

**Soil Organic Matter Dynamics in Cropping Systems of Virginia's Valley Region**

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## Abstract

Soil organic matter (SOM) is a well known indicator of soil quality due to its direct influence on soil properties such as structure, soil stability, water availability, cation exchange capacity, nutrient cycling, and pH buffering and amelioration. Study sites were selected in the Valley region of Virginia with the study objectives to: i) compare the efficiency of density solutions used in recovering free-light fraction (FLF) organic matter; ii) compare different soil organic fractions as sensitive indices of short-term changes in SOM due to management practices; iii) investigate on-farm effects of tillage management on soil organic carbon (SOC) and soil organic nitrogen (SON) stocks; and iv) evaluate the role of SOM in controlling soil available nitrogen (N) for corn uptake. The efficiency of the density solutions sodium iodide (NaI) and sodium polytungstate (SPT) in recovering FLF was the same at densities of 1.6 and 1.8 g cm<sup>-3</sup>, with both chemicals presenting less variability at 1.8 g cm<sup>-3</sup>. The sensitivity of SOM fractions in response to crop and soil management depended on the variable tested with particulate organic matter (POM) being the most sensitive when only tillage was tested, and FLF being the most sensitive when crop rotation and cover crop management were added. The on-farm investigation of tillage management on stocks of SOC and total soil N (TSN) indicated significant increases at 0–15 cm depth by increasing the duration (0 to 10 years) of no-tillage (NT) management ( $0.59 \pm 0.14$  Mg C ha<sup>-1</sup> yr<sup>-1</sup> and  $0.05 \pm 0.02$  Mg N ha<sup>-1</sup> yr<sup>-1</sup>). However, duration of NT had no significant effect on SOC and TSN stocks at 0–60 cm depth. Soil available N as controlled by SOM was modeled using corn (*Zea mays* L.) plant uptake as response and several soil N fractions as explanatory variables. The final model developed for 0–30 cm depth had 6 regressors representing the different SOM pools (active, intermediate, and stable) and a  $R^2$  value of 65%. In summary, this

study provides information about on-farm management affects on SOM levels; measurement of such effects in the short-term; and estimation of soil available N as related to different soil organic fractions.

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## **Attributions**

Several colleagues have assisted with the completion of the research in several chapters of this dissertation. The following is a brief description of their involvement and contribution.

### **Marcus M. Alley** – Crop and Soil Environmental Sciences Department

Dr. Alley served as the major adviser and provided expert advice and guidance during the implementation and completion of the research project and dissertation.

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As an extension agent, Brian Jones had the goodwill in allowing me to use his field experiment which was explored in chapters II and III. He was always efficient in providing details and additional data of the experiment.

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## I. Introduction

Soil organic matter (SOM) is probably the most known and studied indicator of soil quality due to its direct effect on soil properties. Some of the soil quality indicator properties influenced by increasing SOM contents are structure stability, water infiltration, water availability, biological activity, cation exchange capacity, nutrient storage and release, sorption of agrochemicals, metal mobility (chelation), and pH buffering and amelioration (Stevenson, 1994; Weil and Magdoff, 2004). Agronomic management practices such as tillage, crop rotation, cover crop, fertilization, as well as manure application can impact the quantity and quality of SOM and, hence, the related soil quality properties mentioned above. In addition to this well established role of SOM on soil quality, there is an increasing incentive to improve croplands SOM levels as a tool to help mitigate increasing atmospheric concentration of greenhouse gases (GHG) such as carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), and nitrous oxide (N<sub>2</sub>O) (Coppens et al., 2006; Fest et al., 2009; Grace et al., 2006; Purakayastha et al., 2008). Soil OM consists of approximately 50% carbon (C) and 5% nitrogen (N) (Stevenson, 1994); thus, the evaluation of management practices on SOM levels is usually carried out by measuring total soil organic C (SOC) and soil organic N (SON) contents.

Soil preparation through conventional tillage (CT) has always been an important component of traditional agriculture (Lal et al., 2007). Tillage has been defined as the mechanical manipulation of the soil and plant residues to produce grain for human and animal consumption (Reicosky and Allmaras, 2003). In general, farming methods using mechanical tillage can promote SOM losses by disrupting soil aggregates that protect SOM from decomposition, stimulating short-term microbial activity through enhanced aeration, and mixing fresh residues into soil where conditions are more optimum for decomposition (Cerri et al., 2010). Conservation tillage includes a variety of systems, all designed to minimize residue incorporation with the intent of abating soil erosion control (Franzluebbers,

2004). According to the definition of the United States Department of Agriculture (USDA), over 30% residue cover must be on the soil surface immediately after planting for the system to be considered conservation tillage. No-tillage (NT) is a type of conservation tillage that has the potential to increase SOM levels by increasing aggregate stability and, hence, improving the protection of new SOM against soil microbe decomposition; preserving old SOM in long-term formed stable aggregates; and reducing litter decomposition (Paustian et al., 1997). These authors suggested that restoration of soil C to pre-cultivation levels by using NT management may be possible if combined with increasing nutrient and water supply, leading to higher C input. No-tillage systems have been widely adopted in North and South America (Blanco-Canqui and Lal, 2008), with the USA having the largest area, followed by Brazil and Argentina (Cerri et al., 2010). Previous works have shown that adoption of NT generally increases SOC and SON contents in the top soil compared to CT (Calegari et al., 2008; Doyle et al., 2004; Gulde et al., 2008; Huggins et al., 2007). However, the positive effect of management practices on SOM levels as measured by SOC and SON contents is generally not detected in less than 5–7 years.

Soil OM consists of functional pools with different turnover rates that are termed as labile or active, slow or intermediate, and recalcitrant, passive, stable, or inert (von Lützow et al., 2007; Wander, 2004). Typically, soil fractions belonging to the labile pool present turnover rates of days to a few years, while turnover rates for fractions in the intermediate and stable pools range from a few years to centuries (Stevenson, 1994; Wander, 2004). The most labile components of SOM include carbohydrates, amino acids, peptides, amino sugars, and lipids, followed by less readily metabolized structural materials such as waxes, fats, resins, lignin, cellulose, and hemi-cellulose (Tirol-Padre and Ladha, 2004). Stable pool SOM can represent as much as 80% of total SOM and generally consists of humic substances and

other organic macromolecules that are intrinsically resistant to microbial decomposition or physically protected by association with soil minerals (Stevenson, 1994).

To overcome the usual poor short-term sensitivity of total SOC and SON when measuring soil change due to management, the use of soil fractions consisting largely of labile SOM might be appropriate. Both quantity and quality of labile SOM are known to be important considerations for managing soil fertility in conservation systems (Marriott and Wander, 2006a). Quantity is related to the amount of labile SOM substrate available to support microbial activity and promote soil aggregation, and quality is related to SOM dynamics and nutrient (e.g. N) supply (Bending et al., 1998; Bosatta and Ågren, 1999; Marriott and Wander, 2006a). The labile pool of SOM can be readily increased by the addition of plant and animal residues, but it can be easily lost when such addition is reduced or tillage is intensified with associated mineralization (Doyle et al., 2004; Liang et al., 2004; Mirsky et al., 2008). Particulate organic matter (POM), free-light fraction (FLF), Illinois soil N test (ISNT), and mineralizable C are soil fractions that, depending on the system evaluated (e.g. soil type and quality of residue input), can be used to estimate labile SOM and are potentially sensitive indices to changes in soil management practices.

Increasing SOM content will have a great impact on soil available N since over 90% of total soil N occurs in organic forms (Stevenson, 1994). If 1 to 3% of this organic N is mineralized annually, 8 to 120 kg N ha<sup>-1</sup> is released in a plant-available form (Bundy and Meisinger, 1994). In the past half of century, many attempts have been made to develop chemical and biological methods able to predict the plant available N during a growing season (Gianello and Bremner, 1986; Khan et al., 2001; Mulvaney et al., 2001; Serna and Pomares, 1992). Stanford and Smith (1972) developed a biological method that utilized long-term (30 weeks) aerobic incubation where the potentially mineralizable N (N<sub>0</sub>) was determined with intermittent leaching of inorganic N. This incubation is generally recognized

as the best estimator of the mineralizable (active) N pool; however, it is time consuming and not practical for routine use (Sharifi et al., 2007). Other aerobic and anaerobic short-term incubation procedures have been developed but with sometimes conflicting predictions of  $N_0$  (Curtin and Campbell, 2008). Consequently, the search for the ‘holy grail’ of fast and reliable chemical methods to predict  $N_0$  has been the goal of soil scientists for many years. Chemical procedures must detect innate differences among soils or treatments in the quantities of N likely to mineralize during a specific period and must reflect the influence of environmental conditions on the rates and amounts of N mineralized (Bundy and Meisinger, 1994). Meisinger’s (1984) statement that there has been virtually no acceptance of chemical methods into routine soil N evaluation programs is still supported by more recent studies (Mulvaney et al., 2006; Picone et al., 2002; Schomberg et al., 2009; Spargo et al., 2009). Most of the N released during SOM mineralization is provided from fractions belonging to the active pools; however, the role of the other pools, mainly intermediate, cannot be neglected (Seiter and Horwath, 2004). Given the complexity of the soil N cycle and the variability of soil N pools due to a myriad of factors between specific sites, it is likely that predicting available N on a field specific basis may be achieved by evaluating a combination of different soil N fractions.

The overall research objective was to investigate the effects of management practices on soil C and N levels in the Valley region of Virginia. The specific research objectives were to: i) compare the efficiency of sodium iodide (NaI) and sodium polytungstate (SPT) density solutions when adjusted to equal densities in recovering free-light fraction organic matter; ii) compare different soil organic C and N fractions as sensitive indices of short-term changes in SOM status in response to crop rotation, tillage, and cover crop management; iii) investigate, through on-farm observation, the effects of tillage management practices on SOC and SON stocks in the top 60 cm of soil; and iv) evaluate the relationship between widely reported

indices of soil available N and their role in multiple regression models build to predict soil available N as measured by whole-corn plant N uptake.

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## II. Evaluation of Potentially Labile Soil Organic Carbon and Nitrogen Fractionation Procedures

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## Abstract

Particulate organic matter (POM) and light fraction (LF) organic matter are potentially labile (active) fractions of soil organic matter (SOM) that have been shown to be indicators of short-term changes in soil management practices (e.g. tillage, manure and fertilizer applications, and crop rotation). These two fractions consist mainly of partially decomposed plant residues, microbial residues, seeds, and spores forming organo-mineral complexes with soil mineral particles; however, they cannot be used as synonyms because of their different chemical composition and structure. Particulate-OM is recovered by size-based procedures while LF is generally recovered in two distinct fractions [free-LF (FLF) and occluded-LF (OLF)] using density-based solutions in conjunction with soil aggregate disruption. Solutions used in these density based separations have most commonly varied in density from 1.6 to 2.0 g cm<sup>-3</sup>. Sodium iodide (NaI) and sodium polytungstate (SPT) are the chemicals most often used to prepare the density solutions in LF recovery but comparisons of the effectiveness of these two solutions have not been conducted. The objectives of this research were: i) compare the efficiency of similar density solutions of NaI and SPT in recovering FLF; and ii) compare POM, FLF, and OLF as possible sensitive indices of short-term soil changes due to tillage management. Soil samples were collected at 0–15 cm depth from a cropping system experiment conducted on a silt loam Ultisol. Plots selected for sampling had received either reduced till (RT) or no-till (NT), and cropping was continuous corn silage for a period of 3 years prior to sampling. Solutions of NaI and SPT at densities of 1.6 and 1.8 g cm<sup>-3</sup> were used to recover FLF, and OLF was recovered with SPT solution at a density of 2.0 g cm<sup>-3</sup> from the soil pellet remaining after FLF recovery with SPT 1.6 g cm<sup>-3</sup>. The average total soil organic carbon (SOC) content of these samples was 12.7 g kg<sup>-1</sup>, and carbon-POM (C-POM), carbon-FLF (C-FLF), and carbon-OLF (C-OLF) represented 22.4, 5.5, and 5.2% of it, respectively. In general, C-FLF and nitrogen-FLF (N-FLF) contents

recovered did not differ significantly between chemical solutions (NaI or SPT) adjusted to the same density (1.6 or 1.8 g cm<sup>-3</sup>). Increasing the density within a specific solution (NaI or SPT) resulted in significantly higher C-FLF and N-FLF recovery. For instance, C-FLF recovery averaged 637 and 954 mg kg<sup>-1</sup> at 1.6 and 1.8 g cm<sup>-3</sup>, respectively. For both chemicals increasing density from 1.6 to 1.8 g cm<sup>-3</sup> reduced the variability in recovering C-FLF and N-FLF with coefficient of variation values decreasing from a range of 14.9–19.1% for densities of 1.6 g cm<sup>-3</sup> to 6.7–10.4% when densities increased to 1.8 g cm<sup>-3</sup>. In the present work, POM and OLF were more sensitive than FLF to changes in tillage management, with significantly greater amounts of the sensitive fractions in RT samples. A better sensitivity of FLF would be expected if treatments dealing with residue input (e.g. crop rotation and cover crop) were evaluated.

## **2.1. Introduction**

Soil organic matter (SOM) consists of functional pools differing in their turnover rate. Depending on the authors, the pools are termed as labile or active, slow or intermediate, and recalcitrant, passive, stable, or inert (von Lützow et al., 2007; Wander, 2004). Typically, soil fractions belonging to the labile pool present turnover rates of days to a few years, while for fractions in intermediate and passive pools turnover rates range from a few years to centuries (Stevenson, 1994; Wander, 2004). Soil fractions consisting largely of labile SOM are recognized as potential indicators of soil quality changes in the short-term due to variation in management practices (e.g. tillage, fertilizer and manure applications, and crop rotation). Particulate organic matter (POM) and light fraction (LF) are important soil fractions that, depending on the system evaluated (e.g. soil type and quality of residue input), to a large extent consist of labile or active SOM, and are potentially sensitive indicators to changes in soil management practices (Marriott and Wander, 2006b; Sharifi et al., 2008; Yoo and

Wander, 2008). Assuming that sorption is an important mechanism in SOM stabilization (formation of organo-mineral complexes), soil fractions within the sand fraction are allocated to the labile pool and soil fractions in silt and clay fractions to the intermediate and passive pools (von Lützow et al., 2007). These fractions consist mainly of partially decomposed plant residues, microbial residues, seeds, and spores forming organo-mineral complexes with soil mineral particles (Baisden et al., 2002; Gregorich et al., 2006). Despite of their similarity, POM and LF contain different proportions of carbohydrates and other aliphatic compounds, which precludes their use as synonyms (Gregorich et al., 2006).

The POM and LF fractions have been associated with changes in the mineralizable organic nitrogen (N) pool in soils. Sharifi et al., (2008) found that carbon-POM (C-POM) was one of the most sensitive indices to changes in the intermediate mineralizable N pool due to tillage for loam, silt loam, and clay loam soils in Canada. In addition, C-POM and nitrogen-POM (N-POM) had significant but low relationships with potentially mineralizable N ( $N_0$ ) for soils classified as Spodosols, Mollisols, Alfisols, Inceptisols, and Entisols coming from Canada and USA (Sharifi et al., 2007). This suggested varied suitability of these fractions for assessing  $N_0$  from SOM and that the variability is associated with soil, management, and climate characteristics.

Gregorich et al. (2006) defined LF as the organic fraction recovered by density fractionation and POM as the organic fraction recovered by size fractionation alone, or by the combination of size and density fractionation procedures. In this paper, Gregorich et al.'s (2006) nomenclature will be used. In the size-based procedure, the soil sample is dispersed in a sodium hexametaphosphate  $[(NaPO_3)_6]$  solution, and the organic material retained on a 53  $\mu\text{m}$  sieve is operationally defined as POM (Cambardella and Elliott, 1992; Gregorich and Beare, 2008).

The density-based procedure recovers the LF using solutions adjusted to different densities, with a common range between 1.6 to 2.0 g cm<sup>-3</sup> (Gregorich et al., 2006; Wander, 2004). The LF is generally recovered in two distinct pools as either free-LF (FLF) or occluded-LF (OLF) in a two step-fractionation. In the first step the soil sample is gently shaken with a density solution and the supernatant is filtered to obtain FLF. In the second step the remaining pellet from the first step is shaken with more energy with a density solution at the same density used in the previous step, or greater, and the supernatant is filtered to obtain OLF. The greater energy applied in the second step is designed to break stable soil aggregates releasing OLF. The FLF organic matter exists outside of soil aggregates, or is associated with weak aggregates, while OLF organic matter is associated with stable aggregates (Bird et al., 2008). The FLF is considered a more labile fraction of SOM than OLF because it is more closely associated with plants (chemical composition and structure) and exists external to the aggregate; however, OLF is generally considered decomposable when it is released from the aggregate, making the degree of protection to be aggregate-turnover dependent (Gulde et al., 2008).

Sodium iodide (NaI) and sodium polytungstate (SPT) [Na<sub>6</sub>(H<sub>2</sub>W<sub>12</sub>O<sub>40</sub>)] are the most commonly used chemicals to prepare solutions employed to recover FLF and OLF. Sodium polytungstate has become the most used chemical for certain research groups due to lower toxicity and capacity to achieve densities up to 3.1 g cm<sup>-3</sup> (Shang and Tiessen, 2001; Six et al., 1999). Sodium iodide is still used for density fractionation in many studies, but human health issues through chronic exposure (iodism) is a concern, and the highest density achievable with NaI is approximately 1.85 g cm<sup>-3</sup> at 25°C (MSDS, 2009). These two issues are distinct disadvantages for using NaI. However, NaI is significantly less expensive than SPT. These density solutions have been employed in various procedures with both being employed to recover FLF and OLF. In one method the pellet left in the bottle after FLF

recovery is resuspended with the same density solution and higher disaggregation energy is used to disperse soil aggregates (Kölbl and Kögel-Knabner, 2004; Sohi et al., 2001). This sequential recovery of FLF and OLF has also been achieved by the combination of higher disaggregation energy and using the same solution at a higher density to recover OLF after FLF recovery (Bird et al., 2002; Gulde et al., 2008; Marriott and Wander, 2006a; Wander and Yang, 2000).

The use of determined density solution for recovering LF organic matter seems to be based on: i) cost, since NaI is less expensive than SPT; ii) human health concerns, since SPT has lower toxicity than NaI; and iii) the range of densities necessary for specific experiments since these chemicals have different maximum achievable densities. Apparently there has not been much concern if a certain chemical-density solution is more efficient than another in extracting LF. Data have been compared across studies for these chemical density solutions, when adjusted to the same density, which assumes they have the same efficiency in recovering LF. Such an assumption ignores possible solution pH effect on organic substances solubility, different buffering potentials of different density solutions as well as numerous other factors associated with SOM reactions. Data are not available to show that the NaI and SPT solutions at the same density do recover similar amounts of LF SOM.

The objectives of this research were: i) compare the efficiency of NaI and SPT density solutions when adjusted to equal densities in recovering FLF organic matter; and ii) compare POM, FLF, and OLF organic fractions as possible sensitive indices of short-term changes in soil organic carbon (SOC) and soil organic nitrogen (SON) in response to tillage management.

## 2.2. Materials and Methods

Soil samples were collected in July 2008 from a cropping system experiment in the Valley region of Virginia initiated in the spring of 2006 on an Ultisol developed from limestone parent material. The experiment was a split-split-plot design with crop rotation as whole-plot treatment factor, tillage as sub-plot treatment factor, cover crop management as sub-sub-plot treatment factor. The experiment had three replications (blocks). Each plot was 9 m by 37 m. Crop rotation had three levels (continuous corn, corn/alfalfa, and corn/soybean/corn), tillage had two levels (reduced till (RT) and no-till (NT)), and cover crop management had three levels (remaining on the field after killed, removed from the field, and no cover crop planted). The RT management consisted of one pass disc harrow and one pass of field cultivator before planting at approximately 10 cm depth. In this study, only soil samples from plots in RT and NT with continuous corn and with no cover crop planted were sampled, resulting in a total of six plots sampled. Thus, only the effect of tillage management on C and N fractions was evaluated. Fifteen soil cores (3 cm diameter soil probe) were collected at 0–15 cm depth from each selected plot and mixed to form a composite sample. Soil samples were transferred to the lab within 8 h of collection and rapidly air-dried.

### 2.2.1. Objective 1: Comparisons of NaI and SPT density solutions for recovery of FLF

The FLF was recovered from each composite sample in triplicate using NaI density solutions adjusted to 1.6 and 1.8 g cm<sup>-3</sup>, and by SPT density solutions adjusted to 1.6 and 1.8 g cm<sup>-3</sup> (Figure 2.1). No additional adjustments, i.e. pH, were made to the solutions in order to more closely represent solutions used in previous studies in the literature. The fractionation procedure was based on the method described by Marriott and Wander (2006a). Twenty grams of air-dried soil ground to pass a 2 mm sieve were placed in 250 mL centrifuge tubes with 50 mL of one of the solutions described above. Samples were shaken for 2 min on an

orbital shaker at 200 rpm. Material adhered to the sides of the tubes was rinsed into the solution with an additional 20 mL of the density solution initially used. Samples were then centrifuged at 4480 g (5000 rpm) for 15 min. Immediately after centrifugation, the supernatant was filtered through a 20 µm nylon filter under vacuum to collect the FLF. Pre-test evaluation of the procedure indicated that the final volume of 70 mL allowed a better recovery of the floating material (FLF) compared to the final volume of 60 mL proposed in the original procedure. The FLF was rinsed with approximately 200 mL of deionized water followed by 50 mL of 0.01 M CaCl<sub>2</sub> to remove the density solution, transferred to a weighed aluminum tin, and dried at 60°C for 24 h. Oven dry weight of FLF was calculated as the difference between the weight of the tin and the weight of the tin plus FLF. After physical removal of the FLF from the tin, the FLF samples were ground using a mortar and pestle, and total organic C and N concentrations determined by dry combustion at 900°C (VarioMax CNS macro elemental analyzer, Elementar, Hanau, Germany). All dry combustion analyses in this paper were conducted with the same instrument.

#### *2.2.2. Objective 2: Evaluation of POM, FLF, and OLF as sensitive indices of changes in SOC and SON*

The size-based procedure used to recover POM is described by (Gregorich and Beare, 2008). Composite samples from each selected plot were analyzed in triplicate. Twenty five grams of air-dried soil ground to pass a 2 mm sieve were dispersed for 12 h in 100 mL of 0.0082 mol L<sup>-1</sup> sodium hexametaphosphate solution [5 g L<sup>-1</sup> (NaPO<sub>3</sub>)<sub>6</sub>] on a reciprocal shaker at 180 rpm. The suspension was poured onto a 53 µm sieve and rinsed with deionized water until clay and silt-size fractions were completely removed. The retained sand + organic material was dried on the sieve at 60°C for 1 h, transferred into a weighed aluminum tin and

dried at 60°C for 24 h, and weighed. The dried material was ground using a mortar and pestle, and total organic C and N concentrations determined by dry combustion at 900°C.

Light Fraction was recovered in the two-step fractionation to obtain FLF and OLF. The FLF recovered in Objective 1 with SPT at 1.6 g cm<sup>-3</sup> is the same used in Objective 2. Thus, OLF was recovered from the pellet left after FLF extraction with SPT at 1.6 g cm<sup>-3</sup> by resuspending the pellet with 50 mL of SPT solution adjusted to a density of 2.0 g cm<sup>-3</sup>, and then dispersing by shaking for 1 h on a reciprocal shaker at 180 rpm. Material adhering to the sides and lid of the tubes was rinsed into the solution with an additional 20 mL of SPT solution (2.0 g cm<sup>-3</sup>). Samples were then centrifuged at 4480 g (5000 rpm) for 15 min. Immediately after centrifuging, the supernatant was filtered through a 20 µm nylon filter under vacuum to obtain the OLF. The OLF was rinsed with approximately 200 mL of deionized water followed by 50 mL of 0.01 M CaCl<sub>2</sub> to remove the density solution, transferred to a weighed aluminum tin, and dried at 60°C for 24 h. Oven dry weight of OLF was calculated as the difference between the weight of the tin and the weight of the tin plus OLF. The OLF samples were ground using a mortar and pestle, and total organic C and N concentrations determined by dry combustion at 900°C.

### *2.2.3. Statistical Analysis*

Data from Objective 1 were analyzed as a split-plot design with tillage as the whole-plot treatment factor and solution used to recover FLF as the sub-plot treatment factor (SPT 1.6 g cm<sup>-3</sup>, SPT 1.8 g cm<sup>-3</sup>, NaI 1.6 g cm<sup>-3</sup>, and NaI 1.8 g cm<sup>-3</sup>) and three blocks. The response variables were C and N contents, and C-to-N ratio present in the recovered organic fraction. Before statistical analysis, triplicate values were averaged to generate individual plot response values and their respective coefficients of variation (CV). Data analyses were performed using PROC MIXED procedure of SAS software (SAS, 2008). Diagnostic

evaluation for assumptions and outliers were performed in the written order. Non-orthogonal contrasts were created to address the objectives of the experiment ( $P = 0.05$ ) using the Bonferroni correction (Kuehl, 2000).

Data from Objective 2 were analyzed as a randomized complete block design with tillage as the treatment factor and three blocks. The response variables were C and N contents, C-to-N ratio present in the recovered POM, FLF (SPT  $1.6 \text{ g cm}^{-3}$ ), and OLF (SPT  $2.0 \text{ g cm}^{-3}$ ). Before statistical analysis triplicate values were averaged to generate individual plot response values. Data analyses were performed using the PROC MIXED procedure of SAS software (SAS, 2008). Diagnostic for assumptions and outliers were performed in the written order. The two treatment means were compared by t-test ( $P = 0.05$ ).

## **2.3. Results**

### *2.3.1. Comparisons of NaI and SPT density solutions for recovery of FLF*

Some of the general analytical characteristics of the samples used in this study are shown in Table 2.1. The analysis of variance for C-FLF SOM contents and C-to-N ratio of FLF measured in this study showed that the tillage by solution interaction and the tillage treatments were not significant, but at least one of the four solutions (NaI  $1.6 \text{ g cm}^{-3}$ , NaI  $1.8 \text{ g cm}^{-3}$ , SPT  $1.6 \text{ g cm}^{-3}$ , or SPT  $1.8 \text{ g cm}^{-3}$ ) used to recover FLF had a significant effect (Table 2). The analysis of variance for N-FLF soil organic matter contents presented significant tillage by solution interaction (Table 2.2) that leads to comparisons between solutions within each tillage system.

Results for comparisons between NaI and SPT density solutions at the same density ( $1.6$  or  $1.8 \text{ g cm}^{-3}$ ) in recovering C-FLF and N-FLF organic matter are shown in Table 2.3. Sodium iodide and SPT had similar efficiency in recovering C-FLF regardless of density used. Nitrogen-FLF contents recovered differed significantly between NaI and SPT only at

density of  $1.8 \text{ g cm}^{-3}$  in RT system (Table 2.3). Carbon-to-N ratios also differed significantly at density of  $1.8 \text{ g cm}^{-3}$  with SPT presenting a higher C-to-N ratio than NaI. The reason for this observed difference of C-to-N ratio is due to the higher N-FLF content obtained with NaI  $1.8 \text{ g cm}^{-3}$  RT samples.

Effects of increasing the density of solutions from  $1.6$  to  $1.8 \text{ g cm}^{-3}$  for each chemical (NaI or SPT) on amounts of C-FLF and N-FLF recovered are shown in Table 2.4. Values in Table 2.4 are the same as those in Table 2.3 with order and label of the columns changed to better visualize the comparison between densities within each chemical. For both NaI and SPT solutions, an increase in density resulted in an average increase of C-FLF and N-FLF recovered of 50 and 54%, respectively (Table 2.4). For each chemical solution (NaI or SPT) the increase in density did not cause any significant change in C-to-N ratio (Table 2.4), meaning proportional increase of C-FLF and N-FLF recovery by increasing density as mentioned above.

The analysis of variance for the CV for both C-FLF and N-FLF contents showed significant effect only for the solution (NaI  $1.6 \text{ g cm}^{-3}$ , NaI  $1.8 \text{ g cm}^{-3}$ , SPT  $1.6 \text{ g cm}^{-3}$ , or SPT  $1.8 \text{ g cm}^{-3}$ ) used to recover FLF (Table 2.2). The evaluation of the solution effect showed no significant change on CV between NaI and SPT solutions when adjusted to the same density ( $1.6$  or  $1.8 \text{ g cm}^{-3}$ ) (Table 2.3). On the other hand, increasing the density from  $1.6$  to  $1.8 \text{ g cm}^{-3}$  within each chemical solution (NaI or SPT) showed a significant decrease in the CV values for both chemicals (Table 2.4). The decreases in CV values were considerable with an average decrease of 50 and 45% for C-FLF and N-FLF analyses, respectively.

### *2.3.2. Evaluation of POM, FLF, and OLF as sensitive indices of changes in SOC and SON*

Total SOC and SON did not differ significantly between tillage treatments (Tables 2.1 and 2.5). Thus, the average value of total SOC and SON contents across tillage treatments

were 12.7 and 1.3 g kg<sup>-1</sup>, respectively. Carbon-POM, C-FLF, and carbon-OLF (C-OLF) averaged 22.4, 5.5, and 5.2% of total SOC, respectively. On the other hand, N-POM, N-FLF, and nitrogen-OLF (N-OLF) averaged 14.2, 2.8, and 2.4% of total SON, respectively.

The analysis of variance showed that tillage treatment effect was significantly detected by C-POM, N-POM, C-OLF, and N-OLF measurements but not for C-FLF and N-FLF (Table 2.5). In this case, the significant difference indicated that C-POM, N-POM, C-OLF, and N-OLF contents were higher in RT compared to NT (Figure 2.2). The *P*-values for C-POM, N-POM, C-OLF, and N-OLF were 0.012, 0.042, 0.017, and 0.032, respectively, indicating higher sensitivity of C fractions compared to N fractions. The amount of C and N recovered in the POM fraction was approximately four and five times greater, respectively, than that in LF. The C-to-N ratio of these fractions was approximately 15:1 and 22:1 for POM and LF, respectively, with only the C-to-N ratio of OLF being sensitive to tillage treatment (Table 2.5 and Figure 2.2).

## **2.4. Discussion**

### *2.4.1. Comparisons of NaI and SPT density solutions for recovery of FLF*

The values of C-FLF recovered (mg C-FLF kg<sup>-1</sup> soil) by NaI and SPT at 1.6 g cm<sup>-3</sup> in the present work are very close to those reported by other authors using either NaI or SPT at this same density (Marriott and Wander, 2006a; Sohi et al., 2001), and for soils with similar SOM content. Also, Kölbl and Kögel-Knabner (2004) found that SPT adjusted to a density of 1.8 g cm<sup>-3</sup> recovered less than 1000 mg C-FLF kg<sup>-1</sup> for soil types ranging from sandy to loam. Leifeld and Kögel-Knabner (2005) recovered around 1200 mg of C-FLF per kg of soil from loam, sandy loam, and sandy clay loam soils with SPT at 1.8 g cm<sup>-3</sup>, assuming a recovery of 4.3 g of FLF per kg of soil (based on values obtained in the present work). In contrast, Conceição et al. (2007) working with sandy clay loam and clay soils in Brazil reported that

solutions of NaI and SPT adjusted to a density of  $1.8 \text{ g cm}^{-3}$  had different recovery efficiencies for C-FLF organic matter. The amount of C-FLF recovered averaged 159 % more using the SPT solution compared to NaI. Conceição et al. (2007) speculate about possible formation of complexes between the organic fraction in the highly weathered soils and the iodide (I<sup>-</sup>) anion, which might increase the density of the FLF organic matter in the NaI solution with a resulting decrease in flotation and recovery. However, comparisons with other works suggest that the values obtained by Conceição et al. (2007) with SPT are quite high (4305 mg C-FLF per kg of soil) for FLF recovered from whole soil samples. These values are closer to values that would be expected for a residual density fraction composed mainly of organo-mineral complexes with no evidence of organic material in particulate form (Baisden et al., 2002).

The present work indicates a greater recovery of C-FLF as solution density increases from  $1.6$  to  $1.8 \text{ g cm}^{-3}$ . These results agree with Sohi et al. (2001) who recovered more FLF organic matter with NaI solution at a density of  $1.8 \text{ g cm}^{-3}$  than with NaI solutions at  $1.6$  and  $1.7 \text{ g cm}^{-3}$  for a sandy loam soil. However, these researchers did not find significant differences in quantities of FLF organic matter recovered as a result of density increase when working with silty clay loam and clay soils. A higher association of LF with soil particles (organo-mineral complexes) and the presence of more stable aggregates in the two finer texture soils is probably the reason for the lack of sensitivity of FLF to density increase in the work of Sohi et al (2001). Microscopic examination of LF recovered from loamy sand, sandy loam, and loam soils suggested that increasing density can be considered equivalent to an increasing degree of organo-mineral association, combined with a progressive degree of SOM decomposition (Baisden et al., 2002). These authors suggested that a density cutoff of  $\sim 1.6 \text{ g cm}^{-3}$  may more effectively separate FLF from mineral associated SOM than density at  $\sim 2.0 \text{ g cm}^{-3}$ . Finally, a standard density of  $2.0 \text{ g cm}^{-3}$  was suggested by Conceição et al.

(2007) due to the small effect on FLF recovered by increasing the density of SPT solution from 2.0 to 2.2 g cm<sup>-3</sup> when compared to the effect obtained by increasing the density from 1.8 to 2.0 g cm<sup>-3</sup>. These general recommendations would be most applicable by also considering texture, SOM quantity and quality as well as clay mineralogy of samples studied.

In the present work the C-to-N ratio of FLF ranged between 17.8 and 20.4. This similar ratio of FLF recovered at 1.6 and 1.8 g cm<sup>-3</sup> is indicative of similar organic and mineral composition. However, additional qualitative analysis on these fractions would be needed to confirm their nature. In a review of more than 65 studies, Gregorich et al. (2006) reported that the average C-to-N ratio for FLF is between 17 and 22 for density solutions of 1.0–1.8 g cm<sup>-3</sup>. However, the C-to-N ratios narrowed to between 10 and 17 when researchers used solution densities of 1.8–2.2 g cm<sup>-3</sup>. Gregorich et al. (2006) argue that the wider C-to-N ratio at lower densities (<1.8 g cm<sup>-3</sup>) is due to the dominant presence of plant constituents, whereas at higher densities the recovered fractions contain more mineral particles with associated organic matter. The C-to-N ratio generally decreases as labile constituents of SOM are lost and SOM aromaticity increases (Wander, 2004).

Similar CV values between NaI and SPT when adjusted to the same density to recover FLF supports the similarity between these chemical solutions in recovering this organic fraction beyond the fact that the chemicals recovered similar amounts of C-FLF and N-FLF. The decrease in CV for both chemical solutions as density increased from 1.6 to 1.8 g cm<sup>-3</sup> indicates a more precise analysis of FLF concentration by using the density of 1.8 g cm<sup>-3</sup> compared to 1.6 g cm<sup>-3</sup> for the samples used in this study. The decrease in variability by increasing density is possibly due to a reduction in the quantity of FLF organic matter remaining between the pellet at the bottom of the bottle and the floating material at the surface for the higher density solution. The less variable result for the higher density solutions may also reflect the higher density solutions masking variations in the association

of FLF with various soil mineral constituents as well as SOM. Experiments utilizing soils with widely varying textures and SOM contents are needed to reach a more general conclusion regarding the true efficiency of the density solutions for extracting FLF.

#### *2.4.2. Evaluation of POM, FLF, and OLF as sensitive indices of changes in SOC and SON*

The percentage of total SOC and SON represented by these organic fractions were in agreement with those reported by Gregorich et al. (2006) who reported in a literature review that C-POM and C-LF accounted for about 23 and 8% of SOC, respectively; while N-POM and N-LF accounted for about 18 and 5% of SON, respectively. In addition, Carter et al. (2003) found that POM and LF of soils under ten different cropping rotations represented around 22 and 7% of SOC, and 27 and 5% of SON, respectively. Dealing with annual grassland soils, Baisden et al. (2002) found C-FLF to represent about 7–22% of SOC, and mineral-associated fractions (e.g. OLF) to represent about 6–72% of it. These percentages are also soil-aggregate size dependent (e.g. 250–2000  $\mu\text{m}$ ; 53–250  $\mu\text{m}$ ) with larger percentages found in smaller soil-aggregate size samples (Gulde et al., 2008; Lee et al., 2009; Ouédraogo et al., 2006). Generally, about 50–75% of total SOC is associated with clay-sized particles, about 20–40% with silt-sized particles, and less than 10% with sand-sized particles (von Lützow et al., 2007).

The comparison between these organic fractions indicated that for the samples used in the present study POM and OLF were more sensitive than FLF to detect changes in soil due to short-term changes in tillage management. Other works have shown the role of these SOM fractions as land-use sensitive indices (Bird et al., 2002; Carter et al., 2003; Deneff et al., 2007; He et al., 2008; Mirsky et al., 2008; Sharifi et al., 2008; Tan et al., 2007; Yoo and Wander, 2008). Marriott and Wander (2006a) found that the use of organic practices, i.e. application of manure and use of cover crops, significantly increased concentrations of C-

POM, N-POM, N-FLF, C-OLF, and N-OLF compared to conventional farming systems. In addition, Leifeld and Kögel-Knabner (2005) found that C-FLF and C-OLF were not suitable to indicate changes in soil between arable use and grassland for the sandy soils studied. They suggested that a minimum aggregate stability is required to provide a protective environment for LF, and to establish land-use specific proportions of LF in soil. Kölbl and Kögel-Knabner (2004) working with sandy and loam soils stated as a conclusion that at lower soil clay contents significantly lower amounts of OLF are found, whereas the amount of FLF is independent of soil clay content. The lack of response of FLF to tillage in the present work is likely due to the low amount of residue left on the plots after corn-silage harvesting. This reduces the primary source of FLF which is more chemically/structurally related to plant tissue than POM and OLF (Gregorich et al., 2006; Marriott and Wander, 2006a).

The C-to-N ratio of these fractions can also be used as an index of soil change due to management practices. The sensitivity of the OLF C-to-N ratio in the present study to represent tillage management is partially supported by results of Marriott and Wander (2006a) who found that C-to-N ratios of both FLF and OLF were more likely to distinguish between management systems than POM. However, Wander (2004) pointed out that interpretation of C-to-N ratio trends can be difficult due to no clear relationship between the C-to-N ratio of these fractions and the C-to-N ratio of the bulk soil in arable mineral soils. The higher FLF and OLF C-to-N ratio compared to POM C-to-N ratio (Figure 2.2) possibly indicates that FLF and OLF fractions have had similar reduced decomposition compared to POM fraction (Gregorich et al., 2006). However, the similar C-to-N ratio of FLF and OLF cannot be used alone to consider these organic fractions similar, as Kölbl and Kögel-Knabner (2004) found similar C-to-N ratio and different proportions of chemical-group composition between these two fractions. They found enrichment in aryl C and depletion in *O*-alkyl C groups from FLF to OLF which precludes making direct comparisons.

The significantly higher C-POM, N-POM, C-OLF, and N-OLF contents under RT compared to NT (Figure 2.2) may be due to greater corn yield and, hence, greater residue return to soil in RT system. Liebig et al. (2004) found in a long term cropping system experiment higher POM under minimum tillage (MT) (sweep plow in the fall and spring) compared to NT due to greater crop production and, hence, residue input in MT management. Álvaro-Fuentes et al. (2008) found higher C-POM at 0–40 cm depth in NT compared to a conventional till (CT) treatment based on moldboard plowing, and lower C-POM in NT compared to a CT treatment based on a subsoiler at 50 cm depth. These authors suggested that the subsoiler promoted a lower bulk density at soil depth and better conditions for root development compared to NT, leading to greater root biomass and C-POM accumulation. Another reason for the general higher presence of these organic fractions in the RT treatment in the present study is related to the sampling depth of 0–15 cm that eliminated the effect of preferential accumulation of SOM in the top layer (0–5 cm depth). Previous works have reported significantly higher concentration of these fractions at 0–5 cm depth in NT compared to CT, but the inverse result or the absence of difference between tillage treatment below 5 cm depth (Alvaro-Fuentes et al., 2008; Chan et al., 2002; Deneff et al., 2007; Wander and Yang, 2000; Yoo and Wander, 2008).

## **2.5. Conclusions**

For systems with similar characteristics of those found in the present study (e.g. soil type and texture, management, and climate) NaI or SPT solutions would be expected to recover FLF with similar efficiencies at densities of 1.6 and 1.8 g cm<sup>-3</sup>. The selection of a solution can be based on factors such as toxicity and costs. As with previous studies, the use of higher density solution tended to increase amounts of C- and N-FLF recovered. For instance, C-FLF recovery averaged of 637 and 954 mg kg<sup>-1</sup> at 1.6 and 1.8 g cm<sup>-3</sup> densities

respectively. At this point, for both chemicals the use of a density of  $1.8 \text{ g cm}^{-3}$  is preferred over a density of  $1.6 \text{ g cm}^{-3}$  in recovering FLF because the lower variation with density of  $1.8 \text{ g cm}^{-3}$  (8% versus 16% CV for C-FLF) results in a more precise analysis.

The percentage of SOC and SON represented by POM and LF was 22.4 and 5.4%, and 14.2 and 2.6%, respectively. Even though FLF is chemically and structurally closer related to plant residues, the present work found POM and OLF more sensitive to changes in SOC and SON due to the short-term tillage treatment in the sampled experiment. This result could be different if treatments dealing with residue input (e.g. crop rotation and cover crop) were evaluated. Therefore, for systems similar to the one evaluated in the present work, the POM procedure is recommended because it demands much less time and investment to be performed when compared to measuring the FLF.

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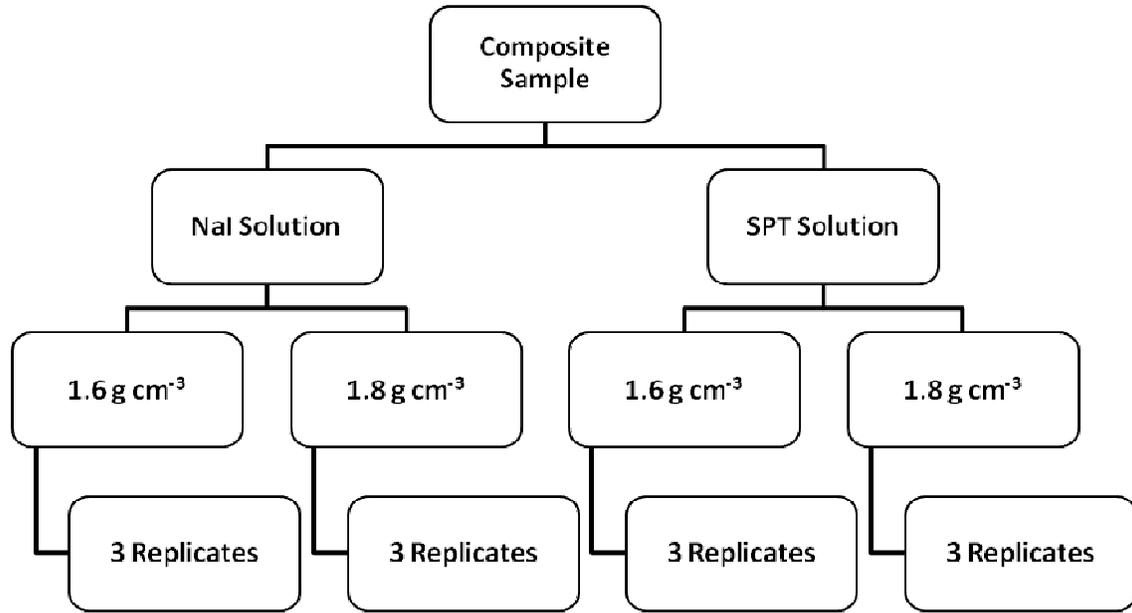
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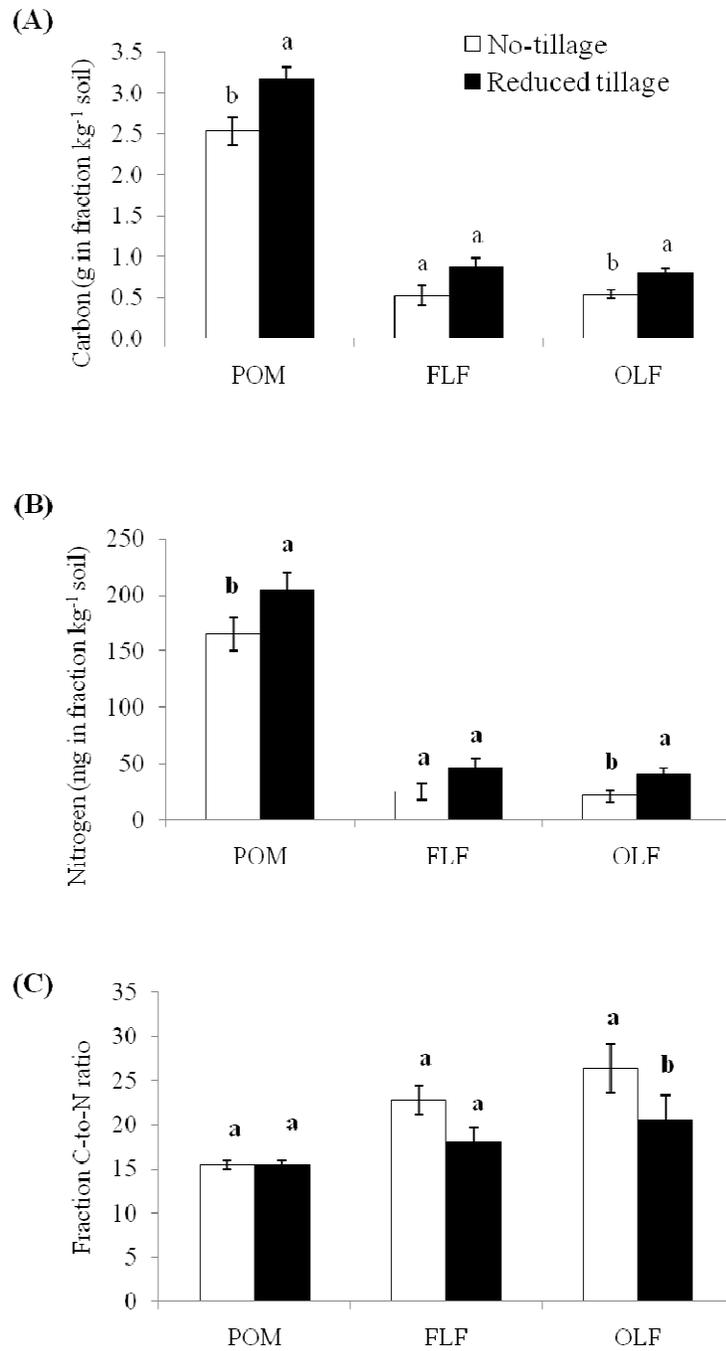
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**Figure 2.1.** Diagram showing the sequence and replication of analysis by NaI and SPT density solutions both adjusted to densities of 1.6 and 1.8 g cm<sup>-3</sup>.



**Figure 2.2.** Carbon (A) and nitrogen (B) contents and C-to-N ratios (C) for particulate organic matter (POM), free-light fraction (FLF) and occluded-light fraction (OLF) in a tillage management experiment sampled in the short-term. Means within a fraction with the same letter are not significantly different ( $P < 0.05$ )

**Table 2.1.** General analytical characteristics of the composite samples collected to represent the plots used from a cropping system experiment in the Valley region of Virginia.

Sample	System	SOC <sup>c</sup>	SON <sup>d</sup>	Clay	Silt	Sand	pH (H <sub>2</sub> O)
-----g kg <sup>-1</sup> -----							
1	RT <sup>a</sup>	13.7	1.4	31	612	357	6.29
2	NT <sup>b</sup>	12.0	1.2	92	517	391	6.56
3	RT	12.3	1.2	96	552	352	6.52
4	NT	12.1	1.2	102	564	334	6.37
5	RT	13.9	1.3	95	656	249	6.19
6	NT	12.1	1.2	96	633	271	6.52

<sup>a</sup>Reduced tillage

<sup>b</sup>No-tillage

<sup>c</sup>Total soil organic carbon

<sup>d</sup>Total soil organic nitrogen

**Table 2.2.** Analysis of variance's *F*-values for the content (mg kg<sup>-1</sup>) and coefficient of variation (%) of C, N, and C-to-N ratio in free-light fraction organic matter.

Source of Variability	Content			CV % <sup>a</sup>		
	Carbon <sup>b</sup>	Nitrogen	C-to-N	Carbon	Nitrogen	C-to-N
Tillage (T)	5.58 <sup>ns</sup>	6.92 <sup>ns</sup>	3.31 <sup>ns</sup>	1.59 <sup>ns</sup>	1.91 <sup>ns</sup>	0.41 <sup>ns</sup>
Solution (S)	36.9 <sup>***</sup>	71.7 <sup>***</sup>	8.32 <sup>**</sup>	7.23 <sup>**</sup>	5.39 <sup>*</sup>	0.56 <sup>ns</sup>
T*S	0.74 <sup>ns</sup>	7.58 <sup>**</sup>	3.08 <sup>ns</sup>	1.62 <sup>ns</sup>	0.79 <sup>ns</sup>	0.90 <sup>ns</sup>

<sup>ns</sup>No significant, \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001.

<sup>a</sup>Coefficient of variation in percentage

<sup>b</sup>Variable log transformed to improve normality and/or equality of variance

**Table 2.3.** C and N contents, C-to-N ratios, and their coefficients of variation (CV) in free-light fraction organic matter recovered by NaI and SPT solutions adjusted to two different densities from a soil under reduced tillage (RT) and no-tillage (NT) managements.

	-----Density of 1.6 g cm <sup>-3</sup> -----		-----Density of 1.8 g cm <sup>-3</sup> -----	
	NaI	SPT	NaI	SPT
	-----mg kg <sup>-1</sup> -----			
Carbon	632.0a	641.2a	971.4a	936.0a
Nitrogen RT	44.0a	46.8a	78.0a	62.9b
Nitrogen NT	26.9a	25.1a	42.5a	37.8a
C-to-N Ratio	19.3a	20.4a	17.8b	20.0a
	-----CV %-----			
Carbon	14.9a	17.6a	6.7a	9.6a
Nitrogen	15.2a	19.1a	8.5a	10.4a
C-to-N Ratio	5.0a	6.3a	7.5a	3.9a

Means in a row within a specific density (1.6 or 1.8 g cm<sup>-3</sup>) with the same letter are not significantly different by t-test ( $P < 0.05$ ).

**Table 2.4.** C and N contents, C-to-N ratios, and their coefficients of variation (CV) in free-light fraction organic matter recovered by NaI and SPT solutions adjusted to two different densities from a soil under reduced tillage (RT) and no-tillage (NT) managements.

	-----NaI-----		-----SPT-----	
	1.6 g cm <sup>-3</sup>	1.8 g cm <sup>-3</sup>	1.6 g cm <sup>-3</sup>	1.8 g cm <sup>-3</sup>
	-----mg kg <sup>-1</sup> -----			
Carbon	632.0b	971.4a	641.2b	936.0a
Nitrogen RT	44.0b	78.0a	46.8b	62.9a
Nitrogen NT	26.9b	42.5a	25.1b	37.8a
C-to-N Ratio	19.3a	17.8a	20.4a	20.0a
	-----CV %-----			
Carbon	14.9a	6.7b	17.6a	9.6b
Nitrogen	15.2a	8.5b	19.1a	10.4b
C-to-N Ratio	5.0a	7.5a	6.3a	3.9a

Means in a row within a specific chemical solution (NaI or SPT) with the same letter are not significantly different by t-test ( $P < 0.05$ ).

**Table 2.5.** Analysis of variance's  $F$ -values for the content (mg kg<sup>-1</sup>) of C and N and C-to-N ratios of bulk soil and soil organic matter fractions as affected by tillage treatment.

	Bulk Soil	POM <sup>a</sup>	FLF <sup>b</sup>	OLF <sup>c</sup>
Carbon	5.68 <sup>ns</sup>	83.25 <sup>*</sup>	3.81 <sup>ns</sup>	59.17 <sup>*</sup>
Nitrogen	4.66 <sup>ns</sup>	22.41 <sup>*</sup>	4.35 <sup>ns</sup>	29.77 <sup>*</sup>
C-to-N ratio	0.12 <sup>ns</sup>	0.02 <sup>ns</sup>	5.31 <sup>ns</sup>	54.14 <sup>*</sup>

<sup>ns</sup>No significant, <sup>\*</sup> $P < 0.05$

<sup>a</sup>Particulate organic matter

<sup>b</sup>Free-light fraction

<sup>c</sup>Occluded-light fraction

### III. Soil Organic Matter Fractions as Indices of Soil Quality Changes

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## **Abstract**

Soil organic matter (SOM) is typically the most used indicator of soil quality with total soil organic carbon (SOC) and nitrogen (SON) typically being the fractions employed to measure changes in SOM caused by management. However, since SOC and SON represent stable SOM pool it would be more appropriate to use labile SOM fractions in order to detect changes in the short-term. The objective of this study was to compare the sensitivity of different SOM fractions as indices of short-term changes in SOM status. In 2006 the experiment was installed as a split-split-plot design with crop rotation (three levels) as whole-plot treatment factor, tillage (two levels) as sub-plot treatment factor, and cover crop management (three levels) as sub-sub-plot treatment factor. Soil samples were collected at 0–15 cm depth in 2008. Particulate organic matter (POM), free-light fraction (FLF), Illinois soil N test (ISNT), and mineralizable C ( $C_{\min}$ ) were tested as possible sensitive indices to SOM change. Carbon-POM (C-POM) with 22.9% and nitrogen-POM (N-POM) with 15.1% were the largest portion of SOC and SON, respectively. The stable fraction SOC was only affected by cover crop management, while C and N contents and C:N ratio of the labile POM and FLF fractions were affected by more management practices. Between POM and FLF, the latter was the most sensitive. On the other hand, the supposedly labile ISNT and  $C_{\min}$  fractions were non-sensitive to any practice shedding doubt on their usefulness as short-term management indices.

## **3.1. Introduction**

Soil organic matter (SOM) is probably the most known and studied indicator of soil quality due to its direct effect on soil properties. Some of the soil quality indicator properties influenced by SOM are structure stability, water infiltration, water availability, biological activity, cation exchange capacity, nutrient storage and release, sorption of agrochemicals,

metal mobility (chelation), and pH buffering and amelioration (Stevenson, 1994; Weil and Magdoff, 2004). Agronomic management practices such as tillage, crop rotation, cover crop, fertilization, as well as manure application can impact the quantity and quality of SOM and, hence, the related soil quality properties mentioned above. Soil OM consists of approximately 50% carbon (C) and 5% nitrogen (N) (Stevenson, 1994); thus, the evaluation of management practices on SOM levels is usually carried out by measuring total soil organic C (SOC) and soil organic N (SON) contents. Previous works have shown that adoption of conservation tillage (e.g. no-tillage), diversification of crop rotation, inclusion of cover crops in the system, and manure application generally increases SOC and SON contents in the top soil compared to conventional till (Doyle et al., 2004; Gulde et al., 2008; Huggins et al., 2007; Lal, 2004; Marriott and Wander, 2006b; Purakayastha et al., 2008; Steenwerth and Belina, 2008).

However, the positive effect of management practices on SOM levels as measured by SOC and SON contents is generally not detectable in less than 5–7 years. Soil OM consists of functional pools with different turnover rates that are termed as labile or active, slow or intermediate, and recalcitrant, passive, stable, or inert (von Lützow et al., 2007; Wander, 2004). Typically, soil fractions belonging to the labile pool present turnover rates of days to a few years, while turnover rates for fractions in the intermediate and stable pools range from a few years to centuries (Stevenson, 1994; Wander, 2004). The most labile components of SOM include carbohydrates, amino acids, peptides, amino sugars, and lipids, followed by less readily metabolized structural materials such as waxes, fats, resins, lignin, cellulose, and hemi-cellulose (Tirol-Padre and Ladha, 2004). Stable pool SOM can represent as much as 80% of total SOM and generally consists of humic substances and other organic macromolecules that are intrinsically resistant to microbial attack or physically protected by association with soil minerals (Stevenson, 1994).

To overcome the poor sensitivity of total SOC and SON when measuring soil short-term change resulting from management, the use of soil fractions consisting largely of labile SOM might be appropriate. Both quantity and quality of labile SOM are known to be important considerations for managing soil fertility in conservation systems (Marriott and Wander, 2006a). Quantity is related to the amount of labile SOM substrate available to support microbial activity and promote soil aggregation while quality is related to SOM dynamics and nutrient (e.g. N) supply (Bending et al., 1998; Bosatta and Ågren, 1999; Marriott and Wander, 2006a). The labile pool of SOM can be readily increased by the addition of plant and animal residues, but it can be easily lost when such addition is reduced or tillage is intensified with associated mineralization (Doyle et al., 2004; Liang et al., 2004; Mirsky et al., 2008). Particulate organic matter (POM), free-light fraction (FLF), amino sugar N as determined by Illinois soil N test (ISNT), and mineralizable C ( $C_{\min}$ ) are soil fractions that, depending on the system evaluated (e.g. soil type and quality of residue input), can be used to estimate labile SOM and are potentially sensitive indices to changes in soil management practices (Blair et al., 1995; Marriott and Wander, 2006b; Sharifi et al., 2008; Yoo and Wander, 2008).

Particulate OM and FLF consist mainly of partially decomposed plant residues, microbial residues, seeds, and spores forming organo-mineral complexes with soil mineral particles (Baisden et al., 2002). Despite of their similarity, POM and LF contain different proportions of carbohydrates and aliphatic compounds, which precludes their use as synonyms (Gregorich et al., 2006). The FLF is more chemically/structurally related to plant tissue than POM (Gregorich et al., 2006; Marriott and Wander, 2006a). Also these fractions will present different organo-mineral associations depending on soil texture and method employed for measurement (Baisden et al., 2002; Sohi et al., 2001). Gregorich et al. (2006) defined LF as the organic fraction recovered by density fractionation and POM as the organic

fraction recovered by size fractionation alone, or by the combination of size and density fractionation procedures. In this paper, Gregorich et al.'s (2006) nomenclature will be used. Many studies have used POM and FLF as indices of labile SOM and these measurements have proved to be sensitive to short-term changes in management practices (Marriott and Wander, 2006a; Mirsky et al., 2008; Sharifi et al., 2008; Yoo and Wander, 2008).

The ISNT was developed by Kahn et al. (2001) to estimate amino sugar N in soils after Mulvaney et al. (2001) attempted to find relationships between different fractions of hydrolysable soil N and corn (*Zea mays* L.) yield response to fertilizer N. Mulvaney et al. (2001), through acid hydrolysis, showed that hydrolysable amino sugar N was highly correlated ( $r = 0.79$ ) with check plot yield and fertilizer N response ( $r = -0.82$ ). In developing the procedure, Kahn et al. (2001) found the ISNT could identify soils that were nonresponsive to N fertilization. They reported the ISNT range of 225–235 mg N kg<sup>-1</sup> in the nonresponsive soils. Amino sugars, which occur in soils as macropolysaccharides including chitin (Stevenson, 1994), have been related to bacterial and fungal biomass and can be used to estimate contributions to the biologically labile pool (Wander, 2004). Chitin is a polymer of *N*-acetylglucosamine that comprises cell walls, structural membranes, and skeletal components of fungal mycelia (Stevenson, 1994). Studies have found ISNT to be related to changes in the labile and intermediate mineralizable organic N pools and to be sensitive to short-term changes in fertilizer management (Marriott and Wander, 2006b; Sharifi et al., 2008; Sharifi et al., 2007).

Loginow et al. (1987) developed a method to simulate enzymatic breakdown of labile SOM by soil microbes. The proposed method had potassium permanganate (KMnO<sub>4</sub>) as the oxidizing agent and was recommended to characterize and evaluate the qualitative changes in SOM due to organic and mineral fertilization and crop rotation. Potassium permanganate under neutral conditions is known to oxidize simple carbohydrates, amino acids, amine/amide

sugars, and C-compounds containing hydroxyl, ketone, carboxyl, double-bond linkages and aliphatic compounds to give a light pink color (Loginow et al., 1987; Weil et al., 2003). The change in concentration of  $\text{KMnO}_4$  is used to estimate the amount of C oxidized, assuming that 1 mol of  $\text{MnO}_4$  is consumed ( $\text{Mn}^{7+}$  to  $\text{Mn}^{2+}$ ) to oxidize 0.75 mol of C. Blair et al. (1995) standardized a method with  $0.333 \text{ mol L}^{-1} \text{ KMnO}_4$  and termed it as labile or easily oxidizable C and, via difference from total C, the non-labile C is estimated. In the present work this fraction will be referred to as  $C_{\text{min}}$ . The use of  $C_{\text{min}}$  has been reported as a successful sensitive index to changes in SOM status for a variety of soil types due to variations in management practices such as crop rotation (Blair et al., 1995; Mirsky et al., 2008; Weil et al., 2003). However, the fact that aromatic structures and humic substances react with  $\text{KMnO}_4$  (Stevenson, 1994; Tirol-Padre and Ladha, 2004) raises doubt on the usefulness of this method in estimating the labile SOM pool (von Lützwow et al., 2007).

The objective of this experiment was to compare total SOC and SON, POM, FLF, ISNT and  $C_{\text{min}}$  as sensitive indices of short-term changes in SOM status in response to crop rotation, tillage, and cover crop management.

## **3.2. Materials and Methods**

### *3.2.1. Experiment description and sampling*

Soil samples were collected in July 2008 from a cropping system experiment in the Valley region of Virginia initiated in the spring of 2006 on a Buchanan silt loam (fine-loamy, mixed, semiactive, mesic Aquic Fragiudults). The composition of clay, silt, and sand was 8.5, 58.9, and 32.6%, respectively ( $n = 18$ ). The soil pH ranged from 6.03 to 6.79 ( $n = 54$ ). The experiment was a split-split-plot design with crop rotation as whole-plot treatment factor, tillage as sub-plot treatment factor, and cover crop management as sub-sub-plot treatment factor. The experiment had three replications (blocks). Each block had an area of 0.6 ha

divided in three whole-plots (0.2 ha) that were randomly assigned to be either continuous corn-silage (CS/CS/CS), corn-silage/alfalfa (*Medicago sativa* L.)/alfalfa (CS/Alf/Alf), or corn-grain/soybean (*Glycine max* L.)/corn-silage (CG/Soy/CS). The whole-plots were divided into two sub-plots (0.1 ha) and they were randomly assigned to be either reduced tillage (RT) or no-tillage (NT). The RT management consisted of one pass of a disc harrow and one pass of a field cultivator before planting at approximately 10 cm depth. The sub-plots were divided into three sub-sub-plots of 333 m<sup>2</sup>, which were randomly assigned to have the cover crop rye (*Secale cereale* L.) remaining on the field after being killed at the early-boot stage (RK), to have cover crop rye harvested from the field at the early-boot stage (RH), or to have no cover crop planted (NC). Fifteen soil cores (3 cm diameter soil probe) were collected at 0–15 cm depth from each plot and mixed to form a composite sample. Before the samples were taken, surface litter was removed. Soil samples were transferred to the lab within 8 h of collection and rapidly air-dried.

### 3.2.2. Soil organic matter fractions

Total SOC and SON of the composite samples were determined in duplicate by dry combustion at 900°C (VarioMax CNS macro elemental analyzer, Elementar, Hanau, Germany). All dry combustion analyses in this paper were conducted with the same instrument.

The size-based procedure used to recover POM is described by Gregorich and Beare (2008). Composite samples from each selected plot were analyzed in duplicate. Twenty five grams of air-dried soil ground to pass a 2 mm sieve were dispersed for 12 h in 100 mL of 0.0082 mol L<sup>-1</sup> sodium hexametaphosphate solution [5 g L<sup>-1</sup> (NaPO<sub>3</sub>)<sub>6</sub>] on a reciprocal shaker at 180 rpm. The suspension was poured onto a 53 µm sieve and rinsed with deionized water until clay and silt-size fractions were completely removed. The retained sand plus organic

material was dried on the sieve at 60°C for 1 h, transferred into a weighed aluminum tin and dried at 60°C for 24 h, and weighed. The dried material was ground using a mortar and pestle, and total organic C and N concentrations determined by dry combustion at 900°C.

The FLF was recovered for each composite sample in duplicate using SPT density solution adjusted to 1.8 g cm<sup>-3</sup> (Sequeira et al., 2011). Twenty grams of air-dried soil ground to pass a 2 mm sieve were placed in 250 mL centrifuge tubes with 50 mL of the density solution. Samples were shaken for 2 min on an orbital shaker at 200 rpm. Material adhered to the sides of the tubes was rinsed into the solution with an additional 20 mL of the density solution. Samples were then centrifuged at 4480 g (5000 rpm) for 15 min. Immediately after centrifugation, the supernatant was filtered through a 20 µm nylon filter under vacuum to collect the FLF. The FLF was rinsed with approximately 200 mL of deionized water followed by 50 mL of 0.01 M CaCl<sub>2</sub> to remove the density solution, transferred to a weighed aluminum tin, and dried at 60°C for 24 h. Oven dry weight of FLF was calculated as the difference between the weight of the tin and the weight of the tin plus FLF. After physical removal of the FLF from the tin, the FLF samples were ground using a mortar and pestle, and total organic C and N concentrations determined by dry combustion at 900°C.

The ISNT was determined for each composite sample in duplicate using the direct diffusion method developed by Khan et al. (2001) and modified by Spargo and Alley (2008). Briefly, 1 g of air-dried soil ground to pass a 2 mm sieve was weighed into Ball jars and spread evenly around the bottom before adding 10 mL of 2 mol L<sup>-1</sup> NaOH. Immediately following the addition of NaOH solution, the jars were fitted with the lid and petri dish apparatus containing 5 mL of 0.647 mol L<sup>-1</sup> (4% w/w) H<sub>3</sub>BO<sub>3</sub>, and tightly secured with a metal screw band. Sealed jars were heated for 15 h in a Precision 815 low temperature incubator (Precision, Winchester, VA) at 50°C to release alkali hydrolysable N as gas ammonia (NH<sub>3</sub>). The released NH<sub>3</sub> was collected in the H<sub>3</sub>BO<sub>3</sub> solution in the petri dish

suspended above the hydrolysate in each jar. The ISNT N was determined by acidimetric titration of the  $\text{H}_3\text{BO}_3$  solution with standardized  $0.01 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$  using a Radiometer TIM 900 Titration Manager and ABU 901 autoburette (Radiometer Analytical S.A., Lyon, France). The soil N ( $\text{mg N kg}^{-1}$ ) was calculated as  $ST$ , where  $S$  is milliliter of titrant and  $T$  is the titer ( $\mu\text{g N mL}^{-1}$ ) of the standardized  $\text{H}_2\text{SO}_4$ .

The  $C_{\min}$  was determined for each composite samples in duplicate using the method developed by Weil et al. (2003). These authors proposed an improvement of the method developed by Blair et al. (1995). Five grams of air-dried soil ground to pass a 2 mm were weighed into 50-mL plastic screw top centrifuge tubes. The soil samples were reacted with 20 mL of solution consisting of  $0.02 \text{ mol L}^{-1} \text{ KMnO}_4$  adjusted to pH 7.2. Samples were shaken for 2 min on a reciprocal shaker at 180 rpm and allowed to settle for 10 min. A 200  $\mu\text{L}$  aliquot of the supernatant was diluted to 10 mL and absorbance was measured using a DU<sup>®</sup> 640 Spectrophotometer (Beckman Coulter, Brea, CA) set at 550 nm wavelength. The amount of C oxidized by  $\text{KMnO}_4$  was determined by the following equation (Blair et al., 1995; Weil et al., 2003):

$$C_{\min} = \{[(0.02) - (a + bz)] * 9 * (0.02/x)\} * 1000 \quad (1)$$

where  $C_{\min}$  is in  $\text{mg C kg}^{-1}$  soil, the first 0.02 of the equation is the initial  $\text{KMnO}_4$  concentration ( $\text{mol L}^{-1}$ ),  $a$  is the intercept of the standard curve,  $b$  is the slope of the standard curve,  $z$  is the absorbance reading of the unknown sample, 9 is the amount of C ( $\text{g mol}^{-1}$ ) oxidized by 1 mol of  $\text{MnO}_4$  (assuming that 1 mol of  $\text{MnO}_4$  is consumed to oxidize 0.75 mol of C), the second 0.02 is the volume (L) of  $\text{KMnO}_4$  solution used, and  $x$  is the weight (kg) of soil sample. Standard curve consisted of three points (0.005, 0.01, and  $0.02 \text{ mol L}^{-1} \text{ KMnO}_4$  adjusted to pH 7.2).

### 3.2.3. Statistical analysis

Data were analyzed as a split-split-plot design with crop rotation as whole-plot treatment factor, tillage as sub-plot treatment factor, and cover crop management as sub-sub-plot treatment factor, using the PROC MIXED procedure of SAS software (SAS, 2008). The response variables were C and N contents, and C:N ratio present in bulk soil, POM and FLF; N content estimated by ISNT; and C content estimated by  $C_{\min}$ . Diagnostic evaluation for assumptions and outliers were performed for each response. No data transformation was needed. Multiple comparisons were performed by the Tukey (Honestly Significant Difference) method ( $\alpha = 0.05$ ). Correlation analysis between response variables was performed using the PROC CORR procedure of SAS software (SAS, 2008).

## 3.3. Results and Discussion

### 3.3.1. Size of organic fractions

Concentration values of the SOM fractions are presented in Table 3.1. Carbon-POM (C-POM) and carbon-FLF (C-FLF) averaged 22.9 and 8.4% of SOC, respectively, while nitrogen-POM (N-POM) and nitrogen-FLF represented 15.1 and 4.4% of SON, respectively. These values are in the same range of those reported by previous works (Carter et al., 2003; Gregorich et al., 2006; Sharifi et al., 2008; Wander, 2004). Dealing with annual grassland soils, Baisden et al. (2002) found C-FLF ranging from 7 to 22% of SOC, and mineral-associated fractions (e.g. occluded-LF) from 6 to 72% of SOC. Generally, about 50–75% of total SOC is associated with clay-sized particles, about 20–40% with silt-sized particles, and less than 10% with sand-sized particles (von Lützow et al., 2007). Mineralizable C ranged from 229–487 mg kg<sup>-1</sup> and accounted for 3.0% total SOC (Table 3.1). The  $C_{\min}$  range found in the present study was lower than 415–681 mg kg<sup>-1</sup> and 360–700 mg kg<sup>-1</sup> reported by Mirsky et al. (2008) and Weil et al. (2003), respectively. In addition, the ISNT accounted for

11.4% of total SON and this value agreed with those reported by other works (Sharifi et al., 2007; Spargo et al., 2009).

### 3.3.2. Evaluation of soil organic fractions as sensitive indices

The analysis of variance for the bulk soil measurements showed that total SOC contents and C:N ratio were significantly affected by cover crop management, while total SON contents were not affected by any treatment (Table 3.2). Soil OC contents were significantly higher for RK cover crop management compared to RH, with intermediate values for NC (Table 3.3). Inclusion of rye cover crop in temperate climate for time periods greater than 5 years is generally related to increased SOM contents compared to no cover crop included due to higher C input (Ding et al., 2006; Steenwerth and Belina, 2008). On the other hand, Sainju et al. (2007) did not find different SOC contents between a variety of cover crop mixes and no cover crop planted after three years for experiments conducted on silt loam soils. The lower SOC content with RH compared to RK is probably related to SOM mineralization that is not balanced by the removal of crop residue with RH. Whalen et al. (2001) found greater SOM mineralization in a silt loam soil when harvesting the above-ground ryegrass (*Lolium multiflorum* L.) compared to not harvesting it. These authors argued that harvesting the above-ground biomass would alter photoassimilate allocation, and the response for it may be to enhance root activity and/or turnover that could stimulate microbial activity and SOM cycling process. The C:N ratio of bulk soil and SOM fractions have often been used as indices of soil quality but interpretation of their trends can be difficult (Wander, 2004). Agricultural use of virgin soils reduces soil C:N ratios from ~20 to the common range ~10 as labile constituents of SOM are lost and SOM aromaticity increases (Stevenson, 1994; Wander, 2004). The treatments with cover crop planted had significantly higher values of bulk soil C:N ratio than the treatment with no cover crop, even with the numerical difference

being relatively small (Table 3.3). Higher C:N ratio suggests SOM relatively less decomposed. This finding disagrees with Ding et al. (2006) who after 10 years of an experiment found no significant effect of cover crop management [no cover crop, rye alone, and combination of hairy vetch (*Vicia villosa* L.) and rye] on soil C:N ratio to a depth of 15 cm. In addition, Marriot and Wander (2006b) found soil C:N ratio to not be sensitive to different farming systems conducted from 3 to 14 years. The higher C:N ratio in treatments with cover crop planted could be due to the input of rye residue with C:N ratio of approximately 80:1; however, Nicolardot et al. (2001) reported that soil C:N ratio reflects characteristics of stable SOM and is not closely related to the quality of the crop residue added to the soil.

The analysis of variance for the POM fraction showed significant effect of cover crop management on C-POM; and significant effect of three-way interaction (crop rotation by tillage by cover crop) on C:N ratio (Table 3.2). No significant effect was detected for N-POM content. Carbon-POM content was significantly higher with RK cover crop management than with both RH and NC (Table 3.3). Plant residues at different stages of decomposition and size are main constituents of POM and FLF (Ding et al., 2006; Gregorich et al., 2006); thus, the effect of cover crop management on C-POM content is explained by the different residue inputs. Assuming the root system contributing with ~20% to the total rye plant weight (Fageria and Moreira, 2011), calculations indicated that the residue input with RK and RH managements were of 4.5 and 0.7 Mg ha<sup>-1</sup>, respectively. Working with sandy loam and sandy clay loam soils, Franzluebbers and Stuedemann (2008) found no difference in C-POM between grazed and ungrazed rye cover crop after three years of experiment. The lack of difference in this case was attributed to the deposition of feces in the grazed treatment that contributed to C-POM. In addition, Dell et al. (2008) found no conclusive effect of rye cover

crop being planted or not on C- and N-POM contents at both 0–5 and 5–10 cm depth in loam, silt loam, silty clay loam, and silty clay soils of Pennsylvania.

The significant three-way interaction for POM C:N ratio made necessary to evaluate the effect of two treatment factors (two-way interaction) at fixed levels of the third factor. In this case, the crop rotation by cover crop interaction was evaluated at fixed levels of tillage due to no significant differences across tillage treatments. Under RT system, the CS/CS/CS rotation showed higher POM C:N ratio than the CS/Alf/Alf rotation with RK cover crop management, with intermediate values for the CG/Soy/CS rotation (Table 3.4). However, with RH management, for both tillage systems, the CG/Soy/CS rotation had higher POM C:N ratio than the CS/Alf/Alf rotation, with intermediate values for the CS/CS/CS rotation (Table 3.4). The reduced POM C:N ratio in the CS/Alf/Alf rotation is likely due to the presence of legume alfalfa for the last two years of the rotation forming residues with low C:N ratio (~12:1), while the other two rotations had corn with its high C:N ratio (~60:1) as the predominant crop. The sensitivity of POM C:N ratio found in the present work does not agree with Marriott and Wander (2006a; 2006b) who reported a lack of sensitivity of POM C:N ratio to distinguish between management systems. Wander (2004) pointed out that interpretation of C:N ratio trends can be difficult due to no clear relationship between the C:N ratio of these fractions and the C:N ratio of the bulk soil in arable mineral soils. Under NT system, NC management resulted in lower POM C:N ratio compared to RH management in the CG/Soy/CS rotation (Table 3.4), which seems to be related to the reduced residue deposition. This difference was not observed under RT system probably due to a higher decomposition of both cover crop residue and POM caused by the moderate mixing of residues into the soil in this system. For both tillage systems, the considerable amount of high and low C:N ratio residues in the CS/CS/CS and CS/Alf/Alf rotations, respectively, likely masked the effect of cover crop managements.

The analysis of variance for FLF organic matter showed significant effects of crop rotation by tillage interaction and crop rotation by cover crop interaction on C-FLF; a significant effect of crop rotation by tillage interaction on N-FLF; and a significant effect of crop rotation by cover interaction, and crop rotation and tillage simple effects on FLF C:N ratio (Table 3.2). Thus, FLF organic matter appears to be the most sensitive fraction to the agronomic practices evaluated. Carbon- and N-FLF contents were higher under RT compared to NT for CS/CS/CS and CG/Soy/CS rotations (Table 3.5), probably due to the greater mix of plant residues into soil under RT than NT.

In the CS/CS/CS and CS/Alf/Alf rotations the RK cover crop management resulted in higher C-FLF contents, while no significant effect of cover crop management was found in the CG/Soy/CS rotation (Table 3.6). The trend of higher C-FLF with RK cover crop management is probably related to its high residue input as indicated above. The fact that FLF is more chemically/structurally related to plant tissue than POM (Gregorich et al., 2006; Marriott and Wander, 2006a) suggests a greater affect of quantity and quality of plant residue on FLF than POM. In a continuous corn rotation, Ding et al. (2006) found higher FLF contents when cover crops were included in the system (rye and rye+vetch) compared to no cover crop included in a 10 years field experiment conducted on a sandy loam soil.

Nitrogen-FLF content was higher in the CS/Alf/Alf rotation compared to the other two rotations under NT management (Table 3.5) due to the enriched-N residue input in the last two years of CS/Alf/Alf rotation. These results agree with Marriott and Wander (2006a) who found significant higher N-FLF contents with cropping systems including legumes in the crop rotation. The evaluation of tillage simple effect on FLF C:N ratio showed significantly higher values for NT compared to RT (Table 3.3), probably due to a higher FLF decomposition under the RT system. Increased soil aggregation under NT is known to promote physical protection to SOM against decomposition (Dell et al., 2008; Huggins et al.,

2007; Liang et al., 2004; Yoo and Wander, 2008). In exploring the significant interaction for FLF C:N ratio, significantly higher values were found in the CS/CS/CS rotation than in the CS/Alf/Alf rotation within RK cover crop management, but within RH cover crop management higher FLF C:N ratio values were found in the CG/Soy/CS compared to the CS/Alf/Alf rotation (Table 3.6). As for POM C:N ratio, the reduced FLF C:N ratio in the CS/Alf/Alf rotation is most likely due to the enriched-N residue input. Marriot and Wander (2006a) also found significantly lower FLF C:N ratio values for systems having legumes involved in the crop rotation compared to systems without legumes.

The ISNT and  $C_{\min}$  organic fractions were not affected by any of the agronomic practices evaluated in the present study. The overall means for ISNT and  $C_{\min}$  were 144 mg N  $\text{kg}^{-1}$  and 396 mg C  $\text{kg}^{-1}$ , respectively (Table 3.1). Both organic fractions have been investigated as sensitive indices for changes in labile SOM due to changes in management, especially ISNT in the last few years, with a mix of satisfactory and unsatisfactory results that sheds doubt on their usefulness for such evaluations (Blair et al., 1995; Marriott and Wander, 2006b; Mirsky et al., 2008; Sharifi et al., 2007; Spargo and Alley, 2008; Tirol-Padre and Ladha, 2004; Weil et al., 2003).

### *3.3.3. Relationship between soil organic fractions*

Correlation analysis between soil organic fractions is presented in Table 3.7. The ISNT and  $C_{\min}$  were highly correlated with total SOC, SON, C-POM, and N-POM, and not significantly correlated with C-FLF and N-FLF. Their high correlation with SOC and SON explains in part the lack of sensitivity of these organic fractions to soil changes due to management in the short-term. Previous research has shown a strong relationship between the ISNT and both total SOC and SON, suggesting that this procedure may extract some recalcitrant forms of N (Marriott and Wander, 2006b; Spargo et al., 2009). The strong

relationship between C-POM and both ISNT and  $C_{\min}$  has been used to suggest similar response of these fractions to management (Mirsky et al., 2008; Sharifi et al., 2007). However, Tirol-Padre and Ladha (2004) found  $C_{\min}$  to lack correlation with water-soluble and microbial biomass carbon, but to be highly correlated to total C. In fact, they suggested  $C_{\min}$  to be a good indicator of the lignified nature of SOM. The positive relationship of  $C_{\min}$  with lignified substance helps to understand why total SOC was sensitive to cover crop management, but  $C_{\min}$  was not. In comparison to ISNT and  $C_{\min}$ , the C-POM and N-POM had weaker correlations with SOC and SON, and stronger correlation with C-FLF and N-FLF. Marriott and Wander (2006b) also found weaker correlation between C-POM and SOC compared to ISNT and SOC. Among all fractions evaluated, FLF had the poorest correlation with total SOC and SON. This poor relationship with total SOC and SON validates the labile nature of FLF which was the most sensitive SOM fraction to changes in management (Table 3.2). The reasonable correlation of C and N contents of POM and LF ( $r = 0.39$  to  $0.60$ ) is supported by the fact that these organic fractions contain different proportions of carbohydrates and aliphatic compounds, which precludes their use as synonyms (Gregorich et al., 2006).

### **3.4. Conclusions**

Across the agronomic practices evaluated in the present work, cover crop management had the greatest effect on SOM fractions with killing and leaving the cover crop rye residue on the field as its most beneficial treatment level resulting in the greatest SOC, C-POM, C-FLF, and N-FLF contents. Thus, this management is shown to be important in improving soil quality which leads to more sustainable land use and crop production. The physical fractions of SOM (POM and FLF) were more sensitive to the agronomic practices evaluated than the other fractions (SOC, SON, ISNT, and  $C_{\min}$ ) with the FLF being the most

sensitive between all fractions. Between all fractions, POM represented the largest portion of total SOC (22.9% on average). The POM and FLF are primarily made up of plant residues in different stages of decomposition which links the sensitivity of these fractions to the cover management leaving residues on the field. Since FLF is chemically/structurally closer to plant residues than POM, the sensitivity rank position of these fractions would probably be at least more similar if only tillage management was considered. In addition, the lack of sensitivity of ISNT and  $C_{\min}$  to any tested management practice is added to previous publications that have raised questions of the representation of the labile SOM pool through these fractions.

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**Table 3.1.** Mean, standard deviation (SD), and range values ( $\text{mg kg}^{-1}$ ) of SOM fractions.

Fraction†	Mean	SD	Minimum	Maximum
Soil C	13061	1591	9630	15500
Soil N	1264	158	862	1618
Soil C:N ratio	10.4	0.4	9.5	11.4
POM C	2991	403	2270	3850
POM N	192	30.99	140	269
POM C:N ratio	15.6	1.1	13.5	17.9
FLF C	1101	197	691	1588
FLF N	56.0	12.4	28.9	82.1
FLF C:N ratio	19.8	2.3	16.3	26.2
ISNT	144	17.1	98.5	182
C <sub>min</sub>	396	51.0	229	487

† Soil C, total soil organic C; Soil N, total soil organic N; POM C, particulate organic matter C; POM N, particulate organic matter N; FLF C, free-light fraction C; FLF N, free-light fraction N; ISNT, Illinois soil N test; C<sub>min</sub>, mineralizable C by 0.02 M KMnO<sub>4</sub>

**Table 3.2.** Analysis of variance's *F*-values for the C and N contents as well as C:N ratio in bulk soil, particulate organic matter (POM), free-light fraction (FLF), Illinois soil N test (ISNT) and mineralizable C ( $C_{min}$ ).

Source of Variability	-----Bulk Soil-----			-----POM-----			-----FLF-----			ISNT	$C_{min}$
	Carbon	Nitrogen	C:N	Carbon	Nitrogen	C:N	Carbon	Nitrogen	C:N		
Rotation (R)	0.12 <sup>ns</sup> †	0.16 <sup>ns</sup>	0.06 <sup>ns</sup>	1.15 <sup>ns</sup>	4.36 <sup>ns</sup>	18.44 <sup>**</sup>	0.84 <sup>ns</sup>	2.50 <sup>ns</sup>	16.43 <sup>*</sup>	2.64 <sup>ns</sup>	0.04 <sup>ns</sup>
Tillage (T)	0.00 <sup>ns</sup>	0.34 <sup>ns</sup>	0.49 <sup>ns</sup>	1.05 <sup>ns</sup>	0.01 <sup>ns</sup>	0.00 <sup>ns</sup>	107.8 <sup>***</sup>	67.43 <sup>***</sup>	29.13 <sup>**</sup>	0.55 <sup>ns</sup>	0.02 <sup>ns</sup>
R*T	1.16 <sup>ns</sup>	0.98 <sup>ns</sup>	1.54 <sup>ns</sup>	5.02 <sup>ns</sup>	1.69 <sup>ns</sup>	1.12 <sup>ns</sup>	35.42 <sup>***</sup>	19.94 <sup>**</sup>	3.67 <sup>ns</sup>	3.32 <sup>ns</sup>	0.20 <sup>ns</sup>
Cover (C)	4.63 <sup>*</sup>	2.31 <sup>ns</sup>	8.62 <sup>**</sup>	7.76 <sup>**</sup>	1.39 <sup>ns</sup>	12.89 <sup>***</sup>	12.66 <sup>***</sup>	2.94 <sup>ns</sup>	3.08 <sup>ns</sup>	0.36 <sup>ns</sup>	2.73 <sup>ns</sup>
R*C	0.91 <sup>ns</sup>	0.82 <sup>ns</sup>	1.53 <sup>ns</sup>	1.07 <sup>ns</sup>	1.32 <sup>ns</sup>	4.66 <sup>**</sup>	6.27 <sup>**</sup>	0.92 <sup>ns</sup>	3.21 <sup>*</sup>	0.91 <sup>ns</sup>	0.40 <sup>ns</sup>
T*C	0.77 <sup>ns</sup>	0.26 <sup>ns</sup>	0.24 <sup>ns</sup>	0.15 <sup>ns</sup>	0.08 <sup>ns</sup>	2.58 <sup>ns</sup>	3.30 <sup>ns</sup>	0.07 <sup>ns</sup>	0.63 <sup>ns</sup>	0.80 <sup>ns</sup>	0.04 <sup>ns</sup>
R*T*C	3.42 <sup>ns</sup>	4.15 <sup>ns</sup>	2.56 <sup>ns</sup>	3.21 <sup>ns</sup>	6.95 <sup>ns</sup>	3.16 <sup>*</sup>	2.71 <sup>ns</sup>	2.40 <sup>ns</sup>	2.77 <sup>ns</sup>	3.39 <sup>ns</sup>	3.53 <sup>ns</sup>

† ns, no significant; \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

**Table 3.3.** Effect, in the short-term, of crop rotation, tillage, as well as cover crop management on total soil organic carbon (SOC) and nitrogen (SON) and soil C:N ratio, C and N contents in particulate organic matter (C-POM and N-POM, respectively), C:N ratio of free-light fraction (FLF), N measured by the Illinois soil N test (ISNT), and mineralizable C ( $C_{\min}$ ) measured by  $0.02 \text{ mol L}^{-1} \text{ KMnO}_4$ .

	-----Crop Rotation‡-----			-----Tillage§-----		-----Cover Crop Management¶-----		
	CS/CS/CS	CS/Alf/Alf	CG/Soy/CS	RT	NT	RK	RH	NC
	-----g kg <sup>-1</sup> -----							
SOC	12.8a†	13.3a	13.1a	13.1a	13.1a	13.6a	12.8b	13.0ab
SON	1.2a	1.3a	1.3a	1.3a	1.3a	1.3a	1.2a	1.3a
Soil C:N	10.4a	10.4a	10.4a	10.4a	10.7a	10.57a	10.44a	10.17b
	-----mg kg <sup>-1</sup> -----							
C-POM	2899a	3176a	2897a	3031a	2951a	3177a	2903b	2892b
N-POM	177.4a	216.0a	180.9a	191.8a	191.1a	196.4a	186.2a	191.7a
FLF C:N	-	-	-	18.8b	20.9a	-	-	-
ISNT	134.9a	154.1a	143.3a	143.0a	145.2a	145.7a	142.8a	143.7a
$C_{\min}$	388.6a	399.2a	393.5a	392.7a	394.9a	399.2a	381.1a	401.0a

† Means in a row with the same letter are not significantly different by the t-test ( $P < 0.05$ )

‡ CS/CS/CS, continuous corn (*Zea mays* L.)-silage; CS/Alf/Alf, corn-silage/alfalfa (*Medicago sativa* L.)/alfalfa; CG/Soy/CS, corn-grain/soybean (*Glycine max* L.)-corn-silage

§ RT, reduced tillage; NT, no-tillage

¶ RK, rye (*Secale cereale* L.) killed; RH, rye harvested; NC, no cover crop

**Table 3.4.** Effect, in the short-term, of crop rotation, tillage, and cover crop management on C:N ratio of particulate organic matter (POM).

Cover Crop	Crop Rotation‡		
	CS/CS/CS	CS/Alf/Alf	CG/Soy/CS
-----POM C:N ratio in reduced tillage-----			
Rye killed	16.9aA†	14.2bA	16.1abA
Rye harvested	16.2abA	14.8bA	17.0aA
No cover crop	15.5aA	14.7aA	15.6aA
-----POM C:N ratio in no-tillage-----			
Rye killed	16.2aA	15.9aA	16.4aAB
Rye harvested	15.8abA	14.4bA	17.1aA
No cover crop	15.5aA	14.5aA	15.2aB

† Means in a row with the same lower case letter and in a column with the same upper case letter are not significantly different by the t-test ( $P < 0.05$ )

‡ CS/CS/CS, continuous corn (*Zea mays* L.)-silage; CS/Alf/Alf, corn-silage/alfalfa (*Medicago sativa* L.)/alfalfa; CG/Soy/CS, corn-grain/soybean (*Glycine max* L.)/corn-silage

**Table 3.5.** Effect, in the short-term, of crop rotation and tillage management on C and N contents in free-light fraction organic matter (C-FLF and N-FLF, respectively).

Tillage	Crop Rotation‡		
	CS/CS/CS	CS/Alf/Alf	CG/Soy/CS
	-----C-FLF mg kg <sup>-1</sup> -----		
Reduced tillage	1296aA†	1114aA	1177aA
No-tillage	953.0aB	1143aA	901.0aB
	-----N-FLF mg kg <sup>-1</sup> -----		
Reduced tillage	66.2aA	60.1aA	63.2aA
No-tillage	41.8bB	61.4aA	42.3bB

† Means in a row with the same lower case letter and in a column with the same upper case letter are not significantly different by the t-test ( $P < 0.05$ )

‡ CS/CS/CS, continuous corn (*Zea mays* L.)-silage; CS/Alf/Alf, corn-silage/alfalfa (*Medicago sativa* L.)/alfalfa; CG/Soy/CS, corn-grain/soybean (*Glycine max* L.)/corn-silage

**Table 3.6.** Effect, in the short-term, of crop rotation and cover crop management on C contents and C:N ratio in free-light fraction (FLF) organic matter.

Cover Crop	Crop Rotation‡		
	CS/CS/CS	CS/Alf/Alf	CG/Soy/CS
	-----C-FLF mg kg <sup>-1</sup> -----		
Rye killed	1214aA†	1185aA	1090aA
Rye harvested	1016aB	1170aAB	1044aA
No cover crop	1144aAB	1030aB	983.3aA
	-----FLF C:N ratio-----		
Rye killed	21.9aA	18.6bA	20.7abA
Rye harvested	19.7abA	18.0bA	21.4aA
No cover crop	20.8aA	18.1aA	19.1aA

† Means in a row with the same lower case letter and in a column with the same upper case letter are not significantly different by the t-test ( $P < 0.05$ )

‡ CS/CS/CS, continuous corn (*Zea mays* L.)-silage; CS/Alf/Alf, corn-silage/alfalfa (*Medicago sativa* L.)/alfalfa; CG/Soy/CS, corn-grain/soybean (*Glycine max* L.)/corn-silage

**Table 3.7.** Correlation analysis between soil organic C and N fractions.

	Soil C	Soil N	Soil C:N	POM C	POM N	POM C:N	FLF C	FLF N	FLF C:N	ISNT	C <sub>min</sub>
Soil C‡	1†										
Soil N	0.94***	1									
Soil C:N	0.15 <sup>ns</sup>	-0.21 <sup>ns</sup>	1								
POM C	0.78***	0.72***	0.20 <sup>ns</sup>	1							
POM N	0.62***	0.69***	-0.11 <sup>ns</sup>	0.90***	1						
POM C:N	0.09 <sup>ns</sup>	-0.16 <sup>ns</sup>	0.56***	-0.18 <sup>ns</sup>	-0.59***	1					
FLF C	0.30*	0.17 <sup>ns</sup>	0.53***	0.60***	0.39**	0.14 <sup>ns</sup>	1				
FLF N	0.19 <sup>ns</sup>	0.08 <sup>ns</sup>	0.33*	0.55***	0.48***	-0.17 <sup>ns</sup>	0.89***	1			
FLF C:N	0.05 <sup>ns</sup>	-0.08 <sup>ns</sup>	0.19 <sup>ns</sup>	-0.30*	-0.50***	0.68***	-0.32*	-0.66***	1		
ISNT	0.82***	0.88***	-0.09 <sup>ns</sup>	0.69***	0.69***	-0.19 <sup>ns</sup>	0.23 <sup>ns</sup>	0.21 <sup>ns</sup>	-0.24 <sup>ns</sup>	1	
C <sub>min</sub>	0.85***	0.87***	-0.13 <sup>ns</sup>	0.68***	0.62***	-0.13 <sup>ns</sup>	0.17 <sup>ns</sup>	0.08 <sup>ns</sup>	-0.07 <sup>ns</sup>	0.73***	1

† ns, no significant; \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

‡ Soil C, total soil organic C; Soil N, total soil organic N; POM C, particulate organic matter C; POM N, particulate organic matter N; FLF C, free-light fraction C; FLF N, free-light fraction N; ISNT, Illinois soil N test; C<sub>min</sub>, mineralizable C by 0.02 M KMnO<sub>4</sub>

#### IV. On-Farm Tillage Management Effects on Soil Carbon and Nitrogen

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## Abstract

Soil organic matter (SOM) is a well known indicator of soil quality and has recently received much attention due to its potential to help mitigate increased atmospheric concentration of greenhouse gases (GHG) such as carbon dioxide (CO<sub>2</sub>). Conversion of natural systems to croplands has caused depletion of 60–70% of initial soil organic carbon (SOC) levels in many soils by using conventional tillage (CT). No-tillage (NT) management is an agronomic technology widely claimed to be superior to CT in sequestering/retaining C in soils and, hence, provide an opportunity for increasing SOM toward pre-cultivated SOM levels. The objective of this study was to investigate the current levels of SOC and total soil N (TSN) in the Valley region of Virginia as a function of tillage management. The majority of the sampled sites had corn (*Zea mays* L.) as the main crop sometimes rotated with wheat (*Triticum aestivum* L.), soybean (*Glycine max* L.), and rye (*Secale Cereale* L.). Soil samples were collected from 13 sites at 0–60cm depth and divided in four depths (0–5, 5–15, 15–30, and 30–60 cm). Tillage managements were CT and NT, where CT consisted in annual plowing and disking. Analyzing the data as an incomplete block design resulted in no significant difference of SOC and TSN levels between tillage management by considering either the entire sampled depth or depth increments. Through regression analysis, SOC and TSN stocks were significantly affected by the duration of NT (0 to 10 years) at 0–5 cm depth with sequestration rates of  $0.44 \pm 0.08 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  and  $0.04 \pm 0.01 \text{ Mg N ha}^{-1} \text{ yr}^{-1}$ ; and at 0–15 cm depth with sequestration rates of  $0.59 \pm 0.14 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  and  $0.05 \pm 0.02 \text{ Mg N ha}^{-1} \text{ yr}^{-1}$ . However, the indicated no significant accumulation of both SOC and TSN over the depths of 0–30 and 0–60 cm, raises doubts about the effectiveness of NT over CT for improving SOM levels. Nevertheless, NT management is a technology that brings other benefits such as erosion control and increasing water availability and, hence, its adoption

must be advocated over CT in areas suitable for it as part of a continuous effort to achieve a more sustainable agriculture.

#### **4.1. Introduction**

Soil organic matter (SOM) is a well known indicator of soil quality due to its direct influence on soil properties such as structure stability, water infiltration, water availability, biological activity, cation exchange capacity (CEC), nutrient storage and release, sorption of agrochemicals, metal mobility (chelation), and pH buffering and amelioration (Stevenson, 1994; Weil and Magdoff, 2004). Besides this well established role of SOM on soil quality, there is an increasing incentive to improve croplands SOM levels as a tool to mitigate increasing atmospheric concentration of greenhouse gases (GHG) such as carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), and nitrous oxide (N<sub>2</sub>O) (Coppens et al., 2006; Fest et al., 2009; Grace et al., 2006; Purakayastha et al., 2008). Greenhouse gases are defined by their radiative force that changes the Earth's atmospheric energy balance (IPCC, 1996) with positive values indicating an increase in the level of energy remaining on the Earth, while a negative value indicates an increase in the level of energy returning to space (Johnson et al., 2007). The main emitters of GHG are energy (86.6%), agriculture (6.3%), industry (4.5%), waste (2.7%), and solvent and product use (1%) (Johnson et al., 2007).

Soil organic carbon (SOC) is the SOM fraction that when increased in soil has the potential to transfer/sequester the atmospheric C into stable soil organic forms, thereby partially mitigating the current increasing atmospheric CO<sub>2</sub> (West and Post, 2002). Agricultural practices with positive effect on SOC are conservation tillage, intensive crop rotation, use of winter cover crops, soil input to increase primary production (e.g. fertilization), and manure application. Lal (2004) stated that soils of well managed ecosystems have the capacity to approximately sequester the cumulative historic C loss

estimated at 55 to 78 Gt, with the current attainable soil C sink capacity being only 50 to 66% of the potential capacity. On the other hand, stable nitrogen (N) retention in soil is credited to the soil organic N (SON) fraction that commonly is highly correlated with SOC (Blanco-Canqui and Lal, 2008; Franzluebbers and Stuedemann, 2008; Wright and Hons, 2005). Soil OM averages about 5%N (Stevenson, 1994) which makes the mineralization of this soil constituent a major component of soil N supply in agricultural systems (Sharifi et al., 2008). If 1 to 3% of this organic N is mineralized annually, 8 to 120 kg N ha<sup>-1</sup> is released in a plant-available form (Bundy and Meisinger, 1994).

Conversion of undisturbed land (e.g. forest) to agricultural systems has caused depletion of SOC by as much as 60% in soils of temperate regions and 75% or more in cultivated tropical soils (Lal, 2004). In general, farming methods using mechanical tillage can promote SOM losses by disrupting soil aggregates that protect SOM from decomposition, stimulating short-term microbial activity through enhanced aeration, and mixing fresh residues into soil where conditions for decomposition are often more favorable than on the soil surface (Cerri et al., 2010). In addition, tillage can make soils more prone to erosion, resulting in further losses of SOM (Magdoff and Weil, 2004). Reduction in C content of surface soil as a result of erosion is due to both the loss of topsoil and the dilution effect from subsoil mixing (Paustian et al., 1997). For instance, the SOC stock of 63.7 Mg ha<sup>-1</sup> under Native Prairie in the Palouse region at 0–20 cm depth was superior to cropland soils under any type of tillage management (Purakayastha et al., 2008). In addition, Huggins et al. (2007) found that in the Midwestern region of United States the SOC stock at 0–45 cm depth decreased from 187 Mg ha<sup>-1</sup> under native tall-grass prairie to 135 Mg ha<sup>-1</sup> under conventional tillage (CT) after 14 years, while under no-tillage (NT) the stock was reduced to 164 Mg ha<sup>-1</sup>.

Soil preparation through CT has always been an important component of traditional agriculture (Lal et al., 2007). Tillage has been defined as the mechanical manipulation of the

soil and plant residues to produce grain for human and animal consumption (Reicosky and Allmaras, 2003). Tillage controls both SOC substrate availability and decomposition rates by: i) affecting the quantity and distribution of C derived from organic materials (e.g. plant residues) in the soil profile, and ii) influencing edaphic factors such as aeration and water content, temperature, soil reaction, and soil aggregation properties (Huggins et al., 2007). Conventional tillage usually consists of moldboard plow or chisel plow in the fall followed by disk harrowing in the spring; one disk plow and one disk harrowing in the fall; one disk harrowing after harvest followed by a chisel plow and a second disk harrowing prior the winter; or a tandem disk and chisel plow in the fall and spring. Each of these tillage operations occurs at depths ranging from 8 to 35 cm.

Conservation tillage includes a variety of systems, all designed to minimize residue incorporation with the intent of controlling soil erosion (Franzluebbers, 2004). According to the definition of the United States Department of Agriculture (USDA), over 30% residue cover must be on the soil surface immediately after planting for the system to be considered conservation tillage. Conservation tillage practices such as NT have the potential to increase SOM levels by increasing aggregate stability and, hence, improving the protection of new SOM against soil microbe attack; preserving old SOM in long-term formed stable aggregates; and reducing litter decomposition (Paustian et al., 1997). Usually, organic residues added to temperate soils decompose rapidly, with approximately one-third of the original C and N persisting as SOM after 1 year, unless edaphic factors, typically physical or chemical extremes, restrict biological activity (Wander, 2004). The SOM level will tend to level off at a maximum amount of C that can be stabilized in organo-mineral complexes, which are resistant to decomposition (Paustian et al., 1997). These authors suggested that restoration of soil C to pre-cultivation levels by using NT management may be possible if combined with increasing nutrient and water supply, leading to higher C input. No-tillage systems have been

widely adopted in North and South America (Blanco-Canqui and Lal, 2008), with the USA having the largest area, followed by Brazil and Argentina (Cerri et al., 2010). However, the effect of NT management on SOM levels cannot be predicted based on a single approach since optimum management varies with crop rotation, fertilization, depth of tillage, soil properties, climatic conditions, and depth of sampling (Franzluebbers, 2004).

Differences in SOM levels between CT and NT are most extreme near the soil surface, primarily due to differences in the distribution of organic matter input between managements where CT mixes organic residues with soil at deeper depths and NT keeps the residue on the soil surface (Blanco-Canqui and Lal, 2008; Franzluebbers, 2004; West and Post, 2002). Consequently, comparisons of tillage effects based on sampling only the top few centimeters of soil can give a misleading and overestimated positive impression of the SOM buildup under NT compared to CT (Paustian et al., 1997; Verhulst et al., 2010). In the top 5 cm, Xu et al., (2007) found higher SOC and SON stocks under NT compared to CT; however, at 5–10 and 10–20 cm depths both organic fractions were higher under CT. In a study conducted during 19 years on a clayey Oxisol in South Brazil, Calegari et al. (2008) found that NT management resulted in significantly higher SOC stock than CT at 0–5 cm depth, lower at 10–20 and 20–30 cm depth, and similar at 5–10, 30–40, and 40–60 cm depth across winter cover crop species. Blanco-Canqui and Lal (2008) listed two other possible reasons for the usual higher SOC and SON amounts in subsoil under CT compared to NT: i) unfavorable root growth into deeper layers under NT due to its near-surface higher water content and favorable temperature; and ii) more favorable formation of organo-mineral complexes of the buried residues with the soil matrix under CT. Baker et al. (2007) defends the idea that not just the entire plow layer but deeper depths should be sampled in order to account for possible differences in root distribution and rhizodeposition between management practices.

Besides SOM accumulation, tillage management affects physical soil quality properties such as soil bulk density. The effect of tillage on soil bulk density is mainly confined to the top soil (plow layer), with similar soil bulk density between CT and NT in deeper soil layers (Verhulst et al., 2010). Bulk density is considered to be a measure of soil quality due to its relationship with properties such as porosity, soil moisture, and hydraulic conductivity (Dam et al., 2005). Working with a silt loam soil at 0–7.5 cm depth, Liebig et al. (2004) found after 17 years soil bulk density values of 1.13 and 1.19 g cm<sup>-3</sup> under NT and CT, respectively. However, Huggins et al. (2007) working with a clay loam soil found no influence of tillage on soil bulk density at 0–7.5, 7.5–15, 15–30, and 30–45 cm depths. In addition, Franzluebbers and Stuedemann (2008) working with sandy loam and sandy clay loam soils in Georgia observed that after one year of conversion from pasture to cropland the soil bulk density was reduced under CT compared to NT at all depths below 6 cm, but was greater under CT at 0–3 cm depth. After 3 years, bulk density was similar between tillage management at 0–3 cm depth, reduced under CT at 3–12 cm depth, and similar again below 12 cm.

The objective of this study was to investigate how tillage management practices employed by farmers in the Valley region of Virginia affect SOC levels, total soil N levels, and soil bulk density in the top 60 cm of soil.

## **4.2. Materials and Methods**

### *4.2.1. Site description and soil sampling and analyses*

Soil samples were collected in April 2009 from 13 sites located in the Valley region of Virginia (Table 4.1). As is commonly practiced in the area of study, most of the sites were cropped to corn (*Zea mays* L.) which was harvested for silage and all sites had manure, poultry litter and/or biosolid applied in previous years. Conventional tillage consisted in

annual plowing and disking for sites using this tillage management, as reported by the farmers. In each site, five undisturbed soil cores were collected at 0–60 cm depth with a hydraulically driven core sampler (3.9 cm diameter soil probe). Soil cores were kept in 60 cm long plastic tubes that were placed inside the soil probe before sampling. Before the samples were taken, surface litter was removed. After sampling, tubes were labeled, sealed with rubber caps of different colors to identify the top and bottom soil, and placed in a container with ice until transferred to the lab. In the lab, samples were stored at 4°C until the start of processing. Coordinate positions of each sample core were recorded using a GPSMAP 76S (Garmin Ltd., Olathe, KS).

Plastic tubes were marked and cut at depths of 0–5, 5–15, 15–30, and 30–60 cm and samples rapidly air-dried and ground to pass a 2 mm sieve. All analyses described below were determined in duplicate. Subsamples from each sample were collected and their weight (~10 g) recorded before and after placing the subsample into an oven at 105°C to determine water content (Gardner, 1986). Bulk density was then obtained by dividing the oven-dry weight of soil inside the core by the volume of sampling tube for each specific depth (Blake and Hartge, 1986). Soil pH was measured on 1:1 soil/water ratio mixtures after equilibration for 30 min. Total SOC and total soil N (TSN) were determined by dry combustion (VarioMax CNS macro elemental analyzer, Elementar, Hanau, Germany) on subsamples ground in an electric grinder (Mortar Grinder RM 200, Retsch, Haan, Germany). For samples with history of biosolid application and/or pH higher than 6.5, any carbonate residue present was removed by pre-treating samples with 1 mol L<sup>-1</sup> hydrochloric acid (HCl) until visible fizzing stopped, washed with deionized water, air-dried, and ground again (Gál et al., 2007); thus, total C was considered equivalent to organic C.

#### 4.2.2 Statistical Analysis

First, data were analyzed as a factorial experiment in an incomplete block design with tillage management and soil depth as treatment factors, and soil series as the blocking factor, using the PROC MIXED procedure of SAS software (SAS, 2008). Tillage management had CT and NT as treatment levels while soil depth had its four depths as treatment levels. There were eight soil series. The response variables were bulk soil C and N levels in both concentration ( $\text{g kg}^{-1}$ ) and area basis ( $\text{Mg ha}^{-1}$ ), and soil bulk density in  $\text{g cm}^{-3}$ . Second, data were analyzed as an incomplete block design with tillage management as the treatment factor and soil series as the blocking factor. The response variables were C and N levels at 0–60 cm depth in area basis. Diagnostic evaluation for assumptions and outliers were performed for each response. Carbon and N levels were log-transformed in order to meet the normal distribution assumption. Multiple comparisons were performed by the Tukey (Honestly Significant Difference) method ( $\alpha = 0.05$ ).

In addition, simple linear regression models were fitted to investigate the relationship between SOC and TSN stocks with the duration of NT management, using the PROC REG procedure of SAS software (SAS, 2008). Diagnostic evaluation for assumptions and outliers were performed for each response. No data transformation was needed.

### 4.3. Results and Discussion

#### 4.3.1. Soil bulk density

The analysis of variance for soil bulk density had the main effects tillage and depth, and the interaction tillage by depth significant which leads to a comparison between depth within fixed tillage management and vice-versa (Table 4.2). Under CT, the lowest bulk density was found at 0–5 cm depth, followed by 5–15, 15–30, and 30–60 cm depth (Table 4.3). No-tillage also promoted the lowest soil bulk density at 0–5 cm depth; however, the

bulk density at 15–30 cm depth did not differ significantly from at 5–15 and 30–60 cm depths (Table 4.3). Soil bulk density tends to increase with soil depth mainly due to a higher mass on deeper layers causing reduced porosity. The reduced differentiation of bulk density between soil layers under NT was due to the tendency of higher soil bulk density in the top 30 cm under NT compared to CT. However, a significantly higher bulk density value under NT than under CT was only found at the 5–15 cm depth for these soils (Table 4.3). These results are somewhat similar to the results after 13 years of NT management on a loam soil where Hernanz et al. (2002) found significantly higher bulk density under NT than under CT from 0 to 10 cm with cereal monoculture, and from 0 to 15 cm in a wheat (*Triticum aestivum* L.)-vetch (*Vicia sativa* L.) rotation. Conducting an experiment from 1991 to 2002 on a loamy sand soil, Dam et al. (2005) found higher bulk density under NT compared to CT at 0–10 cm depth in all years except 1995, and at 10–20 cm depth in 2000 and 2001 only. D’Haene et al. (2008) reported that under temperate climate conditions most researchers found a comparable or higher bulk density in the 0–5 cm soil layer under middle-term (< 11 years) conservation tillage fields with corn, wheat, and soybean (*Glycine max* L.), while under long-term conservation tillage the bulk density was comparable or lower than CT fields. The impact of reduction in tillage on total porosity may be influenced, in part, by the magnitude of axle load of equipment, timing of traffic, and degree of traffic control (Verhulst et al., 2010). The initial negative effect of NT on soil bulk density can be expected to be minimized in the long-term due to residue retention promoting improvement of topsoil SOM levels (D’Haene et al., 2008; Verhulst et al., 2010).

#### 4.3.2. Soil organic C and N concentration

The analysis of variance for SOC and TSN concentrations ( $\text{g kg}^{-1}$ ) had the tillage by depth interaction significant which leads to comparison between tillage managements within

fixed depth management and vice-versa (Table 4.2). However, for both fractions the analysis indicated that differences between depths at a fixed tillage management were the same, and that no difference between tillage management was found at any fixed soil depth (Table 4.4). In other words, the significant interaction for SOC and TSN concentrations in Table 2 did not involve any comparison of research interest. Thus, the analysis of SOC and TSN concentration could be based on the significant main effect depth pooled across tillage management (Table 4.4). Soil OC and TSN concentrations decreased significantly with depth (Table 4.4). The common higher concentration of SOM in the topsoil compared to the subsoil is due to the natural placement of organic materials (plant and animal residues, manure, and soil microbes) in the topsoil. Tillage management resulted in no significant higher SOC and TSN concentrations at 0–5 cm depth under NT, and higher SOC and TSN at 5–15 and 15–30 cm depth under CT, and higher SOC at 30–60 cm depth under CT (Table 4.4). For instance, SOC was 19% higher at 0–5 cm depth under NT than under CT, and 11, 30, and 11% higher at 5–15, 15–30, and 30–60 cm depth, respectively, under CT than under NT. The trend of higher SOM concentration with CT management below 5 cm might be the consequence of turning down crop residues during plowing as well as higher root deposition below upper layers.

The results discussed in the previous paragraph agree with those reported by Wright et al. (2005) who found, after 4 years of continuous corn, no significant effect of tillage on SOC and TSN concentrations measured at any soil depth; and significantly highest content of these fractions was found at 0–2.5 cm depth, followed by 2.5–7.5 and 7.5–20 cm depth. As in the present study, these authors only observed statistically no significant increase in SOC and TSN concentrations under NT than under CT at 0–2.5 cm depth, and under CT than under NT at 2.5–20 cm depth. It is particularly common to observe poor response of total SOC and TSN to tillage management in the short-term due to their characteristic turnover rate ranging

of a few years to centuries (Stevenson, 1994; Wander, 2004). On the other hand, Venterea et al. (2006) found significantly higher SOC and TSN concentrations at 0–5 cm depth under NT than under CT; and higher values under CT than under NT at depths below 20 cm for SOC, and below 30 cm for TSN after 15 years with a corn-soybean rotation. After 10 years conducting an experiment under corn-wheat-soybean rotation, Constantini et al. (2006) observed a tillage by depth interaction for SOC concentration with significantly higher surface (0–5 cm depth) accumulation of SOC under NT than under CT. These authors also observed a more homogeneous distribution of SOC through all depths under CT. In general, it would be expected to find more homogeneous distribution of SOM within the plowed layer under CT than under NT (Franzluebbers, 2004) due to the mix of plant residues and/or manure with soil in CT management.

There are three major reasons that could explain the lack of a tillage effect on SOC and TSN concentrations in the present study even in the topsoil layers (0–5 and 5–15 cm depth): First, the data presented here were not collected from a designed experiment, meaning no control on CT management procedures (time of the year applied, equipment, depth, and intensity) and no control of NT management so that all sites under this management would have same amount of years of conversion to NT. For instance, Gál et al. (2007) in a long-term designed experiment found that 10% higher SOC and TSN concentrations under NT than under CT at 5–15 cm depth was enough to observe statistically significant differences while in the present study, at the same depth, the 11 and 20% higher SOC and TSN concentrations, respectively, under CT than under NT were not significantly different. The second reason could be a higher crop yield and, hence, higher residue input under CT management compared to NT. Reduced crop yields under NT have been reported by several studies for at least the first few years of conversion (So et al., 2009; Venterea et al., 2006; Vetsch and Randall, 2000) with cool and wet conditions, high accumulation of crop residue, and poorly

drained soils being the most important limitations to yield in NT. However, the corn on most of these sites was harvested for silage, which results in a low amount of residue left on the field. This reduced amount of organic material would result in little change in SOM levels that could be detected in stable fractions such as SOC. The third reason is the history of manure, poultry litter, and/or biosolid application to supply crop nutrient (e.g. N) demand in all sampled sites. Manure is often presumed to result in large increases in SOM because it consists of relatively recalcitrant compounds with the most easily oxidized compounds of the original plant tissue being broken down by the digestive systems of animals before excretion (Magdoff and Weil, 2004; Paustian et al., 1997). These authors highlight that effects of manure application on SOM levels can persist for many years after applications have ceased. Thus, continuous application of these materials in the sampled sites could mask the losses of SOM caused by tillage procedures and buildup SOM to levels comparable to NT management. The rate of SOM accumulation is usually highest in the first 10 years of manure application and slowly decreases to a constant rate over time (Sommerfeldt et al., 1988).

#### *4.3.3. Soil organic C and N stock*

The analysis of variance for SOC and TSN stocks on an area basis ( $\text{Mg ha}^{-1}$ ) had tillage by depth interaction significant which leads to a comparison between tillage management within fixed depth and vice-versa (Table 4.2). Calculations of SOM stocks on area basis rather than on mass basis ( $\text{g kg}^{-1}$ ) are preferred in order to account for differences in soil bulk density (Ellert and Bettany, 1995). This can increase apparent mass of SOC and TSN (more mass of soil with higher bulk density) and accurately reflects the difference between tillage managements (Gál et al., 2007; Verhulst et al., 2010). Under CT management, SOC and TSN stocks were significantly higher at 5–15, 15–30, and 30–60 cm depth than at 0–5 cm depth (Table 4.5). Even with SOC and TSN concentrations decreasing

at fixed soil depths (Table 4.4), their stocks were higher below 15 cm due to an increased soil bulk density with depth (Table 4.3). Under NT management, SOC stock was significantly higher at 5–15 cm depth than the other depths, while TSN stock was significantly higher at 5–15 and 30–60 cm depth than 0–5 cm depth with an intermediate value at 15–30 cm depth (Table 4.5). The different SOC and TSN stocks distribution by depth between NT and CT resulted from the different soil bulk density distribution between these two managements (Table 4.3). Similarly as found for SOC and TSN concentrations, SOC and TSN stocks were not significantly affected by tillage management at any soil depth increment as well as to the entire sampled depth (0–60 cm) (Table 4.5).

Working with 11 Major Land Resource Areas (MLRA) in the Eastern USA, Blanco-Canqui and Lal (2008) found higher SOC stock under NT than under CT in 5 MLRAs within the top 10 cm and lower in 3 MLRAs below 10 cm. Analyzing these data at 0–60 cm depth, these authors found the same SOC stock between tillage methods in 8 MLRAs and higher SOC stock under CT than under NT in 3 MLRAs, where more intense cropping systems with higher C input in CT sites was the reason for higher SOC stocks under CT management. In an experiment conducted over 15 years, Venterea et al. (2006) observed significantly higher SOC and TSN stocks under NT compared to CT in the upper 20 to 30 cm and absence of difference between tillage management when considering the entire sampled depth (0–60 cm). These authors also observed that differences in 2000 (10<sup>th</sup> year) and 2005 (15<sup>th</sup> year) were only evident in the upper 10 cm. Additionally, there are other studies that employed sampling to depths below the plow layer and found the similar lack of tillage effect on SOC and TSN stocks (Carter, 2005; Deen and Kataki, 2003; Dolan et al., 2006). However, these results cannot be generalized due to other findings of higher SOC and TSN stocks under NT compared to CT even sampling below the plow layer (Calegari et al., 2008; Gál et al., 2007),

but with a considerably reduced difference between tillage management when compared to results limited to the upper 30 cm.

As previously noted, one probable reason for the lack of tillage effect on SOC and TSN stocks in the present study was the fact that its data were not collected from a designed experiment and, hence, did not have control on the tillage treatment factor (e.g. not having same amount of time of conversion from CT to NT). However, it was possible to observe no significant difference in SOC and TSN accumulation in response to tillage management. To make the most of the inherent source of variation in the data (time of conversion from CT to NT) and, in fact, to use it as an explanatory variable, stocks of SOC and TSN were investigated as a function of the duration of NT management through simple regression analysis. Through this analytical approach, SOC and TSN stocks proved to be significantly affected by the duration of NT at 0–5 cm depth with sequestration rates of  $0.44 \pm 0.08 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  and  $0.04 \pm 0.01 \text{ Mg N ha}^{-1} \text{ yr}^{-1}$ ; and at 0–15 cm depth with sequestration rates of  $0.59 \pm 0.14 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  and  $0.05 \pm 0.02 \text{ Mg N ha}^{-1} \text{ yr}^{-1}$  (Tables 4.6 and 4.7). The regression model developed for the top 5 cm for SOC had a coefficient of determination ( $R^2$ ) value of 33% while for the top 15 cm it was of 23%, meaning an increase of the proportion of unexplained variability in the data by increasing depth. These results agree with West and Post (2002) who, through an extensive literature review, reported that conversion from CT to NT can sequester an average of  $0.57 \pm 0.14 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  in an average soil depth of 22 cm. However, duration of NT had no significant effect on SOC and TSN stocks at 0–30 and 0–60 cm depth (Tables 4.6 and 4.7).

Even though NT management did not affect soil C and N stocks at 0–60 cm depth in the evaluated system, NT management is an agronomic technology that helps to improve soil quality by protecting the soil surface from rainfall impact, reducing water and wind erosion, and improving water infiltration and availability. In addition, NT management can reduce the

costs of production by using less fuel, machinery, and labor. It was not investigated here, but fuel consumption and any other tillage-related CO<sub>2</sub> emission activity could be estimated and used to refine comparisons between managements. The initial reduced yield compared to CT tends to vanish with time and be even higher under NT after a new equilibrium has been reached (So et al., 2009). Thus, its adoption must be advocated over CT in areas suitable for it as a continuous effort to achieve a more sustainable agriculture.

#### **4.4. Conclusions**

The tillage management conducted by farmers in the Valley region of Virginia led to no significant difference between CT and NT for soil organic C and total soil N stocks considering the entire sampled depth (0–60 cm). This same conclusion was reached by two different analytical approaches. In the first approach, the basic observational data were evaluated as an incomplete block design yielding no significant tendencies for the upper 30 cm that followed significant tendencies reported in other studies. The analysis of the entire sampled depth showed no clear trends in SOC and TSN stocks in response to tillage management (~2% difference for both fractions between tillage management) even if differences in the upper layers were found to be significant. In the second approach, the data were explored through regression analysis using the duration of NT management as the explanatory variable. In doing that, it was possible to observe significant increases in SOC and TSN stocks at 0–5 and 0–15 cm depth with increasing duration of NT. For instance, SOC stock increased at a rate of  $0.44 \pm 0.08$  and  $0.59 \pm 0.14$  Mg ha<sup>-1</sup> yr<sup>-1</sup> at 0–5 and 0–15 cm depth, respectively. However, duration of NT had no significant effect on C and N stocks when examined at 0–30 and 0–60 cm depth. Thus, NT management may not in fact improve SOC and TSN stocks compared with CT in the region, cropping systems, and time span

investigated; however, NT still plays a fundamental role in reducing erosion and improving water availability which contributes to achieving a more sustainable agriculture.

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**Table 4.1.** Soil series, classification, crop rotation, years of no-tillage management, and selected soil properties of sites sampled.

Site	Soil series <sup>a</sup>	Crop rotation <sup>b</sup>	Years of no-tillage	Soil organic C (g kg <sup>-1</sup> )				Bulk density (g cm <sup>-3</sup> )				pH <sup>c</sup>
				0–5 cm	5–15 cm	15–30 cm	30–60 cm	0–5 cm	5–15 cm	15–30 cm	30–60 cm	
1	Unison	CC	0	16.13	12.21	8.80	3.78	1.04	1.34	1.45	1.62	6.41
2	Chilhowie	CWS	8	25.99	13.51	7.48	5.57	1.09	1.40	1.47	1.40	6.29
3	Braddock	CC	10	26.79	12.48	4.83	2.42	1.18	1.52	1.53	1.54	5.96
4	Coursey	CWS	5	14.85	9.59	3.94	1.29	1.16	1.57	1.70	1.83	5.79
5	Alonzville	CC	0	12.46	11.52	6.51	2.43	0.88	1.13	1.45	1.65	6.82
6	Caverns	CWS	4	14.57	8.89	4.26	2.17	1.34	1.61	1.71	1.78	5.69
7	Coursey	CS	7	22.32	13.17	4.47	2.16	1.23	1.46	1.60	1.62	6.42
8	Frederick	CC	0	26.49	18.39	6.95	3.21	0.93	1.16	1.49	1.54	6.75
9	Frederick	CR	0	12.77	11.09	6.74	2.39	1.28	1.35	1.52	1.66	6.16
10	Poplimento	CC	0	20.16	13.69	8.58	4.40	1.24	1.30	1.41	1.43	6.71
11	Poplimento	CC	10	19.28	11.92	8.71	5.43	1.22	1.45	1.44	1.49	6.48
12	Poplimento	CC	9	19.42	9.23	4.79	2.26	1.23	1.53	1.58	1.64	6.84
13	Poplimento	CC	0	19.69	11.76	5.59	2.16	1.19	1.27	1.50	1.57	6.85

<sup>a</sup>Unison (loam, mixed, semiactive, mesic Typic Hapludults), Chilhowie (silty clay loam, mixed, semiactive, mesic Typic Hapludalfs), Braddock (loam, mixed, semiactive, mesic Typic Hapludults), Coursey (loam, siliceous, semiactive, mesic Aquic Hapludults), Alonzville (loam, siliceous, semiactive, mesic Typic Hapludults), Caverns (sandy loam, siliceous, semiactive, mesic Ultic Hapludalfs), Frederick (silt loam, mixed, semiactive, mesic Typic Paleudults), and Poplimento (silt loam, mixed, subactive, mesic Ultic Hapludalfs)

<sup>b</sup>CC [continuous corn (*Zea mays* L.)], CWS [corn-wheat (*Triticum aestivum* L.)-soybean (*Glycine max* L.)], CS (corn-soybean), and CR [corn-rye (*Secale cereale* L.)]

<sup>c</sup>pH measured on 1:1 soil/water ratio at 0–5 cm depth

**Table 4.2.** Analysis of variance's *F*-values for C and N levels and soil bulk density.

Source of Variation	-----Carbon-----		-----Nitrogen-----		Bulk Density
	g kg <sup>-1</sup>	Mg ha <sup>-1</sup>	g kg <sup>-1</sup>	Mg ha <sup>-1</sup>	g cm <sup>-3</sup>
Tillage (T)	1.46 <sup>ns</sup>	0.18 <sup>ns</sup>	0.56 <sup>ns</sup>	0.04 <sup>ns</sup>	7.98 <sup>**</sup>
Depth (D)	538 <sup>***</sup>	29.6 <sup>***</sup>	284 <sup>***</sup>	35.6 <sup>***</sup>	162 <sup>***</sup>
T*D	5.94 <sup>***</sup>	8.59 <sup>***</sup>	4.08 <sup>**</sup>	3.57 <sup>*</sup>	9.34 <sup>***</sup>

<sup>ns</sup>No significant, <sup>\*</sup>*P* < 0.05, <sup>\*\*</sup>*P* < 0.01, <sup>\*\*\*</sup>*P* < 0.001.

**Table 4.3.** Effect of tillage management and depth on soil bulk density.

Tillage Management	Depth (cm)			
	0–5	5–15	15–30	30–60
	-----g cm <sup>-3</sup> -----			
Conventional tillage	1.13 dA	1.28 cB	1.49 bA	1.62 aA
No-tillage	1.19 cA	1.48 bA	1.55 abA	1.60 aA

Means in a row followed by the same lowercase letter and in a column followed by the same uppercase letter are not significantly different by *t*-test ( $\alpha = 0.05$ )

**Table 4.4.** Effect of tillage management and depth on soil organic C and total soil N concentrations.

Tillage Management	Depth (cm)			
	0–5	5–15	15–30	30–60
	-----g C kg <sup>-1</sup> -----			
Conventional tillage	17.1 aA	12.6 bA	6.82 cA	2.67 dA
No-tillage	20.3 aA	11.3 bA	5.26 cA	2.41 dA
Pooled value	18.6 a	11.9 b	5.99 c	2.54 d
	-----g N kg <sup>-1</sup> -----			
Conventional tillage	1.90 aA	1.41 bA	0.74 cA	0.38 dA
No-tillage	2.08 aA	1.17 bA	0.60 cA	0.42 dA
Pooled value	1.99 a	1.29 b	0.67 c	0.40 d

Means in a row followed by the same lowercase letter and in a column followed by the same uppercase letter are not significantly different by *t*-test ( $\alpha = 0.05$ )

**Table 4.5.** Effect of tillage management and depth on soil organic C and total soil N stocks.

Tillage Management	Depth (cm)				Sum
	0–5	5–15	15–30	30–60	0–60 cm
	-----Mg C ha <sup>-1</sup> -----				
Conventional tillage	9.56 bA	16.4 aA	15.1 aA	13.2 aA	53.3 A
No-tillage	11.5 bA	16.6 aA	12.1 bA	11.5 bA	54.4 A
	-----Mg N ha <sup>-1</sup> -----				
Conventional tillage	1.03 bA	1.80 aA	1.64 aA	1.82 aA	6.42 A
No-tillage	1.23 cA	1.74 abA	1.39 bcA	1.96 aA	6.58 A

Means in a row followed by the same lowercase letter and in a column followed by the same uppercase letter are

not significantly different by *t*-test ( $\alpha = 0.05$ )

**Table 4.6.** Analysis of variance and parameter estimates of regression analysis used to estimate soil organic C stock (Mg ha<sup>-1</sup>) as function of duration of no-tillage (dNT) management at 0–5, 0–15, 0–30, and 0–60 cm depth.

0–5 cm	Source	<i>df</i> <sup>a</sup>	<i>SS</i> <sup>b</sup>	<i>MS</i> <sup>c</sup>	<i>F</i> -value	<i>Pr</i> > <i>F</i>	<i>R</i> <sup>2e</sup>
	Regression	1	201419	201419	28.99	<.0001	33
	Error	59	409902	6948			
	Total	60	611321				
	Parameter	<i>df</i>	Estimate	<i>SE</i> <sup>d</sup>	<i>t</i> -value	<i>Pr</i> > <i> t </i>	
	Intercept	1	8.85	0.48	18.30	<.0001	
	dNT	1	0.44	0.08	5.38	<.0001	
0–15 cm	Source	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i> -value	<i>Pr</i> > <i>F</i>	<i>R</i> <sup>2</sup>
	Regression	1	355704	355704	17.21	<.0001	23
	Error	59	1219546	20670			
	Total	60	1575250				
	Parameter	<i>df</i>	Estimate	<i>SE</i>	<i>t</i> -value	<i>Pr</i> > <i> t </i>	
	Intercept	1	24.55	0.83	29.42	<.0001	
	dNT	1	0.59	0.14	4.15	0.0001	
0–30 cm	Source	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i> -value	<i>Pr</i> > <i>F</i>	<i>R</i> <sup>2</sup>
	Regression	1	183237	183237	3.65	0.0608	6
	Error	59	2958854	50150			
	Total	60	3142091				
	Parameter	<i>df</i>	Estimate	<i>SE</i>	<i>t</i> -value	<i>Pr</i> > <i> t </i>	
	Intercept	1	39.08	1.30	30.07	<.0001	
	dNT	1	0.42	0.22	1.91	0.0608	
0–60 cm	Source	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i> -value	<i>Pr</i> > <i>F</i>	<i>R</i> <sup>2</sup>
	Regression	1	75561	75561	0.63	0.4323	1
	Error	59	7129783	120843			
	Total	60	7205344				
	Parameter	<i>df</i>	Estimate	<i>SE</i>	<i>t</i> -value	<i>Pr</i> > <i> t </i>	
	Intercept	1	53.43	1.96	27.33	<.0001	
	dNT	1	0.28	0.35	0.79	0.4323	

<sup>a</sup>Degrees of freedom

<sup>b</sup>Sum of squares

<sup>c</sup>Mean square

<sup>d</sup>Standard error

<sup>e</sup>Coefficient of determination in %

**Table 4.7.** Analysis of variance and parameter estimates of regression analysis used to estimate total soil N stock (Mg ha<sup>-1</sup>) as function of duration of no-tillage (dNT) management at 0–5, 0–15, 0–30, and 0–60 cm depth.

0–5 cm	Source	<i>df</i> <sup>a</sup>	<i>SS</i> <sup>b</sup>	<i>MS</i> <sup>c</sup>	<i>F</i> -value	<i>Pr</i> > <i>F</i>	<i>R</i> <sup>2e</sup>
	Regression	1	1722	1722	20.33	<.0001	26
	Error	59	5000	84.75			
	Total	60	6722				
	Parameter	<i>df</i>	Estimate	<i>SE</i> <sup>d</sup>	<i>t</i> -value	<i>Pr</i> > <i> t </i>	
	Intercept	1	0.94	0.05		<.0001	
	dNT	1	0.04	0.01		<.0001	
0–15 cm	Source	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i> -value	<i>Pr</i> > <i>F</i>	<i>R</i> <sup>2</sup>
	Regression	1	2474	2474	6.22	0.0154	9
	Error	60	23877	398			
	Total	61	26351				
	Parameter	<i>df</i>	Estimate	<i>SE</i>	<i>t</i> -value	<i>Pr</i> > <i> t </i>	
	Intercept	1	2.61	0.11	23.26	<.0001	
	dNT	1	0.05	0.02	2.49	0.0154	
0–30 cm	Source	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i> -value	<i>Pr</i> > <i>F</i>	<i>R</i> <sup>2</sup>
	Regression	1	95.04	95.04	0.013	0.7197	<1
	Error	57	41641	731			
	Total	58	41736				
	Parameter	<i>df</i>	Estimate	<i>SE</i>	<i>t</i> -value	<i>Pr</i> > <i> t </i>	
	Intercept	1	4.10	0.15	27.03	<.0001	
	dNT	1	0.01	0.03	0.36	0.7197	
0–60 cm	Source	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i> -value	<i>Pr</i> > <i>F</i>	<i>R</i> <sup>2</sup>
	Regression	1	341	341	0.19	0.6631	<1
	Error	56	99549	1778			
	Total	57	99890				
	Parameter	<i>df</i>	Estimate	<i>SE</i>	<i>t</i> -value	<i>Pr</i> > <i> t </i>	
	Intercept	1	5.93	0.23	25.42	<.0001	
	dNT	1	-0.02	0.04	-0.44	0.6631	

<sup>a</sup>Degrees of freedom

<sup>b</sup>Sum of squares

<sup>c</sup>Mean square

<sup>d</sup>Standard error

<sup>e</sup>Coefficient of determination in %

## V. Statistically Modeling Soil Available Nitrogen as Measured by Corn Uptake

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## Abstract

Nitrogen (N) has a complex biogeochemical cycle in soils making it one of the most difficult plant-essential elements to manage. Over 90% of N in soils is found in organic forms that are subject to mineralization, immobilization, nitrification, and denitrification. The multi-path soil N cycle has provided many challenges to scientists attempting to develop soil N availability indices, with most attempts being unsuccessful. The objectives of this study were: i) evaluate the relationship between indices of soil available N for representative soils of the Valley region of Virginia; and ii) analyze the performance of multiple regression models, which utilize variables associated with soil N pools with varying turnover rates for predicting soil N availability as measured by corn (*Zea mays* L.) N uptake. Soil samples were collected from small plots at 0–60 cm depth before corn was planted at 11 sites located in the Valley region of Virginia. Corn was planted and grown following farmer's schedule and practices, with the exception that no N fertilizer was applied. Soil N determinations were total soil N by dry combustion, exchangeable inorganic N [nitrate (NO<sub>3</sub>-N) and ammonium (NH<sub>4</sub>-N)] by room temperature KCl, soluble and exchangeable NH<sub>4</sub>-N by hot-KCl (HKCl), amino sugar N by Illinois soil N test (ISNT), NH<sub>4</sub>-N produced during 7 days of anaerobic incubation (ANI), inorganic N produced during 56 days of aerobic incubation (AI), and N present in the soil as particulate organic matter (POM). Whole-corn plant samples were collected at R4–R5 growth stage from the plots that did not receive N, dry matter weights determined, and corn tissue N content determined by dry combustion; which allowed calculation of corn total above-ground N uptake. Soil N fractions were highly correlated, and KCl, HKCl, ISNT, ANI, AI, and POM represented 0.3–1.7, 0.3–1.7, 5.0–16.7, 1.2–5.0, 0.9–4.6, and 4.4–35.7% of total soil N. The qualitative variable manure was included in the model with and without interaction with other variables. Full tentative models including soil N fractions and soil clay content (total of 16 regressors) were initially fitted for 0–30 and 0–60 cm depths. Among the

65,536 models developed for each depth, the top 5 models per depth were selected using adjusted coefficient of determination ( $R^2_{Adj}$ ), Mallows'  $C_p$  statistic, and predicted residual sum of squares (*PRESS*) statistic as criteria. The final model selected for 0–30 and 0–60 cm had 6 regressors representing the different SOM pools plus soil clay content, and  $R^2$  values of 65 and 68%, respectively. However, the model developed for the top 30 cm would be the recommended model to predict whole-corn plant N uptake due to its reasonably good prediction power and absence of problems associated with multicollinearity.

## 5.1. Introduction

Nitrogen (N) has a complex biogeochemical cycle in soils making it one of the most difficult plant-essential elements to manage. Most top soils contain 0.06 to 0.3% of N, and over 90% of it occurs in organic forms (Stevenson, 1994), with the rest being present in inorganic forms such as nitrate ( $\text{NO}_3\text{-N}$ ) and ammonium ( $\text{NH}_4\text{-N}$ ). The organic and inorganic forms of N participate in the soil N cycle process of mineralization, immobilization, nitrification, and denitrification that are carried by the soil microbial community. Besides these microbial-dependent processes, the N cycle is affected by  $\text{NO}_3\text{-N}$  leaching,  $\text{NH}_4\text{-N}$  fixation, and ammonia ( $\text{NH}_3\text{-N}$ ) volatilization. This complex microbial driven soil N cycle makes development of routine laboratory N availability indices difficult. This situation contrasts sharply with nutrients that have successful laboratory-test procedures such as other macro and micronutrients that have limited mobility in soils and whose plant availability is largely dictated by inorganic chemical reactions.

Currently, fertilizer N recommendations for nonlegume crops such as corn (*Zea mays* L.) rely on expected yields, with adjustments to account for other N inputs such as legume residues, manures, biosolids, and soil organic matter mineralization (Khan et al., 2001; Mulvaney et al., 2006; Spargo et al., 2009). However, this method does not account for

variations in corn yield response due to soil organic matter (SOM) quantity and quality, and weather and soil spatial variability (Agehara and Warncke, 2005; Mulvaney et al., 2006). Thus, an inaccurate yield-based N recommendation can result in either under- or over-fertilization in any given growing season. Insufficient N application can have economic consequences for farmers, whereas excessive fertilization increases the risk of environmental problems such as NO<sub>3</sub>-N leaching that can increase groundwater NO<sub>3</sub>-N concentrations (Ersahin, 2001; Zhu et al., 2003) and nitrous oxide (N<sub>2</sub>O) emissions that can affect the rate of global warming (Ishikawa et al., 2003; Johnson et al., 2007).

Nitrogen mineralized from SOM is a major component of soil N supply in agricultural systems (Sharifi et al., 2008). For instance, the N content in SOM can range from less than 0.5% to more than 6%, depending on biotic and abiotic ecosystem properties such as climate, soil depth, annual input of organic materials, and soil mineralogy (Hassink, 1997). If 1 to 3% of this organic N is mineralized annually, 8 to 120 kg N ha<sup>-1</sup> are released in a plant-available form (Bundy and Meisinger, 1994). In the past half century, many attempts have been made to develop chemical and biological methods able to predict plant available N during a growing season (Gianello and Bremner, 1986; Khan et al., 2001; Mulvaney et al., 2001; Serna and Pomares, 1992). Stanford and Smith (1972) developed a biological method that utilized long-term (30 weeks) aerobic incubation where the potentially mineralizable N (N<sub>0</sub>) was determined with intermittent leaching of inorganic N. This incubation is generally recognized as the best estimator of the mineralizable (active) N pool; however, it is time consuming and not practical for routine use (Sharifi et al., 2007). Other aerobic and anaerobic short-term incubation procedures have been developed but with sometimes conflicting predictions of N<sub>0</sub> (Curtin and Campbell, 2008). Consequently, the search for the 'holy grail' of fast and reliable chemical methods for predicting N<sub>0</sub> has been disappointing; although soil scientists have pursued this goal for many years. Chemical N availability indices should not

only detect intrinsic organic-N differences among soils or treatments that relate to the quantities of N likely to mineralize, but also reflect the likely influence of environmental conditions on the rate of N mineralization (Bundy and Meisinger, 1994). The 1984 summary statement (Meisinger, 1984), that there has been virtually no acceptance of chemical methods into routine soil N evaluation programs, still holds true today, as supported by the recent studies of Mulvaney et al. (2006), Picone et al. (2002), Schomberg et al. (2009), and Spargo et al. (2009).

The lack of an accurate and rapid method in predicting growing season soil available N to corn from organic sources has made measuring residual soil NO<sub>3</sub>-N the most popular test method for identifying soils where yield response to fertilize N may be limited (Spargo and Alley, 2008). Two soil NO<sub>3</sub>-N tests with different time and depth of sampling have been developed. The preplant NO<sub>3</sub>-N test (PPNT) has been recommended for semi-arid areas and consist of collecting soil samples in the early spring at 0–60 or 0–90 cm depth, and measuring NO<sub>3</sub>-N extracted by 2 mol L<sup>-1</sup> KCl (Bundy and Meisinger, 1994; Khan et al., 2001). The other soil inorganic N test, the pre-sidedress NO<sub>3</sub>-N test (PSNT), was proposed by Magdoff et al., (1984) for use in the humid eastern areas of the USA. The PSNT measures gains in available N due to mineralization of SOM, crop residues, including those from previous legumes, and manures or other organic N sources (Bundy and Meisinger, 1994). The PSNT is currently recommended in Virginia to indentify soils where yield response to fertilizer N may be limited (Evanylo and Alley, 1997). However, the use of PSNT has been limited due to the need to collect soil samples during the growing season at 0–30 cm depth, and by the fact that N fertilization is postponed until after testing. Besides logistical problems, the PSNT is also limited by the inherent variability of soil NO<sub>3</sub>-N due to factors such as the spatial variability of NO<sub>3</sub>-N leaching and denitrification, non-uniform application of sources like manure, and

season-to-season variability in rainfall and temperature (Ersahin, 2001; Khan et al., 2001; Stenger et al., 2002).

Meisinger (1984) suggested that improvements in N evaluation systems would most likely occur by putting more of the “pieces of the puzzle together”, rather than through large single discoveries. This statement is directly linked to the fact that most of soil N (> 90%) is found in organic forms; and that SOM consists of functional pools with different turnover rates that are termed active, intermediate, and stable (von Lützow et al., 2007; Wander, 2004). Typically, the turnover rates of soil fractions belonging to the active pool is of days to a few years, to the intermediate pool is of several years to decades, and to the stable pool is of centuries (Stevenson, 1994). Thus, most of N released during SOM mineralization is provided from fractions belonging to the active pools; however, the role of the other pools, mainly intermediate, cannot be neglected (Seiter and Horwath, 2004). Given the complexity of the soil N cycle and the variability of soil N pools due to a myriad of factors between specific sites, it was chosen to investigate the feasibility of evaluating plant available N on a field specific basis by considering a combination of different soil N fractions.

Therefore, the objectives of this study were: i) evaluate the relationship between widely reported indices of soil available N for representative soils of the Valley region of Virginia; and ii) analyze the performance of multiple regression models including soil N fractions to predict soil available N as measured by whole-corn plant N uptake.

## **5.2. Materials and Methods**

### *5.2.1. Site description*

Soil samples were collected in early April of 2009 and 2010, before corn was planted, from 11 commercial farms located in the Valley region of Virginia (Table 5.1). Sites 1–6 were sampled in 2009 while site 7–11 were sampled in 2010. Each site was under continuous

no-tillage management for at least 5 years. In each site, four small plots were identified, all next to each other and within the same soil type in the field. The plot size varied between sites depending on the type of planter used (6 or 8 rows); thus, plot sizes of 4.5 m by 9 m (6 rows) and 6 m by 9 m (8 rows) were obtained. In each plot, corn was planted and managed with the farmer's schedule and practices, with the exception that no N source (organic and inorganic) was applied either in the fall of the previous year or in the current growing season year. Thus, within the plots the only limiting factor imposed for corn growth was N.

### *5.2.2. Soil sampling and analyses*

The sampling in each plot consisted of collecting three soil cores at the 0–60 cm depth with a hydraulic soil sampler (3.2 cm diameter soil probe) connected to a tractor. Individual core samples were then divided at depths of 0–30 and 30–60 cm and transferred to labeled plastic bags. Before the samples were taken, surface litter was removed. Coordinate positions of each sample core were recorded using a GPSMAP 76S (Garmin Ltd., Olathe, KS). The plastic bags containing the soil samples were kept in a container with ice until transferred to the lab, which took less than 8 h. On returning to the lab samples were rapidly air-dried. Subsamples from each sample were collected in duplicate and their weight (~10 g) recorded before and after placing the sample into an oven at 105°C to determine water content (Gardner, 1986). Bulk density was determined by dividing the weight of soil inside the core on an oven-dry basis by the volume of soil (Blake and Hartge, 1986). After the bulk density determination, subsamples from the original soil samples were collected, mixed, and passed through a 4.76 mm sieve to form composite samples of 0–30 and 30–60 cm depth per plot. Composite samples were formed by mixing equal volume subsamples. The water content of the composite was determined as described above. The soil left after subsampling was ground to pass a 2 mm sieve, and then subsampled to form composite samples of 0–30 and 30–60 cm

depth per plot. Composite samples were formed by mixing equal volume subsamples. All the measurements described below were conducted in duplicate from the composite samples.

Soil pH was measured on 1:1 soil/water ratio after 30 min equilibration. Total soil C and N were determined by dry combustion (VarioMax CNS macro elemental analyzer, Elementar, Hanau, Germany). Soil phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and micronutrients were extracted with Melhich-1, and the elements in the extract were determined by Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) (SPECTRO ARCOS, SPECTRO Analytical Instruments GmbH, Kleve, Germany). For initial soil  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$ , 2 g of air-dried soil ground to pass a 2 mm sieve were placed into 50 mL centrifuge tubes and shaken for 1 h on a reciprocal shaker with 20 mL of 2 mol  $\text{L}^{-1}$  KCl; samples were filtered, and  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  concentrations in the extract were determined colorimetrically using a QuickChem automated ion analyzer (Lachat Instruments, Milwaukee, WI). All colorimetric determinations of  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  in the present study used this equipment.

The chemical method hot-KCl (HKCl) for soluble and exchangeable  $\text{NH}_4\text{-N}$  was determined by heating 3 g of air-dried soil ground to pass a 2 mm sieve with 20 mL of 2 mol  $\text{L}^{-1}$  KCl on a digestion block at 100°C for 4 h (Gianello and Bremner, 1986). After cooling, the suspension was mixed with a Vortex and filtered, and  $\text{NH}_4\text{-N}$  concentration in the extract determined colorimetrically. The  $\text{NH}_4\text{-N}$  produced by heating the soil with 2 mol  $\text{L}^{-1}$  KCl was calculated by subtracting from it the initial  $\text{NH}_4\text{-N}$  described above. Part of the  $\text{NH}_4\text{-N}$  determined in this procedure may also originate from compounds released from lyses of microbial cells (Sharifi et al., 2007).

The chemical method Illinois soil N test (ISNT) for amino sugar N was determined using the direct diffusion method developed by Khan et al. (2001) and modified by Spargo and Alley (2008). Briefly, 1 g of air-dried soil ground to pass a 2 mm sieve was weighed into

Ball jars and spread evenly around the bottom before adding 10 mL of 2 mol L<sup>-1</sup> NaOH. Immediately following the addition of NaOH solution, the jars were fitted with the lid and petri dish apparatus containing 5 mL of 0.647 mol L<sup>-1</sup> (4% w/w) H<sub>3</sub>BO<sub>3</sub>, and tightly secured with a metal screw band. Sealed jars were heated for 15 h in a Precision 815 low temperature incubator (Precision, Winchester, VA) at 50°C to release alkali hydrolysable N as gas NH<sub>3</sub>-N. The released NH<sub>3</sub>-N was collected in the H<sub>3</sub>BO<sub>3</sub> solution in the petri dish suspended above the hydrolysate in each jar. The ISNT N was determined by acidimetric titration of the H<sub>3</sub>BO<sub>3</sub> solution with standardized 0.01 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> using a Radiometer TIM 900 Titration Manager and ABU 901 autoburette (Radiometer Analytical S.A., Lyon, France). The soil N (mg N kg<sup>-1</sup>) was determined as  $ST$ , where  $S$  is milliliter of titrant and  $T$  is the titer (μg N mL<sup>-1</sup>) of the standardized H<sub>2</sub>SO<sub>4</sub>.

The biological method short-term aerobic incubation (AI) followed the procedure described by Curtin and Campbell (2008). Air-dried soil ground to pass a 4.76 mm sieve was weighed (equivalent to about 5 g of dry soil) into 125 mL polypropylene containers. Water was added to adjust the soil water content so that it was equivalent to -10 kPa that is a laboratory approximation to the field capacity water content. Containers were covered with polyethylene film held in place with rubber bands and placed in plastic trays containing water and enclosed in large polyethylene bags to maintain high humidity. Containers were incubated for 56 days in a Precision 815 low temperature incubator (Precision, Winchester, VA) at 25°C. Trays were opened twice a week for ventilation, and their positions in the incubator were randomly changed. After 56 days, the containers were shaken for 1h on a reciprocal shaker with 50 mL of 2 mol L<sup>-1</sup> KCL, samples were filtered, and NO<sub>3</sub>-N and NH<sub>4</sub>-N concentrations in the extract determined colorimetrically. Mineralized N was calculated by subtracting the initial NO<sub>3</sub>-N and NH<sub>4</sub>-N from the NO<sub>3</sub>-N and NH<sub>4</sub>-N at the end of incubation.

The biological method short-term anaerobic incubation (ANI) also followed the procedure described by Curtin and Campbell (2008). Air-dried soil ground to pass a 4.76 mm sieve was weighed (equivalent to about 5 g of dry soil) into a 100 mL plastic centrifuge tube. Ten milliliters of distilled water were added to submerge the soil, tubes were stopped, and incubated for 7 days in a Precision 815 low temperature incubator (Precision, Winchester, VA) at 40°C. After 7 days the tubes were removed from the incubator and 40 mL of 2.5 mol L<sup>-1</sup> KCl added. Tubes were shaken for 1 h on a reciprocal shaker, samples were filtered, and NH<sub>4</sub>-N concentration in the extract was determined colorimetrically. Mineralized N was calculated by subtracting the initial NH<sub>4</sub>-N from the NH<sub>4</sub>-N at the end of incubation.

The physical-based procedure used to recover particulate organic matter (POM) is described by Gregorich and Beare (2008). Twenty five grams of air-dried soil ground to pass a 2 mm sieve were dispersed for 12 h in 100 mL of 0.0082 mol L<sup>-1</sup> sodium hexametaphosphate solution [5 g L<sup>-1</sup> (NaPO<sub>3</sub>)<sub>6</sub>] on a reciprocal shaker at 180 rpm. The suspension was poured onto a 53 µm sieve and rinsed with deionized water until clay- and silt-size fractions were completely removed. The retained sand plus organic material was dried on the sieve at 60°C for 1 h, transferred into a weighed aluminum tin and dried at 60°C for 24 h, and weighed. The dried material was ground using a mortar and pestle, and total organic C and N concentrations determined by dry combustion.

### *5.2.3. Corn plant sampling and analyses*

Whole-corn plant samples were collected at growth stages R4–R5 (ISUE, 2008) from rows 3 and 4 in the 6 rows plots, and from rows 4 and 5 in the 8 rows plots. From each row, whole-corn plants were collected from the center 1.5 m row length and kept in separate bundles. For each plot, plants coming from the same bundle were weighed, counted, and ground together to form one composite sample. A representative subsample was taken from

each composite sample, weighed, and transferred to labeled paper bags. Before grinding, whole-corn samples were checked for soil residues. Samples in paper bags were dried at 60°C until a constant weight, weighed, and ground.

Total N content in tissue samples was determined by dry combustion (VarioMax CNS macro elemental analyzer, Elementar, Hanau, Germany). Plant P, K, Ca, Mg, and zinc (Zn) were extracted by microwave digestion (MARS Xpress, CEM, Matthews, USA) and determined with ICP-AES (SPECTRO ARCOS, SPECTRO Analytical Instruments GmbH, Kleve, Germany).

#### *5.2.4. Statistical analysis*

Correlation analysis between soil N fractions was performed using PROC CORR procedure of SAS software (SAS, 2008). Multiple regression models were fitted to relate soil available N as measured by corn plant uptake with soil N estimated by different methods. Regression analyses were performed with PROC REG procedure of SAS software (SAS, 2008). In the regression analysis, the response variable ( $y$ ) was the whole-corn plant N uptake expressed in  $\text{kg ha}^{-1}$  while the regressors were total soil N ( $x_1$ ),  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  extracted by  $2 \text{ mol L}^{-1}$  KCl ( $x_2$ ),  $\text{NH}_4\text{-N}$  extracted by  $2 \text{ mol L}^{-1}$  HKCl ( $x_3$ ), amino sugar N determined by ISNT ( $x_4$ ),  $\text{NH}_4\text{-N}$  produced during 7 days of ANI ( $x_5$ ),  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  produced during 56 days of AI ( $x_6$ ), and N present in the soil as POM ( $x_7$ ). All soil N fractions were expressed in  $\text{mg kg}^{-1}$ . Two major models were fitted, one with regressor values coming from 0–30 cm depth samples and one with regressors values coming from 0–60 cm depth samples.

Some sites had history of manure application while others had not received manure (Table 5.1); thus, a variable dummy ( $x_8$ ) was included in the model to account for the possible effect of this qualitative variable. The dummy variable had value equal to ‘0’ for sites with history of manure application and the value equal to ‘1’ for sites with no manure history.

Given that manure application could affect the seven soil N fractions cited above, models included interactions between the dummy variable and the soil N fractions. Thus, seven extras regressors were included in the model:  $x_9$  ( $x_1x_8$ ),  $x_{10}$  ( $x_2x_8$ ),  $x_{11}$  ( $x_3x_8$ ),  $x_{12}$  ( $x_4x_8$ ),  $x_{13}$  ( $x_5x_8$ ),  $x_{14}$  ( $x_6x_8$ ), and  $x_{15}$  ( $x_7x_8$ ). In addition, soil clay content ( $\text{g kg}^{-1}$ ) was included in the model ( $x_{16}$ ) to account for a significant soil property other than soil N fractions. Soil clay content was chosen due to its effect on SOM accumulation, water retention, and soil microbial activity that can be influential on soil N mineralization rates (Müller and Höper, 2004). Diagnostic statistical procedures for assumptions and outliers were performed in the written order. For each depth evaluated (0–30 and 0–60 cm), model selection was done following the algorithm proposed by Montgomery et al. (2006) where the coefficient of determination ( $R^2_{Adj}$ ), Mallows'  $C_p$  statistic, and predicted residual sum of squares ( $PRESS$ ) statistic were used as criteria to rank the models. In comparing subset models of a full model, the subset model with highest  $R^2_{Adj}$  is preferred, while small values of  $C_p$  and  $PRESS$  statistic are desired (Montgomery et al., 2006).

### **5.3. Results and Discussion**

#### *5.3.1. Size of N fractions and their relationship*

The mean, standard deviation, and range of values of whole-corn plant N uptake and soil N fractions measured are shown in Table 5.2. The representation of total soil N by each soil N fraction was very similar between the two analyzed depths. The soil N contents estimated by KCl and HKCl were 4.0–33.5 and 4.0–25.3  $\text{mg kg}^{-1}$ , respectively, which gives a proportional range of 0.3–1.7% of both fractions in representing total soil N, agreeing with other finding (Schomberg et al., 2009; Sharifi et al., 2007). Thus, around 99% of total N in the studied soils was found in organic forms, assuming negligible clay-fixed  $\text{NH}_4\text{-N}$ . The probable reason for such a small proportion of inorganic N ( $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$ ) as estimated

by KCl at room temperature is the sampling date (early April) that coincided with the end of a cold and wet winter in the region, leading to low SOM mineralization during the preceding winter months and potential leaching losses of NO<sub>3</sub>-N from the preceding growing season.

The ISNT represented 5.0–16.7% of total soil N, agreeing with the values reported by Sharifi et al. (2007) and Spargo et al. (2009). Amino sugars, which occur in soils as macropolysaccharides including chitin (Stevenson, 1994), have been related to bacterial and fungal biomass and can be used to estimate the contributions to biologically active pools (Sharifi et al., 2008). However, the persistence of elevated amino sugar levels in soils historically amended with manure suggest that, at a minimum, this fraction also includes components that might be more appropriately equated with the intermediate pool of SOM (Wander, 2004). Moreover, the high correlation of this fraction with total soil C and N indicates that ISNT extracts some recalcitrant forms of N (Marriott and Wander, 2006b; Spargo et al., 2009), bringing doubt on the usefulness of the ISNT method in estimating active organic N. The soil N contents estimated by ANI and AI were 19.8–80.4 and 15.0–79.4 mg kg<sup>-1</sup>, respectively. Thus, ANI and AI represented 1.2–5.0 and 0.9–4.6% of total soil N, respectively. Schomberg et al., (2009) working with soils from the Southeastern USA reported 4.3–116 mg N kg<sup>-1</sup> estimated with 7 days ANI, and suggested this procedure to be one of the best biological indicators of potentially soil available N. Working with Australian soils, Wang et al., (2001) reported that during 2 weeks of incubation 1.9–8.4 and 2.3–6.7% of total organic N was mineralized by ANI and AI, respectively. The problem encountered in the present work with short-term aerobic and anaerobic incubations was the lack of standard procedures in the literature with variations in soil/water ratio to incubate, time, temperature, and volume and molarity of KCl solution used in the end of the incubation to extract inorganic N. These variations can lead to differences not attributed to the tested experimental variables. The POM represented the largest portion of total soil N (4.4–35.7%), which was in

agreement with values reported by previous works (Carter et al., 2003; Gregorich et al., 2006; Sharifi et al., 2008; Wander, 2004). Like amino sugars, POM also suffers from multiple identities across SOM pools due to its heterogeneity of materials and perceived multifunctionality (Wander, 2004). In addition, Wander (2004) pointed out that even though there is abundant evidence that POM is biologically and chemically active, measures of POM are commonly used to estimate the size of intermediate mineralizable pool.

Correlation analysis between corn N uptake and contents of soil N fractions and soil clay is presented in Table 5.3. In general, the correlations between all soil N fractions were highly significant ( $P < 0.001$ ) at both analyzed depths. Grouping soils based on manure application history did not improve these relationships. Among the organic fractions, POM was most closely correlated with total soil N in both depths, while HKCl was least but still significantly correlated. These results do not agree with previous studies that have reported a closer relationship of HKCl, ISNT, and ANI than POM with total soil N (Marriott and Wander, 2006b; Schomberg et al., 2009; Sharifi et al., 2007). Such contradiction is probably related to the multi-identity pool of POM with this SOM fraction possibly estimating the intermediate pool of SOM in the present study and the active pool of SOM in the cited studies. Particulate OM consists mainly of partially decomposed plant residues, microbial residues, seeds, and spores forming organo-mineral complexes with soil mineral particles (Baisden et al., 2002). Thus, the above- and below-ground residues left by continuous corn (the predominant cropping system in sampled sites) with its high C:N ratio (~60:1) would generate POM less prone to decomposition than POM generated by residues of legumes, potato (*Solanum tuberosum* L.), cotton, (*Gossypium hirsutum* L.), and cereals that were the main crops in the cited studies.

The relationship of chemical (HKCl and ISNT) and biological methods (ANI and AI) with POM were reasonably strong in both depths but with wider correlation coefficients at 0–

30 cm ( $r = 0.50$  to  $0.78$ ) compared to 0–60 cm ( $r = 0.63$  to  $0.69$ ). In this case, the relationship of HKCl and AI with POM improved at 0–30 and 0–60 cm depth, respectively. In both depths, the correlation among biological methods was better than the correlation among chemical methods, with the relationship of biological methods with ISNT ( $r = 0.69$  to  $0.82$ ) better than those with HKCl ( $r = 0.27$  to  $0.69$ ). These results agree with Schomberg et al. (2009) who found biological methods generally having stronger relationship with each other than chemical methods. The better correlation of HKCl with ANI than with AI in the present study is not in agreement with the result reported by Wang et al., (2001) who found the opposite. The most probable reason for this disagreement is that the AI procedure employed by Wang et al., (2001) was conducted during 14 days with a fixed amount of water added to all soils samples, while in the present study the AI procedure was conducted during 56 days with water added to adjust soil water content to the field capacity for each individual soil. Thus, such differences in procedures may have resulted in the discrepancy between the present study and the work by Wang et al., (2001). The ANI procedures of both studies are not exactly the same but are not as much different as the AI procedures.

### 5.3.2. Modeling soil available N

For both evaluated soil depths (0–30 and 0–60 cm), the full multiple linear regression model initially built to predict soil available N as measured by corn plant N uptake was:

$$y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_4x_4 + \beta_5x_5 + \beta_6x_6 + \beta_7x_7 + \beta_8x_8 + \beta_9x_9 + \beta_{10}x_{10} + \beta_{11}x_{11} + \beta_{12}x_{12} + \beta_{13}x_{13} + \beta_{14}x_{14} + \beta_{15}x_{15} + \beta_{16}x_{16} + \varepsilon \quad (1)$$

where  $y$  denotes soil available N measured by whole-corn plant N uptake (dependent variable),  $x$ 's are regressors (independent variables) described in the materials and methods

section,  $\beta$ 's are the regression coefficients, and  $\varepsilon$  is the error term. The analysis of the model in Eq. (1) for both depths indicated that the assumption of constant variance was violated for  $x_1$ ,  $x_2$  and  $x_7$  at 0–30 cm depth, and for  $x_1$ ,  $x_2$ ,  $x_6$ , and  $x_7$  at 0–60 cm depth. This problem was solved by log transforming these regressors ( $x_n'$ ), where  $n$  is the regressor number. Thus, the full models in Eq. (2) and (3) were created to predict the studied relationship at 0–30 and 0–60 cm depths, respectively.

$$y = \beta_0 + \beta_1x_1' + \beta_2x_2' + \beta_3x_3 + \beta_4x_4 + \beta_5x_5 + \beta_6x_6 + \beta_7x_7' + \beta_8x_8 + \beta_9x_9 + \beta_{10}x_{10} + \beta_{11}x_{11} + \beta_{12}x_{12} + \beta_{13}x_{13} + \beta_{14}x_{14} + \beta_{15}x_{15} + \beta_{16}x_{16} + \varepsilon \quad (2)$$

$$y = \beta_0 + \beta_1x_1' + \beta_2x_2' + \beta_3x_3 + \beta_4x_4 + \beta_5x_5 + \beta_6x_6' + \beta_7x_7' + \beta_8x_8 + \beta_9x_9 + \beta_{10}x_{10} + \beta_{11}x_{11} + \beta_{12}x_{12} + \beta_{13}x_{13} + \beta_{14}x_{14} + \beta_{15}x_{15} + \beta_{16}x_{16} + \varepsilon \quad (3)$$

The analysis of variances of models in Eq. (2) and (3) are shown in Table 5.4. The significant  $F$ -value ( $P < 0.001$ ) indicates that at least one regressor was important in predicting soil available N in both equations. The coefficients of determination ( $R^2$ ) of Eq. (2) and (3) were 68 and 71%, respectively, which is reasonably good for a field observational experiment. However, both models had few significant regressors and problems of multicollinearity indicated by the regressor's variance inflation factor (VIF) values greater than 5 (Graham, 2003; Stine, 1995). Problems of multicollinearity are common in multiple regression analyses of observational experiments using explanatory variables (regressors) that are correlated to each other, causing poor estimation of related regression coefficients (Montgomery et al., 2006). Huang and Liu (1994) stated that after the tentative full models are defined, it is necessary to achieve parsimony through model selection. In other words, it is desirable to select only important (significant) explanatory variables to be included in the

model. Thus, model selection was performed on all possible regressions to overcome or minimize the problems found in the proposed models shown in Eq. (2) and (3). In this procedure, if there are  $K$  candidate regressors, there are  $2^K$  total equations to be estimated and examined assuming that the intercept is included in all equations. Thus, 65,536 regression equations were estimated for each depth [16 regressors in Eq. (2) and (3)].

In the first step of model selection, the top 5 models estimated for each depth were selected using  $R^2_{Adj}$ , Mallows'  $C_p$  statistic, and  $PRESS$  statistic as criteria (Table 5.5). Montgomery et al. (2006) explain that if  $s$  regressors are added to the model,  $R^2_{Adj, p+s}$  will exceed  $R^2_{Adj, p}$  if and only if the partial  $F$  statistic for testing the significance of the  $s$  additional regressors exceeds 1, where  $p$  represents the number of coefficients in the model. They also state that low  $C_p$  and  $PRESS$  statistic values represent models with minimized bias and mean square error of prediction, respectively. Since the  $C_p$  statistic is based on least square estimation, it is very sensitive to outliers and other departures from normality assumption on the error distribution (Çetin and Erar, 2006). These selection criteria are generally more efficient than *Stepwise*, *Backward*, and *Forward* procedures (Pacheco et al., 2009).

In general, equations estimated for both depths had the same number of regressors included, with variables  $x_7'$ ,  $x_9$ ,  $x_{12}$ , and  $x_{16}$  being almost always present (Table 5.5). The general trend observed in these subset models was of small  $C_p$  values related to models with reduced number of regressors and similar  $R^2_{Adj}$  values between models. The next step, following the algorithm proposed by Montgomery et al., (2006), was to perform a thorough analyses on all the selected models presented in Table 5.5. The thorough analyses included diagnostics for assumptions and outliers. All models for both depths had significant  $F$ -values of analysis of variance ( $P < 0.001$ ); however, many of these models did not have all regressors with significant coefficients ( $P < 0.05$ ). Thus, the presence of all regressors with

significant coefficients in the subset model was used as model selection criterion in addition to the criteria mentioned above. The final model selected for each depth is presented in Table 5.6. Models in Table 5.6 can be written as Eq. (4) (0–30 cm depth) and Eq. (5) (0–60 cm depth):

$$\hat{y} = -85.2 - 0.29x_4 + 1.06x_6 + 95.1 x_7' + 0.03x_9 - 0.35x_{12} + 0.71x_{16} \quad (4)$$

$$\hat{y} = -117 + 99.6x_7' + 0.03x_9 + 7.48x_{10} - 1.03x_{12} + 2.40x_{14} + 0.67x_{16} \quad (5)$$

The negative intercepts of Eq. (4) and (5) were caused by the large coefficient of the log-transformed  $x_7'$  regressor and thus has no biological meaning since  $x_7'$  would have value equal to zero if  $x_7$  (POM) was equal to one. The coefficient of regressors  $x_6$  (AI), and  $x_7'$  (log-transformed POM) in Eq. (4), and  $x_7'$  in Eq. (5) had positive signs agreeing with the positive correlations between these organic N fractions with corn N uptake (Table 5.3). On the other hand, the negative coefficient sign of  $x_4$  (ISNT) in Eq. (4) did not agree with the positive correlations of this N fraction with corn N uptake, even though correlations were not highly significant (0.28,  $P < 0.01$  and 0.25,  $P < 0.05$ ). The positive coefficient sign of  $x_9$  (interaction of SON with dummy variable manure) in Eq. (4), and  $x_9$ ,  $x_{10}$  (interaction of KCl with dummy variable manure), and  $x_{14}$  (interaction of AI with dummy variable manure) in Eq. (5) means increases of soil available N as estimated by these fractions with no manure applied compared to manure applied. In contrast, the negative coefficient sign of  $x_{12}$  (interaction of ISNT with dummy variable manure) in Eq. (4) and (5) means reduction of soil available N in the order of 0.35 and 1.03 mg per kg of soil with no manure applied compared to manure applied, respectively. The increased soil available N as measured for certain fractions with no manure applied compared to manure applied can probably be related to a lack of N

enrichment derived from manure into these soil N fractions compared to other fractions such as ISNT. Finally, the coefficient of regressor  $x_{16}$  (clay content in  $\text{g kg}^{-1}$ ) had a positive sign in both equations meaning an increase of soil available N in the order of 0.71 and 0.67 mg per kg of soil by increasing one unit of soil clay content in Eq. (4) and (5), respectively. The clay content effect on these models agrees with the positive correlation between this soil property with corn N uptake (Table 5.3). However, the lack of a significant correlation between clay content and any soil N fraction that could support the effect of this physical soil property on soil available N indicates that perhaps the effect of clay content could be associated with plant available moisture and thus increased N uptake due to increased corn growth.

Models in Eq. (4) and (5) had  $R^2$  values of 65 and 68%, respectively. Previous works have found models with similar power to explain soil available N through the relationship between plant uptake and soil N fractions; however, such works consisted in planned experiments conducted in greenhouse environments (Chalk and Waring, 1970; Gallagher and Bartholomew, 1964; Smith and Li, 1993). In addition, these works had fractions representing the same SOM pool when including multiple fractions in the model and they did not have any established criteria for selecting and recommending a final model. The model in Eq. (4) (0–30 cm depth, Table 5.6) did not present problems of multicollinearity and included four soil N fractions (total soil N, ISNT, AI, and POM) plus soil clay content. On the other hand, the model in Eq. (5) (0–60 cm depth, Table 5.6) had problems of multicollinearity and included five soil N fractions (total soil N, KCl, ISNT, AI, and POM) plus clay content. Thus, the model in Eq. (4) would be the recommended model to predict whole-corn plant N uptake as a function of selected soil N fractions and soil clay content in the Valley region of Virginia. This model contains as regressors total soil N representing the stable pool of SOM; ISNT and POM with their multi-identity in representing both the active and intermediate pools of SOM; and AI representing the active mineralizable pool of SOM.

## **5.4. Conclusion**

Inorganic N represented a small portion of the total soil N measured in the soils used in the present study. Total soil N measured at 0–30 and 0–60 cm depth was of 823–2784 and 1140–4153 mg kg<sup>-1</sup>, respectively, with approximately 99% of it being represented by its organic form at both depths. This number highlights the importance of SOM on soil N cycling. Between the organic fractions evaluated, POM represented the largest portion of total soil N bringing even more attention to this fraction that has been widely used as an indicator of soil quality.

Literature review has indicated that soil scientists have mainly focused on the relationship between a single soil N index and plant N uptake, with less attention given to the relationship between multiple soil N fractions and plant N uptake. The inclusion of soil N fractions representing different SOM pools would have the advantage of accounting for their different turnover rates, being thus more accurate in estimating potentially mineralizable N. The model selection procedure should be based on statistical criteria and on researcher's knowledge regarding the tested relationships to increase the chances of selecting a useful model. The final model selected contained four soil N fractions representing the different SOM pools and, hence, supporting the idea that a proximal-accurate estimation of soil available N through SOM mineralization with a single lab routine index is probably unrealistic.

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**Table 5.1.** Location, soil classification, and selected chemical and physical properties for the sites used in the corn N availability study.

Site	Soil Classification	Crop Rotation	Depth	Total C	Total N	pH	Sand	Silt	Clay	Manure	P	K	Ca	Mg
			cm	g kg <sup>-1</sup>	mg kg <sup>-1</sup>	(H <sub>2</sub> O)	-----g kg <sup>-1</sup> -----				-----mg kg <sup>-1</sup> -----			
1	Aquic Fragiudults	CC <sup>a</sup>	0–30	9.4	1006	6.34	273	604	123	Yes	35.5	84.3	812	106
			30–60	2.9	360	5.49	275	544	181		5.1	27.0	685	108
2	Typic Hapludults	CC	0–30	19.7	2513	7.29	249	619	132	Yes	48.6	252	2417	239
			30–60	5.4	1136	5.79	275	507	218		7.0	55.6	1305	192
3	Typic Paleudults	CB <sup>b</sup>	0–30	14.9	1454	6.82	304	664	32	Yes	68.8	110	1179	198
			30–60	7.0	601	6.76	239	681	80		13.6	59.8	613	115
4	Typic Hapludalfs	CCA <sup>c</sup>	0–30	14.5	2097	5.83	125	611	264	Yes	23.9	102	1766	179
			30–60	4.1	1379	5.50	97	437	466		6.1	53.7	1651	201
5	Typic Paleudults	CWV <sup>d</sup>	0–30	11.9	1129	6.45	172	640	188	Yes	39.6	63.8	830	267
			30–60	5.1	555	5.83	66	472	462		3.0	31.2	688	193
6	Typic Paleudults	CW <sup>e</sup>	0–30	14.8	1458	6.17	313	587	100	Yes	14.0	49.5	797	220
			30–60	2.1	338	5.84	177	447	376		1.9	24.7	732	187
7	Aquic Hapludalfs	CC	0–30	13.5	1310	6.71	379	548	73	No	15.5	83.9	1084	191
			30–60	3.3	505	6.63	337	478	185		14.3	52.5	714	190
8	Ultic Hapludalfs	CC	0–30	13.6	1295	6.63	399	562	39	No	16.3	66.5	711	137
			30–60	2.5	284	6.59	344	546	110		16.3	67.7	589	112
9	Typic Hapludults	CC	0–30	10.8	1150	6.76	222	429	349	No	46.7	124	967	202
			30–60	2.5	321	6.58	154	426	420		3.8	65.4	817	203
10	Typic Hapludults	CSW <sup>f</sup>	0–30	12.5	1155	6.38	263	536	201	No	18.8	75.5	667	160
			30–60	2.1	213	5.75	189	497	314		1.9	36.0	382	136
11	Typic Hapludults	CSW	0–30	12.1	1211	6.97	205	559	236	Yes	26.0	205	987	202
			30–60	3.0	339	6.92	111	426	463		3.1	118	713	220

<sup>a</sup>Continuous corn (*Zea mays* L.)

<sup>b</sup>Corn-barley (*Hordeum vulgare* L.)

<sup>c</sup>Corn-corn-alfalfa (*Medicago sativa* L.)

<sup>d</sup>Corn-wheat (*Triticum aestivum* L.)-vetch (*Vicia villosa* L.)

<sup>e</sup>Corn-wheat

<sup>f</sup>Corn-soybean (*Glycine max* L.)-wheat

**Table 5.2.** Mean, standard deviation (SD), and range of values of whole-corn plant N uptake (kg ha<sup>-1</sup>) soil N fractions (mg kg<sup>-1</sup>) in samples used for the soil N availability study.

Fraction	Depth (cm)	Mean	SD	Minimum	Maximum
Corn uptake	–	122	32.2	65.9	215
Soil N <sup>a</sup>	0–30	1434	467	823	2784
	0–60	1975	809	1140	4153
KCl <sup>b</sup>	0–30	7.3	3.9	4.0	22.7
	0–60	10.3	6.0	5.5	33.5
HKCl <sup>c</sup>	0–30	9.8	3.5	4.0	24.2
	0–60	11.5	3.7	5.1	25.3
ISNT <sup>d</sup>	0–30	139	30.6	73.6	239
	0–60	169	41.7	99.1	326
ANI <sup>e</sup>	0–30	43.1	10.9	19.8	71.3
	0–60	46.2	12.4	23.7	80.4
AI <sup>f</sup>	0–30	28.6	9.1	15.0	65.7
	0–60	31.1	11.5	17.2	79.4
POM <sup>g</sup>	0–30	169	94.0	62.8	511
	0–60	165	117	96.6	600

<sup>a</sup>Total soil N determined by dry combustion

<sup>b</sup>Inorganic N (NO<sub>3</sub>-N and NH<sub>4</sub>-N) extracted with 2 M KCl at room temperature

<sup>c</sup>NH<sub>4</sub>-N extracted with hot 2 M KCl minus NH<sub>4</sub>-N extracted with 2 M KCl at room temperature

<sup>d</sup>Illinois soil N test for amino sugar N

<sup>e</sup>Mineralized N during short-term anaerobic incubation

<sup>f</sup>Mineralized N during short-term aerobic incubation

<sup>g</sup>Particulate organic matter N

**Table 5.3.** Correlation analysis between whole-corn plant N uptake ( $\text{kg ha}^{-1}$ ), and contents of soil N fractions ( $\text{mg kg}^{-1}$ ) and soil clay ( $\text{g kg}^{-1}$ ) at two different depths.

Depth (cm)		Plant N	Soil N	KCl	HKCl	ISNT	ANI	AI	POM
0–30	Plant N	1							
	Soil N <sup>a</sup>	0.51 <sup>***</sup>	1						
	KCl <sup>b</sup>	0.33 <sup>**</sup>	0.80 <sup>***</sup>	1					
	HKCl <sup>c</sup>	0.32 <sup>**</sup>	0.60 <sup>***</sup>	0.51 <sup>***</sup>	1				
	ISNT <sup>d</sup>	0.28 <sup>**</sup>	0.80 <sup>***</sup>	0.70 <sup>***</sup>	0.58 <sup>***</sup>	1			
	ANI <sup>e</sup>	0.49 <sup>***</sup>	0.73 <sup>***</sup>	0.61 <sup>***</sup>	0.58 <sup>***</sup>	0.76 <sup>***</sup>	1		
	AI <sup>f</sup>	0.49 <sup>***</sup>	0.71 <sup>***</sup>	0.48 <sup>***</sup>	0.27 <sup>*</sup>	0.69 <sup>***</sup>	0.74 <sup>***</sup>	1	
	POM <sup>g</sup>	0.55 <sup>***</sup>	0.88 <sup>***</sup>	0.78 <sup>***</sup>	0.78 <sup>***</sup>	0.69 <sup>***</sup>	0.70 <sup>***</sup>	0.50 <sup>***</sup>	1
	Clay	0.39 <sup>***</sup>	-0.02 <sup>ns</sup>	-0.16 <sup>ns</sup>	-0.06 <sup>ns</sup>	-0.14 <sup>ns</sup>	0.06 <sup>ns</sup>	0.11 <sup>ns</sup>	-0.04 <sup>ns</sup>
0–60	Plant N	1							
	Soil N <sup>a</sup>	0.52 <sup>***</sup>	1						
	KCl <sup>b</sup>	0.41 <sup>***</sup>	0.75 <sup>***</sup>	1					
	HKCl <sup>c</sup>	0.27 <sup>*</sup>	0.48 <sup>***</sup>	0.50 <sup>***</sup>	1				
	ISNT <sup>d</sup>	0.25 <sup>*</sup>	0.71 <sup>***</sup>	0.60 <sup>***</sup>	0.65 <sup>***</sup>	1			
	ANI <sup>e</sup>	0.41 <sup>***</sup>	0.68 <sup>***</sup>	0.58 <sup>***</sup>	0.69 <sup>***</sup>	0.82 <sup>***</sup>	1		
	AI <sup>f</sup>	0.50 <sup>***</sup>	0.76 <sup>***</sup>	0.53 <sup>***</sup>	0.35 <sup>***</sup>	0.71 <sup>***</sup>	0.75 <sup>***</sup>	1	
	POM <sup>g</sup>	0.57 <sup>***</sup>	0.86 <sup>***</sup>	0.82 <sup>***</sup>	0.69 <sup>***</sup>	0.68 <sup>***</sup>	0.69 <sup>***</sup>	0.63 <sup>***</sup>	1
	Clay	0.42 <sup>***</sup>	0.04 <sup>ns</sup>	-0.18 <sup>ns</sup>	-0.19 <sup>ns</sup>	-0.29 <sup>**</sup>	-0.10 <sup>ns</sup>	0.11 <sup>ns</sup>	-0.06 <sup>ns</sup>

<sup>a</sup>Total soil N determined by dry combustion

<sup>b</sup>Inorganic N ( $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$ ) extracted with 2 M KCl at room temperature

<sup>c</sup> $\text{NH}_4\text{-N}$  extracted with hot 2 M KCl minus  $\text{NH}_4\text{-N}$  extracted with 2 M KCl at room temperature

<sup>d</sup>Illinois soil N test for amino sugar N

<sup>e</sup>Mineralized N during short-term anaerobic incubation

<sup>f</sup>Mineralized N during short-term aerobic incubation

<sup>g</sup>Particulate organic matter N

**Table 5.4.** Analysis of variance for models to predict corn plant N uptake as described in Eq. (2) and (3).

Model	Source	DF <sup>a</sup>	SS <sup>b</sup>	ME <sup>c</sup>	F-value
Eq. (2)	Regression	16	57620	3601.2	8.69***
	Error	67	27751	414.2	
	Total	83	85370		
Eq. (3)	Regression	16	61239	3827.4	10.16***
	Error	68	25619	376.8	
	Total	84	86858		

\*\*\*  $P < 0.001$

<sup>a</sup>Degrees of freedom

<sup>b</sup>Sum of squares

<sup>c</sup>Mean square

**Table 5.5.** The best five models obtained for each depth evaluated through the all possible regressions procedure and associated statistics.

Number of Regressors in Model	$p^a$	Regressors in Model	$R_p^2$ <sup>b</sup> (%)	$R_{Adj, p}^2$ <sup>c</sup> (%)	$C_p$ <sup>d</sup>	$PRESS_p$ <sup>e</sup>
-----0-30 cm depth-----						
6	7	$x_4x_6x_7'x_9x_{12}x_{16}$	64.54	61.78	3.08	37175
6	7	$x_4x_6x_7'x_9x_{11}x_{16}$	64.51	61.74	3.16	37302
7	8	$x_4x_6x_7'x_8x_9x_{12}x_{16}$	65.42	62.24	3.27	36946
8	9	$x_4x_5x_6x_7'x_8x_9x_{12}x_{16}$	65.84	62.20	4.41	37269
8	9	$x_4x_6x_7'x_8x_9x_{11}x_{12}x_{16}$	65.72	62.06	4.66	37255
-----0-60 cm depth-----						
6	7	$x_7'x_9x_{10}x_{12}x_{14}x_{16}$	68.18	65.74	2.35	31877
7	8	$x_3x_7'x_9x_{10}x_{12}x_{14}x_{16}$	69.26	66.47	1.87	31548
7	8	$x_5x_7'x_9x_{10}x_{12}x_{14}x_{16}$	68.97	66.15	2.53	32333
8	9	$x_5x_6'x_7'x_9x_{10}x_{12}x_{14}x_{16}$	69.64	66.44	3.00	32668
8	9	$x_3x_5x_7'x_9x_{10}x_{12}x_{14}x_{16}$	69.59	66.39	3.10	32407

<sup>a</sup>Number of coefficients in the model

<sup>b</sup>Coefficient of determination

<sup>c</sup>Adjusted  $R^2$

<sup>d</sup>Mallow's  $C_p$  statistic

<sup>e</sup> $PRESS$  statistic

**Table 5.6.** Selected models used to predict soil available N from two sampling depths as measured by whole-corn plant N uptake.

Variable	Coefficient	Standard Error	<i>t</i> -value	<i>P</i> -value	VIF <sup>i</sup>
-----0–30 cm depth-----					
Intercept	-85.2	36.1	-2.36	0.0208	0.00
$x_4^a$	-0.29	0.14	-2.07	0.0421	3.83
$x_6^b$	1.02	0.35	2.89	0.0050	2.11
$x_7'^c$	95.1	22.0	4.32	<.0001	3.47
$x_9^d$	0.03	0.01	4.63	<.0001	3.43
$x_{12}^e$	-0.35	0.06	-5.79	<.0001	3.30
$x_{16}^f$	0.71	0.23	3.11	0.0026	1.19
-----0–60 cm depth-----					
Intercept	-117	28.8	-4.05	0.0001	0.00
$x_7'$	99.6	12.2	8.15	<.0001	1.24
$x_9$	0.03	0.01	5.20	<.0001	3.60
$x_{10}^g$	7.48	3.39	2.20	0.0305	33.7
$x_{12}$	-1.03	0.26	-3.99	0.0001	93.8
$x_{14}^h$	2.40	0.95	2.53	0.0135	36.7
$x_{16}$	0.67	0.21	3.20	0.0020	1.37

<sup>a</sup>Illinois soil N test

<sup>b</sup>Short-term aerobic incubation

<sup>c</sup>Log-transformed particulate organic matter N

<sup>d</sup>Interaction of total soil N with dummy variable used to indicate manure applied (0) or not (1)

<sup>e</sup>Interaction of  $x_4$  with dummy variable used to indicate manure applied (0) or not (1)

<sup>f</sup>Soil clay content

<sup>g</sup>Interaction of inorganic nitrogen extracted with 2 M KCl (25 °C) with dummy variable used to indicate manure applied (0) or not (1)

<sup>h</sup>Interaction of  $x_6$  with dummy variable used to indicate manure applied (0) or not (1)

<sup>i</sup>Variance inflation factor

## VI. Conclusions

This dissertation concentrated efforts to evaluate effects of cropping system practices employed in the Valley region of Virginia on SOM status and functionality. To do so, a designed experiment was used to investigate the sensitivity of different SOM fractions as indices to changes in management practices in the short-term; and two on-farm (observational) experiments used to report SOM stocks as a function of tillage management and the role of SOM in controlling soil available N. These investigations resulted in four individual studies with conclusions presented in the following paragraphs.

The methodological comparison of density solutions of NaI and SPT in recovering FLF resulted in similar efficiency between these chemical solutions at densities of 1.6 and 1.8 g cm<sup>-3</sup>. In this range of density, researchers can with more confidence (when dealing with soil with similar characteristics of those found in the present study) compare information between difference procedures. With both chemicals, the use of higher density solution tended to increase amounts of C- and N-FLF recovered. For instance, C-FLF recovery averaged 637 and 954 mg kg<sup>-1</sup> at 1.6 and 1.8 g cm<sup>-3</sup> densities respectively. At this point, for both chemicals the use of a density of 1.8 g cm<sup>-3</sup> is preferred over a density of 1.6 g cm<sup>-3</sup> in recovering FLF because the lower variation with density of 1.8 g cm<sup>-3</sup> (8% versus 16% CV for C-FLF) results in a more precise analysis.

The data presented in chapters II and III were collected from a field experiment having crop rotation, tillage, and cover crop management as treatment factors. Among these agronomic practices, cover crop management had the greatest effect on SOM fractions with killing and leaving the cover crop rye residue on the field as the most beneficial treatment level to increase contents of organic fractions. This cover crop management is important in improving soil quality which leads to more sustainable land use and crop production. The physically defined fractions of SOM (POM and FLF) were more sensitive to changes in

management than other fractions (SOC, SON, ISNT, and  $C_{\min}$ ) with the FLF being the most sensitive between all fractions. The POM and FLF are fractions that have plant residues in different stages of decomposition as their primary components which links the sensitivity of these fractions to cover crop residue management. However, POM was more sensitive than FLF when only tillage management was evaluated, probably due to the lack of residue input caused by corn-silage harvesting and absence of cover crops. Between all fractions, POM represented the largest portion of total SOC (22.9% on average). In addition, the lack of sensitivity of ISNT and  $C_{\min}$  to any of the tested management practices can be added to previous findings that raise questions on the representation of the labile SOM pool through these two fractions.

The on-farm observation of tillage management conducted by farmers in the Valley region of Virginia revealed no significant difference between CT and NT for soil organic C and total soil N stocks considering the entire sampled depth (0–60 cm). This same conclusion was reached by two different analytical approaches. In the first approach, the observational data were evaluated as an incomplete block design yielding no significant trends for the upper 30 cm. The analysis of the entire sampled depth showed no clear trends (~2% difference for both fractions between tillage management) and would probably be the same even if a difference found in the upper layers was determined to be significant. In the second approach, the data were explored through regression analysis using the duration of NT management as the explanatory variable. In doing that, it was possible to observe significant increases in SOC and TSN stocks at 0–5 and 0–15 cm depth with increasing duration of NT. For instance, SOC stock increased at a rate of  $0.44 \pm 0.08$  and  $0.59 \pm 0.14$  Mg ha<sup>-1</sup> yr<sup>-1</sup> at 0–5 and 0–15 cm depth, respectively. However, duration of NT had no significant effect on C and N stocks when added to 0–30 and 0–60 cm depths. Thus, the largest impact of NT management on SOM in the short-term (< 10 years) was to increase surface and near-surface levels of SOM

compared to CT. On the other hand, the total short-term levels of SOM for 0–30 and 0–60 cm were not increased by NT. Further long-term research (10–30 years) will be required to fully assess the impact of NT vs. CT on SOM and corresponding C sequestration. However, NT still plays a fundamental role in reducing erosion and improving water availability which contributes to achieving a more sustainable agriculture.

The observational data collected to predict soil available N as measured by whole-corn N plant uptake indicated that inorganic N represented a small portion of the total soil N in the soils studied in these experiments. Total soil N measured at 0–30 and 0–60 cm depth was 823–2784 and 1140–4153 mg kg<sup>-1</sup>, respectively, with approximately 99% of it being represented by its organic form at both depths. This highlights the importance of SOM on soil N cycling. Among the organic fractions evaluated, POM represented the largest portion of total soil N, bringing even more attention to this fraction that has been widely used as an indicator of soil quality. Literature reviewed has indicated that soil scientists have mainly focused on the relationship between a single soil N index and plant N uptake, with less attention given to the relationship between multiple soil N fractions and plant N uptake. The inclusion of soil N fractions representing different SOM pools would have the advantage of accounting for their different turnover rates, and being thus more accurate in estimating potentially mineralizable N. The model selection procedure should be based on statistical criteria and on researcher's knowledge regarding the tested relationships to increase the chances of selecting a useful model. The final model selected included four soil N fractions representing different SOM pools and, hence, supporting the idea that a proximal-accurate estimation of soil available N through SOM mineralization with a single lab routine index is probably unrealistic.