crop residue exclusion could underestimate effects due to a previous crop leaving residues with a low C:N ratio.

3.1.2 Materials and Methods

Study site and experimental design

This study was conducted at the University of Kentucky’s Spindletop Research Farm, near Lexington, Kentucky, USA (38°07'36.8"N 84°29'26.4"W). The soil is a Bluegrass-Maury silt loam (fine, mixed, active, mesic Typic Paleudalf), a deep, well-drained soil formed in residuum of phosphatic limestone. Before this study site was established, the baseline soil pH\textsubscript{water} (1:1, w/v) was 6.3, with Mehlich III (Mehlich, 1984) extractable phosphorus (85 mg kg\textsuperscript{-1}), potassium (178 mg kg\textsuperscript{-1}), calcium (1.8 g kg\textsuperscript{-1}), magnesium (110 mg kg\textsuperscript{-1}), and zinc (2.97 mg kg\textsuperscript{-1}). The soil had 12 % sand, 68 % silt and 20 % clay. The location is characterized by a wide variation in mean monthly air temperature from 0 °C in January to 24 °C in July and August and a relatively uniform distribution in mean monthly precipitation, with an annual average precipitation of 1160 mm. Figure 1 shows the cumulative precipitation and mean air temperature at the experiment site for three in situ incubation periods in 2012, including 22 May - 20 June, and 21 June -25 July, and 26 July – 25 September.

The experiment was laid out in a randomized complete blocks design with four replications. The six tillage-rotation system treatments: i) continuous conventional tillage tobacco (TTT-CT); ii) continuous no-tillage tobacco (TTT-NT); iii) 2-yr sod (Festuca
arundinacea Schreb.) and 1-yr conventional tillage tobacco (SST-CT); iv) 2-yr sod and 1-yr no-tillage tobacco (SST-NT), no-tillage corn (Zea mays L.) - soybean (Glycine max L.) - tobacco (CST-NT), and vi) no-tillage soybean-corn-tobacco (SCT-NT); were randomly assigned plots (6.4 m wide by 24.4 m long). These production systems were established in 2007. This laboratory and in situ resin-core soil N mineralization study was begun in spring 2012 after tillage but before transplanting tobacco seedlings. In 2012, every production system was in the last year of the second rotation cycle. There were two tillage comparisons: “TTT-CT versus TTT-NT” and “SST-CT versus SST-NT.” There was also a preceding crop comparison of “CST-NT versus SCT-NT”. Primary conventional tillage consisted of moldboard plowing, with secondary tillage consisting of diskng (twice), followed by a rotary tillage operation before transplanting tobacco seedlings. Other management was recommended by the University of Kentucky Cooperative Extension Service (Seebold and Pearce, 2013).
**Figure 3.1.1** Cumulative precipitation and mean air temperature of the three 2012 *in situ* incubation periods: 22 May - 20 June (1), and 21 June - 25 July (2), and 26 July – 25 September (3).

**Laboratory incubation method**

The long-term aerobic laboratory N mineralization incubation method was based on a procedure described by Hart et al. (1994). Composite soil samples (20 cores per plot) were collected from the 0 to 10 cm and 10 to 20 cm soil depth increments on 16 May 2012 after conventional tillage was done but before N fertilizer was applied and a subsample was immediately removed to determine the baseline levels of soil NO₃-N and NH₄-N. In the soil sample pretreatment of this method, field moist soil was manually passed through a 4 mm sieve. Large pieces of organic material and rocks were removed. Soil water content was determined gravimetrically by oven-drying a subsample at 105 °C. The remaining soil was stored at 4 °C until incubation. To start the incubation, 50 g
of soil was placed in duplicate plastic zip-lock bags. Soil moisture was adjusted to 60% water-filled pore space, which has proved to be the ideal soil moisture for aerobic microbial processes in most soils (Linn and Doran, 1984). Soil moisture content was regularly checked and adjusted as necessary. Soil was incubated at a constant temperature of 25 °C. Periodically, 5 g of soil was removed and KCl was used to extract NO₃-N and NH₄-N. To match the in situ incubation sampling schedule, soils were sub-sampled at 27, 62, and 128 days of incubation.

**In situ resin-core incubation method**

Net N mineralization in the 0 to 20 cm soil depth increment was measured by a modified in situ resin-core procedure (Kolberg et al., 1997). Incubation cores were prepared by driving and removing aluminum conduit (25 cm long with an inner diameter of 4.8 cm) into the soil between tobacco rows before fertilizer N application. The bottom 2.5 cm of soil was removed from each soil core and replaced with a nylon bag containing an equivalent volume of ion-exchange resin beads. The entire assembly was returned to the original hole with a rubber washer surrounding the tube to avoid preferential bypass flow of water along the outside of the tube, which might bring inorganic N from outside the tube close enough to contaminate the resin. The top of the soil core was exposed so as to be subject to field moisture and gas exchange and temperature fluctuations. The resin bag contained equal amounts of cation and anion exchangeable resin (Lanxess Sybron, Birmingham, NJ). The design of the in situ soil resin core is shown in Fig. 3.1.2. Resin-soil cores were allowed to incubate 60 days in field conditions; replacement resin bags were needed for longer incubations (Wienhold et al., 2009). In a preliminary laboratory study, the average inorganic N adsorption capacity N for a resin bag in resin-core tubes
was found to be over 99 % of the inorganic N in leachate, even at the highest rates of N fertilizer (280 kg N ha\(^{-1}\)) and water irrigation (5.9 mm day\(^{-1}\)) conditions during a 30-day period. Intact resin-core tubes were removed and processed and re-installed on 17 and 18 May 2012. Sampling dates were intended to occur at 29, 64, and 127 days of incubation, which was comparable to the laboratory sampling schedule.

To account for suspected high variance in the field conditions, three tubes were used to prepare a composite sample. Therefore, the total number of installed tubes in this study was 216 \((216 = 6(\text{treatments}) \times 4(\text{replications}) \times 3(\text{sampling dates}) \times 3(\text{tubes for a composite sample}))\). On each sampling day, a sample from each composite (three tubes) was oven-dried at 105 °C to determine the gravimetric moisture content. A 10 g subsample of field moist soil was immediately analyzed for NO\(_3\)-N and NH\(_4\)-N. Each composite resin sample was carefully mixed and weighed. Resin samples from different plots exhibited different moisture contents, thus to make result comparable, a subsample equal to 20 g based on original product moisture content was packed into a new nylon bag for NO\(_3\)-N and NH\(_4\)-N assays.
**Figure 3.1.2** Cutaway diagram of soil resin core, in which surficial plant residue and intact soil column (0-20.32 cm depth increment) and one mixed ion-exchange resin bag are incubated. A transparent example of the nylon bag holding the ion-exchange resins is shown. The bottom of the core was covered with nylon mesh (1mm opening) material.

**Laboratory inorganic N analysis procedure**

Each soil subsample was extracted with 25 mL 1M KCl for 1 hr. A 1 mL aliquot was centrifuged at a speed of 3700 rpm for 27 min, equivalent to filtration through a 0.45 μm filter based on a preliminary study. The centrifuged extracts were subjected to NO$_3$-N and NH$_4$-N analysis. The NO$_3$-N analysis was determined colorimetrically according to the procedure described by Crutchfield and Grove (2011), using cadmium brush (Paratech, Lexington, KY). NH$_4$-N analysis was found colorimetrically subsequent to the phenol-
hypochlorite reaction (Weatherburn, 1967; Ngo et al., 1982). Both NO$_3$-N and NH$_4$-N were determined in duplicate. The concentration of the two inorganic N forms in each soil sample were given in mg N kg$^{-1}$ oven-dried soil (105 °C) by adjusting for soil sample gravimetric water content.

The subsample resin bags were serially extracted by shaking in three 50 mL volumes of 1 M KCl for 15, 30, and 60 min, respectively. The three extracts were composited and analyzed for both NO$_3$-N and NH$_4$-N in duplicate, using the same analytical methods described previously for soil sample. The final adsorbed NO$_3$-N and NH$_4$-N concentration was calculated from standard resin extraction recovery equations for NO$_3$-N and NH$_4$-N (Equations. 1 and 2). These equations were developed in a previous resin extraction study utilizing the standard extraction procedure described above.

\[
\text{NO}_3\text{-N (absorbed by resin)} = \text{NO}_3\text{-N (recovered from resin)} \times 1.27 + 258.54 \quad \text{Eqn. 1}
\]

\[
\text{NH}_4\text{-N (absorbed by resin)} = \text{NH}_4\text{-N (recovered from resin)} \times 1.12 - 45.42 \quad \text{Eqn. 2}
\]

**Calculation**

For the laboratory incubation method, soil net mineralized N was determined after correction of initial and final soil inorganic N levels. For the *in situ* resin-core method, net soil mineralization N equaled the sum of resin adsorbed inorganic N and soil inorganic N, corrected for the initial soil inorganic N levels. In this study, the unit of soil net N mineralization is expressed in mg N kg$^{-1}$ oven-dried soil.
**Statistical analysis**

Data were analyzed with the General Linear Model (GLM) procedure in the SAS 9.3 computer package (SAS Institute Inc., Cary, NC). Duplicate measurements on composite soil and resin samples were averaged for statistical analysis of treatments effects. The analysis considered replicate effects to be random, and the effects of treatment to be fixed. Treatment effects were considered significant when the probability of a greater $F$ statistic was less than or equal to 0.1. Means separation was done using the Tukey's HSD (honest significant difference) test at an alpha level of 0.1.

**3.1.3 Results**

**Tillage Effects on Soil Net N Mineralization**

In the comparing of TTT-CT with TTT-NT in the laboratory incubation study (Fig. 3.1.3A), no-tillage soil exhibited significantly higher soil mineralized N than conventional tilled soil from the second sampling date onward, while in the *in situ* resin-core incubation study (Fig. 3.1.3B), there was no significantly different soil N mineralization due to tillage at any sampling date. In comparing SST-CT to SST-NT, laboratory and *in situ* resin-core incubation methods (Fig. 3.1.3C and 3.1.3D) exhibited soil N mineralization trends similar to those for the TTT-CT versus TTT-NT comparison. The coefficients of variance (CV) in the laboratory incubation were 16.1, 12.6, and 10.7 for sampling dates 1, 2, and 3, respectively, while the CV value for the *in situ* incubation study were 40.5, 36.9 and 23.5 for sampling dates 1, 2, and 3, respectively.
Figure 3.1.3 Laboratory and *in situ* resin-core incubation results comparing tillage effects on soil net N mineralization: (A) laboratory incubation results for TTT-CT and TTT-NT; (B) *in situ* resin-core incubation results TTT-CT and TTT-NT; (C) laboratory incubation results for SST-CT and SST-NT; and (D) *in situ* resin-core incubation for SST-CT and SST-CT. Different letters for CT and NT soil net N mineralization within the same incubation method and at the same sampling date indicate a significant difference at an alpha level of 0.1 according to Tukey’s HSD means separation test.
Rotation Effects on Soil Net N Mineralization

In 2012, the previous crop was soybean in the CST-NT production system and corn in SCT-NT production systems. The laboratory incubation method found no significant difference between CST-NT and SCT-NT at any incubation date (Fig. 3.1.4A). However, the in situ resin-core method found that CST-NT resulted in slightly higher N mineralization than SCT-NT at the second sampling date, becoming significantly different by the third sampling date (Fig. 3.1.4B).

Figure 3.1.4 Laboratory and in situ resin-core incubation results comparing previous crop effects on soil net N mineralization: (A) laboratory incubation results for CST-NT and SCT-NT; and (B) in situ resin-core incubation results for CST-NT and SCT-NT. Different letters for CST and SCT soil net N mineralization within the same incubation method and at the same sampling date indicate a significant difference at an alpha level of 0.1 according to Tukey’s HSD means separation test.
3.1.4 Discussion

There have been many studies reporting different results when comparing soil net N mineralization from laboratory and in situ incubation methods (Arnold et al., 2008; Luce et al., 2011). Most of these studies found that there was uncertainty in extrapolating laboratory results to the field application because laboratory biological incubation methods are unable to fully account for the frequent fluctuations in temperature and moisture that occur in the field (Sierra, 1997; Honeycutt, 1999). Data from the present study suggested that the magnitude of soil net N mineralization in laboratory incubation was higher than that found with in situ incubation. This phenomenon was found in other studies (Wienhold, 2007). This reason could be that the aerobic laboratory incubation has the optimal temperature and moisture condition, however, the cumulative precipitation for each incubation period in 2012 for in situ incubation was less than the long-term average (Fig. 3.1.1). The in situ resin-core incubation in this study exhibited higher CV values than the laboratory method, which means more in situ mineralization cores are required to obtain a desired level of precision at certain confidence level per experimental unit (Kolberg et al., 1997). However, all biological incubation methods, including laboratory and in situ methods, can only be considered as soil N available indices due to soil disturbance associated with each method. Rather than pursuing the perfect assessment of soil N availability using these methods, at a high cost, a proper estimate of the relative effect of agronomic practices on soil net N mineralization would be sufficient for evaluation of these practices on soil N fertility management.

Conservation tillage (reduced tillage or no-tillage) is considered an important tool to restore degraded soil and enhance SOM sequestration and soil nutrient cycling (Lal,
Planting land by conservation tillage is continuously increasing worldwide (Holland, 2004; Horowitz et al., 2010). Conservation tillage management is commonly reported to maintain higher SOM than conventional tillage because conservation tillage can reduce SOC and STN turnover rates in large soil aggregates (Cambardella and Elliott, 1993a; Six et al., 2000a). Therefore, the mechanism by which conservation tillage influences soil N mineralization remains importance to agriculture and the surrounding environment. There have been numerous studies on the effects of tillage on soil net N mineralization, and most of these laboratory incubation studies found that no tillage gave higher soil net N mineralization than conventional tillage (Wienhold and Halvorson, 1999; Sainju et al., 2009). From this perspective, one should be able to reduce the N fertilizer rate in no-tillage, relative to conventional tillage production systems. However, no-tillage N fertilizer rate recommendations are usually similar to, even greater than those for conventional tillage. Part of the reason could be that N fertilizer on no-tillage soil surfaces is susceptible to N loss (Beyrouty et al., 1986). However, these present data suggest that there are errors in estimating the relative effect of tillage on soil N mineralization. The laboratory incubation method, which included a sieving pretreatment, could overestimate the net soil N mineralization in no tillage relative to the in situ resin-core method. This is precisely because no-tillage can preserve more SOM than conventional tillage, and most of the soil organic C and N is preserved within aggregates under natural field conditions (Six et al., 2004). The hierarchical structure of aggregates makes the soil organic C and N less susceptible to soil microbial decomposition processes (Tisdall and Oades, 1982). The soil sieving pretreatment in most laboratory incubation methods exposes aggregate-protected SOM to soil microbial activity,
especially for no-tillage soil. Beare et al. (1994b) reported that macroaggregates disruption increased the SOM mineralization in no-tilled soil but had little effect in conventional tilled soil. The reason could be that macroaggregate-associated SOC and STN concentrations in no-tilled soil are usually higher than those in tilled soil. Therefore, preserving field soil structure would be a key concern for properly estimating the relative effect of tillage on net soil N mineralization with biological incubation methods.

In crop rotation systems, N fertilizer recommendations are usually adjusted for the preceding crop because different types and amounts of crop residue have different effects on soil N mineralization, thereby influencing the available soil N supply to the following crop (Fox and Piekielek, 1988; Soon et al., 2001). The quality of crop residue was often characterized as its C:N ratio, can affect N transformations in agro-ecosystems. Generally, high C:N ratio (e.g. wheat and corn) crop residue can increase N immobilization, while low C:N ratio (e.g. alfalfa and soybean) crop residue can enhance N mineralization (Sylvia et al., 2005). Therefore, including crop residue could potentially affect soil net N mineralization. Smith and Sharpley (1990) previously reported that the positive effects of crop residue type on N mineralization generally proceeded in the order alfalfa > peanut > soybean > sorghum >wheat >corn. Unfortunately, crop residue is typically discarded in soil sample pretreatment protocols in most laboratory incubation methods (Stanford and Smith, 1972; Hart et al., 1994). These data further corroborated the hypothesis that laboratory incubations excluding crop residue could underestimate the rotation effect due to low C:N ratio prior crop residue. Discarding prior crop residue in this laboratory method resulted in identical soil net N mineralization in both rotation systems. Therefore, keeping the above- and belowground crop residue in sample would
be a key concern to accurately estimate the relative effect of the previous crop in different crop rotation regimes on net soil N mineralization.

These results also demonstrated that incubation time can play an important role in estimating the relative effects of tillage and rotation on soil net N mineralization. At the first sampling date (27 and 29 incubation days for laboratory and in situ methods, respectively), there was no difference between any treatment for either method. On the second sampling date, the laboratory method started to show a difference due to tillage effect; the in situ resin-core method started to show a difference due to rotation effect on the third sampling date. Therefore, longer term incubation methods could better separate the effect of management on soil net N mineralization. However, what is a sufficient length of incubation? This might depend on the nature of the study. When estimating potential soil N supply for the crop growth season, a season-long incubation period would be relevant; if estimating the N dynamics and N cycling in agro-ecosystems, annual or longer incubation periods would be recommended.

3.1.5 Conclusions

Biological incubation methods can provide different estimates of the relative effect of agronomic practices on net soil N mineralization due to soil sample pretreatments. In estimating the relative effect of tillage these data highlighted the importance of maintaining the inherent soil structure. In estimating the relative effect of crop rotation, it is imperative to include the prior crop residues in the incubation system. The incubation time can also affect the measured influence of agronomic practices on soil net N mineralization. Therefore, more studies are needed to determine suitable incubation
periods that separate the effects of different practices on soil net N mineralization. Clearly, the results of studies on N mineralization can be impacted significantly by choices made during sample collection, preparation, and incubation of soils. The direction and magnitude of these impacts will not be the same for all soil management scenarios, so investigators must carefully consider the potential implications of their experimental methods with due regard to the goals and objectives of the study.
Chapter 3 Part II: Influence of Past and Current N Fertilizer Application on In Situ Net Soil N Mineralization

3.2.1 Introduction

When fertilizer N synthetized by the Haber-Bosch process began to be utilized in agricultural production in the early 20th century (Erisman et al., 2008), the cycling of N in agro-ecosystems was dramatically modified. While the contribution of fertilizer N to soil organic N in agricultural soils is still under debate (Mulvaney et al., 2009; Powlson et al., 2010), one of central points in this argument is whether fertilizer N application affects indigenous N mineralization. In the current drive to improve N use efficiency in production, while reducing undesirable environment impacts, the need to understand the effect of fertilizer N on indigenous N mineralization has become increasingly important.

To address this question, two dimensions in N fertilizer application management should be clarified. One is the effect of previous fertilizer N (PN) on the soil N mineralization rate, which can provide insight into how the mineralizable N pool can be shaped by previous fertilizer N management. The other dimension is the effect of current or “in-season” fertilizer N on soil N mineralization, which can unveil the mechanism behind the priming effect/added nitrogen interaction (ANI) effect on indigenous N mineralization (Jenkinson et al., 1985). Most previous studies on this topic have focused on the effect of previous N fertility management on soil N mineralization because samples were taken either in spring just prior to crop growth, or in the fall after the growth season. Few in situ studies have considered both past and present N fertilizer applications.
Previous research focusing on the effect of PN on soil N mineralization found mixed results, regardless of the *in situ* or laboratory incubation method. Several studies found a positive correlation between PN amounts and soil N mineralization in agricultural production systems (El-Haris et al., 1983a; Singh and Singh, 1994b; Rasmussen et al., 1998; Kolberg et al., 1999; Kanchikerimath and Singh, 2001; Graham et al., 2002; Jordan et al., 2004). Other studies noted no effect of PN on soil N mineralization (Franzluebbers et al., 1994a; Franzluebbers et al., 1994b). Negative PN-mineralization interactions have also been documented (Wienhold and Halvorson, 1999; Carpenter-Boggs et al., 2000). Although results have been mixed, the theory/mechanism that explains these effects is rather uniform. Given a negative effect, lower soil N mineralization at higher PN rates might be due to inorganic N immobilization with greater residue decomposition produced by higher biomass production at higher PN rates (Wienhold and Halvorson, 1999). However, retention of N in root biomass, plant residue, and the active soil organic pool can still be released by mineralization over time, which can explain the positive effect of high PN on soil N mineralization (El-Haris et al., 1983a; Yan et al., 2007). Given this, an investigation of the status of the SOM pool seems critically important when the effect of PN on soil N mineralization is evaluated.

There have been few studies that addressed the effect of current N fertilizer application (CN) on soil N mineralization. The main reason could be the limited number of technical approaches. Laboratory incubation methods based on the procedure of Stanford and Smith (1972) are a classical way to estimate soil N mineralization. In this method, however, leaching of inorganic N with a CaCl$_2$ solution is frequently used. This might cause N fertilizer additions to have a shorter retention period during incubation than
would occur under field conditions. Isotope tracers might seem to be a direct way to test how fertilizer N interacts with indigenous N. However, $^{15}$N labelled fertilizers might have an “apparent” effect, caused by pool substitution or by isotope displacement reactions (Jenkinson et al., 1985). The biggest potential disadvantage for laboratory incubation and isotope methods is that these do not reflect field/ambient temperature and moisture conditions (Carpenter-Boggs et al., 2000). However, these important environment factors affect microbial transformation of organic N (Sierra, 1997). Therefore, in order to make the result relevant to the field, an *in situ* incubation method is desirable.

There are three major *in situ* incubation methods used by researchers to estimate soil N mineralization, including buried bags, covered cylinders, and resin-trap soil cores. Among these, the resin-trap soil core method causes contained soil to experience temperature, moisture, and aeration fluctuations similar to those in the field (Khanna and Raison, 2013). Another advantage to the resin-trap method is the method’s utility for in-season N fertilizer application studies. The core is open at top and bottom, and inorganic N from mineralization and/or fertilizer N addition leached from the soil column during rainfall or irrigation is captured by the resin trap at the bottom (Hanselman et al., 2004). This decreases artificial stimulation of denitrification, especially when the added fertilizer N rate is high. Therefore, in this study, an *in-situ* resin-trap soil core method was used to test the effect of current fertilizer N on soil N mineralization under field conditions.

Temperature and moisture are primary environmental drivers of SOM decomposition (Kirschbaum, 1995; Gabriel and Kellman, 2011), thus substantially affecting microbial-soil N interactions. Unlike the well-controlled conditions of laboratory incubation, *in situ* N mineralization studies can be hard to explain without consideration of these drivers.
Generally, within a certain range in values, moisture and temperature have a positive relationship with soil N mineralization. These two factors can exhibit a positive interaction on N mineralization (Sierra et al., 2015). In this case, N mineralization is more responsive to one factor when the level of the other factor was more favorable. Additionally, except for direct effects, temperature and moisture could have indirect effects on soil aeration, which supports aerobic microbial activity (Sierra, 1997). Previous in situ incubation N mineralization studies found a high correlation between climate factors and N mineralization rate. Singh and Singh (1994a) reported that as much as 80% of the variability in N mineralization rate was explained by the soil moisture content. Kolberg et al. (1997) reported that precipitation, mean air temperature and the interaction between them, gave the best prediction of the daily N mineralization rate.

The present study tests the impacts of N fertilizer rate on in situ soil net N mineralization during the maize growing season, with separation of PN and CN fertilizer effects. Precipitation and temperature data were taken so as to explain field N mineralization conditions. Specifically, the objectives of this study were to: i) measure the influence of past N fertilizer rate on net soil N mineralization; ii) measure the influence of in-season N fertilizer rate on net soil N mineralization; and iii) relate precipitation, air temperature, and soil moisture content to the average daily net soil N mineralization rate.

3.2.2 Materials and Methods

Field Sites and Climatic Information

This study was conducted at the University of Kentucky’s Spindletop Research Farm near Lexington, Kentucky, USA (38°07′18.9″N 84°29′10.6″W). This site has been in a
monoculture corn N rate by tillage study since 1970 (Blevins et al., 1983). The soil is a
Bluegrass-Maury silt loam (fine, mixed, active, mesic Typic Paleudalf) with a 2 to 4
percent slope. This region is characterized by wide variation in mean monthly air
temperature, from 0 °C in January to 24 °C in July and August, but a relatively uniform
distribution in mean monthly precipitation, with an annual average rainfall of 1160 mm.

Experiment Design

Two factors are included in the existing field experiment design: i) N fertilizer rate (0, 84,
168 and 336 kg N ha\(^{-1}\)); and ii) tillage (moldboard plow and no-tillage). There were four
randomized blocks, with the two tillage treatments randomly assigned to one of two strips
within each block. The N fertilizer rates were randomly assigned to one of four strips
within each block, lying perpendicular to/across the two tillage treatment strips. For this
\textit{in situ} incubation N mineralization study, no-tillage soils at three of the four (0, 84 and
336 kg N ha\(^{-1}\)) long-term N rates (PN) were investigated. Three current fertilizer N (CN)
rates (0, 84 and 336 kg N ha\(^{-1}\)) were introduced into each PN rate plot via the incubation
tubes. For example, incubation tubes in the NT-0 kg N ha\(^{-1}\) plot had treatments of 0, 84
and 336 kg N ha\(^{-1}\).

Field Incubation Procedure

Net N mineralization in the 0 to 20 cm soil depth increment was measured by a modified
\textit{in situ} resin-core procedure (Kolberg et al., 1997). Incubation cores were prepared by
driving/removing aluminum conduit (25.4 cm long with an inner diameter of 4.8 cm)
into/from non-trafficked soil between corn rows before fertilizer N application. To
remove intact soil and avoid bypass contamination a hydraulic soil sampler (Giddings 6S
RPS) was gently operated to remove soil cores with little disturbance to the contained and surrounding soil. When the entire core assembly was returned to the original hole, a rubber washer and some soil was used to surround the tube to avoid preferential bypass flow alongside the tube wall. The original plant residue on soil surface was kept in the tube to account for the residue contribution to N mineralization. The entire assembly was returned to the original hole. The top of the soil core was exposed (except as noted below) to facilitate gas exchange and field moisture and temperature fluctuations, allowing inorganic N to leach from contained soil onto the resin as driven by rainfall.

The bottom 2.5 cm of soil was removed from each core and replaced with a nylon bag filled with resin beads. The bottom was then wrapped in 1 mm nylon mesh material to retain the bag. The resin-core incubation tubes are shown in Figure 3.2.1. The resin bag contained equal amounts (total of exactly 35 g, based on original product moisture content) of cation and anion exchange resin (Lanxess Sybron, Birmingham, NJ).

In a preliminary study, the resin was evaluated for retention of adsorbed inorganic N with intensive water flow, and less than 1% of total inorganic N was observed in the leachate. For the CN treatments, a 5 mL aliquot of an NH₄NO₃ solution was evenly placed onto the tube contained soil surface to give rates of 0, 84, and 336 kg N ha⁻¹ according to the tube’s inner soil surface area. To avoid contamination during field N fertilizer application, the tubes were covered during that activity. The tubes were all installed just prior to field N fertilizer application to best mimic the effect of field N fertilizer application on indigenous N mineralization. Given limitations in resin bag durability under field conditions, resin bags needed to be replaced at less than 60 day intervals (Wienhold et al., 2009).
Figure 3.2.1 Cutaway diagram of soil resin-core, in which surficial plant residue and intact soil (0 to 20.32 cm depth increment) and one mixed ion exchange resin bag (35 g) are incubated. A transparent example of the nylon bag holding the ion exchange resins is shown. The bottom of the core was covered with nylon mesh (1 mm opening) material.

Expecting considerable field variance, one observation was a composite of three soil resin-cores. All soil resin-core tubes were randomly assigned. Sampling times were intended to be one and two months after installation and at crop harvest. However, actual sampling dates were adjusted due to weather and soil moisture conditions (Table 3.2.1). Composite soil samples (20 cores plot\(^{-1}\), 0 to 20 cm depth) were taken at incubation initiation to obtain baseline levels of soil NO\(_3\)-N and NH\(_4\)-N. At each sampling time, resin bags and soil cores were removed, immediately transported to the laboratory, mixed
thoroughly, and then frozen until analysis. Dry and wet atmospheric deposition of N was assumed to be small and the same for all treatments, an average 4.7 kg⁻¹ ha⁻¹ yr⁻¹ (National Atmospheric Deposition Program, http://nadp.sws.uiuc.edu/data/ntn/).

Table 3.2.1 *In situ* incubation sampling dates in 2013.

<table>
<thead>
<tr>
<th>Operation</th>
<th>Date</th>
<th>Day of Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initiation date</td>
<td>22-Jun-2013</td>
<td>172</td>
</tr>
<tr>
<td>Sampling Day 1</td>
<td>26-Jul-2013</td>
<td>206</td>
</tr>
<tr>
<td>Sampling Day 2</td>
<td>6-Sep-2013</td>
<td>248</td>
</tr>
<tr>
<td>Sampling Day 3</td>
<td>3-Oct-2013</td>
<td>275</td>
</tr>
</tbody>
</table>

**Laboratory Analyses**

**Soil Sample Analysis**

Gravimetric moisture content of each composite soil sample was determined by weighing and then oven drying (105 °C) a subsample. A 10 g field moist subsample was extracted with 25 mL M KCl for 1 hr. A 1 mL aliquot was centrifuged at a speed of 3700 rpm for 27 min, which is equivalent to filtration through a 0.45 µm filter, based on a preliminary study. The extracts were subjected to NO₃-N and NH₄-N analysis. The NO₃-N was determined colorimetrically according to (Crutchfield and Grove, 2011), using a cadmium brush (Paratech, Lexington, KY). The NH₄-N was found colorimetrically subsequent to the phenol-hypochlorite reaction (Weatherburn, 1967; Ngo et al., 1982). Both NO₃-N and NH₄-N were determined in duplicate. The concentrations of the two inorganic N forms were given in mg N kg⁻¹ oven-dried soil (105 °C) by adjusting for sample gravimetric water content.
Resin Extraction Procedure

Each composite resin sample was mixed and weighed. A subsample equal to 20 g, based on original product moisture content, was packed into a new nylon bag. The subsample resin bags were serially extracted by shaking bags in three 50 mL volumes of 1M KCl for 15, 30, and 60 min, respectively. The three extracts were composited and analyzed for NO$_3$-N and NH$_4$-N, in duplicate, using the same analytical methods described previously. The final adsorbed NO$_3$-N and NH$_4$-N concentration was calculated from standard resin extraction/recovery equations for NO$_3$-N and NH$_4$-N (Equations 1 and 2, respectively). These equations were developed in a previous resin extraction study utilizing the standard extraction procedure described above.

\[
\text{NO}_3\text{-N (adsorbed by resin)} = \text{NO}_3\text{-N (recovered from resin)} \times 1.27 + 258.54 \quad \text{Eqn. 1}
\]

\[
\text{NH}_4\text{-N (adsorbed by resin)} = \text{NH}_4\text{-N (recovered from resin)} \times 1.12 - 45.42 \quad \text{Eqn. 2}
\]

\[
\text{Net Soil N Mineralization} = \text{Inorganic N (t)} - \text{Inorganic N (t$_0$)} - \text{Fertilizer N} \quad \text{Eqn. 3}
\]

Where $t$: sampling date; $t_0$: the initial sampling date.

Calculation

Net soil mineralized N at each sampling date was determined by using the combined amounts of NO$_3$-N and NH$_4$-N in both soil and resin analysis after correction for initial soil inorganic N levels and synthetic N fertilizer input (Eqn. 3). The inorganic N concentration of the soil samples (oven-dry weight basis) and the inorganic N
concentration in resin samples were converted into µg N tube⁻¹ for each tube. Net soil mineralized N at each sampling date equaled the sum of the total inorganic N from soil and resin at each sampling date less the sum of the initial soil inorganic N and synthetic N fertilizer input. The net soil N mineralization was expressed as mg N kg⁻¹ dry soil⁻¹. The average daily net soil N mineralization rate in each incubation period was calculated by dividing total net soil mineralized N by the number of days in that period.

Bulk SOC and STN were determined with a Elementar Vario MAX CNS Analyzer (Elementar Americas Inc., Mount Laurel, NJ). Prior to chemical analysis, visible shoot and root residue was hand removed with a forceps and the subsamples were then ground in a mortar and pestle.

**Statistical Analysis**

Data were statistically analyzed using the General Linear Model (GLM) procedure of the SAS 9.3 computer package (SAS Institute Inc., Cary, NC). Duplicate analyses for the composite soil and resin samples were averaged for statistical analysis of treatment effects. Treatment effects were considered significant at the 95 % level of confidence. Means separation was done using the Tukey's HSD (honest significant difference) test at an alpha level of 0.05.

The regression analyses related precipitation (cumulative amount during each incubation period), temperature (mean air temperature during each incubation period), and soil moisture (gravimetric moisture measured in the soil cores during each incubation period) on average daily net soil N mineralization. Analyses were performed using different
combinations of the independent variables, comparing their predictive ability as measured by the corresponding correlation coefficient ($R^2$).

3.2.3 Results

Effect of Long-Term Fertilizer Applications on SOC and STN

SOC and STN in the surface soil were significantly affected by 43 years under the three different N fertilizer rates (Table 3.2.2). The 336 kg PN ha$^{-1}$ rate exhibited significantly higher SOC, STN, C/N ratio and a significantly lower pH than 0 and 84 kg PN ha$^{-1}$, while there was no difference between 0 and 84 kg PN ha$^{-1}$ in these variates. On the initial day of field incubation there was no difference in inorganic N due to PN rate.

Table 3.2.2 Selected soil properties (0-20 cm depth) at initiation of the in situ incubation study.

<table>
<thead>
<tr>
<th>Past N fertilizer rate (kg PN ha$^{-1}$)</th>
<th>pH†</th>
<th>C/N</th>
<th>Organic C (g kg$^{-1}$)</th>
<th>Total N (mg kg$^{-1}$)</th>
<th>Mineral-N (mg kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.57A‡</td>
<td>8.83B</td>
<td>13.71B</td>
<td>1.55B</td>
<td>4.11A</td>
</tr>
<tr>
<td>84</td>
<td>6.88A</td>
<td>9.06B</td>
<td>13.98B</td>
<td>1.54B</td>
<td>4.63A</td>
</tr>
<tr>
<td>336</td>
<td>6.09B</td>
<td>9.38A</td>
<td>17.13A</td>
<td>1.83A</td>
<td>4.78A</td>
</tr>
</tbody>
</table>

†pH = soil pH$_{water}$ (1:1, w/v).
‡ Values followed by the same letter, within a column, are not significantly different at the 95 % level of confidence according to Tukey’s HSD means separation test.

Effect of Past and Current N Rate and Sampling Date on Net Soil N Mineralization

Net soil N mineralization (NSNM) was significantly (Pr > F ≤ 0.05) influenced by CN rate, PN rate, the CN by PN interaction, sampling date, and the CN by PN by sampling date interaction (Table 3.2.3).
The CN effect on NSNM, at any one PN rate, was not different between 0 and 84 kg CN ha\(^{-1}\). The NSNM were all numerically increased, within each PN rate, when the CN rate increased to 336 kg CN ha\(^{-1}\). However, at 336 kg PN ha\(^{-1}\) and 336 kg CN ha\(^{-1}\) NSNM increased significantly Day 206 and Day 248, but declined on Day 275 (Fig. 3.2.2).

**Table 3.2.3** Analysis of variance for the effects of past (PN) and current (CN) fertilizer N rate, and sampling date on net soil mineralized N (NSNM).

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Past N fertilizer rate (PN)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Current N fertilizer rate (CN)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>PN*CN</td>
<td>0.0031</td>
</tr>
<tr>
<td>Sampling Date (SD)</td>
<td>0.0021</td>
</tr>
<tr>
<td>SD*PN</td>
<td>0.4068</td>
</tr>
<tr>
<td>SD*CN</td>
<td>0.1683</td>
</tr>
<tr>
<td>SD<em>PN</em>CN</td>
<td>0.0184</td>
</tr>
<tr>
<td>Coefficient of Variance</td>
<td>27.5 %</td>
</tr>
</tbody>
</table>

The NSNM was not different between 0 and 84 kg PN ha\(^{-1}\) at any CN rate and on any sampling date. On Days 206 and 248, at 336 kg CN ha\(^{-1}\), the 336 kg PN ha\(^{-1}\) rate exhibited significantly higher NSNM than 0 or 84 kg PN ha\(^{-1}\), but this was not observed on Day 275, because NSNM at 336 kg PN ha\(^{-1}\) and 336 kg CN ha\(^{-1}\) declined 29%.

**Effect of Past and Current N Rates and Incubation Period on Average Daily NSNM**

Average daily NSNM was significantly influenced by PN rate, CN rate and the incubation period. The three-way interaction was also significant (Tables 3.2.4 and 3.2.5). Only at 84 and 336 kg PN ha\(^{-1}\), during the first incubation period, did 336 kg CN ha\(^{-1}\) cause a significantly higher average daily NSNM than 0 and 84 kg CN ha\(^{-1}\). Only at 336 kg CN ha\(^{-1}\), during the first incubation period, did 336 kg PN ha\(^{-1}\) give a significantly
higher average daily NSNM than 0 and 84 kg PN ha\(^{-1}\). Except for 0 kg PN ha\(^{-1}\) at both 0 and 84 kg CN ha\(^{-1}\), the average daily NSNM in the first incubation period (IP) was significantly higher than that observed in the third IP. The average daily NSNM, at each PN rate, also exhibited a declining trend with IP: IP\(_1\) (173-206) > IP\(_2\) (206-248) > IP\(_3\) (248-275).
Figure 3.2.2 The effect of past (PN) and current (CN) fertilizer N rate on net soil mineralized N (NSNM) at three sampling dates in 2013: A) Julian day 206 (July 26); B) Julian day 248 (September 9); C) Julian day 275 (October 3). The NSNM values for different CN rates, within the same PN rate, followed by the same lowercase letter are not significantly different at the 95 % level of confidence according to Tukey’s HSD means separation test. The NSNM values for different PN rates, within the same CN rate, followed by the same uppercase letter are not significantly different at the 95 % level of confidence according to Tukey’s HSD means separation test.
Table 3.2.4  Analysis of variance for the effects of past (PN) and current (CN) fertilizer rate and incubation period (IP) on average daily net soil N mineralization (NSNM).

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Past N fertilizer rate (PN)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Current N fertilizer rate (CN)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>PN*CN</td>
<td>0.249</td>
</tr>
<tr>
<td>Incubation Period (IP)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>IP*PN</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>IP*CN</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>IP<em>PN</em>CN</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Coefficient of Variance</td>
<td>64.3%</td>
</tr>
</tbody>
</table>

Effect of Precipitation, Temperature and Soil Water Content on Average Daily Net Soil N Mineralization

Regression of NSNM against precipitation and temperature, for each PN by CN combination, found that NSNM was driven more strongly by precipitation and temperature than soil water content (SWC) at each sampling date (Table 3.2.6). Overall PN by CN combinations, precipitation was the largest factor influencing NSNM values at this study site, alone accounting for 56 % of NSNM variation. Temperature was the second most important independent variable, accounting for 40 % of NSNM variation. The SWC accounted for 8 % of NSNM variation. Cumulative precipitation and mean air temperature decreased as incubation progressed. Cumulative precipitation, by IP, was 309, 104 and 55 mm for IP₁, IP₂ and IP₃, respectively. Mean air temperature, by IP, was 23.0, 21.7 and 19.2 C for IP₁, IP₂ and IP₃, respectively.
Table 3.2.5  Average daily net soil N mineralization (NSNM) values for the three-way interaction of past (PN) and current (CN) fertilizer N rate and incubation period (IP).

<table>
<thead>
<tr>
<th>kg CN ha⁻¹</th>
<th>0 kg PN ha⁻¹</th>
<th>PN Ave.*</th>
<th>84 kg PN ha⁻¹</th>
<th>PN Ave.</th>
<th>336 kg PN ha⁻¹</th>
<th>PN Ave.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP (Julian Day)</td>
<td>NSNM (mg N kg soil⁻¹ day⁻¹)</td>
<td>NSNM (mg N kg soil⁻¹ day⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>173-206</td>
<td>0.94A²a</td>
<td>0.86Aa$^+$</td>
<td>1.56Ba$^{1#}$</td>
<td>1.12</td>
<td>1.42Ab¹</td>
<td>1.34Ab¹</td>
</tr>
<tr>
<td>206-248</td>
<td>0.21Aa</td>
<td>0.44Aa</td>
<td>-0.03Aa³</td>
<td>0.21</td>
<td>0.21Aa²</td>
<td>0.27Aa</td>
</tr>
<tr>
<td>248-275</td>
<td>-0.03Aa</td>
<td>-0.19Aa</td>
<td>0.31Aa²</td>
<td>0.03</td>
<td>0.04Aa³</td>
<td>-0.19Aa²</td>
</tr>
</tbody>
</table>

* The mean daily NSNM for a given PN by IP combination. $^+$Different NSNM values due to PN rate, within the same CN rate and IP, followed by the same uppercase letter are not significantly different at the 95 % level of confidence according to Tukey’s HSD means separation test. $^1$Different NSNM values due to CN rate, within the same PN rate and IP, followed by the same lowercase letter are not significantly different at the 95 % level of confidence according to Tukey’s HSD means separation test. $^{1\#}$Different NSNM values due to IP, within the same PN rate and the same CN rate, followed by the same superscript number are not significantly different at the 95 % level of confidence according to Tukey’s HSD means separation test.
Table 3.2.6 Correlation coefficients ($R^2$) from regression of average daily net soil N mineralization (NSNM) against precipitation, air temperature and soil water content for the three incubation periods; at each past (PN) and current (CN) fertilizer N rate combination.

<table>
<thead>
<tr>
<th>Current N Rate (kg CN ha$^{-1}$)</th>
<th>0</th>
<th>84</th>
<th>336</th>
<th>0</th>
<th>84</th>
<th>336</th>
<th>0</th>
<th>84</th>
<th>336</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Independent Variables In Model:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>---------</td>
</tr>
<tr>
<td>Precipitation (Precip)$^\dagger$</td>
<td>0.81**</td>
<td>0.68**</td>
<td>0.85**</td>
<td>0.82**</td>
<td>0.87**</td>
<td>0.79**</td>
<td>0.87**</td>
<td>0.70**</td>
<td>0.95**</td>
<td>0.56**</td>
</tr>
<tr>
<td>Temperature (Temp)</td>
<td>0.67**</td>
<td>0.84**</td>
<td>0.37*</td>
<td>0.57**</td>
<td>0.75**</td>
<td>0.49*</td>
<td>0.61**</td>
<td>0.60**</td>
<td>0.65**</td>
<td>0.40**</td>
</tr>
<tr>
<td>Soil Water Content (SWC)</td>
<td>0.39*</td>
<td>0.29</td>
<td>0.12</td>
<td>0.27</td>
<td>0.51**</td>
<td>0.40*</td>
<td>0.08</td>
<td>0.00</td>
<td>0.03</td>
<td>0.08**</td>
</tr>
<tr>
<td>Precip,Temp</td>
<td>0.81**</td>
<td>0.85**</td>
<td>0.94**</td>
<td>0.82**</td>
<td>0.88**</td>
<td>0.82**</td>
<td>0.87**</td>
<td>0.72**</td>
<td>0.96**</td>
<td>0.56**</td>
</tr>
<tr>
<td>Precip,SWC</td>
<td>0.86**</td>
<td>0.75**</td>
<td>0.82**</td>
<td>0.82**</td>
<td>0.88**</td>
<td>0.81**</td>
<td>0.89**</td>
<td>0.75**</td>
<td>0.96**</td>
<td>0.56**</td>
</tr>
<tr>
<td>Temp,SWC</td>
<td>0.70**</td>
<td>0.85**</td>
<td>0.37</td>
<td>0.57*</td>
<td>0.75**</td>
<td>0.56*</td>
<td>0.75**</td>
<td>0.65*</td>
<td>0.69**</td>
<td>0.40**</td>
</tr>
<tr>
<td>Precip,Temp, Precip* Temp</td>
<td>0.81**</td>
<td>0.85**</td>
<td>0.94**</td>
<td>0.82**</td>
<td>0.88**</td>
<td>0.82**</td>
<td>0.87**</td>
<td>0.72**</td>
<td>0.96**</td>
<td>0.56**</td>
</tr>
<tr>
<td>Precip,SWC, Precip*SWC</td>
<td>0.89**</td>
<td>0.89**</td>
<td>0.82**</td>
<td>0.83**</td>
<td>0.88**</td>
<td>0.85**</td>
<td>0.91**</td>
<td>0.75**</td>
<td>0.96**</td>
<td>0.56**</td>
</tr>
<tr>
<td>Temp,SWC,Temp*SWC</td>
<td>0.70**</td>
<td>0.91**</td>
<td>0.47</td>
<td>0.62*</td>
<td>0.85**</td>
<td>0.73*</td>
<td>0.75**</td>
<td>0.65*</td>
<td>0.69*</td>
<td>0.42**</td>
</tr>
<tr>
<td>Precip,Temp,SWC</td>
<td>0.78**</td>
<td>0.85**</td>
<td>0.94**</td>
<td>0.82**</td>
<td>0.88**</td>
<td>0.84**</td>
<td>0.89**</td>
<td>0.76**</td>
<td>0.96**</td>
<td>0.56**</td>
</tr>
<tr>
<td>Precip,Temp,SWC, Precip<em>Temp</em>SWC</td>
<td>0.86**</td>
<td>0.94**</td>
<td>0.95**</td>
<td>0.83**</td>
<td>0.90**</td>
<td>0.86**</td>
<td>0.91**</td>
<td>0.76**</td>
<td>0.96**</td>
<td>0.56**</td>
</tr>
</tbody>
</table>

$^\dagger$ Precip = precipitation received during each incubation period; Temp = mean air temperature during each incubation period; SWC = soil water content measured at the end of each incubation period. *,**, Significant at the 95 and 99 % levels of confidence, respectively.
3.2.4 Discussion

Effect of Long Term Fertilizer N Application on SOC and STN

Soil N mineralization has been reported to be highly correlated to SOC and STN (Schomberg et al., 2009). Therefore, quantification of SOC and STN pools to predict NSNM is important. The 336 kg PN ha\textsuperscript{-1} rate had significantly higher SOC and STN, consistent with other long term N fertilizer studies (El-Haris et al., 1983a; Rasmussen et al., 1998; Graham et al., 2002). The primary reason for a higher SOM pool with greater annual fertilizer N use is likely higher crop production induced by higher fertilizer input, thereby increasing return of shoot and root residues, with a lower C:N ratio, although Six et al. (2002) suggested other possible stabilization mechanisms. While 84 kg PN ha\textsuperscript{-1} is insufficient for maize production on this soil, this PN rate usually caused greater grain yield and N removal than 0 kg PN ha\textsuperscript{-1} (data not shown). Therefore, only slightly higher plant residue and N return might result from the higher grain N removal at 84 kg PN ha\textsuperscript{-1}, resulting in SOC and STN levels similar to those found at 0 kg PN ha\textsuperscript{-1}.

Net soil N mineralization could be influenced by pH (Curtin et al., 1998) and substrate C:N ratio (Janssen, 1996). In this study, soil pH was lower at 336 kg PN ha\textsuperscript{-1} that at 0 or 84 kg PN ha\textsuperscript{-1}, due to acidification resulting from the higher N input. However, agricultural limestone was regularly used to adjust pH in this study (Grove, personal communication), and the pH difference between PN rates was insufficiently large to cause NSNM differences (Dancer et al., 1973). The difference in soil C:N ratio between the three PN rates was relatively narrow as well. This result makes sense because after 44 years of no-till monoculture maize management, the metabolizing soil microbial
population would be essentially similar. Therefore, differences in NSNM among the
different PN rates might not be attributable to soil pH or soil C:N ratio in this study.

**Effect of PN and CN Rate, and Sampling Date, on NSNM**

The effect of PN rate on NSNM reflected SOC and STN pools very well. At any CN rate
and sampling date there was no difference in NSNM between 0 and 84 kg PN ha\(^{-1}\), which
coincides with the similarly sized SOC and STN pools for these two PN rates. The
NSNM at 336 kg PN ha\(^{-1}\) was significantly higher on Days 248 and 275 than that found
at the two lower PN rates. This result indicates that increased SOM due to long term N
fertilizer application could play a role in *in situ* NSNM. These findings might explain
why there have been mixed results regarding the effect of PN rate on NSNM, because
there also have been mixed results regarding whether fertilizer N can result in higher
SOM than the unfertilized controls (Yan et al., 2007; Liu et al., 2013; Brown et al.,
2014). Furthermore, in contrast to fertilizer N application, most studies have reported that
manure or straw amendment can increase SOM (Sommerfeldt et al., 1988; Haynes and
Naidu, 1998; Yan et al., 2007). Therefore, unsurprisingly, most studies have shown that
these amendments can increase NSNM (Singh and Singh, 1994a; Ma et al., 1999; Jordan
et al., 2004; Zhang et al., 2009). Therefore, a hypothesis that impacts of PN on NSNM
will depend on whether PN significantly changes SOM.

From this perspective, a better understanding of the diverse results regarding the effect of
historical N rate on soil N mineralization (Franzluebbers et al., 1994a; Wienhold and
Halvorson, 1999; Carpenter-Boggs et al., 2000). Studies with a single N rate but different
crop management systems may give different NSNM responses (Kolberg et al., 1997).
The influence of CN (in-season) rates on NSNM, is usually referred to as the “priming effect.” Jenkinson et al. (1985) reported that fertilizer N can promote plant growth, and also increase the volume of soil explored by roots, resulting in the priming effect or “added nitrogen interaction.” In this study, plant roots were excluded by herbicide application and discarding tubes with weeds. Therefore, in this study, the CN effect was considered an interaction between inorganic N and soil microorganisms. The in situ study found that the CN or “priming” effect on NSNM had two requirements. First, the priming effect needs sufficient SOM – CN application significantly affected NSNM only at 336 kg PN ha\(^{-1}\), where the SOM was significantly greater than that in the lower PN rate treatments. This result is consistent with an isotopic study on SOM decomposition (Chen et al., 2014), where sucrose or maize straw promoted additional CO\(_2\) respiration.

Second, the priming effect needs sufficient inorganic N input because increasing CN from 0 to 84 kg CN ha\(^{-1}\) in this study did not significantly increase NSNM. This result is also consistent with Chen et al. (2014), who found that when inorganic N was added with sucrose or maize straw, exogenous organic C and inorganic N exerted a synergistic effect on SOM decomposition. (Woods et al., 1987), in an isotope study, also suggested that the “priming effect” on net N mineralization resulted from greater N fertilization when mineral N concentrations were low enough to limit soil microbial activity. These results suggest that both PN and CN need to be considered when evaluating the effect of fertilizer N on indigenous N mineralization in an agroecosystem.
Effect of PN and CN Rate and IP on Average Daily NSNM

The effect of PN and CN on average daily NSNM provided more detailed information for each IP than did total NSNM at each sampling date. During IP₁, only the combination of 336 kg CN ha⁻¹ and 336 kg PN ha⁻¹ gave a significantly higher average daily NSNM than those at 0 and 84 kg PN ha⁻¹. This result confirmed that a significant priming effect on soil N mineralization required both higher CN and the greater SOM resulting from higher PN. In contrast to total NSNM on SD 206 and 248, where 336 kg CN ha⁻¹ gave higher NSNM than 0 or 84 kg CN ha⁻¹ only at 336 kg PN ha⁻¹, the 336 kg CN ha⁻¹ rate gave a significantly higher average daily NSNM than 0 and 84 kg CN ha⁻¹, at both 84 and 336 kg PN ha⁻¹. This result demonstrated that the 84 kg PN ha⁻¹ soil likely had more readily labile organic N or higher SOM quality than the 0 kg PN ha⁻¹ soil, even though total SOC and TSN, and total NSNM were similar (Doran and Parkin, 1994).

Generally, average daily NSNM in IP₁ was significantly greater than that observed in the following incubation periods. The average mean NSNM for each incubation period, at each PN rate, showed the same trend: IP₁ > IP₂ > IP₃. This trend was consistent with that reported by El-Haris et al. (1983a) and Zhang et al. (2009). This change in N mineralization rate can be attributed to the greater amount of readily mineralizable N during the initial incubation period. In the Zhang et al. (2009) study, potentially mineralizable N in the annually N fertilized treatment was significantly higher at the rice transplanting stage than at later stages. In the El-Haris et al. (1983) study, the soil N mineralization rate was higher in the first 4 weeks for soils receiving higher historical fertilizer N than soils where lower historical fertilizer N rates were used. However, after that, soil N mineralization rates were generally unrelated to different historical fertilizer
N rates, and they argued that fertilizer N application contributed greatly to the readily available N pool, with little impact on the intermediately available N pool. Another mechanism that might explain the different soil N mineralization rates would be the different environmental conditions occurring during each IP.

**Effect of Precipitation, Temperature and Soil Water Content on NSNM**

Soil N mineralization can be affected by soil moisture content and temperature (Kirschbaum, 1995; Sierra, 1997). Compared to well-controlled laboratory conditions, estimating soil N mineralization with *in situ* incubation methods might be difficult without consideration of weather data. Regression modeling in this study found that most of the variation in NSNM was explained by precipitation and temperature, consistent with (Kolberg et al., 1997). Cumulative precipitation and mean air temperature gradually decreased from IP₁ to IP₃. Therefore, weather data also helped explain the different patterns in average daily NSNM over the three IPs.

Compared to precipitation and mean air temperature, SWC measured at the end of each IP accounted for less of the NSNM variation, and can be highly affected by rain events just before each sampling date. However, including SWC in the regression models increased predictive capacity. Moreover, the SWC data might help explain the more negative NSNM at the combination of 336 kg PN ha⁻¹ and 336 kg CN ha⁻¹ on Day 275. The SWC on Day 275 at the plots annually receiving 336 kg PN ha⁻¹ was significantly higher than that found in other plots (data not shown). This higher SWC might be due to shading from the greater biomass produced at this greatest PN rate, as well as greater soil
water holding capacity caused by higher SOM. Therefore, these incubation tubes likely
had higher denitrification potential due to higher SWC and high inorganic N levels.

3.2.5 Conclusion

Understanding how fertilizer N application affects indigenous N mineralization will
improve agro-ecosystem N fertilizer management, resulting in improved soil productivity
with reduced adverse environmental impacts. However, the effects of prior and present N
fertilizer application on NSNM require separation so as to achieve clarity. Whether PN
affects NSNM depends on whether the SOM pool has been modified by long term N
application. The priming effect of CN on NSNM depends on both the CN rate and
whether the soil has enough SOM to support/promote microbial decomposition. Further
studies on the effects of N fertilizer on indigenous N mineralization should go beyond
merely evaluating PN and CN rate effects. These future studies should also pay more
attention to the total and readily labile SOM pools induced by long term inorganic or
organic N applications, as well as environmental factors influencing the priming effect.
3.3.1 Introduction

Nitrogen mineralized from soil organic matter (SOM) and crop residue is a major component of soil N supply in agricultural production systems. Estimating net soil N mineralization (NSNM) during a growing season is of considerable importance for maximizing crop N use efficiency from all N sources and minimizing environmental losses (Schomberg et al., 2009). Long term incubation to estimate biological N mineralization has been regarded as the most suitable soil N availability index (Keeney, 1982; Griffin et al., 2007), but the incubation process is time consuming and not practical for routine use. Consequently, rapid biological and chemical methods to estimate NSNM have long been sought. Most studies on this topic have utilized numerous soil samples from a broad region in order to find indices with broad application (Sharifi et al., 2007; Schomberg et al., 2009). However, no single N availability index has proved sufficiently robust for broad acceptance across a wide range of soils (Balkcom et al., 2003; Bushong et al., 2008). The main reason could be that NSNM is a function of many biotic and abiotic factors that are themselves influenced by climate conditions, soil type, cropping history, and soil management (Griffin, 2008). Collecting soil samples from a broad region might better represent soil types, but background information on soil management was often not maintained.

Choosing soil samples from a relatively small geographical area could avoid variance due to climate and soil types, and soil samples from well-designed research sites should
include full information on cropping history and soil management. The data might not be extrapolated to other broader regions, but the predictive value of determined indices of N availability could be greater and might be packaged in routine tests by local soil testing laboratories, because fertilizer N recommendations are commonly developed on a regional basis. Increasing utilization of NSNM while reducing the laboratory effort could take two approaches: (1) decreasing the frequency of NSNM measurement; and (2) determining alternative rapid indices to estimate NSNM. One of major purposes for estimating NSNM in agriculture production is to evaluate the effect of crop and soil management. More efficient N fertility management can then be suggested for different production systems, based on knowledge of these effects (Kolberg et al., 1999; Balota et al., 2004). However, the first objective would be determining the proper frequency of NSNM assays needed to evaluate the effects of crop and soil management. Because NSNM is a component of the biological decomposition of SOM, the second objective would be to evaluate which soil organic C and N fractions are sufficiently robust to predict NSNM.

Different sampling times might give different NSNM magnitudes due to seasonal soil variation or operation error (El-Haris et al., 1983a; Zhang et al., 2009). The relative impacts of crop and soil management practices on NSNM, across different sampling times, would still be valuable knowledge because this can help determine the optimal frequency for NSNM assays. Unfortunately, the literature on this topic is sparse. Using soil organic carbon (C) and N fractions to predict NSNM has been studied extensively in past decades (Keeney and Bremner, 1966; Curtin and Wen, 1999; Sharifi et al., 2007). Soil organic carbon (SOC) and total nitrogen (STN) concentrations have been used as
indices of N availability, with mixed results. Selles et al. (1999) and Schomberg et al. (2009) observed close correlations between STN and/or SOC and NSNM across a wide range of soils, but other researchers reported weak correlations between STN and/or SOC and NSNM (Hassink, 1994). There have been a few studies on the correlation between permanganate oxidizable carbon (POXC) and NSNM, but POXC has been shown to be sensitive to soil management (Culman et al., 2012) and this weakens consequent prediction of crop response to N (Lucas and Weil, 2012). Particulate organic carbon (POC) and nitrogen (PON) were also reported to be closely related to NSNM (Schomberg et al., 2009).

In this study, six well defined tobacco rotation-tillage systems at a single research site were used. The objectives were to: (1) test the temporal stability of the effect of six tobacco rotation-tillage systems on NSNM; and (2) evaluate five soil C and N fractions as predictors of NSNM.

3.3.2 Methods and Materials

Site Description and Climate

This study was conducted from 2011 to 2013 at the University of Kentucky Spindletop Research Farm near Lexington, Kentucky, USA (38°07'36.8"N 84°29'26.4"W). The soil is a Bluegrass-Maury silt loam (fine, mixed, active, mesic Typic Paleudalf), a deep well-drained soil formed in phosphatic limestone residuum. Before this study site was established, the baseline soil pH$_{\text{water}}$(1:1, w/v) was 6.3, with Mehlich III (Mehlich, 1984) extractable nutrient levels were 85, 178, 1800, 110 and 3 mg kg$^{-1}$ for phosphorus, potassium, calcium, magnesium, and zinc, respectively. The soil texture was 12 % sand,
68 % silt and 20 % clay. The location is characterized by a wide variation in mean monthly air temperatures, from 0 °C in January to 24 °C in July and August, but a relatively uniform distribution in mean monthly precipitation, with an annual average annual rainfall of 1160 mm.

**Experiment Design and Field Sampling**

Treatment plots (6.4 m wide by 24.4 m long) were arranged in four randomized complete blocks. The six tillage-crop sequence treatments were: i) conventional tillage continuous tobacco (TTT-CT); ii) no-tillage continuous tobacco (TTT-NT); iii) 2-yr fescue (*Festuca arundinacea Schreb.*) sod and 1-yr conventional tillage tobacco (SST-CT); iv) 2-yr fescue sod and 1-yr no-tillage tobacco (SST-NT); v) no-tillage corn, soybean and tobacco (CST-NT); and vi) no-tillage soybean, corn and tobacco (SCT-NT). These production systems were established in 2007.

Composite soil samples (20 cores per plot) were collected from the 0 to 10 and 10 to 20 cm depth increments on 13 May 2011, 16 May 2012 and 14 May 2013, respectively when tillage was implemented, but prior to fertilizer application. The samples were used to measure NSNM, POXC, POC, PON, SOC and STN. Conventional tillage refers to moldboard plowing, followed by disking twice and a soil finisher operation before transplanting tobacco seedlings. Other agronomic management was applied according to recommendations from the University of Kentucky Cooperative Extension Service (Seebold and Pearce, 2013).
Laboratory Analysis

Net Soil Nitrogen Mineralization (NSNM)

The aerobic laboratory N mineralization incubation method was based on a procedure described by Hart et al. (1994). After composite soil samples were collected, a field moist subsample was immediately extracted with 1M KCl to determine baseline levels of soil NO$_3$-N and NH$_4$-N. The remaining field moist soil was manually passed through a 4 mm sieve. Large pieces of organic material and rocks were removed. Soil water content was determined gravimetrically by oven-drying a second subsample. The remaining soil was stored at 4 °C until incubation. To start the incubation, 50 g soil was placed in duplicate sealable plastic bags. Soil moisture was adjusted to 60 % water-filled pore space, which has proved to be optimal for aerobic microbial processes in most soils (Linn and Doran, 1984). Soil moisture content was regularly checked and adjusted as necessary. Soil was incubated at a constant temperature of 25 °C. Periodically, 5 g of soil was removed and 1M KCl was used to extract NO$_3$-N and NH$_4$-N. The NO$_3$-N analysis was determined with a microplate cadmium brush (Paratech, Lexington, KY) reduction method (Crutchfield and Grove, 2011). The NH$_4$-N analysis was determined with the phenol-hypochlorite reaction (Weatherburn, 1967; Ngo et al., 1982). The cumulative incubation days were 127, 128, and 119 days for samples taken in 2011, 2012 and 2013, respectively. The NSNM was calculated after correction for final and initial inorganic soil N. The final concentration of inorganic N in each sample is reported in mg N kg$^{-1}$ oven-dried (105 °C) soil after adjusting for sample gravimetric water content. The NSNM analysis was duplicated for each sample, and the mean of the duplicates was used for statistical analysis.
Permanganate Oxidizable Carbon (POXC)

The POXC analysis was based on the procedure described by Weil et al. (2003) and modified by Stiles et al. (2011). Field moist soil was manually passed through a 4 mm sieve to remove coarse debris and then passed through a 2 mm sieve and air-dried for 2 to 3 weeks. For POXC analysis, 2.5 g of air-dried soil were weighed into 50 mL screw-top plastic centrifuge tubes. The soil was reacted with 20 mL of a 0.02 mol L\(^{-1}\) KMnO\(_4\) solution in 0.1 mol L\(^{-1}\) CaCl\(_2\) by shaking for exactly 2 min on a reciprocating shaker at 180 rpm. After shaking, tubes were removed from the shaker and allowed to settle for exactly 10 min (shaking and settling times are very important, so sample batches were limited to 10 samples or less). A 0.5 mL aliquot of the supernatant was transferred into a second 50 mL centrifuge tube and mixed with 49.5 mL deionized water by hand shaking. The solution absorbance at 550 nm was measured by a Genesys 20 Spectrophotometer (Thermo Fisher Scientific, Inc.). To determine residual KMnO\(_4\) concentrations, sample absorbance was compared with a standard curve that ranged from 0.005 to 0.02 mol L\(^{-1}\) KMnO\(_4\). Sample POXC was calculated as in Weil et al. (2003) and Blair et al. (1995) as follows:

\[
\text{POXC (mg kg}^{-1}\text{)} = [0.02 \text{ mol L}^{-1} - (a + bz)] \times (9000 \text{ mg C mol}^{-1}) \times \left(\frac{0.02 \text{ L solution}}{0.0025 \text{ kg soil}}\right)
\]

Eqn 1

where 0.02 mol L\(^{-1}\) is the initial KMnO\(_4\) concentration, \(a\) is the intercept and \(b\) the slope of the standard curve, \(z\) is the absorbance of the unknown, 9000 mg is the amount of C oxidized by 1 mole of MnO\(_4\) (Mn\(^{7+}\) reduction to Mn\(^{4+}\)), 0.02 L is the volume of KMnO\(_4\) reaction solution, and 0.0025 kg is the mass of soil used in the reaction. The POXC
analysis was duplicated for each sample, and the mean of the duplicates used for statistical analysis.

**Particulate Organic Carbon (POC) and Nitrogen (PON)**

Particulate organic matter fractions were isolated by a particle size fractionation method described by Cambardella and Elliott (1992) and Cambardella and Elliott (1993a). Field moist soil was manually passed through a 4 mm sieve to remove coarse debris and then passed through a 2 mm sieve and air-dried for 2 to 3 weeks. For physical separation, 50 g subsamples were dispersed in 150 mL of a 5 % (w/v) solution of sodium hexametaphosphate by shaking 15 h on a reciprocal shaker. The dispersed samples were passed through a 53 µm sieve. After rinsing several times with water, the material retained on the sieve was transferred to an aluminum pan and dried at 50 °C. The dried sample was ground with a mortar and pestle and analyzed for carbon and nitrogen by dry combustion. The POC and PON values are given in mg C (or N) kg\(^{-1}\) soil.

**Soil Organic Carbon (SOC) and Soil Total Nitrogen (STN)**

The SOC and STN concentrations were determined by dry combustion of air-dry 2-mm-sieved whole soil samples from each depth with an Elementar Vario Max CN analyzer (Elementar Co.). The SOC and STN values are given in mg C (or N) kg\(^{-1}\) soil.

**Statistical Analysis**

Data were analyzed with the General Linear Model (GLM) and Pearson correlation procedures in the SAS 9.3 computer package (SAS Institute Inc., Cary, NC). Duplicate sample measurements were averaged for statistical analysis. The analysis considered
replicate effects to be random and treatments effects to be fixed. Treatment effects were considered significant at the 95% level of confidence. Means separation was done using the Tukey's HSD (honest significant difference) test at an alpha level of 0.05. Graphical presentations were developed using SigmaPlot 12.3 (Systat Software Inc., San Jose, CA).

3.3.3 Result

The effect of tobacco tillage and rotation systems on NSNM from 2011 to 2013

The NSNM was significantly affected by year, system, depth, depth by system, and depth by year, but the system by year and system by year by depth interactions were not significant (Table 3.3.1). POXC, PON, POC, STN, and SOC were affected by the similar variances as to those observed for NSNM. The system by year interaction for NSNM was used to evaluate the first objective - evaluating the temporal stability of the effect of the six tobacco rotation-tillage systems on NSNM. The system by year interactions for POXC, PON, POC, STN, and SOC were not significant.

Soil depth significantly affected NSNM (Table 3.3.1), so the non-significant system by year was separated into the two sampled depths. At 0 to 10 cm, the NSNM values for the SST-NT, SST-CT and TTT-CT treatments were stably ranked as 1st, 5th, and 6th, respectively (Figure 1). Although the rank order for NSNM values for the TTT-NT, CST-NT and SCT-NT treatments varied somewhat from year to year, these NSNM values were not significantly different from one another in any year. At 10 to 20 cm (Figure 2), only NSNM values for SST-CT had a consistent rank (1st) across the three years, other treatments gave NSNM values that ranked randomly each year. Furthermore, there was no significant difference in NSNM due to treatment, in any year.
Table 3.3.1 Analysis of variance summary for the effects of different tobacco tillage-rotation systems on NSNM, POXC, PON, POC, STN and SOC.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF †</th>
<th>SNM</th>
<th>POXC</th>
<th>PON</th>
<th>POC</th>
<th>STN</th>
<th>SOC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-------</td>
<td>------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Probability of a greater F value</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replicate</td>
<td>3</td>
<td>0.5217</td>
<td>0.0051</td>
<td>0.223</td>
<td>0.2392</td>
<td>0.0021</td>
<td>0.0003</td>
</tr>
<tr>
<td>Year (Y)</td>
<td>2</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.0002</td>
<td>0.0008</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Treatment (S)</td>
<td>5</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.1248</td>
<td>0.0150</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Treatment*Year</td>
<td>10</td>
<td>0.1290</td>
<td>0.1020</td>
<td>0.6218</td>
<td>0.3591</td>
<td>0.2689</td>
<td>0.0948</td>
</tr>
<tr>
<td>Depth (D)</td>
<td>1</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Treatment*Depth</td>
<td>5</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.070</td>
<td>0.0743</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Year*Depth</td>
<td>2</td>
<td>0.016</td>
<td>&lt;.0001</td>
<td>0.001</td>
<td>0.0214</td>
<td>0.0136</td>
<td>0.1887</td>
</tr>
<tr>
<td>Treatment<em>Year</em>Depth</td>
<td>10</td>
<td>0.9729</td>
<td>&lt;.0001</td>
<td>0.6769</td>
<td>0.7342</td>
<td>0.7128</td>
<td>0.8202</td>
</tr>
</tbody>
</table>

†DF = degrees of freedom; NSNM = net soil nitrogen mineralization; POXC = permanganate oxidizable carbon; PON = particulate organic nitrogen; POC = particulate organic carbon; STN = total soil nitrogen; SOC = soil organic carbon. Statistically significant relationships are in bold.
Figure 3.3.1 Effect of six tobacco tillage-rotations on net soil N mineralization (NSNM) at 0 to 10 cm in 2011 (A), 2012 (B) and 2013 (C). The NSNM values are given above each vertical bar. Different letters indicate values are significantly different at the 95 % level of confidence, according to Tukey’s HSD means separation test. Note: Treatments were numerically ranked from lowest to highest in X axis.
Figure 3.3.2  Effect of six tobacco tillage-rotation systems on net soil N mineralization (NSNM) at 10 to 20 cm in 2011 (A), 2012 (B) and 2013 (C). The NSNM values are given above each vertical bar. Different letters indicate values are significantly different at the 95 % level of confidence, according to Tukey’s HSD means separation test. Note: Treatments were numerically ranked from lowest to highest in X axis.
Indices related to soil nitrogen mineralization

The correlation coefficient (r) between the NSNM values and potential indicator indices (across both the 0 to 10 and 10 to 20 cm depth increments) varied from 0.609 to 0.863 (Table 3.3.2). The rank order for the coefficients of correlation between NSNM and these other indices was SOC > POXC > POC > STN > PON. For Table 3.3.3, a randomized complete block design, using the SAS GLM procedure, was fitted to each response variable, and the residuals were output. Then, a correlation among the residuals found for each response variable was conducted using the SAS Pearson correlation procedure. The correlation coefficients (r) among the response variable residuals ranged from 0.191 to 0.463 (Table 3.3.3). The rank order for the coefficients of correlation between these residuals for NSNM and the soil carbon and nitrogen fractions was SOC > STN > POC > PON > POXC. Except for the coefficients of correlation among residuals for PON versus POC and SOC versus STN, all other coefficients of correlation among variate residuals were lower than the corresponding coefficients of correlation among variate values.

Table 3.3.2 Pearson correlation coefficients (r) for correlations among NSNM values and the values for other soil carbon and nitrogen indices (n=144).

<table>
<thead>
<tr>
<th>Parameter†</th>
<th>NSNM</th>
<th>POXC</th>
<th>PON</th>
<th>POC</th>
<th>STN</th>
<th>SOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSNM</td>
<td>1‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POXC</td>
<td>0.693</td>
<td>1‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PON</td>
<td>0.609</td>
<td>0.316</td>
<td>1‡</td>
<td></td>
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<tr>
<td>POC</td>
<td>0.667</td>
<td>0.417</td>
<td>0.960</td>
<td>1‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STN</td>
<td>0.632</td>
<td>0.350</td>
<td>0.552</td>
<td>0.538</td>
<td>1‡</td>
<td></td>
</tr>
<tr>
<td>SOC</td>
<td>0.863</td>
<td>0.638</td>
<td>0.684</td>
<td>0.724</td>
<td>0.773</td>
<td>1‡</td>
</tr>
</tbody>
</table>

‡NSNM = net soil nitrogen mineralization; POXC = permanganate oxidizable carbon; PON = particulate organic nitrogen; POC = particulate organic carbon; STN= soil total nitrogen; SOC= soil organic carbon. ‡All correlations are significant (p < 0.001).
Table 3.3.3 Pearson correlation coefficients (r) for correlations among NSNM residuals and the residuals for other soil carbon and nitrogen indices (n=144).

<table>
<thead>
<tr>
<th>Parameter ( ^\dagger )</th>
<th>r(^\NSNM )</th>
<th>r(^\POXC )</th>
<th>r(^\PON )</th>
<th>r(^\POC )</th>
<th>r(^\STN )</th>
<th>r(^\SOC )</th>
</tr>
</thead>
<tbody>
<tr>
<td>rNSNM ( ^\dagger )</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rPOXC</td>
<td>0.191</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rPON</td>
<td>0.332</td>
<td>0.029</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rPOC</td>
<td>0.382</td>
<td>0.072</td>
<td>0.942</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rSTN</td>
<td>0.438</td>
<td>0.109</td>
<td>0.291</td>
<td>0.299</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>rSOC</td>
<td>0.463</td>
<td>0.238</td>
<td>0.343</td>
<td>0.369</td>
<td>0.830</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^\NSNM = \text{net soil nitrogen mineralization}; \ POXC = \text{permanganate oxidizable carbon}; \ PON = \text{particulate organic nitrogen}; \ POC = \text{particulate organic carbon}; \ STN = \text{soil total nitrogen}; \ SOC = \text{soil organic carbon}. \(^\dagger \)r refers to the residual for each response variable that resulted from fitting the model. \(^\dagger \)All correlations are significant (p < 0.05) except for r\(^\POXC \) versus r\(^\PON \), r\(^\POXC \) versus r\(^\POC \), and r\(^\POXC \) versus r\(^\STN \).

3.3.4 Discussion

Soil N mineralization estimated by biological incubation methods is considered an effective index of soil N availability (Binkley and Hart, 1989; Bundy and Meisinger, 1994); these incubations have also proved to have a high correlation with crop N uptake or yield response (Stanford et al., 1973). For most studies, the main purpose for estimating NSNM has been to evaluate the impact of agronomic practices (including crop sequences, tillage practices, and N fertilizer application) on soil N dynamics to cope with both agriculture production and environment concerns (Campbell et al., 1995; Ju et al., 2009). The classic long-term aerobic laboratory incubation methods require substantial time and equipment (Stanford and Smith, 1972). There has been much effort to simplify NSNM methods and to find alternative but rapid biological and chemical N availability indices (Griffin, 2008). Two questions, related to the need to reduce NSNM incubation work and raise time efficiency, were examined in this study. Question 1 was whether
NSNM assay frequency could be reduced. Question 2 was whether SOC or another soil C or N fraction could serve as an NSNM predictor.

For objective 1, the system by year interaction was not statistically significant, which indicates that the effects of the six tobacco rotation-tillage treatments on NSNM were stable across years. This finding suggests that long-term laboratory incubations to estimate NSNM are not necessary every year. In this study, crop and soil management systems significantly affected NSNM, consistent with many other studies (El-Haris et al., 1983a; Carpenter-Boggs et al., 2000). However, the individual rotation systems were stable – similarly ranked across several years even though the absolute amount of NSNM varied from year to year. Soil depth significantly affected NSNM. Surface soil usually contains much SOM and has higher soil microbial activity, thus the 0 to 10 cm depth increment exhibited higher NSNM than the 10 to 20 cm depth increment and contributes most to soil profile NSNM (Cassman and Munns, 1980). Most crop sequence and tillage management choices affect SOM mainly in surface soil (Post and Kwon, 2000). Therefore, the temporally stable NSNM pattern among the six tobacco tillage-rotation systems gives confidence to a decision to reduce the frequency of NSNM assays.

No-tillage can increase NSNM estimates because soil is homogenized for laboratory incubation. No-tillage can increase the surface SOM pool by reducing the macroaggregate turnover rate (Six et al., 2000a). The system by depth interaction on NSNM could be due mainly to a tillage effect. All samples were taken after tillage. Therefore, soil was homogenized in the 0 to 10 and 10 to 20 cm depth increments in the TTT-CT and SST-CT systems, and there were only slight differences in NSNM between
depths in these two systems. However, for the other four no-tillage systems, NSNM at the 0 to 10 cm depth was much higher than in the 10 to 20 cm soil depth.

Although the relative rankings of NSNM values for the six systems were stable across the three years, the year significantly affected absolute NSNM values. This phenomenon could be due to soil substrate status differences or subtle changes in the incubation procedures. Zhang et al. (2009) reported that soil samples taken at different rice growth stages gave different NSNM values because the most labile mineralizable organic N pool was mineralized quickly. Although soil samples were taken at the same time each year, different weather conditions (e.g. precipitation and temperature) ahead of each year’s sampling date could cause differences in the mineralizable organic N pool. Subtle random operation errors include small differences in sample pretreatment, such as adjusting moisture. Others have noted the challenge in accurately obtaining a realistic estimate of NSNM for agricultural soils with current techniques (Nadelhoffer et al., 1991; Knoepp and Vose, 2007). However, the difference in absolute values between years was not the focus of this study, which was, rather, to determine the relative impact of the different tillage-rotation systems on NSNM, which could influence decisions regarding N fertility management. As similar to NSNM, the system by year interactions for POXC, PON, POC, STN, and SOC were not significant, which indicates that the effects of tobacco rotation-tillage systems on these parameters are temporally stale as well.

For objective 2, coefficients for correlations between NSNM and the other five soil C and N fractions used as indices in this study indicated that all five could be effective NSNM predictors at 0 to 10 cm (r > 0.6 and significant; P < 0.001). However, coefficients for correlations between residuals from the GLM model for NSNM and those of the other
indices were lower. The Pearson correlation between actual NSNM values and the indices only shows the relationship between the two datasets without full consideration of how the data were collected, or the experimental design, which is a suitable way to select predictors when using numerous samples from a wide range of soil types.

Alternative analysis of the residuals was done to better account for the experimental design, according to Kutner et al. (2004). Correlating residuals instead of the actual values can mitigate spurious estimation of correlation coefficients. Although there were a large number of samples (n = 144) in the study, all were collected from the same site. The same composite soil samples were used to determine NSNM, POXC, PON, POC, STN and SOC. In these samples, those properties would be inherently correlated due to several reasons: (1) these measured variates all reflect certain SOC or STN fractions; (2) soil samples were collected from the small plot areas; and (3) they belonged to one experimental study site. These reasons caused the measurements to be similarly affected by similar sources of variance, as shown in Table 3.3.1.

Therefore, simply using Pearson correlation without taking the background information into account might result in overestimation of the coefficients between the five indices and NSNM. For example, POXC was reported to be as sensitive to changes in management as NSNM (Soon et al., 2007; Culman et al., 2012). The Pearson correlation coefficient between values of POXC and NSNM was 0.693 (Table 3.3.1), but the Pearson correlation coefficient between POXC and NSNM residuals was only 0.191 (Table 3.3.2). Thus, using the residuals from the GLM model can reduce the influence of known variance, e.g. year, depth and systems, and thereby more precisely unveil the relationship between NSNM and the five indices.
3.3.5 Conclusion

An efficient and simple index of NSNM would improve N fertilizer recommendations and minimize adverse environmental impacts due to excessive N availability. This study evaluated the frequency of NSNM (estimated by a season-long soil N mineralization laboratory incubation method) evaluation needed to effectively compare the tillage-rotation systems and determine the predictive success of other common measurements that would serves as indices of NSNM. Among six tobacco tillage-rotation systems, NSNM was relatively stable across three years, indicating that NSNM need not be measured annually. The SOC was a reliable index of NSNM across the six tillage-rotation systems and three years in this study. Because SOC is commonly an option in routine soil tests done by many testing laboratories, SOC is arguably the best proxy to use for predicting NSNM among different tillage-rotation systems.
4.1 Introduction

Since no-tillage commercial corn production was introduced in Kentucky in 1962 (Phillips and Young Jr, 1973), the no-tillage system has been quickly adopted for grain crop production around the world (Derpsch et al., 2010). In 2008, 21% of maize (*Zea mays* L.) and 41% of soybean (*Glycine max* (L.) Merrill) planted acres were in no-tillage production in the United States according to the Crop Residue Management Survey (http://www.ctic.purdue.edu/CRM/). Burley tobacco, known as a major cash crop in the southeastern US, has been considered a conservation plan problem because tobacco traditionally required clean cultivation and is grown in a season when intense rains raise the erosion hazard. Recently, more growers have adopted conservation plans for burley tobacco production, especially as the tobacco industry has promoted good agriculture practices for future tobacco production. No-tillage tobacco offers several advantages compared to conventional production. It can reduce soil erosion, reduce field preparation time, and save fuel cost relative to mechanical tillage (Pearce and Zeleznik, 1996a). The presence of ground cover at harvest might result in cleaner cured tobacco leaf than with bare soil (Wood and Worsham, 1986). Although no-tillage burley tobacco production research was attempted in the early 1970’s (Phillips and Zeleznik, 1989). The recent appearance of a modified no-tillage transplanter and successful weed control options made no-tillage tobacco production economically viable (Morrison et al., 1973; Pearce and Zeleznik, 2003; Zou, 2013). However, past research provides inadequate detail comparing no-till and tilled burley tobacco production with respect to seasonal
agronomic response, agronomic performance, nitrogen nutrition management, and cured leaf chemical composition.

Nitrogen nutrition management is an important component of tobacco production. With appropriate N management, it may be possible to limit excessive NO$_3^-$N accumulation in leaves, optimize fertilizer N use efficiency, and reduce the potential for ground and surface water pollution without compromising burley tobacco leaf yield and quality (MacKown et al., 1999). In addition to fertilizer N, N mineralization from the soil organic matter (SOM) is another important N source. However, soil N mineralization may be affected by tillage method. No-tillage can have higher soil N mineralization potential than conventional tillage (El-Haris et al., 1983b; Wienhold and Halvorson, 1999; Pandey et al., 2010) because no-tillage tends to preserve surface soil carbon (C) and N pools (Six et al., 1998; Six et al., 1999; Wright and Hons, 2005b) and maintain higher surface soil moisture levels (Blevins et al., 1971). Most of these studies were conducted under laboratory conditions and might not reflect field conditions. One strategy to approximate field environments is to use *in situ* resin core incubation methods to estimate soil mineral-N dynamics during the tobacco growth season. Compared to laboratory incubations, the *in situ* approach may better predict differences in plant available nitrogen (PAN) supply due to different tillage systems during the growth season (Hübner et al., 1991; Kolberg et al., 1997; Khanna and Raison, 2013).

Tobacco agronomic performance might also be influenced by the effect of tillage on soil properties such as surface structure (Arshad et al., 1999), moisture (De Vita et al., 2007), penetration resistance (Lampurlanés and Cantero-Martínez, 2003), nutrient distribution in the soil profile (Franzluebbers and Hons, 1996; Duiker and Beegle, 2006; Wright et al.,
2007), and the rhizosphere environment (Andrade et al., 2003; Yadav and Tarafdar, 2004; Thomas et al., 2007). In tobacco tillage studies, Phillips and Zeleznik (1989) found that the killed sod mulch on no-tillage soil reduced water evaporation compared to tilled soil for the first 60 days after transplanting. Tobacco growth rates were higher with no-tillage than with tillage. Ritchey (2010) found that penetrometer resistance in tobacco fields over the 0 to 10 cm depth was significantly lower with tillage than no-tillage. Zartman et al. (1976) reported that tobacco root density over the 0 to 15 cm depth increment was higher with tillage than no-tillage. The effects of different tillage systems on burley tobacco cured leaf yield have been mixed. Zeleznik and Phillips (1990) reported that over a five year period there were no significant differences in cured leaf yield and quality with tillage or no-tillage when 336 kg N ha\(^{-1}\) fertilizer was applied. In contrast, Zartman et al. (1976) reported that burley tobacco growth rates were lower with no-tillage than conventional tillage when only 90 kg N ha\(^{-1}\) fertilizer was applied. Link (1984) in Virginia reported that no-tillage burley yields were equal to conventional tillage yields for 2 of the 5 years tested and significantly less in the remaining years.

With increasing health consciousness and regulation of tobacco products, the chemical constituents of the cured leaf are as important as yield. Alkaloids, including nicotine, nornicotine, anabasine, and anatabine are precursors to carcinogenic nitrosamines (Andersen et al., 1986). Tobacco specific nitrosamines (TSNAs) have been reported as carcinogenic compounds in tobacco for many years (MacKown et al., 1984). Since the first report documenting the presence of N-nitrosonornicotine in unburned tobacco and induced malignant tumors in mice, rats, and hamsters (Hoffmann and Hecht, 1985), there have been many studies that evaluated the effects of genotypes (Guo et al., 2013),
chemical applications (Li et al., 2013), and the regulation of humidity and temperature during the curing process on the formation of TSNAs (Burton et al., 1989a; Chamberlain and Chortyk, 1992; Padmavathy et al., 2011; de Godoy Lusso et al., 2014). Liming, N fertilizer rate, irrigation management and cultivar on reducing sugars and nicotine concentration and leaf quality have also been investigated (Karaivazoglou et al., 2007; Çakir and Çebi, 2010; Kaleb Rathbone et al., 2010). It is well known that tobacco alkaloid synthesis is located in the root system (Dawson and Solt, 1959), which might be greatly affected by the soil physical and chemical conditions resulting from tillage practice. However, few studies have discussed the potential impacts of tillage on tobacco leaf chemical constituents.

With more emphasis on conservation tillage, in a context of sustainable agricultural systems, more information is needed to further improve N management in relation to no-tillage tobacco agronomic performance and leaf quality. Therefore, the objectives of this study were to determine if tillage method and N fertilizer rate: (i) influenced tobacco yield; (ii) affected plant available N supply during tobacco growth season; and (iii) altered cured tobacco leaf chemical composition.

4.2 Materials and Methods

Site Description and Climate Information

This research was conducted at the University of Kentucky Spindletop Research Farm, near Lexington, Kentucky, USA (38°07′36.8″N 84°29′26.4″W). The soil is a Bluegrass-Maury silt loam (fine, mixed, active, mesic Typic Paleudalf), with a 2 to 4 % slope at the study site. A long-term study was established in 2007 to evaluate the yield response of
burley tobacco to tillage method. When study site was established in 2007, the baseline soil pH<sub>water</sub> (1:1, w/v) was 6.3, with Mehlich III extractable phosphorus (85 mg kg<sup>-1</sup>), potassium (178 mg kg<sup>-1</sup>), calcium (1.8 g kg<sup>-1</sup>), magnesium (110 mg kg<sup>-1</sup>), and zinc (3 mg kg<sup>-1</sup>). The soil had 12 % sand, 68 % silt and 20 % clay. The location is characterized by a wide variation in mean monthly air temperature from 0 °C in January to 24 °C in July and August and a relatively even distribution in mean monthly precipitation with a total average annual precipitation of 1160 mm.

**Seasonal Weather Conditions**

Monthly mean air temperature and cumulative precipitation for the 2012 and 2013 tobacco growing seasons are shown in Figures 4.1 and 4.2, respectively. These two seasons had dramatically different climate relative to the long term mean as a standard. Total precipitation during the tobacco growing period was 432 mm in 2012 and 706 mm in 2013 with 515 mm being the long term mean. Average air temperature during the tobacco growing period in 2012 was warmer than 2013, especially in July and August when tobacco enters into the rapid growing stage. Therefore, the tobacco growth season in 2012 was considered to have a warm and dry pattern, whereas 2013 was considered to have a cool and wet pattern.
Figure 4.1  Monthly mean air temperature for 2012 and 2013 tobacco growing seasons and for the long term average mean (1971-2013) at the experiment site. (Source: Kentucky Agricultural Weather Center, http://wwwagwx.ca.uky.edu/)
Figure 4.2 Monthly total precipitation for 2012 and 2013 tobacco growing seasons and for the long term average mean (1971-2013) at the experiment site. (Source: Kentucky Agricultural Weather Center, http://wwwagwx.ca.uky.edu/)

Experiment Design

The tillage comparison study was established with four replications in 2007. Nitrogen fertilizer rate (280 kg N ha\(^{-1}\)) was applied each year to both tillage treatments. Conventional tillage in this study consisted of moldboard plowing followed by disking twice and soil finishing operation with a rototiller, prior to tobacco seedling transplanting. Beginning in 2012, three N fertilizer rates (0, 140, and 280 kg N ha\(^{-1}\)) were introduced as split-plots. Therefore, the field trial had a two factor factorial split-plot design; tillage was included as main plot and N fertilization as sub-plot. Tillage treatments (no-tillage and conventional tillage) were randomly assigned in main plots (6.40 m x 24.40 m). Three N fertilizer rates were randomly assigned to split plots (6.40 m x 8.10 m) within each main
Broadcast applications were applied by hand before transplanting tobacco seedlings. The population density was 18,286 plants per hectare and individual plant spacing was 0.54 by 1.07 m. Winter wheat (*Triticum aestivum* L.) was planted after tobacco harvest each season as a winter cover crop and was chemically killed approximately four weeks prior to transplanting. Tobacco production practices followed recommendations of the University of Kentucky Cooperative Extension Service (Sebold and Pearce, 2013), except for treatments imposed. Before 2012, only total cured tobacco leaf yield data was collected. The dates of important agronomic operation in 2012 and 2013 are shown in Table 4.1.

**Table 4.1** Dates of fertilizer application, transplanting, topping, and harvest.

<table>
<thead>
<tr>
<th>Year</th>
<th>Fertilizer Application</th>
<th>Transplanting</th>
<th>Topping</th>
<th>Harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>22-May</td>
<td>6-Jun</td>
<td>30-Aug</td>
<td>3-Oct</td>
</tr>
<tr>
<td>2013</td>
<td>4-Jun</td>
<td>15-Jun</td>
<td>9-Sep</td>
<td>30-Sep</td>
</tr>
</tbody>
</table>

**Variables Measured**

**Agronomic Parameters**

Tobacco cured leaf yield, leaf chlorophyll, leaf tissue NO$_3$-N and total N concentrations were determined. After harvest, tobacco was cured according to standard air curing procedures. Tobacco leaves were then manually removed and placed into “grades” (flyings, lugs, reds, and tips), from the bottom to the top of the stalk, respectively (Tso, 1990). Only total cured leaf yield is reported in this paper. Leaf chlorophyll was estimated using a chlorophyll meter (Minolta SPAD-502 Konica Minolta, Osaka, Japan) at topping (removal of flowers at top of the plant). The measurement was done, avoiding
the central vein, on the middle position of the last fully expanded leaf, commonly the fourth leaf from the apex. The same leaves were subsequently removed, oven-dried (55 °C), and ground to pass a 1-mm sieve for NO₃-N and TN determination.

*In Situ* Plant Available Nitrogen (PAN)

Plant available inorganic nitrogen in the 0 to 20 cm soil depth was measured by a modified *in situ* resin core procedure (Kolberg et al., 1997). Incubation cores were prepared by driving and removing aluminum conduit (25.40 cm long with an inner diameter of 4.80 cm) into the soil between tobacco rows before fertilizer N application. The bottom 2.54 cm of soil was removed from each soil core and replaced with the same volume of ion-exchange resin beads in a nylon bag. The entire assembly was returned to the original hole with a rubber washer surrounding the tube to avoid preferential bypass flow of surface runoff water potentially bringing inorganic nitrogen from outside the tubes to contaminate the resin bags. The top of the core was open to the atmosphere to in order to simulate field conditions. The resin bag consisted of equal amounts of cation and anion exchangeable resins (Lanxess Sybron, Birmingham, NJ). The design of the *in situ* soil resin core is shown in Fig. 4.3. Resins were allowed to incubate 60 days in field conditions; replacement resin bags were needed for longer incubations. For fertilizer N treatment application, N fertilizer solution (5 mL NH₄NO₃) was evenly sprayed into the inner soil surface of tubes at rates of 0, 140, and 280 kg N ha⁻¹ equivalent based on the inner area of the tubes. Tillage operations were performed before the sampling date 1 (SD1). On SD1 we removed a 0 to 20 cm soil sample to analyze inorganic N as baseline, installed the tubes, and put fertilizer N treatment into the tubes. Additional sampling dates were intended to occur at 30 days after transplanting (DAT), 60 DAT, and at
tobacco harvest. Actual sampling dates were adjusted due to weather and field soil moisture conditions (Table 4.2).

For laboratory analysis, each composite (three tubes) field soil sample dry weight was determined by weighing the field moist sample and determining the gravimetric moisture content of a subsample. A 10 g field moist sub-sample was immediately analyzed for NO$_3$-N and NH$_4$-N. Each composite resin sample was carefully mixed and weighed. Resin samples in different plots exhibited different moisture contents. A subsample for each plot was removed to adjust to the same weight based on original product moisture and recent moisture percentage. The subsample resin bags were serially extracted by shaking bags in three separate volumes of 50 mL each of 1M KCl for three different periods (15, 30, and 60 min). The three liquid samples were composited. The final recovered NO$_3$-N and NH$_4$-N concentrations were calculated by equations (1) and (2), respectively, derived from a previous extraction study following the standard extraction procedure described above.

\[
\text{NO}_3\text{-N (absorbed by resin)} = \text{NO}_3\text{-N (recovered from resin)} \times 1.30 + 57.15 \quad \text{Eqn. 1}
\]

\[
\text{NH}_4\text{-N (absorbed by resin)} = \text{NH}_4\text{-N (recovered from resin)} \times 1.12 + 45.42 \quad \text{Eqn. 2}
\]

The analysis of inorganic N for resin and soil samples followed the same analytical method. The NO$_3$-N analysis was determined by cadmium reduction method (Paratech, Lexington, KY) (Crutchfield and Grove, 2011). The NH$_4$-N analysis was determined by
phenol-hypochlorite reaction (Weatherburn, 1967; Ngo et al., 1982). Each sample was analyzed in duplicate and mean data was used for statistical analysis. PAN supply during the incubation period was calculated as the sum of inorganic N extracted from soil and absorbed from resin, presented as µg inorganic N g⁻¹ dry soil.

**Figure 4.3** Cutaway diagram of soil resin core, in which surficial plant residue and intact soil column (0-20.32 cm) and one mixed-media ion-exchange resin bag are incubated inside a core tube. A transparent example of an ion-exchange resin nylon bag is shown with a nylon mesh (1mm opening) as a bottom cover.
Table 4.2  Sampling dates for plant available nitrogen assays in 2012 and 2013.

<table>
<thead>
<tr>
<th>Operation</th>
<th>2012</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling 1</td>
<td>22-May</td>
<td>29-May</td>
</tr>
<tr>
<td>Sampling 2</td>
<td>20-June</td>
<td>9-July</td>
</tr>
<tr>
<td>Sampling 3</td>
<td>25-July</td>
<td>12-Aug.</td>
</tr>
<tr>
<td>Replacement of resin bag</td>
<td>30-July</td>
<td>3-Sept.</td>
</tr>
<tr>
<td>Sampling 4</td>
<td>25-Sept.</td>
<td>30-Sept.</td>
</tr>
</tbody>
</table>

Air-Cured Leaf Chemistry Parameters

After air curing was complete, the fourth leaf from the apex of each stalk was collected and used to quantify NO$_3$-N, alkaloids (including nicotine, nornicotine, anabasine, and anatabine), and TSNAs concentrations on a leaf dry matter basis. Air dried samples were ground to pass a 1-mm sieve and all leaf analyses were done in triplicate. NO$_3$-N concentration was determined by cadmium reduction method (Paratech, Lexington KY) (Crutchfield and Grove, 2011). Nicotine, nornicotine, anabasine, and anatabine were quantitatively analyzed by flame ionization gas chromatography (GC) (Perkin-Elmer Autosystem XL with Prevent) according to the LC-Protocol (Jack and Bush, 2007). Alkaloids of ground tobacco samples were extracted by methyl tert-butyl alcohol (MTBE) and aqueous sodium hydroxide. The MTBE extract was injected into the GC, and quantification of alkaloids was compared to chemical standards. Samples were injected in splitless mode at 250 °C. The carrier gas was helium, and the flow rate was 20 ml min$^{-1}$. Temperature of flame ionization detector was 250 °C. GC column was DB-5 (30m (L) × 0.53mm (D) × 1.5µm (FT)) (J&W Scientific). TSNAs analysis were performed following methylene chloride extraction (Morgan, 2004).
Statistical Analysis

Data were analyzed with the Mixed Model procedure of the SAS 9.3 computer package (SAS Institute Inc., Cary, NC). Tillage treatment was a whole-plot factor, whereas the N fertilization rate was a sub-plot factor. Duplicate measurements on composite soil and resin samples were averaged for statistical analysis of treatments effects. Treatment effects were considered significant at the 90% level of confidence (P > F ≤ 0.1). Mean separation was done using the Tukey-Kramer adjustment at an alpha level of 0.1. All quadratic regression analyses were conducted by using Sigma Plot 12.3.

4.3 Results

Long Term No Tillage Tobacco Yield Performance

Tobacco cured yield at 280 kg N ha⁻¹ for both tillage treatments and cumulative growing (May to September) precipitation from 2007 to 2013 are shown in Table 4.3. Conventional tillage tobacco had significantly higher yield than no tillage tobacco in 2007, 2008, and 2012 when cumulative precipitation was lower than the long-term average (515 mm). The relationship between the NT/CT yield ratio and cumulative precipitation was significant (r² = 0.84, P=0.0254, n=7) (Fig. 4.4), further indicating that no-till tobacco productivity approached that of tilled tobacco only in wet seasons.
Table 4.3  Tobacco cured leaf yield as related to at a 280 kg N ha⁻¹ application rate

<table>
<thead>
<tr>
<th>Year</th>
<th>Yield</th>
<th>Tillage</th>
<th>Pr &gt; F</th>
<th>Cumulative precipitation (May-September)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Conventional</td>
<td>No-till</td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>3508</td>
<td>2647</td>
<td>0.0197</td>
<td>351</td>
</tr>
<tr>
<td>2008</td>
<td>2128</td>
<td>1366</td>
<td>0.0017</td>
<td>330</td>
</tr>
<tr>
<td>2009</td>
<td>2725</td>
<td>2680</td>
<td>0.5999</td>
<td>688</td>
</tr>
<tr>
<td>2010</td>
<td>2866</td>
<td>2373</td>
<td>0.1347</td>
<td>523</td>
</tr>
<tr>
<td>2011</td>
<td>1998</td>
<td>2146</td>
<td>0.3822</td>
<td>610</td>
</tr>
<tr>
<td>2012</td>
<td>3293</td>
<td>2891</td>
<td>0.0853</td>
<td>432</td>
</tr>
<tr>
<td>2013</td>
<td>2756</td>
<td>2896</td>
<td>0.1559</td>
<td>706</td>
</tr>
</tbody>
</table>

Figure 4.4 Relationship between the NT/CT yield ratio and May to September cumulative precipitation from 2007 to 2013
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yield</td>
<td>SPAD at TD†</td>
<td>TN at TD</td>
</tr>
<tr>
<td>Tillage</td>
<td>0.0187</td>
<td>0.0469</td>
<td>0.0002</td>
</tr>
<tr>
<td>N Rate</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Tillage × N Rate</td>
<td>0.0868</td>
<td>0.0001</td>
<td>0.0098</td>
</tr>
<tr>
<td>Tillage</td>
<td>0.2196</td>
<td>0.8549</td>
<td>0.8286</td>
</tr>
<tr>
<td>N Rate</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.0283</td>
</tr>
<tr>
<td>Tillage × N Rate</td>
<td>0.9472</td>
<td>0.8805</td>
<td>0.1679</td>
</tr>
</tbody>
</table>

N/A‡ refers to not applicable in 2012, there was only one composite soil baseline sample for all three N fertilization rates because N treatment was not applied before that day.

†TD† refers to measure made at tobacco topping day.

§SD§ refers to sampling date.

Table 4.4 Analysis of variance (P >F) for agronomic parameters, plant available nitrogen supply, and leaf chemistry parameters.
Agronomic Parameters

The response of tobacco cured leaf yield to tillage and N fertilization rate was not the same for 2012 as in 2013 (Table 4.4). In 2012, total yield was significantly affected by both tillage and N fertilization rate, and there was a significant tillage by N rate interaction (Fig. 4.5). At 0 kg N ha⁻¹, leaf yield with tillage was significantly higher than with no-tillage, while there was no difference due to tillage at 140 or 280 kg N ha⁻¹. In 2012 other agronomic parameters, including SPAD reading, leaf TN, and leaf NO₃-N at topping, had a similar response to tillage and N fertilization rate as leaf yield (Table 4.4, Figures 4.6a, 4.7a, and 4.8a). However, in 2013, only N fertilization rate significantly affected leaf yield (Fig. 4.5b). There was no significant tillage effect and there was no tillage by N rate interaction. The 2013 SPAD readings showed a similar response pattern (Fig. 4.6b). The 2013 leaf TN showed no difference between no-till and tilled tobacco at any N rate (Fig. 4.7b). The 2013 leaf NO₃-N for tilled tobacco was significantly higher only at 280 kg N ha⁻¹ (Fig. 4.8b). In general, these agronomic parameters were numerically higher in 2012 than in 2013.
**Figure 4.5** Tobacco leaf yield response to tillage and fertilizer N rate in 2012 (a) and 2013 (b). Values followed by the same letter are not significantly different ($\alpha \leq 0.1$).

**Figure 4.6** SPAD reading at topping response to tillage and fertilizer N rate in 2012 (a) and 2013 (b). Values followed by the same letter are not significantly different ($\alpha \leq 0.1$).
Figure 4.7  Leaf TN at topping response to tillage and fertilizer N rate in 2012 (a) and 2013 (b). Values followed by the same letter are not significantly different ($\alpha \leq 0.1$).

Figure 4.8  Leaf NO$_3$-N at topping response to tillage and fertilizer N rate in 2012 (a) and 2013 (b). Values followed by the same letter are not significantly different ($\alpha \leq 0.1$).
Plant Available Nitrogen (PAN) Supply

The response of PAN supply to tillage and N fertilizer rate is shown in Table 4.4 and Figure 4.9. Tillage had a significant effect on PAN only at SD 1 in both years. Except for SD 1, PAN supply was significantly affected largely by N fertilizer rate during the tobacco growth period. The relationships between no-till and tilled tobacco yield and PAN supply in 2012 and 2013 are illustrated in Figure 4.10. These relationships were all well modeled by a quadratic polynomial. In general, 2012 tobacco had greater yield potential but numerically lower PAN than 2013 tobacco. Tilled tobacco exhibited generally greater yield than no-till tobacco in 2012, but in 2013 there was evidence of substantial tobacco yield ‘overlap’ between the two tillage treatments (Fig. 4.10).
Figure 4.9 Plant available nitrogen supply for conventional tillage and no tillage during the tobacco growth period in 2012(a) and 2013 (b). Values within a single sampling date, for a given year, followed by the same letter are not significantly different ($\alpha \leq 0.1$).

Note: the scales for plant available nitrogen are different for 2012 and 2013.
Figure 4.10  Relationship between tobacco yield and plant available nitrogen supply for no tillage and conventional tillage production in 2012 and 2013.

Air-Cured Leaf Chemistry Parameters

The responses of air-cured leaf chemical contents to tillage method and N fertilization rate are shown in Table 4.4 and Figures 4.11 to 4.14. In 2012, tillage significantly affected air cured leaf NO₃-N concentration, and there was also a significant interaction between tillage and N rate on leaf alkaloid and nicotine levels (Table 4.4). Tobacco-specific N-nitrosamines (TSNAs) was significantly affected only by N fertilizer rate (Table 4.4). Like the agronomic parameters, in 2012 no-tillage at 0 kg N ha⁻¹ gave the lowest values for the four cured leaf chemical parameters (Figs. 4.11-4.14). In 2013, there was no significant tillage effect on cured leaf NO₃-N, alkaloid, or nicotine concentrations.
A significant tillage by N rate interaction on TSNAs was observed in 2013 (Table 4.4). In general, cured leaf chemical concentrations were numerically lower in 2013 than in 2012 (Figs. 4.11-4.14).

**Figure 4.11** Cured leaf NO3-N concentration response to tillage and fertilizer N rate in 2012 (a) and 2013 (b). Values followed by the same letter are not significantly different ($\alpha \leq 0.1$).
Figure 4.12  Cured leaf alkaloid concentration response to tillage and fertilizer N rate in 2012 (a) and 2013 (b). Values followed by the same letter are not significantly different ($\alpha \leq 0.1$).

Figure 4.13  Cured leaf nicotine concentration response to tillage and fertilizer N rate in 2012 (a) and 2013 (b). Values followed by the same letter are not significantly different ($\alpha \leq 0.1$).
Figure 4.14 Cured leaf TSNAs concentration response to tillage and fertilizer N rate in 2012 (a) and 2013 (b). Values followed by the same letter are not significantly different ($\alpha \leq 0.1$).

4.4 Discussion

Agronomic Parameters

No-till burley tobacco production tended to result in lower cured leaf yield in dry seasons. This result differs from that observed with no-till summer annual grain production (De Vita et al., 2007). In summer annual grain production, no-tillage tends to be better than tillage in dry seasons because no-tillage preserves more moisture (Blevins et al., 1971). The same conservation of soil moisture has also been reported for no-tillage tobacco (Phillips and Zeleznik, 1989), but this benefit did not result in greater cured leaf yield, relative to tilled tobacco. In the dry 2012 season, cured leaf yield was significant higher with tillage than with no-tillage at the 0 kg N/ha fertilizer rate. With increasing fertilizer N rate, the yield difference due to tillage became smaller. The same response
pattern was found leaf SPAD readings, and leaf NO$_3$-N and TN concentrations at topping. Taken together, the agronomic parameters indicate that no-till tobacco suffered greater N deficiency at 0 kg N ha$^{-1}$ in the dry 2012 season (MacKown and Sutton, 1998; MacKown et al., 1999). However, in the moist 2013 season tillage did not affect cured leaf yield, leaf SPAD value, or leaf TN concentrations at any N fertilizer rate.

The relationship between yield and PAN supply showed that tilled tobacco had higher N use efficiency than no-till tobacco in 2012. *In situ* incubation results showed that there were no significant differences in PAN supply between tillage methods in either year. Because tillage did not significantly affect N supply, there must be other reasons why greater N deficiency was observed in no-till tobacco at 0 kg N ha$^{-1}$ in 2012. One potential explanation could be that the tobacco root system was limited by higher surface soil bulk density with no-tillage (Unger and Kaspar, 1994). Unlike soybean and corn, tobacco plants have extensively branched non-symmetrical rooting systems, developing adventitious roots at many angles from the plant base due to transplanting (Bruner, 1932).

Zartman et al. (1976) found that tilled burley tobacco root density tillage was significantly higher than that for no-till tobacco, especially in the 0 to 15 cm depth increment. The authors’ unpublished data from roots collected during the 2014 season also supports the hypothesis that greater tobacco root length and surface area occur with tillage. Most inorganic N moves to plant roots via mass flow as the plant absorbs water for transpiration (Barber, 1995). Therefore, in dry seasons, no-till tobacco N uptake might be limited by reduced root density/surface area. Greater water availability during wet seasons can make inorganic N more equally accessible to tobacco plants despite root
density differences caused by tillage. This explains the increased NT/CT cured leaf yield ratio with increasing cumulative precipitation during the tobacco growing season.

**Plant Available Nitrogen (PAN) Supply**

Tilled soil exhibited higher PAN than no-till soil at the first sampling date. The reason for this is that these baseline samples were taken immediately after tillage. Tillage exposed soil organic matter to mineralization by soil microbes. However, there was no significant difference in PAN supply due to tillage at later sampling dates. With plant species that do not cause biological N fixation, the field PAN supply consists of fertilizer N and N mineralized from soil organic matter. In this study, where the same fertilizer N rates were applied in both tillage systems, the equality in PAN supplies with tillage indicates that divergent trends in mineralization-immobilization turnover in soil and fertilizer N resulted in this equivalency. This observation, likely fortuitous, differs from many other reports (El-Haris et al., 1983b; Franzluebbers et al., 1994a; Wienhold and Halvorson, 1999). These reports argue that the no-till SOM pool is typically higher than the pool in tilled soil, resulting in a higher no-till soil N mineralization potential. Most of these were laboratory studies using disturbed/sieved samples. Sieving can accelerate SOM decomposition, resulting in higher no-till soil N mineralization potential (Oorts et al., 2006). The *in situ* resin core method better reflects field conditions, especially soil structure (Raison et al., 1987a), preserving physical sequestration of labile SOM. Consequently, although no-till soil has more SOM than tilled soil (Bernacchi et al., 2005), intact conditions might keep aggregate SOM physically protected from microorganisms and enhanced aeration. Rice and Smith (1984) and Rice et al. (1986) also reported equal N availability in both no-till and tilled soil using a $^{15}$N dilution method.
The greater no-till SOM pools do not necessarily guarantee greater soil N mineralization under field conditions, as those organic N pools may not be accessible to soil microbes.

The PAN supply was higher during the 2013 tobacco growth season. This difference could be due to seasonal precipitation. There were 432 mm and 706 mm of cumulative (May to September) precipitation in 2012 and 2013, respectively. Increasing soil moisture would promote N mineralization (Jin et al., 2013). However, tobacco yield was higher in 2012 than 2013. Though there could be several reasons for this seasonal response pattern, including other weather factors, the main limiting factor to tobacco yield was N nutrition. Given this, the result suggests that the in situ resin method overestimates PAN supply. The function of a resin bag at the bottom of the soil core is to capture inorganic N leaching from the soil column. But during heavy rainfall events the inorganic N captured by resin bag would otherwise have been leached from the tobacco root zone. A greater fraction of inorganic N was absorbed by the resin, rather than contained in soil above the resin, in 2013 than in 2012.

Air-Cured Leaf Chemistry Parameters

Cured leaf chemical composition is an important determinant of tobacco leaf usability. Most tobacco alkaloids are synthetized in the root, especially in elongating root tips (Solt, 1957; Dawson and Solt, 1959; Flores et al., 1999). Tso (1990) suggested that soil conditions during root development could also affect alkaloid levels. Changes in structure, moisture, penetration resistance, and the rhizosphere caused by different tillage practices might affect alkaloid levels. In 2012, tillage significantly affected cured leaf NO₃-N concentration and the influence of tillage on alkaloid and nicotine levels
depended also on N fertilizer rate. This might be the result of the low N uptake capacity caused by no-tillage, as tillage had no significant effect on cured leaf NO3-N, alkaloid, and nicotine concentrations in 2013. The N fertilizer rate significantly affected these three leaf chemical components in both years. This result suggests that fertilizer N management is more crucial than tillage in alkaloid synthesis, and that root development differences due to tillage might be not be enough to affect secondary tobacco metabolites. Tobacco TNSAs levels were significantly affected by N rate in 2012, but not in 2013, probably because TSNAs concentrations are relatively low compared to NO3-N, nicotine, and alkaloids. Also, TSNAs formation occurs mainly during leaf curing (Burton et al., 1989b), which is dependent on humidity, temperature, and the design of the tobacco curing barn. This makes very difficult the identification of agronomic management effects on TSNAs without large scale data collection.

4.5 Conclusion

Unlike no-till summer grain crop production, no-till burley tobacco production did not derive a yield benefit from soil moisture conservation in dry seasons. Instead, the low tobacco root density caused by higher soil bulk density and penetration resistance in no-tillage can limit tobacco N uptake capacity in dry seasons, subsequently resulting in tobacco N deficiency. Although tobacco cured leaf yield and chemistry were mainly controlled by N fertilizer rate management, tillage could affect tobacco cured leaf yield and chemistry by influencing N uptake. The impact of this effect is especially noticeable at low N fertilizer rates in dry seasons. This N deficiency could be overcome by appropriate N fertilizer intervention, such as N side dressing or banding, and other agronomic practices like strip tillage or irrigation. The effects of tillage on tobacco root
development, at different N fertilization rates and under different weather conditions, should be further examined to improve N nutrition management for no-tillage tobacco production.
Chapter 5: Conclusion

Grower profit and environmental protection are two major concerns for sustainable agricultural production. Soil N dynamics bridge these two concerns because N is a major element to plant nutrition and is also a greenhouse gas and surface and groundwater pollutant component. Conservation tillage, crop rotation, and proper N fertilizer rate management are effective at regulating soil N dynamics in sustainable agro-ecosystems. To better understand the influence of agronomic practices on crop N nutrition, three mechanisms were proposed: (1) an influence on soil organic matter; (2) an influence on soil N availability; and (3) an influence on crop N uptake capacity. A burley tobacco tillage and rotation study established in 2007 and a corn tillage by fertilizer N rate study established in 1970 were used in this research to evaluate the three mechanisms.

Conservation tillage, crop rotation, and N fertilization rate affected SOM sequestration differently. No-tillage and rotation increased SOM (Chapter 2), while the effect of N fertilization on SOM depended on both application time and amount. In burley tobacco production, a one-year N fertilizer application difference (0 versus 280 kg N ha\(^{-1}\)) did not cause differences in SOM (Chapter 2). In the corn study, 42 years of N fertilizer rate difference did not cause differences in SOM between 0 and 84 kg N ha\(^{-1}\), while 336 kg N ha\(^{-1}\) significantly increased SOM (Chapter 3, part II).

Increased SOM did not always contribute to soil N availability. Although conservation tillage can increase SOC and TSN stocks in surface soil, no-till did not necessarily increase PAN supply under field conditions (Chapter 3, part I and Chapter 4). The
increased SOM resulting from conservation tillage was mainly found in macroaggregates (Chapter 2), which can cause that SOM to be resistant to microbial decomposition.

Soil N availability might not be the only factor affecting crop N nutrition and yield that was influenced by agronomic practices. In Chapter 4, similar PAN supply between the two tillage methods suggested that tobacco N deficiency was due mainly to N uptake capacity differences. In 2014, tobacco root architecture analysis confirmed that the higher no-till soil bulk density could result in less root soil exploration. Thus, banding or other N nutrition management practices should be recommended for no-till tobacco production.

Systematically evaluating these three mechanisms gave insight on how agronomic practices affect crop N nutrition and illustrated improved strategies to balance farmer profit with environmental benefits.

**Other Lessons from This Dissertation**

1. Different crop residues might have different effects on NSNM because the quality (C:N ratio) of the residue can influence the direction of NSNM (Chapter 3, part I). Therefore, including previous crop residues would be beneficial to soil N dynamic evaluations for different rotation regimes.

2. To study the effect of fertilizer N application on NSNM, the separation of previous and current fertilizer N rate management was required because these affect NSNM differently (Chapter 3, part II). Previous fertilizer N application influences background SOM/labile SOM levels, while the current fertilizer N application might have a priming effect on NSNM, depending on the current N rate and the SOM level.
3. From a plant nutrition management standpoint, crop differentiation may be beneficial when conservation plans are applied to agricultural production systems. Different crops have different root architectures and response plasticity to imposed soil management, which can affect root nutrition uptake capacity.

A Proposed ABIT Model to Understand Factors Affecting Soil N Mineralization

Figure 5.1 The ABIT model proposed to understand factors affecting soil N mineralization

That soil N mineralization can be affected by abiotic and biotic factors and their interactions is well known. However, there is benefit to visualizing this in a diagram. The crop and soil management impacts on soil N mineralization affect not only a single factor in the ABIT model framework, but simultaneously change several factors. For an example, no-tillage management could affect mineralization substrate (SOM), soil temperature, and soil moisture, all at the same time. Figure 5.1 can clarify factors influenced by agronomic practices. Furthermore, this diagram illustrates the reliability of
methods estimating NSNM and the hazards of interpreting the results. Soil sample pretreatment during NSNM estimation can artificially affect some of these factors, as compared to natural field soil conditions.

In this diagram abiotic factors include (but are not limited to) temperature, moisture, aeration, pH and fertilizer addition; biotic factors include (but are not limited to) substrate, microorganism quantity and quality, fauna, enzyme quantity and quality and rhizosphere quantity and quality. There are three types of interactions: interactions among abiotic factors; interactions among biotic factors; and interactions between abiotic and biotic factors. The ABIT framework also includes time as a factor. Different biological incubation times can give different results when comparing management practices (Chapter 3, part I). Therefore, the proper incubation period length in NSNM studies should be taken into account to limit flawed decisions in soil N fertility management.


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