

## ABSTRACT

KAWA, SAMBA ANSUMANA. Impact of Tillage and Management Practices on Soil Organic Carbon and Nitrogen Dynamics in Diverse Agroecosystems. (Under the direction of Dr. Michael G. Wagger.)

Agricultural management practices impact the sources and sinks of carbon dioxide (CO<sub>2</sub>) and other greenhouse gases attributed to global warming. Application of long-term no-till (NT) practices to sequester soil C has been recognized in C trading and agroecosystem sustainability. Contributions by conservation management practices (e.g. no-till, cover cropping, and organic amendments) in sequestering C can be estimated at about one-fourth to one-third of annual atmospheric CO<sub>2</sub> enrichment; whereas conventional tillage (CT) and management practices such as crop residue removal and low-input agriculture contribute to soil organic carbon (SOC) and nitrogen (SON) loss. This research examines the impacts of tillage and management induced changes on SOC and SON dynamics in diverse agroecosystems. The objectives of the research were to investigate tillage effects on water stable aggregation (WSA) and C and N distribution in bulk soil, aggregates, and particle-size fractions; and to evaluate various soil factors controlling WSA and SOC using a principal component analysis (PCA) method. Soil samples used in the investigation were from the surface 0- to 10-cm depth of NT and CT systems in a Wickham sandy loam (fine-loamy, mixed, semi active, thermic Typic Hapludult), Delanco fine-sandy loam (fine-loamy, mixed, mesic, Aquic Hapludult), and Wedowee sandy clay loam (fine, kaolinitic, thermic Typic

Kanhapludult); representing North Carolina Coastal Plain, Mountain, and Piedmont locations, respectively. Water stable aggregation was determined on wet-sieved aggregates. Silt+clay fractions were fractionated according to particle size. Carbon, nitrogen, and free and non-crystalline aluminum (Al) and iron (Fe) (hydr)oxides were determined and enrichment factors for C (EC) and N (EN) (mass C or N per mass fraction/mass C or N per mass bulk soil) calculated. Relative to CT, NT increased WSA and SOC/N and MBC/N. In the Mountain, C and N pools in bulk soil and aggregates were 1.6-2.6 times greater under organic-amended NT (NTO) than CT. In the Piedmont, NT increased WSA by 1.4-2.0 times, SOC and N by 1.5-2.0 times, and aggregate associated C and N by 1.4-3 times. Average SOC in equivalent soil mass was 1.5 and 1.8 times greater under NT in the Mountain and Piedmont, respectively; and 1.1 times greater in the fallow system in the Coastal Plain. Under NT and CT, silt+coarse clay proportion was 3-12 times greater than fine clay but C and N concentrations and enrichment factors were 1.1-3 times greater in fine clay than silt+coarse clay across locations. Silt+coarse clay and fine clay C and N concentrations were 1.1 and 1.6 times greater under NT than CT, while EC and EN were 1.3 and 1.1-1.2 times greater under CT than NT, in the Mountain and Piedmont, respectively. The EC was 1.1 times greater under CT than NT in the Coastal Plain. Significant relationships existed between MWD and non-crystalline Fe, hot water extractable C (HWEC; which measures potentially bioavailable C), and SOC and bulk density in the Coastal Plain, Mountain, and Piedmont, respectively; whereas SOC related with clay in the Coastal Plain, humic matter in

the Piedmont, and HWEC in all locations. These results illustrate greater enhancement of WSA and sequestration of C and N in bulk soil, aggregates, and the fine clay fraction under NT in the soils studied. The results further demonstrate the usefulness of the physical fractionation procedure used to differentiate SOC and N distribution in bulk soil, aggregates, and particle-size fractions, and of principal components in estimating WSA and SOC, under different tillage and management practices in diverse agroecosystems.

Impact of Tillage and Management Practices on Soil Organic Carbon and Nitrogen Dynamics  
in Diverse Agroecosystems

by  
Samba Ansumana Kawa

A dissertation submitted to the Graduate Faculty of  
North Carolina State University  
in partial fulfillment of the  
requirements for the degree of  
Doctor of Philosophy

Soil Science

Raleigh, North Carolina

2010

APPROVED BY:

---

T. Jot Smyth

---

Daniel W. Israel

---

Greg D. Hoyt

---

Chris Reberg-Horton

---

Michael Waggoner  
Chair of Advisory Committee

## **DEDICATION**

I dedicate this work to God; to my mom (Mamie Ngasoh Ansumana; RIP); and to my wife (Massah) and children.

## BIOGRAPHY

Samba grew up with his mom in Tawey, a tiny village near Mobai in the Mandu Chiefdom of the tiny West African nation of Sierra Leone. He attended primary and secondary schools in the Eastern Province (Methodist and Roman Catholic Primary Schools in Segbwema and Mobai, respectively, and Holy Ghost Secondary School in Segbwema); Southern Province (Roman Catholic Primary Schools in Yamandu and Koribondo); and Northern Province (Saint Francis Secondary School, Makeni) of Sierra Leone. Samba holds a B.Sc. degree in Agriculture General from the Njala University College (NUC), Southern Province; a M.Sc. in Soil Science from the Zhejiang Agricultural University in Hangzhou, Zhejiang province, China; and a M.Sc. in Education from Mercy College in New York, USA. Samba's interests in soil science perhaps started early in his life, as he spent much of his early childhood years with his mom, who grew vegetables and swamp rice and processed palm oil in their tiny village. Samba had more exposure when a high school teacher, who introduced the subject of Agricultural Science at Holy Ghost Secondary School, taught Samba and other male students to grow cassava, peanuts, and other crops, while their female counterparts prepared the farm produce, for the school's lunch program. With these experiences and an undergraduate education, Samba also introduced the subject to the Richard Allen Girls High School in Freetown (the Sierra Leone capital), where he was a teacher between 1986 and 1987. Samba's employment as a Variety Maintenance Officer

and, later on, Production Assistant, at the Seed Multiplication Project (SMP) enhanced his interest and experience in cultivating the soil to grow crops. Samba attended several training programs at the Rice Research Station and the then West Africa Rice Development Association, both in Rokupr, Northern Sierra Leone; as well as at the International Institute for Tropical Agriculture in Ibadan, Nigeria. Samba was also happy to have traveled in 1994 to China where, as part of his studies for the M.Sc. program, he visited several farms and learned more about soil cultivation using appropriate technology. In 2005, Samba embarked on a doctoral program at the North Carolina State University. Prior to this, he lived in New York City and worked as a Skills Development Trainer at the New York League Center of the Association for the Rehabilitation for Retarded Children (AHRC) for a year and as a science teacher at the Rafael y Molina School (Intermediate School 184) in South Bronx, New York City, for three years.

## ACKNOWLEDGMENTS

I thank God for his love. I would like to express profound thanks and gratitude to Dr. Michael Wagger, who has been more than my advisor. He has provided me with much needed support in diverse ways, including helping to keep me on track despite several unfortunate incidences that threatened my continued study in the university. I am most grateful for support from Dr. Hoyt, who took much valuable time to ensure that my sample collection event at the Mountain Horticultural Research station was successful; to Drs. Smyth, Israel, and Reberg-Horton who were always ready to respond to my numerous questions about this research. These people were the best research committee I could possibly get. Dr. Arellano Consuelo, in the Statistics Department, provided immense help in making sense of my data. I also thank Robert Walters, Ken Fager, and Melissa Bell; they helped me greatly during my sample collection exercises. Robert was also involved in many other aspects of this research, from driving with me to the fields to helping me locate materials for the experiments to providing assistance in analyzing my results. I also thank Dr. Robarge and his staff in the Analytical Services Lab at North Carolina State University for their immense help in analyzing my samples. Dr. Joel Gruver, Assistant Professor (Soil Science/Sustainable Agriculture) in the Agriculture Department at Western Illinois University (Macomb, IL), was very instrumental at the planning stage of this research; when

we were both college and house mates. I also want to thank Sheri Cahill, Emily Dell, and my other friends at NCSU. Finally, I thank my family for their love and great support; I love and adore them too, because they put up with my “not-so-good” moments. Massam, my youngest son, was a special inspiration.

## TABLE OF CONTENTS

<b>LIST OF FIGURES.....</b>	<b>x</b>
<b>LIST OF TABLES .....</b>	<b>xii</b>
<b>CHAPTER I: TILLAGE EFFECTS ON WATER STABLE AGGREGATES AND AGGREGATE ASSOCIATED MICROBIAL BIOMASS CARBON AND NITROGEN .....</b>	<b>1</b>
Abstract .....	1
Introduction.....	3
Materials and Methods .....	4
Site description and soil sampling.....	4
Aggregate fractionation.....	5
Sample analysis.....	7
Calculations and statistical analysis .....	8
Results and Discussion .....	8
Size distribution and water stable aggregation.....	8
Soil organic and microbial biomass C and N .....	11
Aggregate associated organic and microbial biomass C and N.....	15
Conclusions.....	22
References.....	23
<b>CHAPTER II: TILLAGE EFFECTS ON C AND N DYNAMICS IN DIVERSE AGROECOSYSTEMS: SOIL C AND N STRATIFICATION AND SEQUESTRATION .....</b>	<b>45</b>
Abstract .....	45
Introduction.....	46
Materials and Methods .....	49
Description of study sites .....	49
Experimental design .....	52
Soil sampling.....	53
Soil physical, chemical, and microbiological analyses.....	54
Calculations and statistical analysis .....	55

Results and Discussion .....	58
Soil mass, bulk density, and additional thickness required to achieve equivalent mass	58
Soil organic carbon and nitrogen .....	60
Labile pools of carbon and nitrogen.....	62
Soil organic and microbial biomass C and N stratification ratios.....	67
Soil organic carbon sequestration rates and factors influencing C sequestration .....	69
Conclusions.....	73
References.....	75
<b>CHAPTER III: TILLAGE EFFECTS ON THE DISTRIBUTION OF ORGANIC CARBON AND NITROGEN IN SOIL PARTICLE-SIZE FRACTIONS .....</b>	<b>100</b>
Abstract .....	100
Introduction.....	102
Materials and Methods.....	103
Soils and soil sampling.....	103
Particle size fractionation .....	104
Determination of mineral associated and particulate organic matter .....	106
Calculations and statistical analysis .....	108
Results and Discussion .....	109
Distribution of primary particle-size fractions .....	109
Carbon and nitrogen composition of particle-size fractions.....	112
Concentrations of C and N in particle-size fractions within aggregates .....	116
Enrichment of particle-size fractions .....	118
Carbon to nitrogen ratios .....	120
Conclusions.....	120
References.....	122
<b>CHAPTER IV: MULTIVARIATE ANALYSIS OF SOIL FACTORS INFLUENCING AGGREGATE STABILITY AND ORGANIC CARBON CONTENT IN DIVERSE AGROECOSYSTEMS .....</b>	<b>141</b>
Abstract .....	141
Introduction.....	143
Materials and Methods.....	145

Soil sampling and analysis .....	145
Principal component and statistical analyses .....	151
Results and Discussion .....	154
Interpretation of PCs based on values for MWD .....	157
Interpretation of PCs based on values for SOC .....	163
Multiple regression analysis .....	166
Variables distinguishing between tillage effects on MWD and SOC across locations .	167
Conclusions.....	168
References .....	170

## LIST OF FIGURES

### **Chapter I. Tillage effects on water stable aggregates and aggregate associated microbial biomass carbon and nitrogen**

- Fig. 1. Aggregate size class distribution and mean weight diameter of wet-sieved samples from three long-term experimental sites ..... 31
- Fig. 2. Soil organic C and N and microbial biomass C and N concentrations in bulk soil for different tillage systems in the Coastal Plain, Mountain, and Piedmont locations..... 32
- Fig. 3. Microbial biomass C and N concentrations in bulk soil for different tillage systems in the Coastal Plain, Mountain, and Piedmont locations..... 33
- Fig. 4. Organic C concentrations in soil aggregates under different tillage systems in the Coastal Plain, Mountain, and Piedmont locations ..... 34
- Fig. 5. Organic N concentrations in soil aggregates under different tillage systems in the Coastal Plain, Mountain, and Piedmont locations ..... 35
- Fig. 6. Contributions of aggregate associated C to SOC under different tillage systems in the Coastal Plain, Mountain, and Piedmont locations..... 36
- Fig. 7. Contributions of aggregate associated N to SON under different tillage systems in the Coastal Plain, Mountain, and Piedmont locations..... 37
- Fig. 8. Microbial biomass C quotients in aggregates under different tillage systems ..... 38
- Fig. 9. Microbial biomass N quotients in aggregates under different tillage systems..... 39

### **Chapter II. Tillage effects on C and N dynamics in diverse agroecosystems: Soil C and N stratification and sequestration**

- Fig. 1. Soil bulk density values in diverse tillage systems in the Coastal Plain, Mountain, and Piedmont locations ..... 81
- Fig. 2. Soil organic C and N stocks in equivalent soil mass for tillage systems in Coastal Plain, Mountain, and Piedmont locations..... 82
- Fig. 3. Microbial biomass C and N stocks in equivalent soil mass for tillage systems in Coastal Plain, Mountain, and Piedmont locations ..... 83
- Fig. 4 Percent SOC contributed by microbial biomass C, water soluble C, and hot water extractable C in Coastal Plain, Mountain, and Piedmont locations ..... 84
- Fig. 5 Carbon sequestration rates in different tillage systems in Coastal Plain, Mountain, and Piedmont locations ..... 85
- Fig. 6 Corn yields in the experimental plots in the Mountain for 2007 and 2008 ..... 86

**Chapter III. Tillage effects on the distribution of organic carbon and nitrogen in soil particle-size fractions**

Fig. 1. Aggregate size class distribution and mean weight diameter of wet-sieved samples from three long-term experimental sites ..... 126

Fig. 2 Particle-size fraction distribution (not including sand particles) outside of macro- and micro-aggregates..... 127

Fig. 3. Distribution of mineral associated and particulate organic matter ..... 128

Fig. 4. Carbon concentrations in particle-size fractions in the Coastal Plain, Mountain, and Piedmont locations..... 129

Fig. 5. Nitrogen concentrations in particle-size fractions in the Coastal Plain, Mountain, and Piedmont locations ..... 130

Fig. 6. C concentrations in silt+clay- and sand-sized particles associated with macro- and micro-aggregates in the Coastal Plain, Mountain, and Piedmont locations ..... 131

Fig. 7. N concentrations in silt+clay- and sand-sized particles associated with macro- and micro-aggregates in the Coastal Plain, Mountain, and Piedmont locations ..... 132

Fig. 8. Enrichment factors for C in the Coastal Plain, Mountain, and Piedmont locations133

Fig. 9. Enrichment factors for N in the Coastal Plain, Mountain, and Piedmont locations134

Fig. 10. Enrichment factors for C in particle size fractions contained in aggregates ..... 135

Fig. 11. Enrichment factors for N in particle size fractions contained in aggregates ..... 136

**Chapter IV. Multivariate analysis of soil factors influencing aggregate stability and organic carbon content in diverse agroecosystems**

Fig. 1 Distribution of soil organic C and mean weight diameter in the Coastal Plain, Mountain, and Piedmont locations..... 179

Fig. 2. Biplot of the first and second PCs showing their relationships to MWD ..... 180

Fig. 3. Biplot of the first and second PCs showing their relationships to SOC..... 181

Fig. 4. Biplot of the third and fourth PCs showing their relationships to MWD ..... 182

Fig. 5. Biplot of the third and fourth PCs showing their relationships to SOC..... 183

Fig. 6. Biplot of the third and fourth PCs showing their relationships to HWEC ..... 184

Fig. 7. Biplot of the second and third PCs showing their relationships to HWEC ..... 185

## LIST OF TABLES

### **Chapter I. Tillage effects on water stable aggregates and aggregate associated microbial biomass carbon and nitrogen**

Table 1. Tillage systems and selected chemical and physical properties of the 0 to 10 cm depth of soils studied .....	40
Table 2. Description of crops grown in the plots studied at the experimental sites .....	41
Table 3. Organic and synthetic amendments at the experimental sites .....	43
Table 4. C:N ratios in soil and aggregate associated organic matter and microbial biomass under different tillage systems in the Coastal Plain, Mountain, and Piedmont locations .	44

### **Chapter II. Tillage effects on C and N dynamics in diverse agroecosystems: Soil C and N stratification and sequestration**

Table 1. Description of crops grown in the plots at the experimental sites .....	87
Table 2. Organic and synthetic amendments used at the experimental sites.....	89
Table 3. Tillage systems and characteristics of the 0 to 10 cm soil depth at the three locations studied .....	91
Table 4. Masses of soil and various pools of C and N per area in equivalent soil mass .....	92
Table 5. Soil C and N pools, expressed as concentration and equivalent mass, for different tillage systems in the Coastal Plain, Mountain, and Piedmont.....	94
Table 6. Type 3 tests of fixed effects for SR for C and N pools in different tillage systems	95
Table 7. Stratification ratios of C and N pools under different tillage systems .....	96
Table 8. Differential stratification ratios of soil organic C and N and microbial biomass C and N under different tillage systems.....	97
Table 9. Correlations between SOC and soil properties affecting SOC sequestration in the Coastal Plain, Mountain, and Piedmont.....	98

### **Chapter III. Tillage effects on the distribution of organic carbon and nitrogen in soil particle-size fractions**

Table 1. Tillage systems and selected chemical and physical properties for the 0 to 10 cm depth in soils studied .....	137
Table 2. Distribution of SOC and SON among aggregates and particle-size fractions.....	138
Table 3. Main correlations among soil organic C and N and the C and N contents of, and proportions of SOC and SON contributed by, particle-size fractions .....	139

Table 4. C/N ratios for organic matter associated with particle-size fractions within and outside aggregates from the 0 to 10 cm depth in soils studied ..... 140

**Chapter IV. Multivariate analysis of soil factors influencing aggregate stability and organic carbon content in diverse agroecosystems**

Table 1. Tillage systems and selected soil physical and chemical properties for the 0 to 10-cm depth of the experimental locations..... 186

Table 2. Correlation matrices of the PCA for MWD and SOC ..... 187

Table 3. Eigen values of the correlation matrices of the PCAs for MWD and SOC showing orthogonal components explaining the variations among 11 soil variables examined in the PCAs ..... 188

Table 4. Principal component loadings after Varimax rotation ..... 189

Table. 5. Selected soil physical and chemical properties for the 0 to 10-cm depth of soils at the experimental sites studied..... 190

Table 6. Pearson correlations among selected soil properties at the experimental sites 191

Table 7. Multiple linear regression equations of mean weight diameter and soil organic C on PC defining variables ..... 194

## CHAPTER I

# TILLAGE EFFECTS ON WATER STABLE AGGREGATES AND AGGREGATE ASSOCIATED MICROBIAL BIOMASS CARBON AND NITROGEN

### Abstract

No-till (NT) can improve water stable aggregation (WSA) and alter long-term soil organic C (SOC) and N (SON) dynamics. Tillage effects on WSA and soil and aggregate associated microbial biomass C (MBC) and N (MBN) in the surface 10 cm depth of NT and conventional tillage (CT) systems were examined in three experiments varying in duration and soil type. The soils were a Wickham sandy loam (fine-loamy, mixed, semi active, thermic Typic Hapludult), Delanco fine-sandy loam (fine-loamy, mixed, mesic, Aquic Hapludult), and Wedowee sandy clay loam (fine, kaolinitic, thermic Typic Kanhapludult) in the Coastal Plain, Mountain, and Piedmont, respectively. In the Piedmont, NT increased WSA by 1.4 to 2.0 times, compared to CT. The SOC concentrations were 1.5 and 2.0 times greater under NT and organic-amended NT (NTO) than CT in the Piedmont and Mountain, respectively. Average SON in the Mountain and Piedmont was 2 times greater under NT than CT, respectively. In the Mountain, MBC was 1.5 times greater under NT than CT systems; macro- and micro-aggregate C and N were 2 times greater under NTO than CT. In the Piedmont, macro- and micro-aggregate C and N were 2-3 times greater under NT than CT; macro- and micro-aggregate MBC and MBN were 1.4 and 1.9, and 1.7 and 1.8, times

greater under NT than CT, respectively. These results demonstrate that NT systems, alone or with organic amendments, can enhance WSA and organic and microbial biomass C and N in aggregates and bulk soil in the locations studied.

## Introduction

Tillage practices that disrupt aggregates cause increased breakdown of soil organic matter (SOM) (Adu and Oades, 1978) and affect the constituent SOM fractions (Parton et al., 1987; Angers et al., 1992; Cambardella and Elliott, 1994). Conventional tillage systems result in SOC loss (Dalal and Mayer, 1986) and soil aggregate destruction (Tisdale and Oades, 1982) via exposure of physically protected SOM to microbial attack (Beare et al., 1994). Comparison of stable versus unstable aggregates has been used to identify factors that influence aggregate stability (Puget et al., 1999). Relative to microaggregates (< 0.250 mm), stable macroaggregates (> 0.250 mm) are characterized as containing higher amounts of organic C (Elliott, 1986; Puget et al., 1995) and microbial biomass (Degens et al., 1994). Reduced aggregation in CT systems has been attributed to soil mixing and inversion (Chan and Mead, 1988). In a study of soil components affecting aggregation, Chesters et al. (1957) concluded that microbial gum had the most important effect on soil aggregate formation in the surface 15 cm of a Spodosol. Studying the differences in microbial biomass in a NT chronosequence previously under continuous maize cropping (0 to 20 yr) on a silt loam Alfisol, Staley et al. (1988) reported that microbial biomass C in the top 7.5 cm of soil was about 60% greater under NT compared to CT. Hernández-Hernández and Hernández (2002) observed greater amounts of macroaggregate associated MBC under NT than CT systems in a Venezuelan Ultisol and suggested that tillage impacts on the hierarchical distribution of soil aggregates are caused by changes in microbial biomass size and activity in

macroaggregates. The objective of the present study was to examine the effects of tillage systems on the distributions of organic and microbial biomass C and N associated with aggregates in soils of diverse agroecosystems. It was hypothesized that tillage intensities will differentially influence water stable aggregation and organic and microbial biomass C and N associated with aggregates.

## **Materials and Methods**

### **Site description and soil sampling**

This study was conducted at three locations: 1) Center for Environmental Farming Systems (CEFS; 35° 23' 5" N, 77° 59' 35" W) located near Goldsboro, Wayne County, 2) Mountain Horticultural Crops Research Station (MHCRS; 35° 25' 50" North, 82° 30' 5" West) near Mills River, Henderson County, and 3) Upper Piedmont Research Station (UPRS; 36° 21' 17" N, 79° 39' 53" W) near Reidsville, Rockingham County. These sites are located in the Coastal Plain, Mountain, and Piedmont physiographic regions of NC, respectively. Experimental designs at each site were randomized complete block, with 3 replications per treatment at CEFS and 4 replications each at MHCRS and UPRS. This study examined 4 treatments at CEFS [conventional tillage (CT) and no-till (NT), an organic system under CT (CTO), and a successional or forest regrowth system(S)]. At MHCRS, the five treatments included synthetic (fertilizers, herbicides, etc.) amended plots under CT (CTS) and NT (NTS), organic-amended plots under CT (CTO) and NT (NTO), and a control CT system receiving no

inputs (CT). The three treatments at UPRS consisted of a moldboard plow/disk (MBP), spring chisel plow only (SCP), and no-till (NT). At CEFS, soil samples were taken around previously geo-referenced points, using a 2.5 cm diameter by 30 cm deep long probe, within plant rows from the surface 10 cm. At the other locations, sampling was done within plant rows from 3 to 6 randomly assigned and demarcated sampling locations selected within defined soil-landscape positions in each replication. Samples were taken from the same 10 cm soil depth using a 7.5 cm diameter by 30 cm deep long core auger. At all locations, three samples were taken around each sampling location, composited, placed into ziplock bags, and transported to the laboratory. Table 1 describes selected properties of the soils.

### **Aggregate fractionation**

The modification by Haynes (1993) of the Yoder (1936) wet sieving method was used to separate soil samples into three water stable aggregate size classes: macroaggregates (4.00-0.250 mm diameter), microaggregates (0.250-0.053 mm diameter), and silt+clay (< 0.053 mm diameter). The device used to accomplish the separations consisted of four nests of three 12.5-cm diameter sieves, each nest consisting of a 2.00-mm, 0.250-mm, and 0.053-mm aperture sieve.

Thirty-five gram samples (oven dry equivalent) of field moist samples, previously crushed gently by hand and passed through 8- and 4-mm aperture sieves and stored in zip-lock bags at 4°C, were placed on the 2-mm sieve, pre-wetted for 5 min, and mechanically

raised and lowered on each nest of sieves through a distance of about 3.5 cm at a rate of 34 oscillations  $\text{min}^{-1}$  for 15 min. The sieves were allowed to drain and a strong jet of distilled water from a wash bottle used to backwash the contents on each sieve into small, aluminum trays. The contents were weighed and placed in a freeze dryer (VirTis Freeze Mobile 12XL from The VirTis Company, Gardiner, N.Y. 12525). The material that passed through the smallest sieve, containing materials  $<0.053$  mm, was kept for further fractionation into silt+coarse clay and fine clay fractions in a separate study. After freeze-drying, the 4.00-2.00 mm and 2.00-0.250 mm materials were composited and labeled macroaggregates, so that each sample was now divided into macro- (4.00-0.250 mm) and micro-aggregates (0.250-0.053 mm).

The percentage of soil remaining on each sieve was calculated after excluding the material  $> 4$  mm. The respective values obtained were designated as the water stable aggregate size distribution between 4.00 to 2.00, 2.00 to 0.250, and 0.250 to 0.053mm. Mean weight diameter (MWD) values were used as another way to express aggregate size distribution (Youker and McGuiness, 1957) and were calculated as follows:

$$\text{MWD} = \sum(\text{proportion of sample retained on sieve} \times \text{size class midpoint or mean diameter of the size fraction})$$

The size class midpoints (mean intersieve diameters) were calculated as follows:

$((4.00+2.00)/2)$ ,  $((2.00+0.250)/2)$ ,  $((0.250+0.053)/2)$ , and  $((0.053+0)/2)$ , or 3.0, 1.125, 0.152, and 0.027 mm, respectively. The macro and microaggregates were stored between 0 to 4°C until further analysis.

### **Sample analyses**

The chloroform fumigation extraction method according to Vance et al. (1987) was used to determine soil microbial biomass C and N in aggregates without the subtraction of controls, using the method of handling small sample sizes of aggregates suggested by Kandeler et al. (1999). Aggregate size materials were finely ground and total C and N concentrations, as well as those in bulk soil, were determined by direct combustion in a Perkin–Elmer 2400 CHN analyzer. Soil particle size analysis was according to the hydrometer method of Gee and Bauder (1986). Soils in this study were generally at pH 6.5 or less and low in carbonates. Therefore, total C was assumed equal to organic C. Aggregate weights and C and N concentrations were corrected for sand according to Wick et al. (2009) and Deneff et al. (2001). Gravimetric moisture content was measured and found to range between 12 and 14% in the Coastal Plain, 10 to 15% in the Mountains, and 8 to 12% in the Piedmont location. The samples were stored for between 90 and 150 days at 4°C before biochemical analyses.

## Calculations and Statistical Analysis

Contributions of organic or microbial biomass C and N in an aggregate size class relative to organic C and N in bulk soil were calculated according to Paul et al. (2008):

$$RC_{\text{fraction}} = (C_{\text{fraction}}/C_{\text{bulk soil}}) \times 100,$$

where:  $RC_{\text{fraction}}$  (%) is the contribution of C or N in an aggregate size class relative to organic C and N in bulk soil;  $C_{\text{fraction}}$  ( $\text{g C kg}^{-1}$  soil) is the absolute quantity of soil C or N in an aggregate size class per kg bulk soil; and  $C_{\text{bulk soil}}$  ( $\text{g C kg}^{-1}$  soil) is the quantity of soil C or N per kg bulk soil.

Tillage and region effects were evaluated by using the mixed procedure (Proc Mixed) model of the Statistical Analytical Software (SAS Inst., Cary, NC) to compare treatment means, aggregate size classes, and particle size fractions. Pearson's correlation coefficients were determined for the correlation matrix of variables measured in aggregates and fractions.

## Results and Discussion

### Size distribution and water stable aggregation

Across all locations, percent recovery of samples relative to bulk soil that was fractionated ranged from 87 to 92%. Total sand free water stable aggregates ranged between 18 and 27%, 35 and 44%, and 21 and 30% in the Coastal Plain, Mountain, and Piedmont locations, respectively; but tillage did not significantly impact the distribution of

these aggregate size classes in these locations (Fig. 1). Tillage had no effect on mean weight diameter (MWD), an index of water stable aggregation (WSA) in the Coastal Plain and Mountain locations; but did impact ( $p = 0.0034$ ) MWD in the Piedmont location. In the Piedmont location, MWD under the no-till system (NT) was 1.4 times greater than under the spring chisel plow (SCP) and twice that under moldboard plow (MBP); and MWD was 1.4 times greater under SCP than MBP (Fig. 1). The greater MWD under NT (1.0 mm) than in the disturbed or cultivated soils (0.7 mm for SCP, and 0.5 mm for MBP) illustrates the adverse effects of cultivation and the positive influence of NT on soil aggregation; while the greater MWD under SCP than MBP suggests further that the adverse effects of cultivation on WSA increases with intensity of tillage. Crop residues and other soil cover under NT can have a mulching effect as well as contribute to organic matter replenishment, which in turn enhances WSA (Tisdall and Oades, 1980; Blanco-Canqui and Lal, 2004). In contrast, biomass removal under CT results in bare soils that are susceptible to surface soil erosive forces. The topography of the Piedmont region is rolling to hilly, with the hills and valleys separated by a few hundred meters of elevation ranging from about 90 to about 460 m (Daniels et al., 1999). Furthermore, binding agents that bind micro- and macro-aggregates are sensitive to cultivation (Stott et al., 1999). Cultivation disrupts soil aggregates, thereby reducing their MWD. Thus while tillage operations under CT expose soil and cause soil aggregate destruction (Tisdale and Oades, 1982); NT enhances aggregation (Zotarelli et al., 2005).

The absence of significant tillage effects on aggregate size distribution and MWD in

the Coastal Plain and Mountain locations suggests that the duration a given tillage system has been in place may be a factor determining aggregation in the surface depth in these soils. Six et al. (2000) wet-sieved field moist soils to measure MWD in the 0 to 5 cm depth in NT and CT systems of a sandy loam Alfisol and regarded the “young age” of the then 9-yr experiment as a probable cause for similarities in MWD values between NT and CT. Also, Bayer et al. (2000) observed SOC and N accumulation to proceed in a step-by-step depth-wise distribution for a sandy clay loam Paleudult previously plowed, converted to NT, and amended with crop residue. The authors observed SOC and N accumulation in the 0 to 2.5, 0 to 7.5, 0 to 12.5, and 0 to 17.5 cm depths only after 3, 5, 9, and 11 years of NT. At the time of sampling in the present study, the experimental plots in the Coastal Plain and Mountain had been established for 9 and 14 yr, respectively, compared to 24 yr for the plots sampled in the Piedmont. In a previous study, Overstreet (2005) observed greater macroaggregate content in the top 15 cm of the NTO plots compared to the other systems of the same MHCRS experimental site in this study. Differences in aggregation between the former and current findings may be related to differences in sampling depth (top 15 cm in study by Overstreet (2005) versus top 10 cm in current study). Greater aggregation has been observed in the 5 to 20 than 0 to 5cm depth in NT systems in a Duroc loam (fine-silty, mixed, mesic Pachic Haplustoll) (Six et al., 1998) and in the 10 to 20 cm than 0 to 10 cm depth in native, perennial, and annual pasture systems in a Typic Cryrendoll (Li et al., 2007).

## Soil organic and microbial biomass C and N

Tillage significantly influenced SOC concentrations in the Coastal Plain ( $p = 0.0328$ ), Mountain ( $p = 0.0006$ ), and Piedmont ( $p = 0.0006$ ) locations (Fig. 2). In the Coastal Plain, SOC was approximately 10% greater in the successional (S;  $10.7 \text{ g kg}^{-1}$  soil) than no-till (NT;  $9.9 \text{ g kg}^{-1}$  soil) or conventional tillage (CT;  $9.5 \text{ g kg}^{-1}$  soil); but did not differ between S and organic (CTO;  $10.2 \text{ g kg}^{-1}$  soil). The SOC concentrations under CTO, NT, and CT did not differ. On average, undisturbed (NT and successional) systems contained  $10.3 \text{ g SOC kg}^{-1}$  soil, compared to  $9.8 \text{ g SOC kg}^{-1}$  soil in systems with tillage (organic and CT). In the Mountain location, SOC concentrations were of the order  $\text{NTO} > \text{NTS} = \text{CTO} = \text{CTS} = \text{CT}$ . The SOC concentrations under NT averaged  $13.4 \text{ g kg}^{-1}$  soil, 1.5 times greater than under CT. In the Piedmont location, SOC was significantly greater under NT ( $10.6 \text{ g kg}^{-1}$  soil) than either SCP ( $6.7 \text{ g kg}^{-1}$  soil) or MBP ( $5.2 \text{ g kg}^{-1}$  soil). On average, the concentration of SOC under NT was 1.8 times greater than under CT (SCP and MBP). Concentrations of SON in the Mountain and Piedmont locations followed patterns similar to SOC in those locations; with SON concentrations averaging 1.6 and 1.7 times greater under undisturbed (NT) than disturbed (tilled) systems, respectively. Tillage did not impact SON in the Coastal Plain.

The greater concentration of SOC under the successional system (S), compared to concentrations under NT and CT, may be attributed to the nearly steady addition of organic matter, lack of tillage-induced SOC loss, and lack of SOC loss (such as in crop harvests) in the S system. This illustrates the positive effects of S systems on the enhancement of SOC levels

in soil. Based on the mean values for various types of turkey litter (<http://www.soil.ncsu.edu/publications/Soilfacts/AG-439-05/>), it was calculated that the high rate (3900–13500 Mg ha<sup>-1</sup>) of turkey litter added to the CTO system in the Coastal Plain location supplied between 81 and 282 Mg N ha<sup>-1</sup> (Table 2). Assuming that only 50% of the N in the turkey litter was available for plant uptake during the growing season, this would translate to between 40 and 140 Mg N ha<sup>-1</sup> from the turkey litter, compared to 0.20 Mg N ha<sup>-1</sup> from urea and N:P:K fertilizers in the CT system. It might be expected, therefore, that SON would be significantly greater under CTO than the other systems in the Coastal Plain. However, it is possible that a large proportion of the excess N in the turkey litter was lost to leaching, ammonia volatilization, and uptake by the previous (2006) soybean crop.

The significance of organic amendments is illustrated in the Mountain location, where the difference between the two no-till systems (NTO and NTS) was greater SOC content under NTO than NTS. In addition to crop residues left on the soil surface with both no-tillage systems, the NTO system received more organic amendments. This resulted in greater SOC content in the NTO than NTS and other systems in the location. The greater SON under NTO and CTO in the Mountain location also indicates the positive influence of organic amendments on SON enhancement. Assuming 100% solubility for soybean meal applied, the organic-amended (NTO and CTO) systems in the Mountain each received about 0.02 Mg N ha<sup>-1</sup>; whereas the synthetic-amended systems (NTS and CTS) systems received about 0.06 Mg N ha<sup>-1</sup> from ammonium nitrate fertilizer added (Table 2). However,

increased organic residues from cover crops and previous crops have possibly resulted in increased SON under NTO. The different types of cover crops in the organic- and synthetic-amended systems may make up for the difference in SON concentrations, since soil N contribution is expected to be greater under the leguminous (hairy vetch (*Vicia sativa* L.)) than non-leguminous (rye (*Secale cereale* L.) cover crop. Sainju and Singh (2008) observed greater N concentrations in hairy vetch than rye cover crop with or without N fertilization application under NT and CT. Organic amendments in conjunction with leguminous cover crop under NT seemed to increase SOC in the Mountain. Overstreet (2005) observed greater SOC and SON in organic-amended than synthetic-amended systems under NT than under synthetic-amended tilled systems in similar plots from the same experimental site. The NT, SCP, and MBP systems in the Piedmont location each received  $0.06 \text{ Mg N ha}^{-1}$ ,  $0.03 \text{ Mg P ha}^{-1}$ , and  $0.03 \text{ Mg K ha}^{-1}$ . However, crop residues that were left on the soil surface, together with the lack of soil disturbance, are responsible for the higher SOC and SON under NT than SCP or MBP.

Tillage did not impact MBC in the Coastal Plain and Piedmont locations; but a tillage effect was observed in the Mountain location, with MBC concentrations of the order  $\text{NTO} > \text{CTO} = \text{CTS} = \text{CT} = \text{NTS}$ . Averaged across tillage systems for the Mountain location, MBC was 1.5 times greater under NT than CT (Fig. 3). The greater concentration of MBC in the NTO than CTO suggests the positive influence of no-till, while the greater concentration under organic-amended (NTO or CTO) than synthetic-amended systems suggests the positive

influence of organic amendments on enhancing MBC in soil.

Tillage influenced MBN concentrations in all locations (Fig. 3). In the Coastal Plain, the MBN concentration did not differ under S ( $0.03 \text{ g kg}^{-1} \text{ soil}$ ), NT ( $0.02 \text{ g kg}^{-1} \text{ soil}$ ), and CT ( $0.02 \text{ g kg}^{-1} \text{ soil}$ ) but MBN was significantly greater under CTO ( $0.03 \text{ g kg}^{-1} \text{ soil}$ ) than under these other systems. Average MBN concentration was 1.4 times greater under NT than CT systems in the Mountain, where the order for MBN was the same as that for MBC. In the Piedmont, the MBN concentration under NT ( $0.04 \text{ g kg}^{-1} \text{ soil}$ ) was twice the concentration under SCP ( $0.02 \text{ g kg}^{-1} \text{ soil}$ ) or MBP ( $0.02 \text{ g kg}^{-1} \text{ soil}$ ); while MBN under SCP and MBP did not differ. These results suggest the positive impacts of organic amendments, no-till and/or organic amendments, and no-till systems to MBN enhancement in the Coastal Plain, Mountain, and Piedmont locations, respectively. This study thus observed greater C and N concentrations under undisturbed systems [SOC in S, NTO, and NT in the Coastal Plain, Mountain, and Piedmont, respectively; SON in NTO and NT in the Mountain and Piedmont locations, respectively; MBC (NTO in the Mountain location); and MBN (NTO and NT in the Mountain and Piedmont locations, respectively)] and under disturbed systems that received organic amendments [MBC under CTO in the Mountain location) and MBN under CTO in both Coastal Plain and Mountain locations). Other authors (Overstreet, 2005; Muruganandam, 2007; Gruver, 2007) have reported similar results about greater SOC and N and MBC and N contents under NT compared to CT in the same experimental Mountain and Piedmont plots. The results also

support evidence for the positive impacts of NT on organic C and N accumulation reported elsewhere in the literature. The results provide further evidence for the increased aggregation with decreasing tillage intensity observed in the Piedmont location, where greater SOC and SON under NT (Fig. 2) corresponded to greater MWD under same system (Fig. 1). Zhang et al. (2004) observed higher MBC and MBN in NT and organic than CT systems in the Coastal Plain location. Under NT, continuous residue addition to the soil surface occurs, resulting in increased organic C and N concentrations (Tisdall and Oades, 1980). Gradual decomposition of these residues on the soil surface by the soil microbial biomass enhances SOC and SON concentrations in NT systems. Relative to NT, organic matter loss is greater under CT because the soil is more severely disturbed by cultivation (Adu and Oades 1978).

#### **Aggregate associated organic and microbial biomass C and N**

There were no significant effects due to tillage on aggregate associated C and N (organic C and N in aggregates) in the Coastal Plain location, nor were there any tillage and aggregate size interactions (Fig. 4). However, there was a significant effect due to aggregate size in this location; as organic C was two times greater in macro- ( $118.8 \text{ g C kg}^{-1}$  aggregates) than micro-aggregates ( $59.1 \text{ g C kg}^{-1}$  aggregates) in the successional or forest regrowth system (S). Tillage significantly affected aggregate associated C and N in the Mountain location; and there were also significant effects due to aggregate size, but not

due to tillage by aggregate size interaction (Fig. 4). In this location, organic C concentration in macroaggregates was about two times greater under NTO than under CTO, CTS, or CT; whereas it did not differ between NTO and NTS or between NTS and CTO, CTS, and CT. Microaggregate associated C was two times greater under NTO than CT but not significantly different between NTO and NTS, CTO, and CTS or between CT and NTS, CTO, and CTS. In the Piedmont, tillage significantly affected aggregate associated C but there were no aggregate size or tillage  $\times$  aggregate size interaction effects (Fig. 4). Macroaggregate associated C was two times greater under NT than SCP and three times greater than under MBP; the concentrations under SCP and MBP did not differ. Microaggregate associated C was two times greater under NT than SCP or MBP.

Tillage, aggregate size, and tillage  $\times$  aggregate size effects on aggregate associated N in all locations were similar to those observed for aggregate associated C in the respective locations; except in the Mountain location where aggregate size effects on organic N in aggregates did not differ (Fig. 5). Organic N was two times greater in macro- ( $10.6 \text{ g C kg}^{-1}$  aggregates) than micro-aggregates ( $4.7 \text{ g C kg}^{-1}$  aggregates) in the successional or forest regrowth system (S) in the Coastal Plain location. In the Mountain location, aggregate associated N in macroaggregates was two times greater under both NTO and NTS than under CT. Concentrations of aggregate associated N did not differ between NTO or NTS and CTO and CTS. Microaggregate associated N was 2 times greater under NTO than CT. In the Piedmont location, macro- and micro-aggregate associated N concentrations were two

times greater under NT than SCP or MBP, while concentrations did not differ under SCP and MBP.

In their study of crop rotation effects on roots, hyphae, and organic matter and water-stable aggregates in the 0 to 10 cm depth in a red-brown earth in Australia over five decades, Tisdall and Oades (1980) attributed the higher organic matter content of macroaggregates than microaggregates to the occurrence of decomposing roots and hyphae in intimate association with inorganic materials. The authors suggested that tillage operations accelerated the destruction of the roots and hyphae, thereby disrupting the association and rendering macroaggregates unstable. The authors also observed that microaggregates contained less organic C than macroaggregates. In another study involving the 0 to 30 cm depth of a loam soil (fine, silty, mixed, mesic Pachic Haplustolls) in Nebraska, Elliott (1986) explained that the higher organic C contents of macroaggregates vs microaggregates were due to the organic C contents of microaggregates that are bound together to form macroaggregates (microaggregates within macroaggregates). In the present study, the concentrations of macroaggregate associated C and N were 2 or 3 times greater under NT and NTO than CT (SCP and MBP) and CT in the Piedmont and Mountain locations, respectively. The greater C and N concentrations are likely due to C and N concentrations of decomposing plant materials, crop residues on the soil surface and plant roots and hyphae in the undisturbed NT systems relative to the tilled systems. These results support the findings of Tisdall and Oades (1980) and illustrate the positive influence of NT

and/or organic-amended NT systems, and the adverse effects of CT and no-amended CT systems, on aggregate C and N enhancements.

It is not clear why there were no significant tillage effects on aggregate associated organic C and N in the Coastal Plain location. This result may, however, be related to the age of the experiment (9 yr) at this location, relative to 13 and 24 yr in the Mountain and Piedmont locations, respectively. According to Angers et al. (1992), tillage-induced changes in cultivated native soils are observed first with aggregate size distribution and stability before changes in SOC are observed. This premise was supported in the present study by the lack of evidence for significant differences in MWD in the Coastal Plain and Mountain locations (Fig. 1). It is likely that the experiment at the Mountain location, 4 yr older than the Coastal Plain location at the time this study was undertaken, has begun to show observable changes in SOC content and will subsequently show differences in aggregate stability. The experiment at the Piedmont location was 15 and 11 yr older than those at Coastal Plain and Mountain locations, respectively. It is also possible that some inherent properties, such as clay or sand content, may account for the absence of significant effects in the Coastal Plain contrary to what was observed in the Mountain. The Coastal Plain contained approximately 50% less clay, but 1.2 times more sand, than either the Mountain or Piedmont location (data shown in Chapter IV of this dissertation).

Aggregate associated MBC and MBN concentrations were unaffected by tillage or aggregate size in the Coastal Plain and Mountain locations. In contrast, tillage significantly

impacted aggregate associated MBC and MBN in the Piedmont location (Fig. 4). In the Coastal Plain, mean MBC associated with macroaggregates was 1.3 and 1.7 g kg<sup>-1</sup> soil under NT and CT, respectively; while MBC associated with microaggregates averaged 0.9 and 1.2 g kg<sup>-1</sup> soil under NT and CT, respectively. Corresponding values for MBN in this location were 0.1 g kg<sup>-1</sup> soil for both macro- and micro-aggregates under CT and 0.2 g kg<sup>-1</sup> soil for both aggregate types under NT. In the Mountains, mean concentrations of macro- and micro-aggregate associated MBC were 0.7 and 0.5 g kg<sup>-1</sup> soil under CT and 0.8 and 0.7 g kg<sup>-1</sup> soil under NT. Average concentrations of MBN associated with macroaggregates were 0.2 g kg<sup>-1</sup> soil under both NT and CT and 0.1 g kg<sup>-1</sup> soil under NT and near 0 g kg<sup>-1</sup> soil under CT for MBN associated with microaggregates. In the Piedmont, the concentrations of MBC and MBN were greater under NT than either SCP or MBP (Fig. 4). In this location, the concentrations of macroaggregate associated MBC and MBN were, respectively, 1.4 and 1.9 times greater under NT than CT; while concentrations of microaggregate associated MBC and MBN were 1.7 and 1.8 times greater, respectively. Other authors (Costantini et al., 1996; Wright et al., 2005) have reported significant tillage effects on microbial biomass in the 0 to 2.5 and 0 to 5cm depths, but not deeper. Wright et al. (2005) observed no significant MBC differences in the 2.5 to 20 cm depth in corn under NT or CT, and no differences in MBN for the 0 to 20 cm depth.

The proportions of aggregate associated C in SOC were significantly impacted by tillage and aggregate size effects in the Coastal Plain, where the contribution of aggregate

associated C in SOC was significantly greater in NT than CT by 1.4 times; and greater in macro- than micro-aggregates under all tillage systems in the location by 2-3 times (Fig. 6). There were no significant effects due to interaction between tillage and aggregate size in this location. In the Mountain, there were no significant effects due to tillage or aggregate size but there were significant effects due to interaction between tillage and aggregate size. In the Piedmont, there were no significant tillage or aggregate size effects but there were significant tillage × aggregate size effects. On average, organic C contributions by macro- and micro-aggregates to SOC were, respectively, 57 and 19% under NT and 53 and 21% under CT in the Coastal Plain; 54 and 24% under NT and 60 and 28% under CT in the Mountain; and 63 and 24% under NT and 34 and 37% under CT in the Piedmont location. Contributions to SON by organic N in macro- and micro-aggregates averaged, respectively, 63 and 22% under NT and 53 and 24% under CT in the Coastal Plain; 54 and 25% under NT and 59 and 30% under CT in the Mountain; and 60 and 22% under NT and 34 and 34% under CT in the Piedmont. The tillage × aggregate size interactions in the different locations suggest that tillage effects on aggregate C (or N) contributions in SOC (or SON) depended on aggregate size.

Aggregate associated MBC and MBN quotients, which express the proportions of SOC and SON contributed by aggregate associated MBC and MBN, were not significantly different under both NT and CT in the three locations (Fig. 7). There were significant aggregate size effects on MBC quotients in the Coastal Plain, where aggregate C

contributions to SOC were 4 and 3 times greater in macro- than micro-aggregates under CT and NT; and in the Mountain, where the contributions were 6 times greater in macro- than micro-aggregates under CTO. There were tillage × aggregate size interactions in the Piedmont location. Contributions to SON were 3 times greater in macro- than micro-aggregates under CT in the Coastal Plain.

Soil microbial biomass quotient has been suggested as an index to measure the efficiency of conversion of organic C into microbial C and to measure SOC losses during decomposition, with a suggested range of 1-5% of SOM (Sparling, 1992). The values obtained in the present study ranged from about 1 to 2% (Fig. 8). The similar and relatively narrow C/N ratios in microbial biomass (Table 4) suggest that similar sizes and nature of microorganisms were present in both macro- and micro-aggregates in the different tillage systems at each location. The MBC/MBN ratios all seem to be approximately in the range (10 to 12) for fungal MBC/MBN ratio (Jenkinson and Ladd, 1981). Soil microbial biomass quotient, which has also been suggested as an index to measure SOM response to management changes (Ross et al. 1982), has been found to vary from 0.27 to 7.0% across different soil management systems, sampling times, and analytical methodology (Anderson and Domsch, 1989).

Soil, aggregate, and microbial biomass C/N ratios were not significantly different in all locations (Table 4). However, the microbial biomass C/N ratios were generally below the soil C/N ratios, suggesting that there was no net immobilization of N by microbial biomass.

## Conclusions

After 9, 13, and 24 yr of continuous tillage systems in the Coastal Plain, Mountain, and Piedmont location, respectively, NT practices increased water stable aggregation in the Piedmont but not in the other locations. Among other factors, the duration a given tillage system has been in place and the intensity of the tillage system may be determining factors of aggregation in the surface depth of the soils studied. Due to the accumulation of organic residues on the soil surface, undisturbed systems (with or without organic amendments) increased SOC and SON concentrations in the bulk soil at all locations. Aggregation increases with decreasing tillage intensity and greater SOC and SON contents indicated greater MWD under NT systems. Relative to NT, organic matter loss is greater under CT because the soil is more severely disturbed by cultivation under CT. No-till systems have positive effects on the enhancement of organic C and N in aggregate because no-till systems increase organic C and N concentrations in macro- and micro-aggregates, likely due to C and N concentrations of decomposing plant materials and crop residues on the soil surface in undisturbed systems. Relative to CT, NT systems (with or without organic amendments) can enhance organic and microbial biomass C and N contents of aggregates and bulk soil; likely because continuous residue addition to the soil surface occurs under NT.

## References

- Adu, J.K. and J.M. Oades. 1978. Physical factors influencing decomposition of organic materials in soil aggregates. *Soil Biol. Biochem.* 10: 109-115.
- Abid, M. and R. Lal. 2008. Tillage and drainage impact on soil quality I. Aggregate stability, carbon and nitrogen pools. *Soil & Till. Res.* 89-98.
- Ahl, C., R.G. Joergensen, E. Kandeler, B. Meyer, V. Woehler. 1998. Microbial biomass and activity in silt and sand loams after long-term shallow tillage in central Germany. *Soil & Tillage Research* 49: 93-104.
- Anderson, T.H. and K.H. Domsch. 1989. Ratio of microbial biomass carbon to total organic carbon in arable soils. *Soil Biol. Biochem.* 21: 471-479.
- Angers, D. A., A. Pesant, and J. Vigneux. 1992. Early cropping-induced changes in soil aggregation, organic matter, and microbial biomass. *Soil Sci. Soc. Am. J.* 56:115-119.
- Aoyama, M., D.A. Angers, and A. Dayegamiye. 1999. Particulate and mineral-associated organic matter in water-stable aggregates as affected by mineral fertilizer and manure applications. *Can. J. Soil Sci.* 79: 295–302.
- Ashman, M.R., P.D. Hallett, and P.C. Brookes. 2003. Are the links between soil aggregate size class, soil organic matter and respiration rate artefacts of the fractionation procedure? *Soil Biology & Biochemistry* 35: 435–444.
- Bayer, C., L. Martin-Neto, J. Mielniczuk, and C.A. Ceretta. 2000. Effect of no-till cropping systems on soil organic matter in a sandy clay loam Acrisol from Southern Brazil monitored by electron spin resonance and nuclear magnetic resonance. *Soil Till. Res.*, 53: 95–104.
- Bayer, C., J. Mielniczuk, E. Giasson, L. Martin-Neto, and A. Pavinato. 2006. Tillage Effects on Particulate and Mineral-Associated Organic Matter in Two Tropical Brazilian Soils. *Communications in Soil Science and Plant Analysis* 37 (3): 389-400.
- Beare, M.H., P.F. Hendrix, and D.C. Coleman. 1994. Water-stable aggregates and organic matter fractions in conventional and no tillage soils. *Soil Sci. Soc. Am. J.* 58: 777–786.
- Birch, H.F. 1960. Nitrification in soils after different periods of dryness. *Plant & Soil* 12: 81-96.

- Blanco-Canqui, H., and R. Lal. 2004. Mechanisms of carbon sequestration in soil aggregates. *Crit. Rev. Plant Sci.* 23:481–504.
- Bossuyt, H., K. Denef, J. Six, S.D. Frey, R. Merckx, K. Paustian. 2001. Influence of microbial populations and residue quality on aggregate stability. *Applied Soil Ecology* 16: 195–208.
- Cambardella, C.A. and E.T. Elliott. 1992. Particulate organic matter changes across a grassland cultivation sequence. *Soil Sci. Soc. Am. J.* 777-783.
- Cambardella, C.A., Elliott, E.T., 1993. Carbon and nitrogen distribution in aggregates from cultivated and native grassland soils. *Soil Biol. and Biochem.* 57: 1071-1076.
- Cambardella, C.A. and E. Elliott. 1994. Carbon and nitrogen dynamics of soil organic matter fractions from cultivated grassland soils. *Soil Sci. Soc. Am. J.* 58:123-130.
- Caravaca, F. , A. Lax, and J. Albaladejo. 2001. Soil aggregate stability and organic matter in clay and fine silt fractions in urban refuse-amended semiarid soils. *Soil Sci. Soc. Am. J.* 65:1235–1238.
- Celik, I, I. Ortas, and S. Kilic. 2004. Effects of compost, mycorrhiza, manure and fertilizer on some physical properties of a Chromoxerert soil. *Soil & Till. Res.* 78: 59–67.
- Chan, K.Y. 2001. Soil particulate organic carbon under different land use management. *Soil Use and Management* 17: 217-221.
- Chan, K. Y., and J.A. Mead. 1988. Surface physical properties of a sandy loam soil under different tillage practices. *Aust. J. Soil Res.* 26, 549-59.
- Chaney, K. and R.S. Swift. 1984. The influence of organic matter on aggregate stability in some British soils. *Journal of soil Science* 35: 223-230.
- Chesters, G., O. J. Attoe, and O. N. Allen. 1957. Soil aggregation in relation to various soil constituents. *Soil Sci Soc Am J.* 21: 272–277.
- Costantini, A., D. Cosentino, and S. Andrea. 1996. Influence of tillage systems on biological properties of a Typic Argiudoll soil under continuous maize in central Argentina. *Soil & Till. Res.* 38: 265-271.
- Dalal, R.C. and R.J. Mayer. 1986. Long-term trends in fertility of soils under continuous

- cultivation and cereal cropping in Southern Queensland. II. Total organic carbon and its rate of loss from the soil profile. *Aust. J. Soil Res.* 24: 281-292.
- Daniels, R.B., S.W. Buol, H.J. Kleiss, and C.A. Ditzler. 1999. Soil Systems in North Carolina. *Tech. Bull.* 314. North Carolina State University. Soil Sci. Dept. Raleigh, NC 27695-7619.
- Degens, B.P., G.P. Sparling, L.K. Abbott. 1994. The contribution from hyphae, roots and organic carbon constituents to the aggregation of a sandy loam under long term clover-based and grass pastures. *European J. Soil Sci.* 45: 459-468.
- Denef, K., J. Six, H. Bossuyt, S.D. Frey, E.T. Elliott, R. Merckx, and K. Paustian. 2001. Influence of dry-wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. *Soil Biol. Biochem.* 33: 1559-1611.
- Dick, W.A., R.L. Blevins, W.W. Frye, S.E. Peters, D.R. Christenson, F.J. Pierce, and M.L. Vitosh. 1998. Impacts of agricultural management practices on C sequestration in forest-derived soils of the eastern Corn Belt. *Soil & Tillage Research* 47: 235-244.
- Elliott, E.T., 1986. Aggregate structure and carbon, nitrogen and phosphorus in native and cultivated soils. *Soil Science Society of America Journal* 50: 627-633.
- Elliott, E.T., K. Paustian., and S. D. Frey. 1996. Modeling the measurable or measuring the modelable: a hierarchical approach to isolating meaningful soil organic matter fractionations. In: Powlson, D.S., Smith, P., Smith, J.U. (Eds.), *Evaluation of Soil Organic Matter Models Using Existing Long-term Datasets*, NATO ASI Series. Springer Verlag, Berlin, pp. 161–179.
- Evrendilek, F, I. Celik, and S. Kilic. 2004. Changes in soil organic carbon and other physical soil properties along adjacent Mediterranean forest, grassland, and cropland ecosystems. *J. Arid Environ* 59:743–752.
- Fonte, S. J., Y. Edward, P. Ofori, G. W. Quansah, B. Vanlauwe, and J. Six. 2009. Fertilizer and residue quality effects on organic matter stabilization in soil aggregates. *Soil Sci. Soc. Am. J.* 73:961-966.
- Gallardo, A. and W. H. Schlesinger. 1994. Factors limiting microbial biomass in the mineral soil and forest floor of a warm-temperate forest. *Soil Biol. Biochem.* 26: 1409–1415.

- Gee, G.W. and J.W. Bauder. 1986. Particle size analysis. pp. 383-441. In: A. Klute (ed.), *Methods of Soil Analysis, Part 1*, 2<sup>nd</sup> Edition. ASA and SSSA Publ., Madison, WI, USA.
- Green, V.S., D.E. Stott, J.C. Cruz, and N. Curi. 2007. Tillage impacts on soil biological activity and aggregation in a Brazilian Cerrado Oxisol. *Soil and Till Res.* 92 (1-2): 114-121.
- Gregorich, E. G., M. H. Beare, U. F. McKim, and J. O. Skjemstad. 2006. Chemical and Biological Characteristics of Physically Uncomplexed Organic Matter. *Soil Sci. Soc. Am. J.* 70:975–985.
- Gruver, Joel Brookes. 2007. Impact of Management and Texture on Soil Organic Matter Fractions (Doctoral dissertation). North Carolina State University, Raleigh, NC. 312 p.
- Hamblin, A.P. 1980. Changes in Aggregate Stability and Associated Organic Matter Properties after Direct Drilling and Ploughing on some Australian Soils. *Aust. J. Soil Res.* 18: 27-36.
- Hassink, J. 1997. The capacity of soils to preserve organic C and N by their association with clay and silt particles. *Plant and Soil* 191: 77–87.
- Haynes, R.J. and G.S. Francis. 1993. Changes in microbial biomass C, soil carbohydrate composition and aggregate stability induced by growth of selected crop and forage species under field conditions. *Journal of Soil Science*, 44, 665–675.
- Hernández-Hernández, R.M., D. López-Hernández. 2002. Microbial biomass, mineral nitrogen and carbon content in savanna soil aggregates under conventional and no-tillage. *Soil Biol. Biochem.* 34: 1563–1570.
- Jastrow, J.D. 1996. Soil aggregate formation and the accrual of particulate and mineral-associated organic matter. *Soil Biol. Biochem.* 28 (4/5): 665-676.
- Jenkinson, D. S. and D.S. Powlson. 1976. The effects of biocidal treatments on metabolism in soil – V A method for measuring soil biomass. *Soil Biol. Biochem.* 8: 209-213.
- Jenkinson, D. S., and J. N. Ladd. 1981. Microbial biomass in soil: measurement and turnover. *In 'Soil Biochemistry'*. (Eds. E. A. Paul and J. N. Ladd.) Vol. 5, pp. 415-417. (Marcel Dekker: New York.)
- Kanazawa, S., S. Asakawa, Y. Takai. 1988. Effect on fertilizer and manure application on microbial numbers, biomass, and enzyme activities in volcanic ash soils. I. Microbial

- numbers and biomass carbon. *Soil Sci. Plant Nutr.* 34: 429–439.
- Kandeler, Ellen, M. Stemmer, and E-M Klimanek. 1999. Response of soil microbial biomass, urease and xylanase within particle size fractions to long-term soil management. *Soil Biol. Biochem.* 31: 261-273.
- Laird, D A., P. Barak, E.A. Nater, and R.H. Howdy. 1991. Chemistry of smectitic and illitic phases in interstratified soil smectite. *Soil Sci. Soc. Am. J.* 55: 1499-1504.
- Laird, D A., P. Y. Yen, W. C. Koskinen, T. R. Steinhelmer, and R. H. Dowdy. 1994. Sorption of atrazine on soil clay components. *Environ. Sci. Technol.* 28: 1054-1061.
- Li, X. G., F. M. Li, R. Zed, Z. Y. Zhan, and B. Singh. 2007. Soil physical properties and their relations to organic carbon pools as affected by land use in an alpine pastureland. *Geoderma* 139: 98-105.
- Marquez, C.O., V.P. Garcia, C.A. Cambardella, R.C. Schultz, and T.M. Isenhardt. 2004. Aggregate-size stability distribution and soil stability. *Soil Sci. Soc. Am. J.* 68: 725–735.
- Mazzarino, M.J., Szott, L., Zimenez, M., 1993. Dynamics of soil total C and N, microbial biomass, and water soluble C in tropical agroecosystems. *Soil Biol. Biochem.* 25: 205–214.
- Mikha, M. M. and C. W. Rice. 2004. Tillage and manure effects on soil and aggregate-associated carbon and nitrogen. *Soil Sci. Soc. Am. J.* 68: 809-816.
- Muruganadam, S. 2007. Soil aggregate-associated microbial community structure and nitrogen transformations in three different tillage systems. Dissertation. North Carolina State University. 138 p.
- Oades, G.M. and A.G. Waters. 1991. Aggregate hierarchy in soils. *Aust. J. Soil Res.* 29: 815-828.
- Overstreet, Laura Flint. 2005. Relationships between soil biological and physical properties in a long-term vegetable management study. (Doctoral dissertation). North Carolina State University, Raleigh, NC. 187 p.
- Parton W J, D. S. Schimel, C. V. Cole and D. S. Ojima. 1987 Analysis of factors controlling soil organic matter levels in Great Plains grasslands. *Soil Sci. Soc. Am. J.* 51: 1173–1179.

- Paul, S., G. O. Martinson, E. Veldkamp, and H. Flessa. 2008. Sample pretreatment affects the distribution of organic carbon in aggregates of tropical grassland soils. *Soil Sci. Soc. Am. J.* 72:500-506.
- Piovanelli, C., C. Gamba, G. Brandi, S. Simonini, and E. Batistoni. 2006. Tillage choices affect biochemical properties in the soil profile. *Soil Tillage Res.* 90, 84–92.
- Powelson, D.S. and D.S. Jenkinson. 1976. The effects of biocidal treatments on metabolism in soil - II. Gamma irradiation, autoclaving, air-drying and fumigation with chloroform or methyl bromide. *Soil Biol. Biochem.* 8: 179-188.
- Puget, P., C. Chenu, and J. Balesdent. 1995. Total and young organic matter distributions in aggregates of silty cultivated soils. *European J. Soil Sci.* 46, 449-459.
- Puget, P., D.A. Angers, and C. Chenu. 1999. Nature of carbohydrates associated with water-stable aggregates of two cultivated soils. *Soil Biology and Biochemistry* 31: 55-63.
- Puget, P., C. Chenu, and J. Balesdent. 2000. Dynamics of soil organic matter associated with particle size fractions of water stable aggregates. *European J. Soil Sci.* 51: 595-605.
- Rosell, R.A., J.C. Gasparoni, and J.A. Galantini. 2001. Soil organic matter evaluation. *In* Assessment methods for soil carbon. Lal, R., J.M. Kimble, R.F. Follet, B.A. Stewart (eds.) CRC Press Boca Raton, FL 33431. pp. 311-322.
- Ross, D. J., T.W. Speir, K.R. Tate, A. Cairns, K.F. Meyrick, and E.A. Pansier. 1982. Restoration of pasture after topsoil removal: effects on soil carbon and nitrogen mineralization, microbial biomass and enzyme activities. *Soil Biol. Biochem.* 14: 575-581.
- Sainju, U.M. and B.P. Singh. 2008. Nitrogen storage with cover crops and nitrogen fertilization in tilled and nontilled soils. *Agron. J.* 100: 619-627.
- Schimel, D.S., D.C. Coleman, and K.A. Horton. 1985. Soil organic matter dynamics in paired rangeland and cropland toposequences in North Dakota. *Geoderma* 36: 201-214.
- Seech, A.G. and E.G. Beauchamp. 1988. Denitrification in soil aggregates of different Sizes. *Soil Sci. Soc. Am. J.* 52:1616-1621.
- Six, J., E.T. Elliott, K. Paustian, and J.W. Doran. 1998. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. *Soil Sci. Soc. Am. J.* 62: 1367–1377.

- Six, J., E.T. Elliott, and K. Paustian. 2000. Soil structure and soil organic matter: II. A normalized stability index and the effect of mineralogy. *Soil Sci. Soc. Am. J.* 64: 1042–1049.
- Sparling, G. P. 1992. Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter. *Aust. J. Soil Res.* 30: 195-207.
- Staley, T.E., W.M. Edwards, C.L. Scott, and L.B. Owens. 1988. Soil microbial biomass and organic component alterations in a no-tillage chronosequence. *Soil Sci. Soc. Am. J.* 52:998-1005.
- Stenberg, B., M. Johansson, M. Pell, K. Sjö Dahl-Svensson, J. Stenström, and L. Torstensson. 1998. Microbial and activities in soil as affected by frozen and cold storage. *Soil Biol. Biochem.* 30 (3): 393-402.
- Stott, D.E., A.C. Kennedy, and C.A. Cambardella. 1999. Impact of soil organisms and organic matter on soil structure. *In* Lal, Rattan (ed.) CRC Press, Inc. 1999. Pp. 57-73.
- Sugihara, S., S. Funakawa, M. Kilasara, and T. Kosaki. 2010. Effect of land management and soil texture on seasonal variations in soil microbial biomass in dry tropical agroecosystems in Tanzania. *Applied Soil Ecology* 44: 80–88.
- Tisdall, J. M. 1980. Stabilization of soil aggregates by plant roots. Ph.D. Thesis. University of Adelaide, Adelaide, Australia.
- Tisdall, J.M., and J.M. Oades. 1980. The effect of crop rotation on aggregation in a ed-brown Earth. *Aust. J. Soil Res.* 18, 423-33.
- Tisdall, J.M., and J.M. Oades. 1982. Organic matter and water stable aggregates in soils. *J. Soil Sci.* 33: 141–163.
- Tu, C., F. J. Louws, N. G. Creamer, J. P. Mueller, C. Brownie, K. Fager, M. Bell, and S. Hu. 2006. Response of soil microbial biomass and N availability to transition strategies from conventional to organic farming systems. *Agriculture, Ecosystems, and Environment* 113: 206-215.
- Vance, E.D., P.C. Brookes, and D.S. Jenkinson. 1987. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19(6): 703-707.
- Van Gestel, M., R. Merckx, and K. Vlassak. 1996. Spatial distribution of microbial biomass in

- microaggregates of a silty-loam soil and the relation with the resistance of microorganisms to soil drying. *Soil Biol. Biochem.* Vol. 28: 503-510.
- Wardle, D.A. 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. *Biol. Rev.* 67: 321–538.
- Whalen, J. K. and C. Chang. 2002. Macroaggregate characteristics in cultivated soils after 25 annual manure applications. *Soil Sci. Soc. Am. J.* 66:1637–1647.
- Wick, A. F., I. J. Lachlan, and P. D. Stahl. 2009. Aggregate and organic matter dynamics in reclaimed soils as indicated by stable carbon isotopes. *Soil Biol. Biochem.* 41: 201-209.
- Wright, A. L., F.M. Hons, and J. E. Matocha Jr. 2005. Tillage impacts on microbial biomass and soil carbon and nitrogen dynamics of corn and cotton rotations. *Applied Soil Ecology* 29: 85–92.
- Yoder, R.E. 1936. A direct method of aggregate analysis of soils and a study of the physical nature of erosion losses. *J. Am. Soc. Agron.* 28: 337-351.
- Youker, R.E., and J.L. McGuinness. 1957. A short method of obtaining mean weight diameter values of aggregate analyses of soils. *Soil Sci.* 83:291–294.
- Zak, D.R., D.F. Grigal, S. Gleeson, and D. Tilman. 1990. Carbon and nitrogen cycling during old-field succession: constraints on plants and microbial biomass. *Biogeochem.* 11: 111–129.
- Zhang, Wei-Jian, Jin-Xia Feng, J. Wu, and K. Parker. 2004. Differences in soil microbial biomass and activity for six agroecosystems with a management disturbance gradient. *Pedosphere* 14 (4): 441-447.
- Zotarelli, L., B. J. R. Alves, S. Urquiaga, E. Torres, H. P. dos Santos, K. Paustian, R. M. Boddey, and J. Six. 2005. Impact of Tillage and Crop Rotation on Aggregate-Associated Carbon in Two Oxisols. *Soil Sci. Soc. Am. J.* 69:482–491.

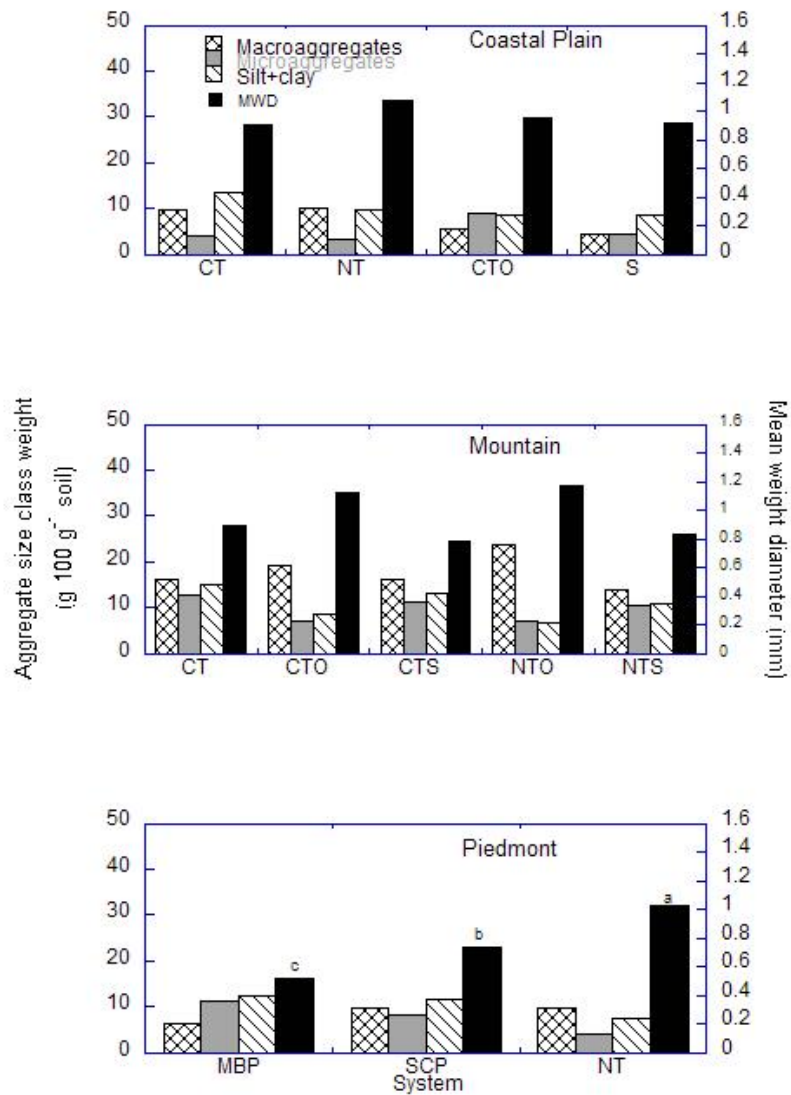


Fig. 1. Aggregate size class distribution and mean weight diameter of wet-sieved samples from three long-term experimental sites. Columns with the same or no letters indicate no significant difference between tillage systems ( $p < 0.05$ ). Coastal Plain systems / CT, NT: conventional tillage and no-till practices, respectively; CTO: Organic-amended CT; S: successional system. Mountain systems / CT: CT with no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic-amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; SCP: spring chisel plow; NT: no-till.

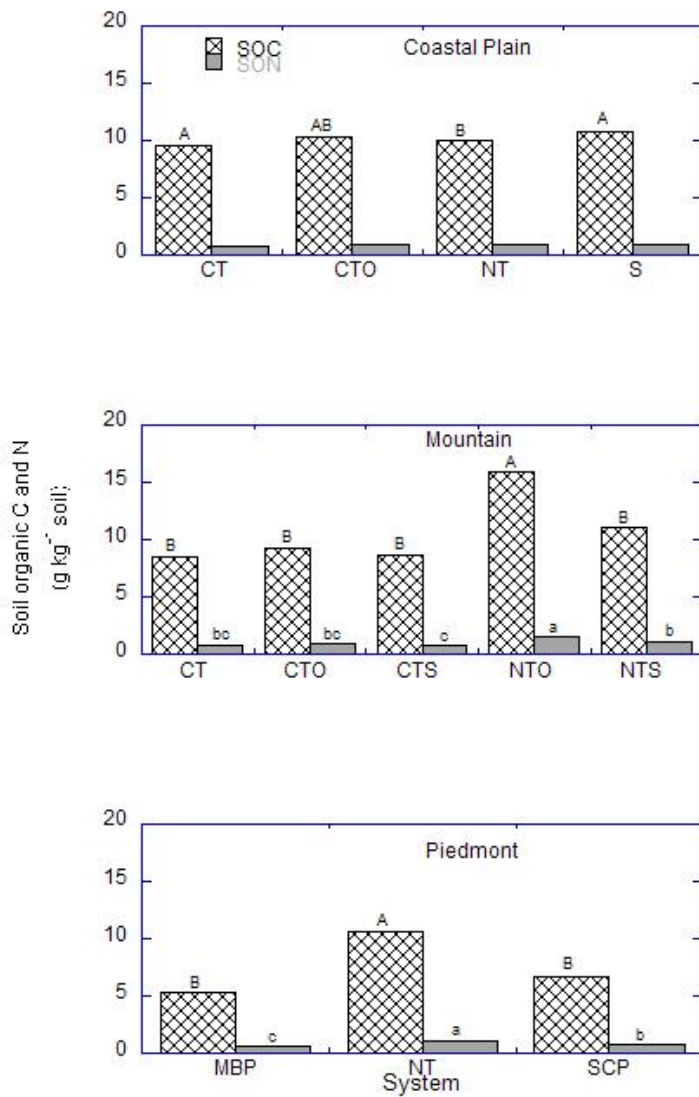


Fig. 2. Soil organic C and N concentrations in bulk soil for different tillage systems in the Coastal Plain, Mountain, and Piedmont locations. Columns with the same (upper/lower case) or no letter indicate no significant difference between tillage systems at ( $p < 0.05$ ). Coastal Plain systems / CT, NT: conventional tillage and no-till practices, respectively; CTO: Organic-amended CT; S: successional system. Mountain systems / CT: CT with no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic- amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; SCP: spring chisel plow; NT: no-till.

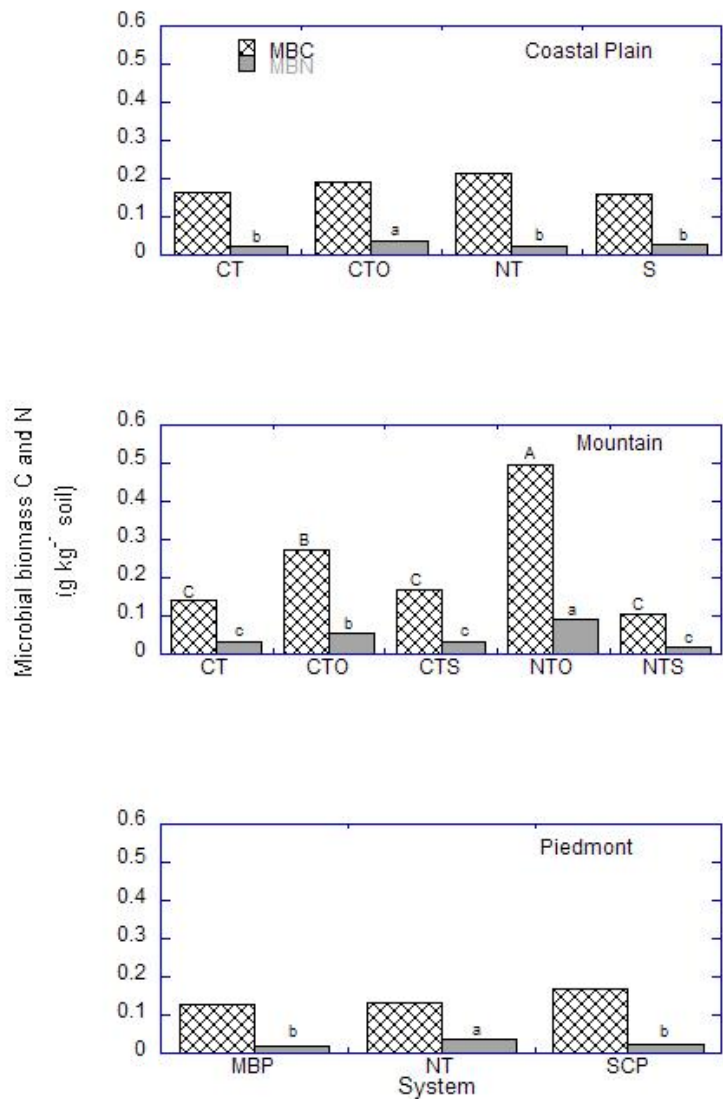


Fig. 3. Microbial biomass C and N concentrations in bulk soil for different tillage systems in the Coastal Plain, Mountain, and Piedmont locations. Columns with the same (upper/lower case) or no letter indicate no significant difference between tillage systems at ( $p < 0.05$ ). Coastal Plain systems / CT, NT: conventional tillage and no-till practices, respectively; CTO: Organic-amended CT; S: successional system. Mountain systems / CT: CT with no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic- amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; SCP: spring chisel plow; NT: no-till.

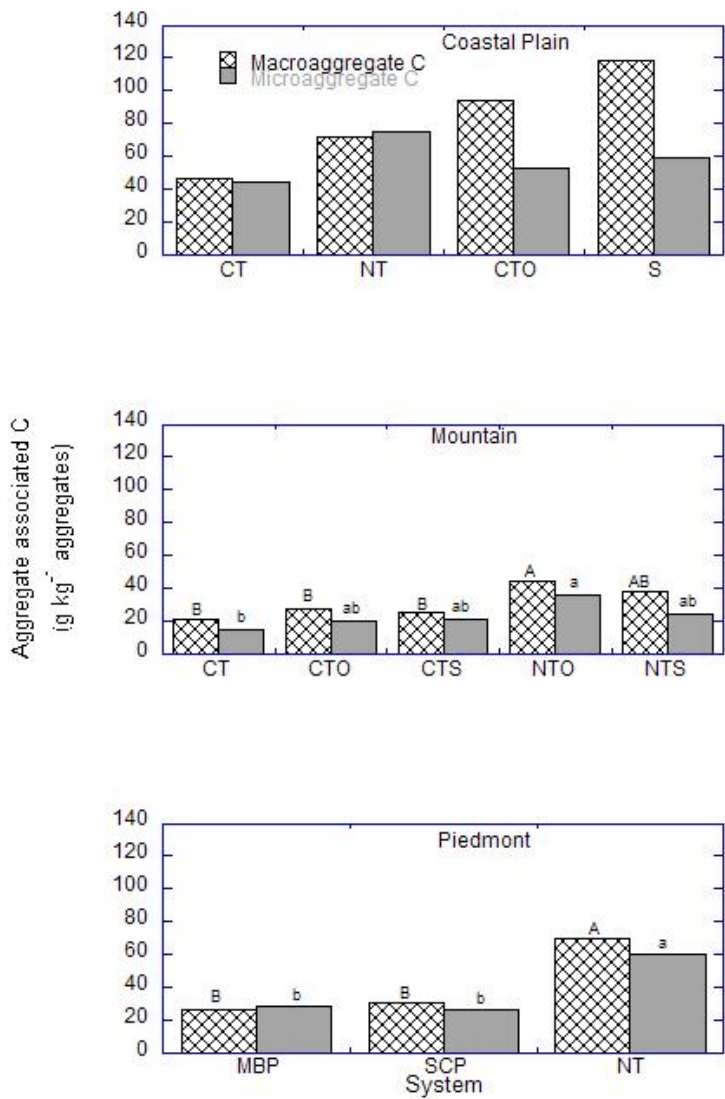


Fig. 4. Organic C concentrations in soil aggregates under different tillage systems in the Coastal Plain, Mountain, and Piedmont locations. Columns with same or no letter indicate no significant difference between tillage systems at ( $p < 0.05$ ): upper and lowercase letters for macro- and micro-aggregate associated C. Coastal Plain systems / CT, NT: conventional tillage and no-till practices, respectively; CTO: Organic-amended CT; S: successional system. Mountain systems / CT: CT with no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic- amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; SCP: spring chisel plow; NT: no-till.

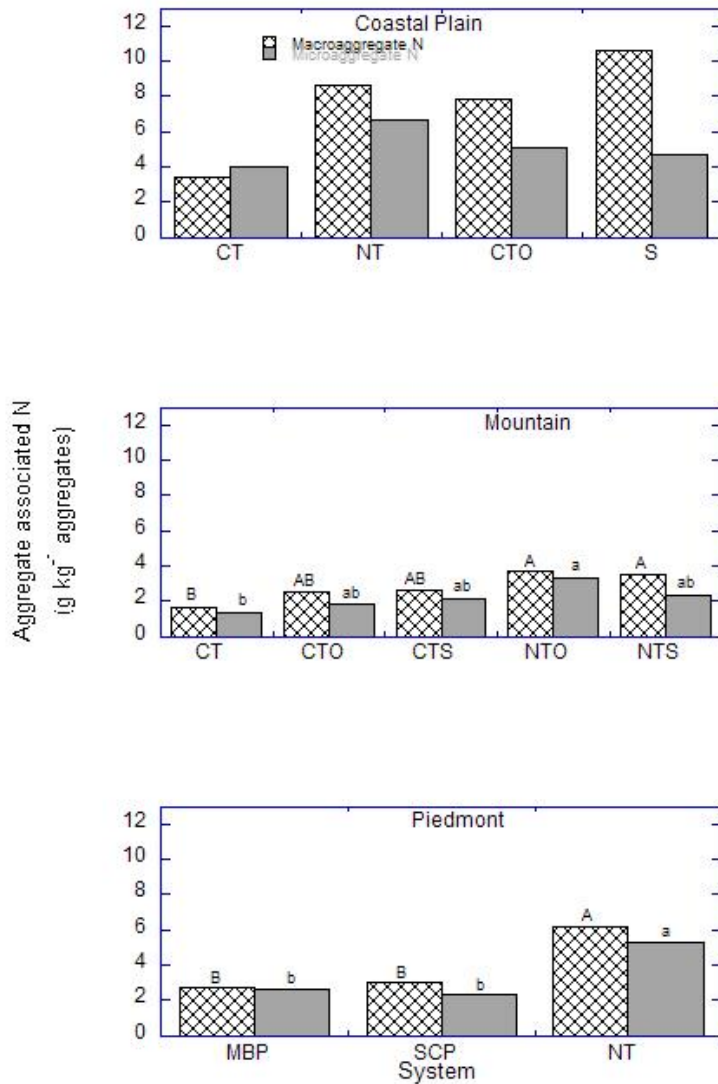


Fig. 5. Organic N concentrations in soil aggregates under different tillage systems in the Coastal Plain, Mountain, and Piedmont locations. Columns with same or no letter indicate no significant difference between tillage systems at ( $p < 0.05$ ): upper and lowercase letters for macro- and micro-aggregate associated N. Coastal Plain systems / CT, NT: conventional tillage and no-till practices, respectively; CTO: Organic-amended CT; S: successional system. Mountain systems / CT: CT with no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic- amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; SCP: spring chisel plow; NT: no-till.

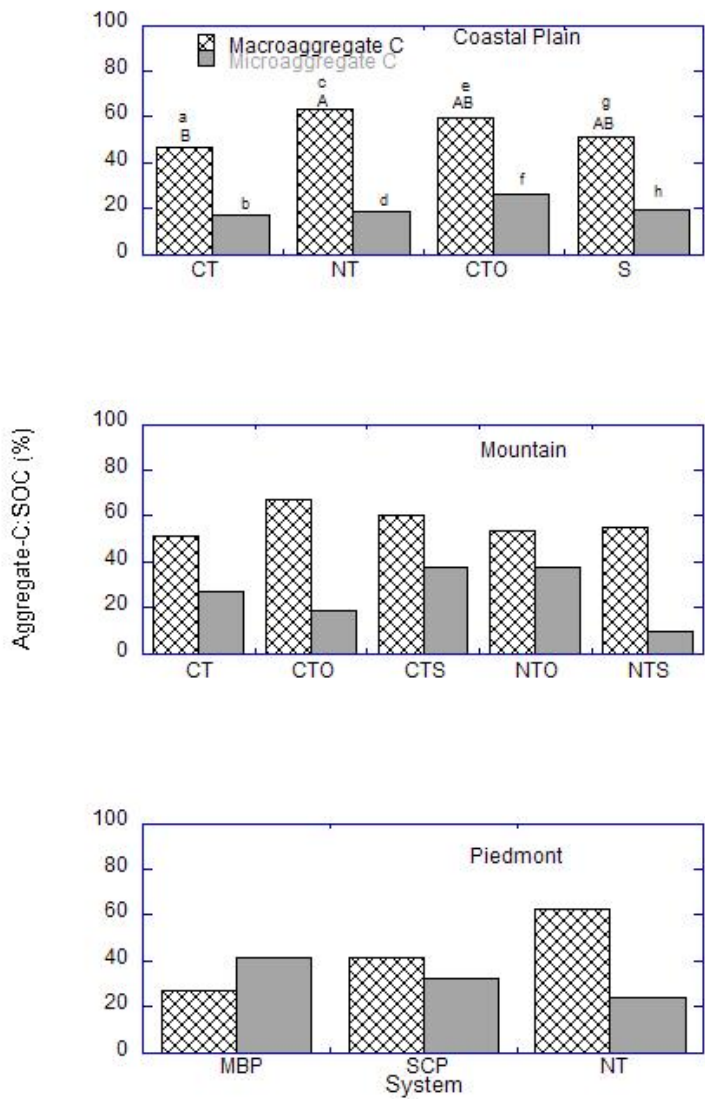


Fig. 6. Contributions of aggregate associated C to SOC under different tillage systems in the Coastal Plain, Mountain, and Piedmont locations. Columns with the same or no letters indicate no significant difference between aggregate associated C contributions ( $p < 0.05$ ): upper and lower case letters compare differences between systems and aggregates, respectively. Coastal Plain systems / CT, NT: conventional tillage and no-till practices, respectively; CTO: Organic-amended CT; S: successional system. Mountain systems / CT: CT with no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic-amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; SCP: spring chisel plow; NT: no-till.

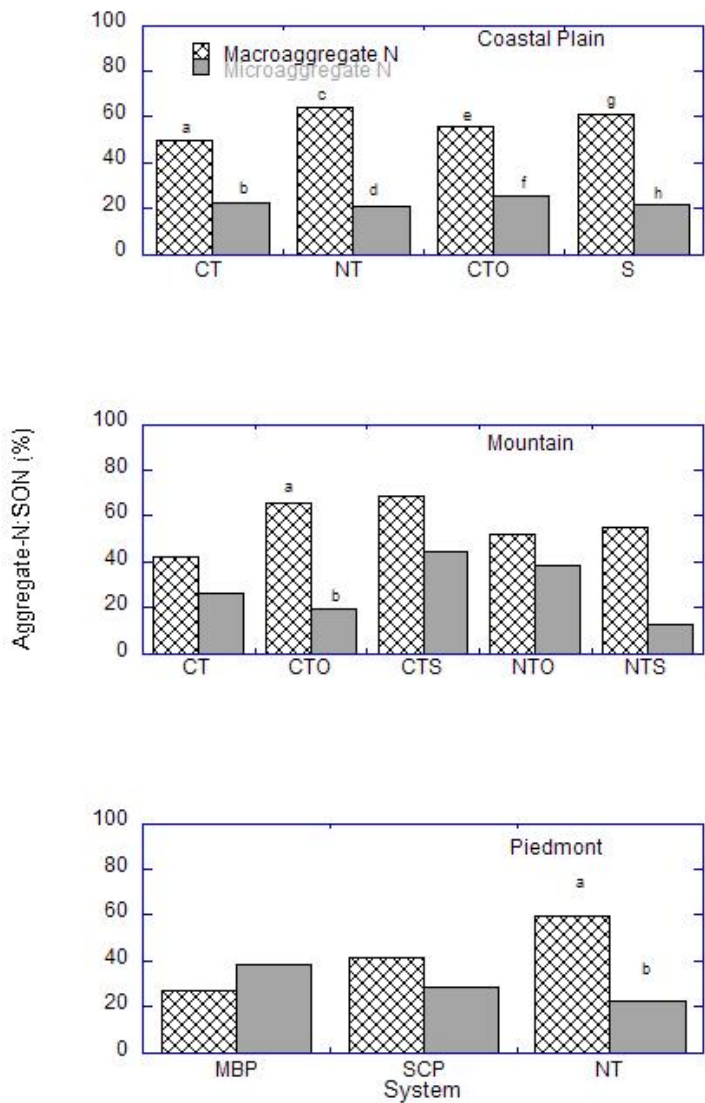


Fig. 7. Contributions of aggregate associated N to SON under different tillage systems in the Coastal Plain, Mountain, and Piedmont locations. Columns with the same or no letters indicate no significant difference between aggregate type contributions ( $p < 0.05$ ). Coastal Plain systems / CT, NT: conventional tillage and no-till practices, respectively; CTO: Organic-amended CT; S: successional system. Mountain systems / CT: CT with no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic-amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; SCP: spring chisel plow; NT: no-till.

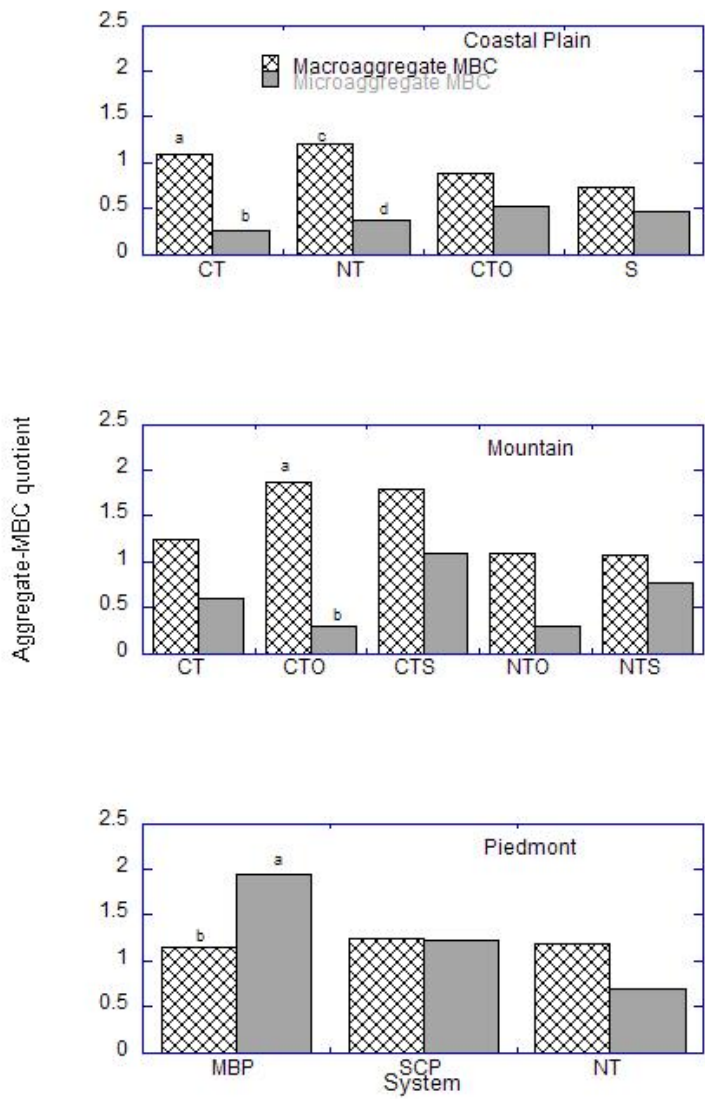


Fig. 8. Microbial biomass C quotients in aggregates under different tillage systems. Columns with the same or no letter indicate no significant difference between aggregate associated MBC ( $p < 0.05$ ). Coastal Plain systems / CT, NT: conventional tillage and no-till practices, respectively; CTO: Organic-amended CT; S: successional system. Mountain systems / CT: CT with no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic-amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; SCP: spring chisel plow; NT: no-till.

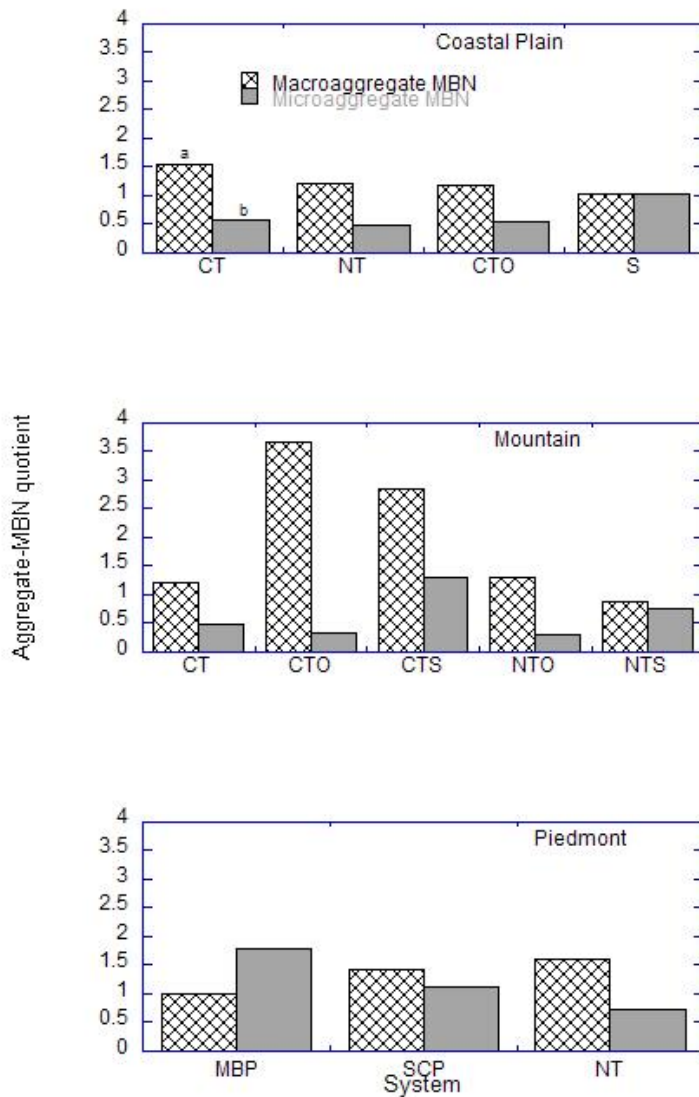


Fig. 9. Microbial biomass N quotients in aggregates under different tillage systems. Columns with the same or no letter indicate no significant difference between aggregate associated MBN ( $p < 0.05$ ). Coastal Plain systems / CT, NT: conventional tillage and no-till practices, respectively; CTO: Organic-amended CT; S: successional system. Mountain systems / CT: CT with no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic-amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; SCP: spring chisel plow; NT: no-till.

Table 1. Tillage systems and selected chemical and physical properties of the 0 to 10 cm depth of soils studied.

System†	Duration yr	pH <sub>water</sub>	CEC cmol <sub>c</sub> kg <sup>-1</sup>	Sand	Silt %	Clay
<u>Coastal Plain</u>						
CT	9	6.1 a	5.6 a	67.3	23.6	9.1
CTO	9	5.9 a	6.4 a	70.2	20.3	9.4
NT	9	5.9 a	6.0 a	64.7	24.6	10.7
S	9	4.8 b	4.2 b	74.2	17.2	8.6
<u>Mountain</u>						
CT	13	5.9 b	6.9 b	60.8	19.8	19.4
CTO	13	6.3 a	7.4 b	53.0	23.2	23.8
CTS	13	6.1 ab	6.8 b	57.6	20.2	22.1
NTO	13	6.9 a	10.5 a	52.1	26.6	21.3
NTS	13	6.0 b	7.6 b	50.9	26.9	22.2
<u>Piedmont</u>						
MBP	24	5.4 b	4.6 b	50.7	26.9	22.4
NT	24	5.9 a	6.1 a	56.9	26.1	17.1
SCP	24	6.1 a	5.9 a	58.1	18.3	23.6

†Coastal Plain systems/ CT, NT: conventional tillage and no-till practices, respectively; CTO: Organic system under conventional tillage; S: successional or natural vegetation re-growth system. Mountain systems/ CT: conventional tillage (CT) with no inputs; CTO: organic-amended system under CT; CTS: synthetic-amended system under CT; NTO: organic-amended system under no-till; NTS: synthetic-amended system under no-till. Piedmont systems/ MBP: moldboard plow; SCP: spring chisel plow; NT: no-till.

Table 2. Description of crops grown in the plots studied at the experimental sites.

Year(s)/Period	Treatments (Tillage systems and crops grown)			
	Coastal Plain			
	<u>CT</u> <sup>†</sup>	<u>NT</u>	<u>CTO</u>	<u>S</u>
1999	Corn ( <i>Zea mays</i> L.)	Corn	Soybean	Fallow
2000	Peanut ( <i>Arachis hypogaea</i> L.)	Peanut	Sweet potato ( <i>Ipomea batatas</i> L.)	Fallow
2001	Cotton ( <i>Gossypium spp.</i> L.)	Cotton	Wheat-Cabbage ( <i>Brassica oleracea</i> L.)	Fallow
2002	Corn	Corn	Corn	Fallow
2003	Peanut	Peanut	Soybean	Fallow
2004	Corn	Corn	Corn	Fallow
2005	Corn	Corn	Corn	Fallow
2006	Grain sorghum ( <i>Sorghum bicolor</i> L.) and wheat ( <i>Triticum aestivum</i> L.)	Grain and wheat	Biculture: Cowpea ( <i>Vigna sinensis</i> L.) or soybean and crimson clover ( <i>Trifolium incarnatum</i> L.) or wheat	Fallow
2007	Soybean ( <i>Glycine max</i> L.)	Soybean	Soybean	Fallow

<sup>†</sup>CT, NT: conventional tillage and no-till practices, respectively; CTO: Organic system under conventional tillage; S: successional or natural vegetation re-growth system.

Table 2 continued

Year(s)/Period	Treatments (Tillage systems and crops grown)				
	Mountain				
	<u>CTS</u> <sup>†</sup>	<u>NTS</u>	<u>CTO</u>	<u>NTO</u>	<u>CT</u>
1995-2006	Staked tomatoes ( <i>Solanum lycopersicum</i> ) with rye ( <i>Secale cereale</i> L.) as cover crop		Staked tomatoes with hairy vetch ( <i>Vicia sativa</i> L.) as cover crop		Staked tomatoes with wheat or crimson clover ( <i>Trifolium incarnatum</i> L.) as cover crop
2007	Corn only with rye as cover crop		Corn only with vetch as cover crop		Corn only with wheat or crimson clover as cover crop
	Piedmont				
	<u>MBP</u> <sup>†</sup>		<u>SCP</u>		<u>NT</u>
1984-1989	Corn only				
1990-2005	Corn-Soybean rotation				
2006-2007	Corn only				

†Mountain systems/ CT: conventional tillage (CT) with no inputs; CTO: organic-amended system under CT; CTS: synthetic-amended system under CT; NTO: organic- amended system under no-till; NTS: synthetic-amended system under no-till. Piedmont systems/ MBP: moldboard plow; SCP: spring chisel plow; NT: no-till.

Table 3. Organic and synthetic amendments at the experimental sites.

Amendment	Application method	Rate	Element supplied		
			N	P	K
(Mg ha <sup>-1</sup> )					
<u>Coastal Plain</u>					
Turkey litter	Mechanically incorporated	3900 – 13500	81-282	51-176	52-176
TSP (0-46-0)			-	0.11	-
Potash (0-0-60)			-	0	0.14
Urea	Banded in NT, mechanically incorporated in CT	0.23	0.11	0	0
N:P:K 10-34-0			0.02	0.08	0
N:P:K 30-0-0			0.07	0	0
Gypsum, for liming peanut	Broadcast and mechanically incorporated	0.22 to 8.75			
<u>Mountain</u>					
Soybean meal (N:P:K, 7:2:1)		0.23	0.02	0.005	0.002
ammonium nitrate (NH <sub>4</sub> NO <sub>3</sub> )		0.17	0.06		
TSP (0-46-0)	Banded in NT, mechanically incorporated in CT, at planting.				
KCl (0-0-60)					
RP (0-30-0)					
Sul-Po-Mag (0-0-22)	Surface broadcast prior to planting winter cover crops.				
<u>Piedmont</u>					
N:P:K 10-20-20	At planting. Banded in NT, mechanically incorporated in MBP and SCP.	0.13	0.01	0.03	0.03
NH <sub>4</sub> NO <sub>3</sub>	Applied as side-dressing about one month after planting. Banded in NT, mechanically incorporated in MBP and SCP.	0.15	0.05		

TSP: triple superphosphate; RP: rock phosphate; KCl: potassium chloride

Table 4. C:N ratios in soil and aggregate associated organic matter and microbial biomass under different tillage systems in the Coastal Plain, Mountain, and Piedmont locations.

System†	Bulk soil C:N	Bulk soil MBC:MBN	Aggregate associated C:N			
			M_OM	m_OM	MMB	mMB
<u>Coastal Plain</u>						
CT	15 (1)††	8 (1)	14 (1)	11 (1)	11 (3)	8 (3)
NT	13 (1)	10 (1)	12 (1)	11 (1)	16 (3)	9 (3)
CTO	12 (1)	6 (1)	12 (1)	11 (1)	8 (3)	9 (3)
S	13(1)	7 (1)	11 (1)	13 (1)	9 (3)	7 (3)
<u>Mountains</u>						
CT	10 (0)	4 (1)	12 (1)	11 (1)	13 (2)	13 (2)
CTO	11 (0)	5 (1)	11 (1)	11 (1)	8 (2)	11 (2)
CTS	11 (0)	6 (1)	10 (1)	10 (1)	10 (2)	10 (3)
NTO	12 (0)	6 (1)	11 (1)	10 (1)	15 (2)	12 (3)
NTS	11 (0)	5 (1)	12 (1)	11 (1)	9 (3)	11 (3)
<u>Piedmont</u>						
MBP	10 (0)	8 (1)	10 (1)	10 (1)	12 (1)	12 (1)
SCP	10 (0)	8 (1)	10 (1)	11 (1)	10 (1)	11 (1)
NT	11 (0)	5 (1)	11 (1)	11 (1)	8 (1)	10 (1)

†Coastal Plain systems/ CT, NT: conventional tillage and no-till practices, respectively; CTO: Organic system under conventional tillage; S: successional or natural vegetation re-growth system. Mountain systems/ CT: conventional tillage (CT) with no inputs; CTO: organic-amended system under CT; CTS: synthetic-amended system under CT; NTO: organic- amended system under no-till; NTS: synthetic-amended system under no-till. Piedmont systems/ MBP: moldboard plow; SCP: spring chisel plow; NT: no-till.

††Values in parentheses are standard errors of means. M\_OM, m\_OM: macro- and micro-aggregate associated organic matter; MMB/mMB: macro- or micro-aggregate associated microbial biomass, respectively.

## CHAPTER II

### TILLAGE EFFECTS ON C AND N DYNAMICS IN DIVERSE AGROECOSYSTEMS: SOIL C AND N STRATIFICATION AND SEQUESTRATION

#### Abstract

No-till (NT) practices conserve organic matter and enhance C sequestration. This study assessed tillage impacts on soil organic C (SOC) and N (SON) dynamics in the 0 to 10 cm depth of a Wickham sandy loam (fine-loamy, mixed, semi active, thermic Typic Hapludult), Delanco fine-sandy loam (fine-loamy, mixed, mesic, Aquic Hapludult), and Wedowee sandy clay loam (fine, kaolinitic, thermic Typic KanHapludult) in Coastal Plain, Mountain, and Piedmont locations, respectively. Mean SOC under NT was 18 and 14 Mg ha<sup>-1</sup> in the Mountain and Piedmont respectively, 1.5 and 1.8 times greater than under conventional tillage (CT). Average SOC under both NT and CT in the Coastal Plain was 13 Mg ha<sup>-1</sup>. Under NT and CT, SON ranged from 1 to 2 Mg ha<sup>-1</sup> in all locations. Stratification ratios (SR), which express the ratio C (or N) in surface soil depth to that in lower depths, generally averaged 2 under NT and 1 under CT for both SOC and SON in the upper depth ratio for all locations. The difference between SR in the lower and upper depth ratios was generally 1 in the Coastal Plain and Piedmont, and zero in the Mountain. These results illustrate greater accumulation and stratification of SOC and SON under NT; and that stratification ratios can reflect SOC and SON dynamics across tillage systems and locations.

## Introduction

Soils contain the largest pool of organic C in the terrestrial ecosystem and are important to offsetting global warming (Lal, 2007). Benefits of high levels of SOC in farming systems include ease of cultivation, root penetration, greater aggregate stability, reduced bulk density, and improved water holding capacity (Carter and Stewart, 1996; Lal, 2002). Perturbations such as tillage influence SOC maintenance and enhancement (Jarecki and Lal, 2003; Lal, 2004) and, therefore, long-term ecosystem sustainability (Percival et al., 2000). Farmers adopt no-till (NT) and other conservation tillage systems mainly because the practices are energy efficient and cost effective, sequester C, conserve soil moisture and organic matter, reduce erosion, and deliver other benefits related to improvement of wild life habitat and soil biodiversity (Ahl et al., 1998; Dick et al., 1998; Kushwaha et al., 2001; Mitchell et al., 2007). By enhancing soil aeration contact between soil and crop residues, and exposing organic matter protected by aggregates to attack by microbial biomass, conventional tillage (CT) systems result in SOC loss (Dalal and Mayer, 1986; Beare et al., 1994). Adoption of conservation tillage practices causes modifications in soil properties which in turn results in a new equilibrium distribution of soil organic C and N (Dick et al., 1998). Organic matter attenuation, through mixing of surface with lower soils horizons, and decomposition due to frequent tillage operations (Angers et al., 1992) are some of the causes of tillage-induced decline in SOC content of native soils (Tiessen et al., 1982; Elliott, 1986). In the southeastern region of the United States, about 42.5% of the cropped land

was under no-till or other forms of conservation tillage in 2002 (Franzluebbers, 2005).

Soil organic matter (SOM) comprises 50 to 58% C (Nelson and Sommers, 1982) and is the main source of N in native soils (Bauer and Black, 1994). Soil microorganisms obtain most of their energy from SOM and, therefore, control SOM composition (Sparling, 1985; Jenkinson, 1988), in addition to the source of the organic material. Soil organic matter influences key soil processes and properties including nutrient cycling, soil structure formation, water retention, C sequestration, bulk density, infiltration rate, cation exchange capacity, aggregate stability, and biological activity (Mitchell et al., 2000; Olk and Gregorich, 2006). Soil microbial biomass responds more rapidly to changes in the below-ground quality of SOM, compared to the quantity of SOM present (Powlson et al., 1987; Saffigna et al., 1989). It is, therefore, often used as an early and highly responsive indicator of tillage-induced changes in soil (Lynch and Panting, 1980; Drury et al., 1991). Staley et al. (1988) observed that microbial biomass C (MBC) levels were 60% greater in the 0 to 7.5 cm soil depth of NT than under CT systems in a NT chronosequence previously under continuous maize (*Zea mays*) cropping (0 to 20 yr) on a silt loam Alfisol.

By influencing the extent of soil mixing and crop residue incorporation, tillage affects organic matter distribution and microbial biomass activity within the soil (Carter, 1986). Conventional tillage disperses plant and other organic residues in an even manner and enhances greater soil-residue contact and decomposition in a uniform soil environment. This more uniform distribution of OM promotes soil aeration, organic C and N distribution

in the plow layer, and microbial activity (Piovanelli et al., 2006). However, these benefits fade with time. These soil conditions stimulate the mineralization of native SOM and added residue (Wander and Yang, 2000), which slows down soil C sequestration (Piovanelli et al., 2006; Wander and Yang, 2000). In conservation tillage, crop residue accumulates near the surface of the soil (Franzluebbers, 2002; Deen and Kataki, 2003; Piovanelli et al., 2006) and, because most of the residue is humified, its accumulation reduces SOM mineralization, thereby increasing C sequestration (Piovanelli et al., 2006). There are, however, no evidences suggesting that conservation tillage systems are superior to CT systems regarding the amount of C sequestered in the entire soil profile. In an on-farm assessment of C sequestration in a 0 to 60 cm soil depth in paired fields under long-term (>4 yr) NT and CT systems across 11 representative Major Land Resource Areas (MLRAs) in Ohio, Kentucky, and Pennsylvania, Blanco-Canqui and Lal (2008) concluded that on the whole, NT does not store SOC more than CT for the entire soil profile.

There are few studies that have examined the influence of management practices on SOC across physiographic regions in the southeastern US (Causarano et al., 2008). Also, assessments of SOC storage in soils under varying tillage and management systems in different regions on an equivalent soil mass basis has been reported to be more appropriate and accurate (Ellert and Bettany, 1995; Yang and Wander, 1999; Wuest, 2009). In a comparative evaluation of various methods of calculating nutrient storage, Ellert and Bettany (1995) found that expressing SOC and other nutrients on an equivalent soil mass

basis can account for differences in soil bulk density and, therefore, for soil mass effects on estimates of SOC storage. With these factors in mind, the objectives of this study were to use the equivalent mass method to assess the impacts of different tillage systems on SOC and N dynamics in long-term experiments of different agroecosystems. The results will be used to test the following hypotheses: 1. Changes in soil organic and microbial biomass C and N in the 0-to 10 cm soil depth will be associated with changes in tillage intensity. 2. Differential stratification ratios (SR), which indicate the differences between SR for an element (e.g. SOC or SON) in different depth ratios (e.g. the 0-10:10-20 cm and 0-10:20-30 cm depth ratios examined in this study), can serve as an index of tillage intensity and SOC and SON dynamics.

## **Materials and Methods**

### **Description of study sites**

This study was conducted in three long-term experiments on research stations, two farmer-managed fields, and three forests adjacent to each long-term experimental site. The experimental sites are each located in one of the three physiographic regions of North Carolina, namely, the Coastal Plain, Piedmont, and Mountain regions, which represent approximately 45, 39, and 16% of the state's land area, respectively. Detailed descriptions of the regions are given in Daniels et al. (1999) and Medina et al. (2004). The Center for Environmental Farming Systems (CEFS; 35° 23' 5" N, 77° 59' 35" W) was established in 1994

and is located near Goldsboro, Wayne County. The Mountain Horticultural Crops Research Station, MHCRS; (35° 25' 50" North, 82° 30' 5" West) is situated near Mills River, Henderson County. The Upper Piedmont Research Station; UPRS (36° 21' 17" N, 79° 39' 53" W) is near Reidsville, Rockingham County. Long term mean annual temperatures and precipitations at the experimental sites in the Coastal Plain, Mountain, and Piedmont are 16°C and 953 mm, 13°C and 1050 mm, and 15°C and 1066 mm, respectively (State Climate Office of North Carolina). Comparative studies of tillage and management practices in the experimental plots in the Coastal Plain, Mountain, and Piedmont locations started 9, 14, and 24 years, respectively before the current study was undertaken. Further details about each of the experimental sites can be obtained at the following web sites:

<http://www.cefs.ncsu.edu/farmingsys.htm>, <http://www.ces.ncsu.edu/fletcher/>, and <http://www.agr.state.nc.us/research/uprs.htm>. Table 1 shows the different crops grown since establishment of experiments in the three locations. Adjacent to each experimental site, a natural forest was included as a reference ecosystem. Two farmer-managed fields were also studied in Faison, Duplin County (Coastal Plain location) and Reidsville, Rockingham County (Piedmont location).

Tillage systems evaluated at CEFS included a Best Management Practices (BMP) cropping system subdivided into NT and CT, an organic production system under CT (CTO), and an old field succession ecosystem under fallow (S). Conventional tillage in both organic and CT with BMP systems generally consisted of fall disking followed by chisel plowing in

early spring. At MHCRS, tillage systems included the following: 1) moldboard plowing, followed by disking and harrowing, receiving organic inputs and a fall planted hairy vetch cover crop (CTO); 2) moldboard plowing, followed by disking and harrowing and receiving fertilizers and other synthetic amendments (e.g. herbicides and fungicides) and a fall planted rye cover crop (CTS); 3) moldboard plowing, followed by disking and harrowing and receiving no inputs or cover crops (CT); 4) no-till, receiving chemical fertilizers and other synthetic amendments and a rye cover crop (NTS); and 5) no-till, receiving organic fertilizers and other amendments and a hairy vetch cover crop (NTO). Tillage systems studied at UPRS were part of a larger experiment investigating nine tillage systems. However, the present study evaluated only three of these systems: no-till (NT); spring moldboard plow followed by disking (MBP); and spring chisel plow (SCP; chisel plowing in the spring without disking). Depths of tillage in the moldboard, chisel, and disk operations, where applicable at each research station, were approximately 25, 25, and 12 cm, respectively.

Various synthetic and organic fertilizers or amendments were applied in the plots at each location, reflecting the different farming systems and needs for the various crop types (Table 2). It was assumed that over 90% of the P and K in turkey litter were available for plant uptake during the growing season. The applied quantities of turkey litter were assumed to provide a fertilizer N, P, and K equivalents of 141 to 488, 153 to 530, and 89 to 308 kg ha<sup>-1</sup>, respectively. Litter application was mainly for supplying N, so that P and K were apparently over-applied. Maximum (100%) solubility was assumed for soybean meal

applied, while a 3% solubility was assumed for rock phosphate. In the synthetic treatments, disease and insect control treatments were applied according to recommendations from the NC Ag Chemical Manual. In the organic treatments disease and insect control in the organic treatments were according to generally approved methods for organic production systems. Table 2 describes the amendments applied at the experimental sites as well as the timing and frequency of applications of inputs.

For the on-farm sites, the Piedmont farmer had practiced no-till for at least 20 yr that included cover cropping (a mixture of rye, hairy vetch, and crimson clover) and crop rotation. Fertilizer (18-46-0) was surface applied at  $113 \text{ kg ha}^{-1}$  for corn, as well as cattle manure at  $25 \text{ Mg ha}^{-1}$ . The organic farm at Faison, ridged and planted to sweet potato, had been in operation for about 15 yr and received mainly poultry manure at about  $6\text{-}7 \text{ Mg ha}^{-1}$ , corresponding to  $220$  to  $250 \text{ kg N ha}^{-1}$ .

### **Experimental designs**

Experimental plots consisted of replicated tillage treatments ( $n = 3$  at CEFS and  $n = 4$  at both UPRS and MHCRS) in randomized complete block designs. The experimental design at CEFS comprised five treatments in each replication. The soils at this (CEFS) site are mainly Wickham sandy loam (fine-loamy, mixed, semi active, thermic Typic Hapludult), although there was some area classified as a Tarboro loamy sand (mixed, thermic, Typic Udipsamment). Plots vary in size from  $0.8$  to  $4.5 \text{ ha}$  (Bell, 2002). Plot dimensions at MHCRS

were 24 by 12 m (Overstreet, 2005) and 16 by 5.8 m at UPRS (Gruver, 2007). The soil type at MHCRS was a Delanco fine-sandy loam (fine-loamy, mixed, mesic, Aquic Hapludult) and at UPRS a Wedowee sandy clay loam (fine, kaolinitic, thermic Typic Kanhapludult). Table 3 describes selected physical and chemical properties of the soils studied.

### **Soil sampling**

Sampling at all locations occurred between May and August 2008, beginning at CEFS and ending at the MHCRS plots. Sampling in natural ecosystems was on the same day(s) that samples were taken at the research station in the region. Planting had already occurred prior to sampling at the UPRS, but not at the CEFS and MHCRS experimental sites. Sampling at CEFS was near or around 5 to 6 points that have been previously marked or geo-referenced for sampling purposes. At other locations, 3 to 6 randomly assigned and demarcated sampling locations were selected within defined soil-landscape positions in each replication or farm. Samples were taken within plant rows for the 0-10, 10-20, and 20-30 cm soil depths using a 2.5-cm diameter by 30-cm deep long soil probe (at CEFS) or a 7.5-cm diameter by 30-cm deep long soil probe at UPRS, MHCRS, and farmer fields). Around each sampling location, three samples were taken and combined in plastic buckets according to the three soil depths. Composite samples were placed into ziplock bags and stored in coolers containing ice for transport to the laboratory.

A subsample from each composite depth samples was placed in moisture cans for

determination of gravimetric soil water content. Once at the lab, and within 6 to 24 h after sample collection, field moist samples were gently crushed by hand and sieved to pass, first through an 8-mm sieve, and then a 4-mm sieve. A set of the sieved samples was stored in a refrigerator (4°C) while another set was air-dried and ground to pass a 2-mm sieve screen.

### **Soil physical, chemical, and microbiological analyses**

Particle size distribution was determined according to Gee and Bauder (1986). The surface soil (0-10 cm depth) was sampled with an Uhland core sampler (ring diameter of 7.6cm by 7.6 cm in length) for bulk density determinations (Blake and Hartge, 1986) at locations in the Piedmont region. For the experimental sites at research stations in the two other regions, bulk density was measured in the previous year (2007). The soil maximum field capacity was determined according to Schachtschabel et al., 1998). Determination of CEC, soil pH, and exchangeable bases were performed by the North Carolina Department of Agriculture and Consumer Services. The chloroform fumigation extraction method according to Vance et al. (1987) was used to determine soil microbial biomass C (MBC) on moist samples, previously stored at 4°C, without the subtraction of values from controls or unfumigated samples. Mineralizable C and N were determined according to Anderson (1982) and Parfitt et al. (2001). Whole soil and aggregate size materials were finely ground and their total C and N concentrations determined by direct combustion in a Perkin–Elmer 2400 CHN analyzer. The methods of Ghani et al. (2003) and Chan and Heenan (1999) were

used to determine water soluble C (WSC) and hot water extractable C (HWEC). Soils in this study were generally at pH 6.5 or less and low in carbonates. Therefore, total C was assumed equal to organic C.

### Calculations and statistical analysis

Correction for artifacts of sampling equipment and conditions are crucial for across-region comparisons (Wuest, 2009). Sample adjustment to equivalent soil mass removes sensitivities associated with these artifacts (McGarry and Malafant, 1987; Ellert and Bettany, 1995). Therefore, SOC and N stocks for tillage systems in each ecosystem were calculated on an equivalent mass basis according to Ellert and Bettany (1995):

- a. Calculating soil mass for each treatment at each site from:

Soil mass per unit area ( $\text{Mg ha}^{-1}$ ) = soil depth (m)  $\times$  bulk density ( $\text{Mg m}^{-3}$ )  $\times$   $10000 \text{ m}^{-2} \text{ ha}^{-1}$ .

- b. Identifying the equivalent soil mass: In this study, the mass of the most disturbed system (in the 0- 10-cm depth) in each location was denoted as the equivalent mass ( $M_{\text{equiv}}$ ) per unit area.
- c. Accounting for between-sample variations, by calculating the additional thickness ( $T_{\text{add}}$ ) or the layer additional to the 0-10 cm soil depth that is required to attain the equivalent mass:

$$T_{\text{add}} = ((M_{\text{equiv}} - M_{0-10}) \times 0.0001 \text{ ha m}^{-2}) / \rho_b$$

where  $T_{add}$  and  $M_{equiv}$  are as defined above,  $M_{0-10}$  is the soil mass in the 0-10 cm depth, and  $\rho_b$  is the bulk density at the 0-10 cm depth of the soil to be adjusted.

- d. Expressing SOC (or N) pools on mass per unit area in equivalent soil mass basis:

$$\text{SOC (or N) (Mg ha}^{-1}\text{)} = \text{C (or N) (kg Mg}^{-1}\text{ soil)}/M_{equiv}.$$

According to Ellert and Bettany (1995), it is not so much the definitive value chosen as the  $M_{equiv}$  that is critical, but the use of a single reference value for comparing SOM storage at a particular site. In the present investigation,  $M_{equiv}$  values were used for within-region comparisons only. Sampling was done in 10-cm increments up to 30 cm; but only the first 10 cm depth was analyzed for masses of SOC and SON per area in equivalent soil mass.

Natural ecosystems can serve as references for experimental treatments in managed systems (Dalal and Mayer, 1986) since they may be considered the standard ecosystem of “climax steady-state” (Puget et al., 2005), with nearly steady addition of organic matter and no anthropogenic perturbations or exports of elements (e.g. in harvests or tillage). For this reason, the difference between C and N pools in forests and those in managed systems can serve as indicators of each managed system’s potential to sequester C (Degryze et al., 2004). Ellert and Bettany (1995), however; seem to urge caution regarding comparisons of managed and natural systems which are clearly not subjected to similar experimental conditions. This study considered Ellert and Bettany’s (1995) caution and, therefore, did not include data from both natural and farmer-managed sites into the statistical analysis for

data from the experimental sites. The data from both natural and farmer-managed sites were averaged within locations and included for non-statistical comparisons only.

Sequestered SOC (or SON) was taken to be the mass of SOC (or SON) per unit area ( $\text{Mg ha}^{-1}$ ) in the equivalent mass of soil for each location. Soil organic C sequestration rates (mass of SOC per unit area in an equivalent soil mass per duration of experiment) were calculated as follows:

$$\text{SOC sequestration rate} = \text{SOC}_{\text{System; in equiv. soil mass}} / \text{duration of experiment}$$

where  $\text{SOC}_{\text{System; in equiv. soil mass}}$  is the mass of SOC per unit area in the equivalent soil mass for the tillage system under consideration and duration is the number of years for which the tillage system has been in place.

According to the definition by Franzluebbers (2002), the stratification ratio (SR) of C or N in the present study is the amount of C (or N) in the surface (0- to 10-cm depth) divided by the amount of C (or N) in the 10 to 20 or 20 to 30cm depth. Differential stratification ratio (DSR) is thus defined in this study as the difference between SR at the lower depth of stratification and SR at the upper depth of stratification. Since bulk densities measurements were conducted for the 0 to 10 cm soil depth only, SR C and N concentrations (instead of masses) were used to calculate SR1&2 and DSR.

a.  $DSR\ C\ (or\ N) = SR2\ C\ (or\ N)_{0-10:20-30\ cm} - SR1\ C\ (or\ N)_{0-10:10-20\ cm}$

where  $DSR\ C\ (or\ N)$  = differential stratification for C (or N).

b.  $SR1\ C\ (or\ N) = C\ (or\ N)$  concentration in the 0 to 10 cm depth divided by C (or N) concentration in the 10-20 cm depth; and

c.  $SR2\ C\ (or\ N) = C\ (or\ N)$  concentration in the 0-10 cm depth divided by C (or N) concentration in the 20 to 30 cm depth.

Tillage effects and comparison of treatment means were evaluated by using the mixed procedure (Proc Mixed) model of the Statistical Analytical Software (SAS Inst., Cary, NC).

## Results and Discussion

### Soil mass, bulk density, and additional soil thickness required to attain equivalent mass

Tillage effects on bulk density ( $Db$ ) were significant in the Coastal Plain and Mountain but not in the Piedmont (Fig. 1). Soil  $Db$  values ranged from 1.2 to 1.5, 1.1 to 1.3, and 1.3 to 1.4  $Mg\ m^{-3}$  in the Coastal Plain, Mountain, and Piedmont locations, respectively. This study observed significantly greater  $Db$  values in less disturbed systems (NT and S) compared to CT in the Coastal Plain, suggesting that tillage reduced  $Db$  in the location. Relative to the conventional tillage system with no inputs (CT), lower  $Db$  values were observed in both conventional tillage and no-till systems with organic (CTO, NTO) or

synthetic (CTS, NTS) amendments in the Mountain, suggesting that organic or synthetic amendments decreased Db in this location. On average, Db values were 1.1 times greater under no-till than conventional tillage systems in the Coastal Plain location and 1.1 times greater under conventional than no-tillage in the Mountain location. There were no significant tillage differences in Db among tillage systems in the Piedmont. Reports about tillage effects on Db are varied in the literature. Osunbitana et al. (2005) observed increased Db with increased tillage intensity in the 0 to 15 cm depth in an Oxic Tropudalf, while Yang and Kay (2000) did not observe any differences in Db between NT and CT. In contrast, Wander et al. (1998) and Pierce et al. (1994) found greater Db values for NT relative to CT. Other authors have reported that Db in both NT and CT is also affected by row position. Da Silva et al. (1997) and Vervoot et al. (2001) observed greater Db in trafficked than untrafficked interrows in three different Alfisols.

Tillage effects on soil mass per area followed similar patterns as soil Db; with significant effects occurring in the Coastal Plain and Mountain locations but not in the Piedmont (Table 4). Tillage effects on soil mass were in the order NT = S > CT = CTO in the Coastal Plain; and CT > CTS = CTO = NTO = NTS in the Mountain. Soil mass per area ranged from 1231 to 1501, 1133 to 1323, and 1252 to 1412 Mg ha<sup>-1</sup> in the Coastal Plain, Mountain, and Piedmont, respectively. Additional soil thickness (T<sub>add</sub>) required to attain the equivalent soil mass differed significantly in the Coastal Plain; being significantly greater in CTO than NT and S but not significantly different from CT. In the Mountain location, T<sub>add</sub>

was significantly lower in CT but not different in the other systems. The values for T<sub>add</sub> did not differ among tillage systems in the Piedmont (Table 4). In all tillage systems, the patterns for T<sub>add</sub> were opposite those for soil mass and Db, suggesting negative correlations of T<sub>add</sub> with soil mass and Db.

### **Soil organic carbon and nitrogen**

Tillage significantly affected SOC stocks in surface soils of all locations (Fig. 2). In the Coastal Plain location, SOC was significantly greater under the successional system (S; 14.0 Mg ha<sup>-1</sup>) than no-till (12.9 Mg ha<sup>-1</sup>) and conventional tillage under best management practices (CT; 12.3 Mg ha<sup>-1</sup>). The SOC stock did not, however, differ between S and CTO, the conventional system with organic amendments (13.3 Mg ha<sup>-1</sup>). In the Mountain location, SOC under no-till with organic amendments (NTO; 21.0 Mg ha<sup>-1</sup>) was significantly greater than SOC under no-till with synthetic amendments (NTS; 14.6 Mg ha<sup>-1</sup>), CTO (12.2 Mg ha<sup>-1</sup>), conventional tillage with synthetic amendments (CTS; 11.4 Mg ha<sup>-1</sup>), and conventional tillage receiving no inputs (CT; 11.1 Mg ha<sup>-1</sup>). The SOC in these latter systems (NTS, CTO, CTS, and CT) were not significantly different. In the Piedmont, the no-till system (NT) contained a significantly greater stock of SOC (13.9 Mg ha<sup>-1</sup>) than either the spring chisel plow (SCP; 8.8 Mg ha<sup>-1</sup>) or moldboard plow (MBP; 6.8 Mg ha<sup>-1</sup>). The SOC stocks under SCP and MBP did not differ. Averaged across tillage systems, SOC stocks did not differ between less disturbed and disturbed systems in the Coastal Plain but were 1.5 and 1.8 times greater

under less disturbed than disturbed system. Soil organic N stocks were not significantly affected by tillage systems in the Coastal Plain; but patterns in SON distribution in the Mountain and Piedmont were similar to those for SOC (Fig. 2). In the Mountain, SON was significantly greater under NTO ( $2.0 \text{ Mg ha}^{-1}$ ) than under the other tillage systems which averaged approximately  $1.0 \text{ Mg SON ha}^{-1}$ . In the Piedmont, SON was significantly greater under NT ( $1.3 \text{ Mg ha}^{-1}$ ) than SCP ( $0.9 \text{ Mg ha}^{-1}$ ), which was in turn greater than SOC under MBP ( $0.7 \text{ Mg ha}^{-1}$ ). The SOC stock averaged 1.1, 1.6, and 1.7 times greater under less disturbed than disturbed systems in the Coastal Plain, Mountain, and Piedmont locations, respectively.

The above findings indicate that undisturbed systems in the Coastal Plain, such as the successional or forest re-growth system, and conventional tillage systems receiving organic amendments, generally increased SOC and SON stocks. In the Mountain, organic amendments also increased SOC and SON; while in the Piedmont, no-till increased SOC and SON. Relative to disturbed systems, less disturbed soils contain more C and N in the surface layers (Doran, 1987; Bossuyt et al., 2002) and are often covered on the surface by crop residues which protect surface soil from erosion and SOC from exposure to biotic and abiotic degradative processes (Dumontet et al, 2001). Organic amendments also increase SOC in soils (Tirol-Padre et al., 2007). Relative to less disturbed systems, SOC oxidation is greater under conventional tillage systems due to increased soil aeration, soil-crop residue contact, and exposure of aggregate-protected organic C to biotic and abiotic degradation

(Elliott, 1986; Beare et al., 1994; Al-Kaisi and Yin, 2005). Soil organic C is also greater under conventional tillage systems due to increased soil erosion under these systems (Chaney and Swift, 1984). Overstreet (2005) and Gruver (2007) have reported greater SOC amounts in conservation tillage systems compared to disturbed systems in the Mountain and Piedmont locations, respectively.

### **Labile pools of carbon and nitrogen**

Soil microbial biomass C stocks were significantly affected by tillage in the Mountain ( $p < 0.0001$ ) and Piedmont ( $p < 0.0001$ ) locations but not in the Coastal Plain location (Fig. 3). Soil microbial biomass C averaged 0.23 and 0.24 Mg ha<sup>-1</sup> under no-till and conventional tillage systems in the Coastal Plain. In the Mountain, MBC stocks were of the order NTO (0.65 Mg ha<sup>-1</sup>) > CTO (0.37 Mg ha<sup>-1</sup>) > CTS (0.22 Mg ha<sup>-1</sup>) = CT (0.18 Mg ha<sup>-1</sup>) = NTS (0.13 Mg ha<sup>-1</sup>). The MBC stocks in the Piedmont were in the order of SCP (0.22 Mg ha<sup>-1</sup>) > NT (0.17 Mg ha<sup>-1</sup>) = MBP (0.16 Mg ha<sup>-1</sup>). Averaged over tillage systems within a location, MBC did not differ under NT and CT in the Coastal Plain and Piedmont but was 1.5 times greater under NT than CT in the Mountain. Microbial biomass N stocks did not differ among tillage systems in the Coastal Plain and Piedmont. In the Mountain, MBC stocks were of similar order to MBN: NTO (0.12 Mg ha<sup>-1</sup>) > CTO (0.08 Mg ha<sup>-1</sup>) > CTS (0.04 Mg ha<sup>-1</sup>) = CT (0.04 Mg ha<sup>-1</sup>) = NTS (0.03 Mg ha<sup>-1</sup>) (Fig. 3).

This study did not find any differences in MBC and MBN among tillage systems in the

Coastal Plain. Zhang et al. (2004) reported higher amounts of MBC and MBN in the NT and organic systems than CT and successional systems for the plots at this location. Among other reasons, such as the time or season of the year when sampling was conducted, the discrepancy between the observation in this study and that of Zhang et al. (2004) may possibly be related to differences in the sampling depths (15 cm in the study by Zhang et al. (2004) versus 10 cm in the present study). The amounts of C or N in the relatively shallow depth in the present study may not have been subjected to greater dilution as may occur when samples from deeper down the soil profile are mixed with the surface soil. This study also considered the possibility of differences due to the methods of expressing amounts of C and N in the two studies, namely; on concentrations basis by Zhang et al. compared to an equivalent soil mass basis in the present study. According to Ellert and Bettany (1995), differences in soil Db and thickness influence nutrient storage in soil. In the present study Db, which was greater under NT than CT systems (Fig. 1), correlated highly and positively with soil mass ( $r \geq 0.99$ ;  $p < 0.0001$ ), but highly and negatively with T<sub>add</sub>, the additional soil thickness required to achieve equivalent mass ( $r \geq -0.99$ ;  $p < 0.0001$ ). This suggests that between two soils differing in Db, the lower Db soil would require greater additional thickness to achieve an equivalent mass of the denser soil. Therefore, it is speculated that the (CT) system with the lower Db would require greater additional thickness to achieve an equivalent mass of the denser (NT) system, resulting in increased MBC under CT and subsequent elimination of significant differences in MBC stocks between the two systems.

In the 0-10 cm depth considered in the present study, expressing C and N pools on concentration as well as equivalent soil mass basis did not, however, drastically change the order of the different pools (Table 5); suggesting that the two methods of expressing C and N amounts will not differ to any great extent in this depth. In the present study, additional thickness required in a tillage system to attain the equivalent soil mass ranged from -0.01 to 0.03 m (Table 4), which was not expected to introduce any appreciable errors or differences in estimating element masses in equivalent soil mass as may occur when estimates have to be made for deeper depths.

In the Mountain location, organic amendments in the NTO and CTO systems resulted in greater MBC and MBN stocks (Fig. 3). The significantly greater stock of MBC under SCP than NT at the Piedmont location (Fig. 3) may be due to transient benefits of long-term tillage, viz a viz slight increases in soil temperature (Dao, 1998) which, in turn, enhance microbial processes (Jackson et al., 2003) and biomass increase. This study noted, however, that increases in MBC and MBN have been observed due to (N) fertilizer applications (Raiesi, 2004) and organic amendments (Kandeler et al., 1999; Tu et al., 2006). Microbial biomass quotient (MBQ), reflecting the percentage of SOC that was microbial biomass C, ranged from 1 to 3% and was not affected by tillage in the three locations (Fig. 4). The MBQ range was within the (1 to 5% ) range for soils, used as an index reflecting management-induced changes in SOM rather than SOC alone (Sparling, 1992).

Tillage significantly influenced mineralizable C in the Coastal Plain ( $p < 0.0001$ ) and

Mountains ( $p = 0.0053$ ) locations but not in the Piedmont (Table 4). Mineralizable C stocks in the Coastal Plain were of the order  $S > NT > CT > CTO$ . In the Mountain, mineralizable C was greater under NTS than all other systems; but the other systems did not differ in mineralizable C stock. Mineralizable C stocks were 2.6, 1.7, and 1.5 times greater under NT than CT systems in the Coastal Plain, Mountain, and Piedmont locations, respectively. The lower stock of mineralizable C under CT, relative to NT, systems in all locations may be attributable to stimulated microbial activity during tillage operations. Tillage did not influence the mineralizable N pool in all locations (Table 4).

Water soluble (WSC) and hot water extractable (HWEC) C stocks were significantly influenced by tillage systems in all locations studied (Table 4). Tillage significantly affected the WSC stocks in the Coastal Plain ( $p = 0.0021$ ), Mountains ( $p = 0.0098$ ), and Piedmont ( $p = 0.0032$ ). In the Coastal Plain, WSC was significantly greater in the successional system than all other systems. The stock of WSC did not differ among these other systems. In the Mountain location, WSC stocks did not differ between no-till systems and between conventional tillage systems; but WSC stocks were greater under no-till than conventional tillage systems. In the Piedmont, WSC was greater under no-till than either SCP or MBP but not different between SCP and MBP. Averaged by tillage systems within locations, WSC stocks were 1.4, 1.6, and 1.6 times greater under less disturbed than disturbed systems in the Coastal Plain, Mountain, and Piedmont, respectively. Stocks of HWEC were also significantly affected by tillage systems in the Coastal Plain ( $p = 0.0377$ ), Mountain ( $p =$

0.0004), and Piedmont ( $p = 0.0004$ ), respectively (Table 4). In the Coastal Plain and Piedmont locations, the orders for HWEC were similar to the orders for WSC in the respective locations. However, HWEC stocks did not differ between S and CTO in the Coastal Plain. In the Mountain, HWEC was significantly greater under NTO than other systems; but HWEC did not differ among these other systems. Average HWEC was 1.1, 2.0, and 2.1 times greater under less disturbed than disturbed systems in the Coastal Plain, Mountain, and Piedmont locations, respectively. Under less disturbed and disturbed systems, the percentage of SOC that was contributed by WSC averaged 1.8 and 1.3% in the Coastal Plain, 1.7 and 1.6% in the Mountain, and 1.5 and 1.7% in the Piedmont. The corresponding percentages for HWEC were 4.9 and 4.6% in the Coastal Plain, 3.5 and 2.8% in the Mountain, and 5.0 and 4.2% in the Piedmont. Puget et al. (1999) observed that HWEC made up about 2% of SOC in a Typic Hapludalf, while Ghani et al. (2003) extracted 3 to 6% of SOC as HWEC in allophanic soils.

Water soluble C reflects readily soluble C from recent liming materials and animal manures or plant residues (Ghani et al., 2003). Hot water extractable C is another measure of potentially bio-available materials (Zsolnay and Gorlitz, 1994; Ghani et al., 2003) and is highly capable of differentiating between treatments within and across ecosystems (Ghani et al., 2003). The greater WSC and HWEC values under less disturbed than disturbed systems in all three locations were likely due to little or no dilution into a larger soil volume of these labile C pools and to the presence of plant residues and/or organic amendments

under the less disturbed systems. Relative to MBC, HWEC seemed to better separate the soils on the basis of tillage intensity. For instance, MBC did not differentiate among tillage systems in the Coastal Plain (Fig. 3) but HWEC did in all three locations (Table 4). There were significant correlations between C pool with SOC and HWEC in the Coastal Plain ( $r = 0.62$ ;  $p = 0.0315$ ), Mountain ( $r = 0.96$ ;  $p < 0.0001$ ), and Piedmont ( $r = 0.94$ ;  $p < 0.0001$ ), respectively, support this statement. These results support the findings of Ghani et al. (2003), indicating that HWEC was a sensitive indicator of changes in SOC.

### **Soil organic and microbial biomass C and N stratification ratios**

The effects of tillage, stratification ratios (SR), and SR by tillage interactions are shown in Table 6. There were no significant tillage effects on SOC, SON, MBC, and MBN stratification ratios (SR) in the Coastal Plain. There were significant SR effects on SOC, SON, and MBN but no interactions for all SRs. In the Mountain location, there were significant effects due to tillage, SR, and the SR by tillage interaction. In the Piedmont, there were significant tillage effects on SOC, SON, and MBN; and there were significant effects due to SR for SOC and SON. Where there were significant SR effects, on SOC, SON, MBC, and MBN, SR was significantly greater in the lower (0-10 cm minus 20-30 cm) than upper (0-10 cm minus 10-20 cm) soil depth ratios.

In the Coastal Plain, SR for SOC in the upper soil depth ratio (0 to 10 cm:10 to 20 cm depth ratio) ranged from 1.5 to 2.0; while the SR for the lower depth ratio (0 to 10 cm:20 to

30 cm depth ratio) ranged from 2.6 to 3.0. In the Mountain, SR for SOC in both soil depth ratios were of the order  $NTO > NTS > CTO = CT = CTS$  (Table 7). In the Piedmont location, the SR for SOC in the upper soil depth ratio did not differ. The SR for the lower depth ratio was significantly greater for both NT and SCP than MBP; but the SR under NT and SCP did not differ. In this location, the SR for SOC was greater in the lower than upper soil depth ratios in the SCP and NT systems (Table 7). In both Coastal Plain and Mountain locations, SR orders for SON in both depth ratios were similar to the orders in the SR for SOC. However, the SRs in the lower depth ratio were greater than those in the upper depth ratio. In the Piedmont, the SR for SON in the upper depth ratio was of the order  $NT = SCP > MBP$ ; whereas in the lower depth ratio it was  $NT > SCP > MBP$ . Under NT, the SR for SON was greater in the lower than upper depth ratio. The SRs for MBC and MBN in both depth ratios did not differ among tillage systems in the Coastal Plain and Piedmont locations. In the Mountain, the SRs for MBC and MBN were greater under NTS than the other tillage systems (NTO, CTS, CTO, and CT). The SR for MBC in the upper depth ratio did not differ for these other systems; while in the lower depth, the SR for MBC under CTO was significantly greater than NTO, CTS, CTO, and CT. The pattern for the SR for MBN was similar to the pattern in the SR for MBC.

Under NT and SCP systems in the Piedmont, SRs for SOC in the lower soil depth ratio were greater than in the upper soil depth ratio (Table 7), suggesting that SOC stock decreased with soil depth in this location. Similarly, SON decreased with soil depth under NTO and NTS in the Mountain location and under NT in the Piedmont location. In these

minimally disturbed systems, most C or N is concentrated near the soil surface, whereas in plowed systems, a greater proportion of soil C and N is mixed into lower depths, thus narrowing differences in C and N contents between depths. The range of SR for SOC and SON for the 0-10 cm:20-30 cm depth in CT and NT systems in some of the systems in the present study is comparable to the ranges observed in the 0-5:12.5-20 cm depths in similar systems in the southeastern US (Causarano et al, 2008) and to NT, chisel plow and MBP systems for the 0-5 cm:15-30 cm depths in two Alfisols studied by Jarecki and Lal (2005).

Larger differences between SR for C or N pools (SOC, SON, MBC, or MBN) in lower depths and SR for those pools in upper soil depths (differential SR) will indicate greater quantities of C (or N) pools in the upper depth ratio. Therefore, higher differential SR are expected in NT than CT systems. However, significant differences between differential SR for these C and N pools occurred only in the Mountain location, where differences were significantly greater under the NTO than other systems. Differences in SR in the lower and upper soil depth ratios did not differ among these other tillage systems in the Mountain (Table 8); differential SR also did not differ in the Coastal Plain and Piedmont locations. These results suggest that SR can, but that differential SR cannot, indicate SOC and SON dynamics across tillage systems and locations in these soils.

### **Soil organic carbon sequestration rates and factors influencing C sequestration**

Tillage systems significantly affected the rate of SOC sequestration in all locations

(Fig. 5). In the Coastal Plain, C sequestration rate under the successional system (S;  $1.6 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ) did not differ from the rate under CTO ( $1.5 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ) but was significantly greater than the NT ( $1.4 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ) and CT ( $0.9 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ) systems; while the sequestration rate did not differ among CTO, NT, and CT. In the Mountain, the order for C sequestration rate was NTO ( $1.6 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ) > NTS ( $1.1 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ) = CTO ( $0.9 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ) = CTS ( $0.9 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ) = CT ( $0.9 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ). In the Piedmont, C sequestration rate was significantly greater under NT ( $0.6 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ) than either SCP ( $0.4 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ) or MBP ( $0.3 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ); but sequestration rates under SCP and MBP did not differ. On average, sequestration rates were 1.1, 1.5, and 1.8 times greater under undisturbed or less disturbed (no-till) than disturbed (conventional tillage) systems in the Coastal Plain, Mountain, and Piedmont, respectively. Values for C sequestration rates for the natural forests in this study were 1.7, 1.4, and  $1.0 \text{ Mg ha}^{-1} \text{ yr}^{-1}$  for the Coastal Plain, Mountain, and Piedmont, respectively; while rates for the farmer-managed conventional tillage with organic amendments in the Coastal Plain and no-till system with organic amendments in the Piedmont were 0.7 and  $1.2 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ , respectively. The values for C sequestration rates observed in the present study (Fig. 5) are above those observed by Jarecki and Lal (2005), who reported sequestration rates of 0.15 and  $0.20 \text{ Mg ha}^{-1} \text{ yr}^{-1}$  for NT systems in two Alfisols in Ohio.

Significantly greater sequestration rates occurred under the undisturbed successional system (S;  $1.6 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ) and conventional tillage system with organic

amendments (CTO;  $1.5 \text{ Mg ha}^{-1}\text{yr}^{-1}$ ) in the Coastal Plain; under no-till with organic amendments (NTO;  $1.6 \text{ Mg ha}^{-1}\text{yr}^{-1}$ ) in the Mountain; and under no-till (NT;  $0.6 \text{ Mg ha}^{-1}\text{yr}^{-1}$ ) in the Piedmont (Fig. 5), suggesting that undisturbed systems accumulate more C than disturbed systems. As is obvious, these high sequestration rates, relative to rates in the other systems, correspond to high SOC storage in these systems (Fig. 2). The greater C sequestration rate under CTO in the Mountain also suggests that organic amendments to no-till or conventional tillage systems can also increase C sequestration rates in this soil. Higher yields would be expected from these systems with the highest C sequestration rates. This is supported in Fig. 6, where the NTO system in the Mountain location had the highest corn yield ( $13.6 \text{ Mg ha}^{-1}$ ) in 2007, the year before this study was undertaken. According to Gruver (2007), the no-till (NT) system in the Piedmont location also showed the highest yield ( $10.2 \text{ Mg ha}^{-1}$ ) in the 2006 growing season, compared to  $6.1 \text{ Mg ha}^{-1}$  under MBP. The yield of  $9.3 \text{ Mg ha}^{-1}$  under SCP did not differ significantly from those in both NT and MBP. Tu et al. (2006) reported higher yields of cabbage ( $4.1 \text{ Mg ha}^{-1}$ ) under CTO, compared to  $1.4 \text{ Mg ha}^{-1}$  under CT, between 2000 and 2002 in the Coastal Plain.

Several factors may explain the higher sequestration rates observed under these systems. First, relatively high levels of nutrients were added via organic amendments in the organic system in the Coastal Plain (Table 2). Based on the mean values calculated in this study for various types of turkey litter(<http://www.soil.ncsu.edu/publications/Soilfacts/AG-439-05/>), turkey litter provided between  $81$  to  $282 \text{ Mg N ha}^{-1}$ ,  $51$  to  $176 \text{ Mg P ha}^{-1}$ , and  $52$

to 176 Mg K ha<sup>-1</sup>. The conventional tillage systems in this location received about 0.2 Mg N ha<sup>-1</sup> from urea and N:P:K fertilizers added. The NTO and CTO system in the Mountain each received, from the 0.23 Mg soybean meal (7:2:1) ha<sup>-1</sup> applied, about 0.02 Mg N ha<sup>-1</sup>, 0.005 Mg P ha<sup>-1</sup>, and 0.002 Mg K ha<sup>-1</sup>. The conventional tillage systems in this location received about 0.06 Mg N ha<sup>-1</sup> from ammonium nitrate fertilizer. The no-till in the Piedmont location received 0.06 Mg N ha<sup>-1</sup>, 0.03 Mg P ha<sup>-1</sup>, and 0.03 Mg K ha<sup>-1</sup>, as did the SCP and MBP systems. A second factor to consider is that the successional system and the no-till treatments received no or only minimal disturbance by way of tillage since the inception of the different experimental sites. Thus aggregate stability, which has been shown in this study (Chapter 1 and the current chapter) and other studies to increase SOC storage, would be greater in these systems relative to the disturbed systems. Finally, results from experiments in Chapter 4 of this dissertation also showed that SOC accumulation is influenced by soil properties such as clay in the Coastal Plain, humic matter (HM) in the Piedmont, and hot water extractable C (HWEC; a measure of potentially bioavailable C) in all three locations. The correlations among SOC (and sequestration rate) and the selected soil properties are shown in Table 9. The significant correlations of SOC with HWEC and clay in the Coastal Plain; with HWEC and MWD in the Mountain; and with HWEC, MWD, and HM in the Piedmont suggest links between SOC accumulation (and C sequestration rates) and these soil properties. Additionally, significant correlations between SOC and stratification ratios for SOC in the 0-10:10-20 (SOCSR1) and 0-10:20-30 cm (SOCSR2) soil depth ratios, as

well as the difference between these ratios (SOCSR1 subtracted from SOCSR2), were observed in the Mountain and Piedmont locations. These latter correlations suggest that spatially descriptive parameters such as stratification ratios (Franzluebbers, 2002) may differentiate tillage systems with regard to their capability to sequester C.

### **Conclusions**

This study examined the sequestration and stratification of organic C and N under no-till and conventional tillage systems in three locations representing the physiographic regions of North Carolina. Within the 0-10 cm soil depth studied for C and N sequestration, there was little or no difference in expressing amounts of C or N on either concentration basis or mass per area in an equivalent soil mass. Carbon and nitrogen pools (SOC, SON, MBC, and MBN) seemed to influence bulk density under the different tillage systems studied. Soil bulk density was generally greater under NT than CT in all locations. Because they are less disturbed, no-till systems generally resulted in greater pools of C and N, especially in the Mountain and Piedmont locations. Depth ratios used in this study can potentially distinguish tillage effects on SOC and SON stratification in all locations. However, the differences between stratification ratios in different depth ratios may distinguish between tillage effects only in the Mountain location possibly because of the greater SOC contents in the region. Accumulation of SOC in the three locations was primarily controlled by the amount of bioavailable materials, especially labile C extracted by hot water. Soil OC

accumulation in the Coastal Plain was linked to clay content; while in the Mountain it was also linked to water stable aggregation and to depth stratification of SOC. In the Piedmont, SOC accumulation was linked to humic matter content and depth stratification. The differences in depth stratification can also possibly influence SOC storage in no-till and/or organic systems in the Mountain location. Spatially descriptive parameters can, therefore, differentiate tillage systems with regard to their capability to sequester C.

## References

- Al-Kaisi, M.M. and X. Yin. 2005. Tillage and crop residue effects on soil carbon and carbon dioxide emission in corn–soybean rotations. *J. Environ. Qual.* 34, 437–445.
- Allison, F.E. 1973. Loss of nitrogen from soils. *In* Soil organic matter and its role in crop production (Developments in Soil Science 3). Published by Amsterdam, New York, Elsevier Scientific Pub. Co. pp 254-274.
- Anderson, J.P.E. 1982. Soil respiration. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), *Methods of Soil Analysis. Part 2, 2nd ed. Agronomy Monograph 9.* ASA and SSSA, Madison, WI, pp. 837–871.
- Angers, D.A. and C. Chenu. 1998. Dynamics of soil aggregation and C sequestration. In: Lal, R., Kimble, J.M., Follett, R.F., Stewart, B.A. (Eds.), *Soil Processes and the Carbon Cycle. Advances in Soil Science.* CRC Press, Boca Raton, FL, pp. 199–206.
- Arvidsson, J. 1998. Influence of soil texture and organic matter content on bulk density, air content, compression index and crop yield in field and laboratory compression experiments. *Soil & Tillage Research* 49: 159-170.
- Bauer, A. and A. L. Black. 1994. Quantification of the effect of soil organic matter content on soil productivity. *Soil Sci. Soc. Am. J.* 58:185-193.
- Bell, M. C. 2002. A multidisciplinary approach to assessing changes in the soil quality of diverse farming systems. M.Sc. Thesis. North Carolina State University, Raleigh, NC.
- Blake, G.R. and K.H. Hartge. 1986. Bulk density. pp. 363-375. In: A. Klute (ed.), *Methods of Soil Analysis, Part 1, 2<sup>nd</sup> Edition.* ASA and SSSA Publ., Madison, WI, USA.
- Blanco-Canqui, H. and R. Lal. 2008. No-tillage and soil-profile carbon sequestration: An on-farm assessment. *Soil Sci. Soc. Am. J.* 72:693-701.
- Bossuyt, H., J.Six, and P. F. Hendrix. 2002. Aggregate-protected carbon in no-tillage and conventional tillage agroecosystems using carbon-14 labeled plant residue. *Soil Sci. Soc. Am. J.* 66:1965–1973.
- Causarano, H. J., A. J. Franzluebbers, J. N. Shaw, D. W. Reeves, R. L. Raper, and C. W. Wood. 2008. Soil organic carbon fractions and aggregation in the Southern Piedmont and

- Coastal Plain. Soil Sci. Soc. Am. J. 72: 221-230.
- Chan, K.Y. and D.P. Heenan. 1998. Microbial-induced soil aggregate stability under different crop rotations. Biol. Fert. Soils 30: 29-32.
- Chaney, K., and Swift, R.S. 1984. The influence of organic matter on aggregate stability in some British soils. Journal of Soil Science 35: 223-230.
- Clapp, C.E., S.A. Stark, D.E. Clay, and W.E. Larson. 1986. Sewage sludge organic matter and soil properties. *In* Chen, Y. and Y. Avnimelech (e.d.) The Role of organic matter in modern agriculture. Dordrecht ; Boston : Martinus Nijhoff. pp 209-254.
- CTIC. 2002. National Crop Residue Management Survey. Conservation Technology Information Center.  
<http://www.ctic.purdue.edu/Core4/CT/ctsurvey/2002/RegionalSynopses.html>.
- Dalal, R.C. and R.J. Mayer. 1986. Long-term trends in fertility of soils under continuous cultivation and cereal cropping in Southern Queensland. II. Total organic carbon and its rate of loss from the soil profile. Aust. J. Soil Res. 24: 281-292.
- Daniels, R.B., S.W. Buol, H.J. Kleiss, and C.A. Ditzler. 1999. Soil Systems in North Carolina. Tech. Bull. 314. North Carolina State University. Soil Sci. Dept. Raleigh, NC 27695-7619.
- Dao, T.H. 1998. Tillage and crop residue effects on carbon dioxide evolution and carbon storage in a Paleustoll. Soil Sci. Soc. Am. J. 62, 250–256.
- DeGryze, S., J. Six, K. Paustian, S. J. Morris, E. A. Paul, and R. Merckx. 2004. Soil organic carbon pool changes following land-use conversions. Global Change Biology 10: 1120-1132.
- Dick, W.A., R.L. Blevins, W.W. Frye, S.E. Peters, D.R. Christenson, F.J. Pierce, and M.L. Vitosh. 1998. Impacts of agricultural management practices on C sequestration in forest-derived soils of the eastern Corn Belt. Soil & Tillage Research 47: 235-244.
- Doran, J.W. 1987. Microbial biomass and mineralizable nitrogen distributions in no-tillage and plowed soils. Biology and Fertility of Soils 5: 68–75.
- Dumontet, S., A. Mazzatura, C. Casucci, and P. Perucci . 2001. Effectiveness of microbial indexes in discriminating interactive effects of tillage and crop rotations in a Vertic

- Ustorthens Biol. and Fert. Soils 34(6): 411-416.
- Ellert, B.H. and J.R. Bettany. 1995. Calculation of organic matter and nutrients stored in soils under contrasting management regimes. *Can. J. Soil Sci.* 75: 529-538.
- Feller, C., and M. H. Beare. 1997. Physical control of soil organic matter dynamics in the tropics. *Geoderma* 79, 69–116.
- Franzluebbers, A.J. 2002. Soil organic matter stratification ratios as an indicator of soil quality. *Soil Tillage and Res.* 66: 95-106.
- Franzluebbers, A. F. 2004. Tillage and residue management effects on soil organic matter. *In* Soil organic matter in sustainable agriculture. Fred Magdoff and Ray R. Weil (ed.). Boca Raton: CRC Press. pp. 227-268.
- Franzluebbers, A.J. 2005. Soil organic carbon sequestration and agricultural greenhouse gas emissions in the southeastern USA *Soil & Tillage Research* 83: 120–147.
- Gee, G.W. and J.W. Bauder. 1986. Particle size analysis. pp. 383-441. In: A. Klute (ed.), *Methods of Soil Analysis, Part 1, 2<sup>nd</sup> Edition*. ASA and SSSA Publ., Madison, WI, USA.
- Ghani, A, M. Dexter, and K.W. Perrott. 2003. Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. *Soil Biology & Biochemistry* 35 1231–1243.
- Goyal, S., M.M. Mishra, I.S. Hooda, and R. Singh. 1992. Organic matter-microbial biomass relationships in field experiments under tropical conditions: effects of inorganic fertilization and organic amendments. *Soil Biol. Biochem.* 24, 1081–1084.
- Gruver, J. B. 2007. Impact of Management and Texture on Soil Organic Matter Fractions (Doctoral dissertation). North Carolina State University, Raleigh, NC.
- Haynes, R.J. 1993. Effect of sample pretreatment on aggregate stability measured by wet sieving or turbidimetry on soils of different cropping history. *J. Soil Sci.* 44: 261-270.
- Haynes, R. J. and R. Naidu. 1998. Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review. *Nutrient Cycling in Agroecosystems* 51, 123-137.
- Jackson, L.E., F.J. Calderon, K.L. Steenwertha, K.M. Scowc, and D.E. Rolston. 2003.

- Responses of soil microbial processes and community structure to tillage events and implications for soil quality. *Geoderma* 114: 305–317.
- Jarecki, M. K. and R. Lal. 2005. Soil organic carbon sequestration rates in two long-term no-till experiments in Ohio. *Soil Sci.* 170: 280-291.
- Johnston, A.E. 1986 Soil organic matter effects on soils and crops. *Soil Use Management* 2: 97–105.
- Lynch, J.M. and L.M. Panting. 1982. Effects of season, cultivation and nitrogen fertilizer on the size of the soil microbial biomass. *J. Sci. Food Agric.* 33, 249–252.
- Medina, M.A., J.C. Reid, and R.H. Carpenter. 2004. Physiography of North Carolina. Modified from 1991 Generalized Geologic Map. Digital representation. North Carolina Geological Survey, Division of Land Resources, 1612 Mail Service Center, Raleigh, North Carolina 27699-1612. On the web at: [http://www.geology.enr.state.nc.us/proj\\_earth/pdf/color\\_physiography\\_600dpi.pdf](http://www.geology.enr.state.nc.us/proj_earth/pdf/color_physiography_600dpi.pdf)
- Mitchell, J. P., K. Klonsky, A. Shrestha, R. Fry, A. DuSault, J. Beyer, and R. Harben. 2007. Adoption of conservation tillage in California: current status and future perspectives. *Austr. J. Exp. Agric.* 47: 1383–1388.
- Muruganandam, S. 2007. Soil aggregate-associated microbial community structure and nitrogen transformations in three different tillage systems. Dissertation. North Carolina State University. 138 p.
- Nelson, D.W. and L.E. Sommers. 1982. Total carbon, organic carbon, and organic matter. In: *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties* (ed. A.L. Page, R.H. Miller & D.R. Keeney), pp. 539–579. American Society of Agronomy, Madison, WI.
- Osunbitana, J.A., D.J. Oyedele, K.O. Adekalu. 2005. Tillage effects on bulk density, hydraulic conductivity and strength of a loamy sand soil in southwestern Nigeria *Soil & Tillage Research* 82: 57–64.
- Overstreet, L. F. 2005. Relationships between soil biological and physical properties in a long-term vegetable management study. (Doctoral dissertation). North Carolina State University, Raleigh, NC.

- Parfitt, R.L., G.J. Salt, and S. Saggar. 2001. Effect of leaching and clay content on carbon and nitrogen mineralization in maize and pasture soils. *Aust. J. Soil Res.* 39: 535-542.
- Powers, W.L., G.W. Wallingford, and L.S. Murphy. 1975. Research status on effects of land application of animal wastes. EPA-660/2-75-010. USEPA. Washington DC.
- Powlson, D.S. and D.S. Jenkinson. 1981. A comparison of the organic matter, biomass, adenosine triphosphate and mineralizable nitrogen contents of ploughed and direct-drilled soils. *J. Agric. Sci. (Camb.)* 97: 713-721.
- Puget, P., D.A. Angers, and C. Chenu. 1999. Nature of carbohydrates associated with water-stable aggregates of two cultivated soils. *Soil Biol. Biochem.* 31: 55-63.
- Puget, P., R. Lal, C. Izaurralde, M. Post, and L. Owens. 2005. Stock and distribution of total and corn-derived soil organic carbon in aggregate and primary particle fractions for different land use and soil management practices. *Soil Sci.* 170: 256-279.
- Raiesi, F. 2004. Soil properties and N application effects on microbial activities in two winter wheat cropping systems. *Biol Fertil Soils* 40: 88-92.
- Reicosky, D.C., W.D. Kemper, G.W. Langdale, C.L. Douglas Jr., and P.E. Rasmussen. 1995. Soil organic matter changes resulting from tillage and biomass production. *J. Soil Water Conserv.* 50, 253- 261.
- Reicosky, D.C., W.A. Dugas, and H.A. Torbert. 1997. Tillage-induced soil carbon dioxide loss from different cropping systems. *Soil Tillage Res.* 41, 105- 118.
- Rice, C.W., Smith, M.S., Blevins, R.L. 1986. Soil nitrogen availability after long-term continuous NT and CT corn production. *Soil Science Society of America Journal* 50, 1206-1210.
- Saggar, S., G.W. Yeates, T.G. Shepherd. 2001. Cultivation effects on soil biological properties, microfauna and organic matter dynamics in Eutric Gleysol and Gleyic Luvisol soils in New Zealand. *Soil & Till. Res.* 58: 55-68.
- Schachtschabel, P., H.P. Blume, G. Brümmer, K.H. Hartge, U. Schwertmann, K. Auerswald, L. Beyer, W.R. Fischer, I. Kögel-Knabner, M. Renger, and O. Strebel. 1998. *Lehrbuch der Bodenkunde*. Ferdinand Enke Verlag, Stuttgart, Germany.
- Sparling, G. P. 1992. Ratio of microbial biomass carbon to soil organic carbon as a sensitive

- indicator of changes in soil organic matter. *Aust. J. Soil Res.* 30: 195-207.
- State Climate Office of North Carolina, Centennial Campus, Box 7236, North Carolina State University, Raleigh, North Carolina 27695-7236.
- Tester, C. F. 1990. Organic amendment effects on physical and chemical properties of a sandy soil. *Soil Sci. Soc. Am. J.* 54:827-831.
- Tirol-Padre, A., J. K. Ladha, A. P. Regmi, A. L. Bhandari, and K. Inubushi. 2007. Organic amendments affect soil parameters in two long-term rice-wheat experiments. *Soil Sci. Soc. Am. J.* 71:442–452.
- Torn, M.S., S.E. Trumbore, O.A. Chadwick, P.M. Vitousek, and D.M. Hendricks. 1997. Mineral control of soil organic carbon storage and turnover. *Nature* 389:170–173.
- Tu, C., F. J. Louws, N. G. Creamer, J. P. Mueller, C. Brownie, K.F., M. Bell, and S. Hu. 2006. Response of soil microbial biomass and N availability to transition strategies from conventional to organic farming systems. *Agriculture, Ecosystems, and Environment* 113: 206-215.
- West, T.O. and W.M. Post. 2002. Soil organic carbon sequestration rates by tillage and crop rotation: a global data analysis. *Soil Sci. Soc. Am. J.* 66, 1930–1946.
- White, P.F. 2004. The influence of alternative tillage systems on the distribution of nutrients and organic carbon in some common West Australian wheatbelt soils. *Aust. J. Soil Sci.* 28: 95-116.
- Yang, X-M. and M. M. Wander. 1999. Tillage effects on soil organic carbon distribution and storage in a silt loam soil in Illinois. *Soil & Tillage Research* 52: 1-9.
- Yoder, R.E. 1936. A direct method of aggregate analysis of soils and a study of the physical nature of erosion losses. *J. Am. Soc. Agron.* 28: 337-351.
- Zotarelli, L, B.J.R. Alves, S. Urquiaga, E Torres, H. P. dos Santos, K Paustian, R. M. Boddey, and J. Six. 2005. Impact of tillage and crop rotation on aggregate-associated carbon in two oxisols. *Soil Sci. Soc. Am. J.* 69: 482-491.
- Zsolnay, A., and H. Gorlitz. 1994. Water-extractable organic matter in arable soils: effects of drought and long-term fertilization. *Soil Biol. Biochem.* 26, 1257–1261.

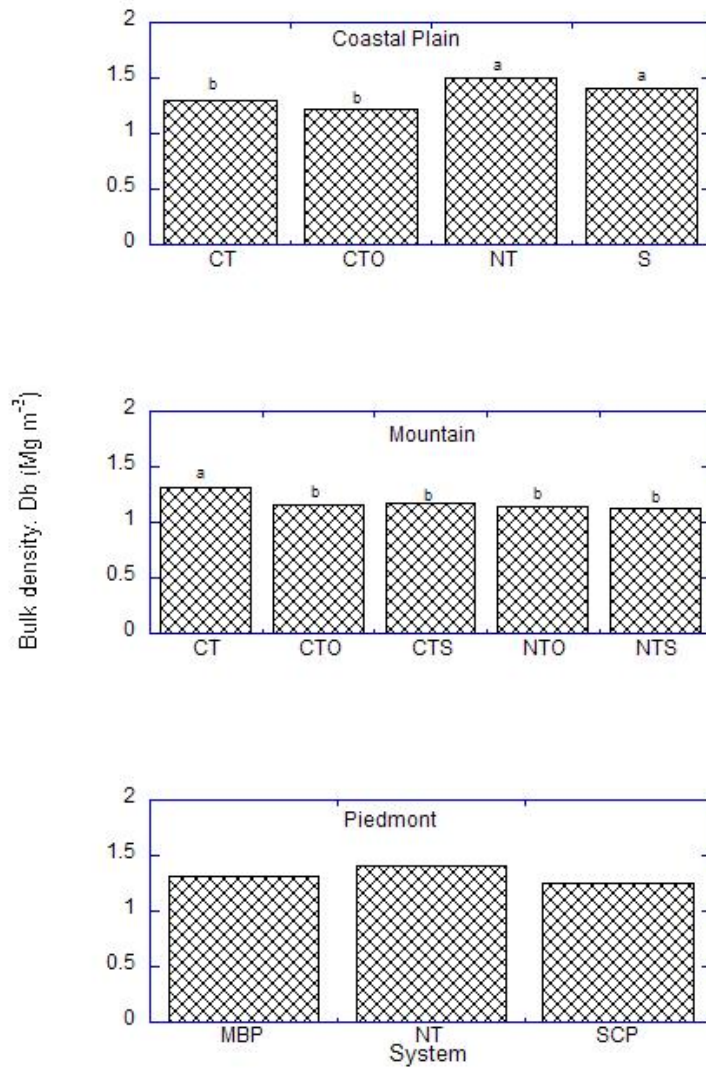


Fig. 1. Soil bulk density values in diverse tillage systems in the Coastal Plain, Mountain, and Piedmont locations. Values with same or no lower- or upper-case letters indicate no significant difference at  $p < 0.05$ . Coastal Plain systems/ CT: Conventional tillage (CT) under Best Management Practices (BMP); NT: no-till under BMP; CTO: organic system under CT; S: old field succession ecosystem under fallow. Mountain systems/ CT: Conventional tillage (CT) receiving no inputs; CTO: CT receiving organic amendments; CTS: CT receiving synthetic amendments; NTO: no-till receiving organic amendments; NTS: no-till receiving synthetic amendments. Piedmont systems/ MBP: moldboard plow; NT: no-till system; SCP: spring chisel plow.

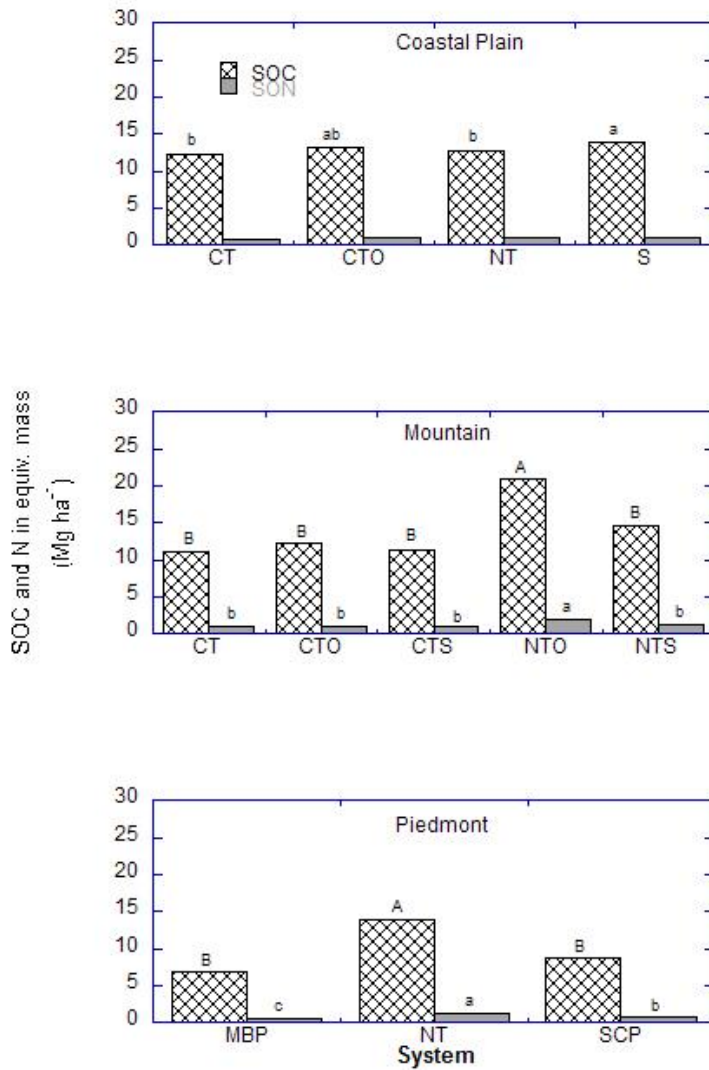


Fig. 2. Soil organic C and N stocks in equivalent soil mass for tillage systems in Coastal Plain, Mountain, and Piedmont locations. Values with same or no letters indicate no significant differences between tillage systems,  $p < 0.05$ . Coastal Plain systems/ CT: Conventional tillage (CT); NT: no-till; CTO: organic-amended CT; S: successional system. Mountain systems/ CT: CT receiving no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic-amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; NT: no-till system; SCP: spring chisel plow.

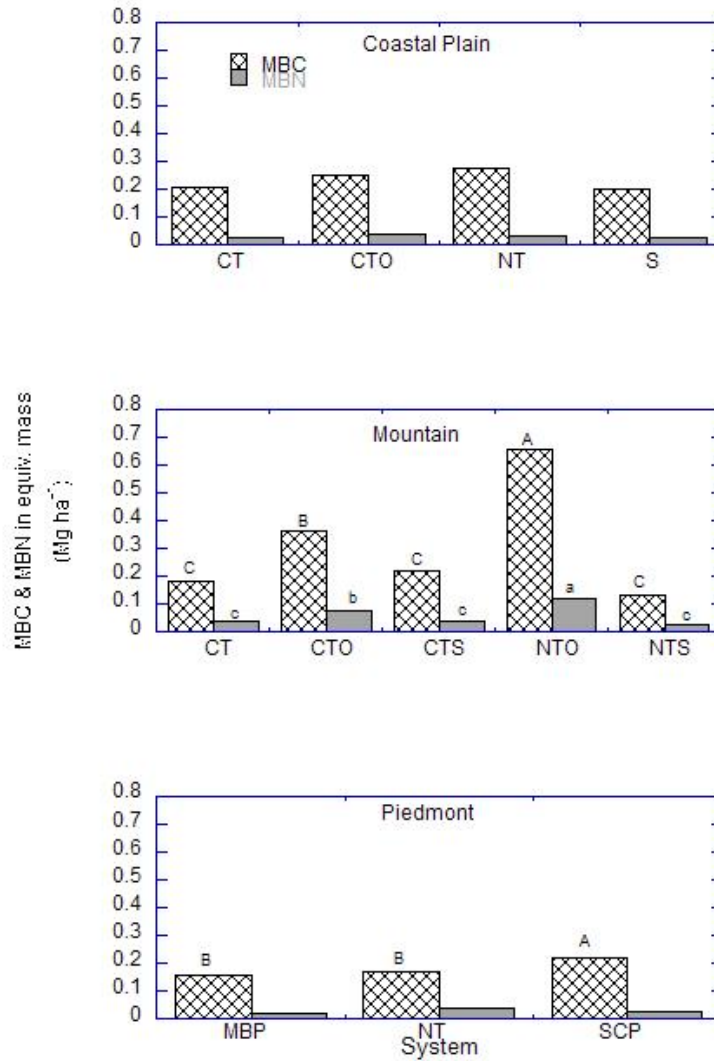


Fig. 3. Microbial biomass C (MBC) and N (MBN) stocks in equivalent soil mass for tillage systems in Coastal Plain, Mountain, and Piedmont locations. Values with same or no lower- or upper-case letters indicate no significant difference at  $p < 0.05$ . Coastal Plain systems/ CT: Conventional tillage (CT); NT: no-till; CTO: organic-amended CT; S: successional system. Mountain systems/ CT: CT receiving no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic-amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; NT: no-till system; SCP: spring chisel plow.

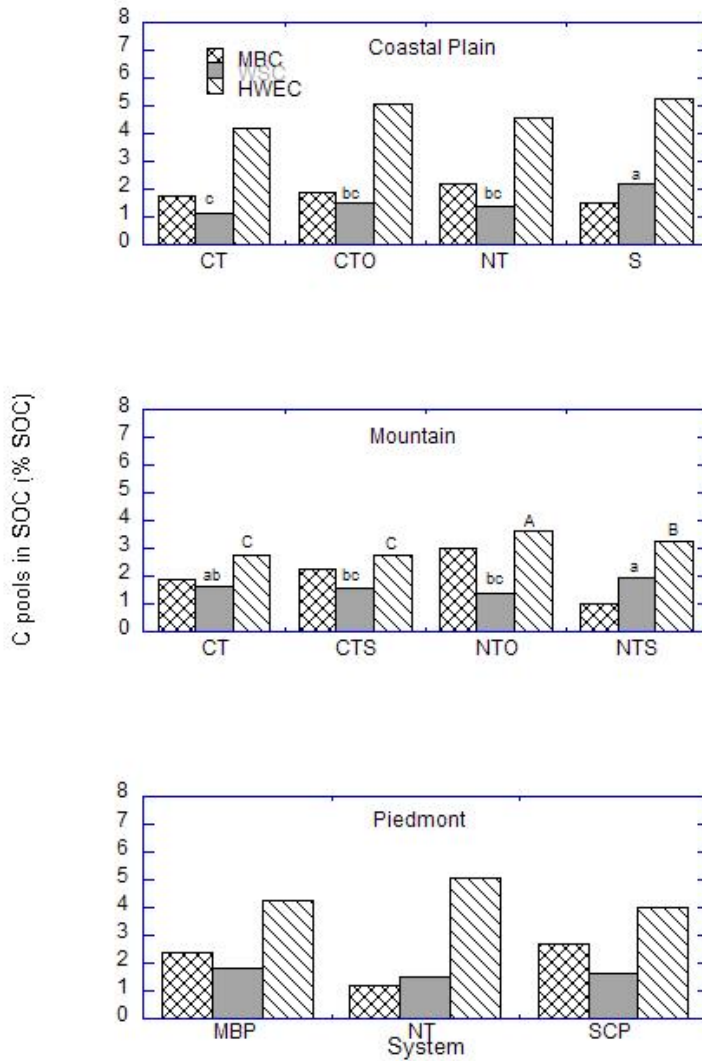


Fig. 4 Percent SOC contributed by microbial biomass C (MBC), water soluble C (WSC), and hot water extractable C (HWEC) in Coastal Plain, Mountain, and Piedmont locations. Values with same or no letters indicate no significant difference at  $p < 0.05$ . Coastal Plain systems/ CT: Conventional tillage (CT); NT: no-till; CTO: organic-amended CT; S: successional system. Mountain systems/ CT: CT receiving no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic-amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; NT: no-till system; SCP: spring chisel plow.

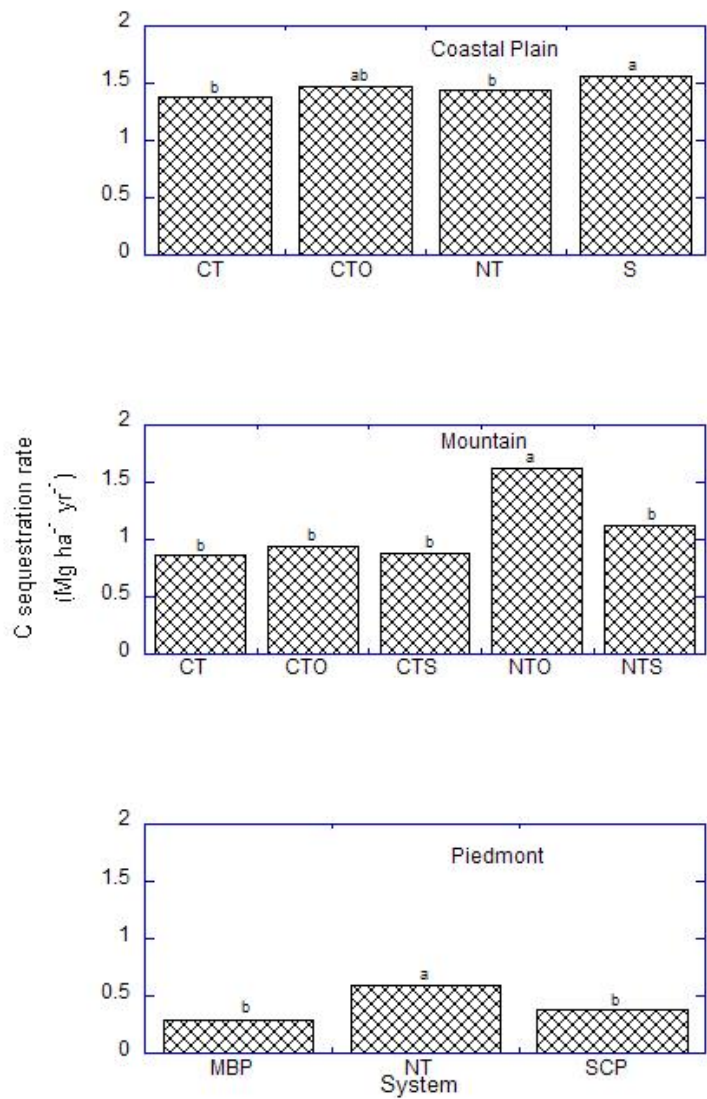


Fig. 5 Carbon sequestration rates in different tillage systems in Coastal Plain, Mountain, and Piedmont locations. Values with same or no letters indicate no significant difference at  $p < 0.05$ . Coastal Plain systems/ CT: Conventional tillage (CT); NT: no-till; CTO: organic-amended CT; S: successional system. Mountain systems/ CT: CT receiving no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic-amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; NT: no-till system; SCP: spring chisel plow.

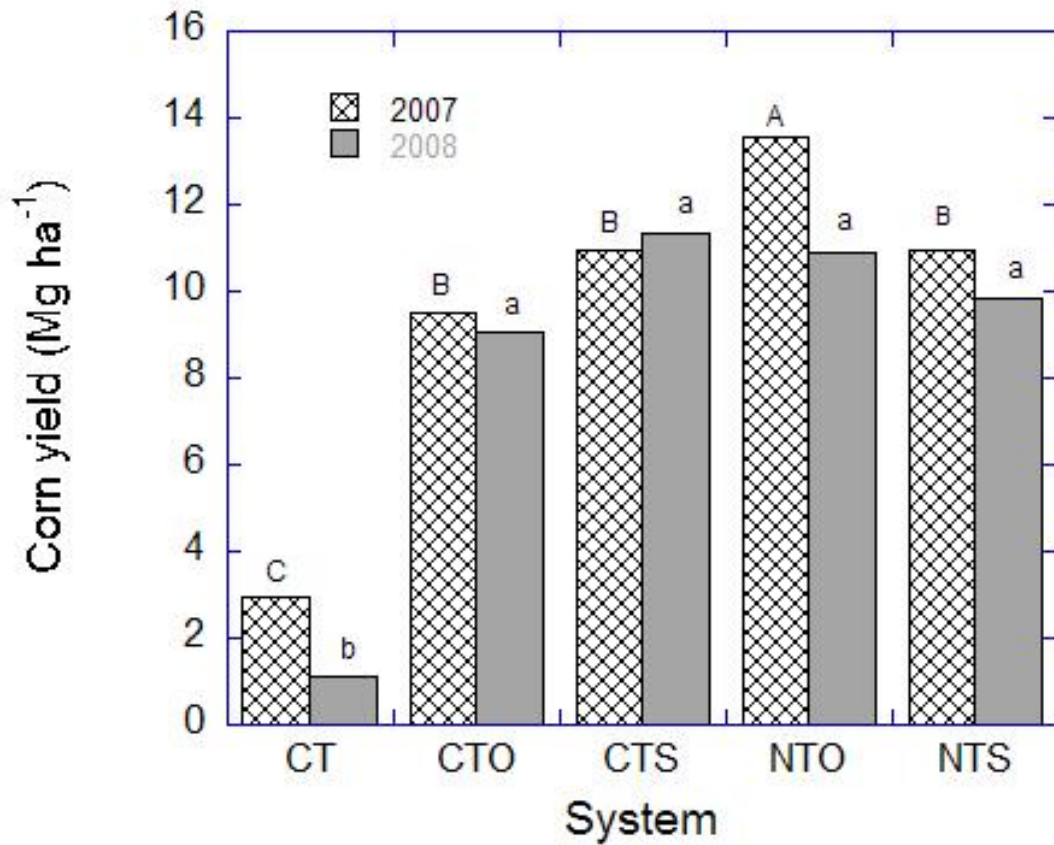


Fig. 6 Corn yields in the experimental plots in the Mountain for 2007 and 2008. The 2008 yields are for the year following the end of this study. Values with same or no letters indicate no significant difference at  $p < 0.05$ . CT: Conventional tillage (CT) receiving no inputs; CTO: CT receiving organic amendments; CTS: CT receiving synthetic amendments; NTO: no-till receiving organic amendments; NTS: no-till receiving synthetic amendments.

Table 1. Description of crops grown in the plots at the experimental sites.

Year(s)/Period	Treatment			
	Coastal Plain			
	CT	NT	CTO	S
1999	Corn ( <i>Zea mays</i> L.)	Corn	Soybean	Fallow
2000	Peanut ( <i>Arachis hypogaea</i> L.)	Peanut	Sweet potato ( <i>Ipomea batatas</i> L.)	Fallow
2001	Cotton ( <i>Gossypium spp.</i> L.)	Cotton	Wheat-Cabbage ( <i>Brassica oleracea</i> L.)	Fallow
2002	Corn	Corn	Corn	Fallow
2003	Peanut	Peanut	Soybean	Fallow
2004	Corn	Corn	Corn	Fallow
2005	Corn	Corn	Corn	Fallow
2006	Grain sorghum ( <i>Sorghum bicolor</i> L.) and wheat ( <i>Triticum aestivum</i> L.)	Grain and wheat	Biculture: Cowpea ( <i>Vigna sinensis</i> L.) or soybean and crimson clover ( <i>Trifolium incarnatum</i> L.) or wheat	Fallow
2007	Soybean ( <i>Glycine max</i> L.)	Soybean	Soybean	Fallow

Table 1 continued

Year(s)/Period	Treatment				
Mountain					
	CTS	NTS	CTO	NTO	CT
1995-2006	Staked tomatoes ( <i>Solanum lycopersicum</i> ) with rye ( <i>Secale cereale</i> L.) as cover crop		Staked tomatoes with hairy vetch ( <i>Vicia sativa</i> L.) as cover crop		Staked tomatoes with wheat or crimson clover ( <i>Trifolium incarnatum</i> L.) as cover crop
2007	Corn only with rye as cover crop		Corn only with vetch as cover crop		Corn only with wheat or crimson clover as cover crop
Piedmont					
	MBP	SCP	NT		
1984-1989	Corn only				
1990-2005	Corn-Soybean rotation				
2006-2007	Corn only				

Table 2. Organic and synthetic amendments used at the experimental sites.

Amendment	Application method	Rate	Element supplied		
			N	P	K
Mg ha <sup>-1</sup>					
<u>Coastal Plain</u>					
Turkey litter	Mechanically incorporated	3900 – 13500	81-282	51-176	52-176
TSP (0-46-0)			-	0.11	-
Potash (0-0-60)			-	0	0.14
Urea	Banded in NT, mechanically incorporated in CT	0.23	0.11	0	0
N:P:K 10-34-0			0.02	0.08	0
N:P:K 30-0-0			0.07	0	0
Gypsum, for liming peanut	Broadcast and mechanically incorporated	0.22 to 8.75			
<u>Mountain</u>					
Soybean meal (N:P:K, 7:2:1)		0.23	0.02	0.005	0.002
ammonium nitrate (NH <sub>4</sub> NO <sub>3</sub> )	Banded in NT, mechanically incorporated in CT, at planting.	0.17	0.06		
TSP (0-46-0)					
KCl (0-0-60)					
RP (0-30-0)					
Sul-Po-Mag (0-0-22)	Surface broadcast prior to planting winter cover crops.				

TSP: triple superphosphate; RP: rock phosphate; KCl: potassium chloride

Table 2. Continued.

Amendment	Application method	Rate	Element supplied		
			N	P	K
Mg ha <sup>-1</sup>					
<u>Piedmont</u>					
N:P:K 10-20-20	At planting. Banded in NT, mechanically incorporated in MBP and SCP.	0.13	0.01	0.03	0.03
NH <sub>4</sub> NO <sub>3</sub>	Applied as side-dressing about one month after planting. Banded in NT, mechanically incorporated in MBP and SCP.	0.15	0.05		

TSP: triple superphosphate; RP: rock phosphate; KCl: potassium chloride

Table 3. Tillage systems and characteristics of the 0 to 10 cm soil depth at the three locations studied.

System†	Duration yr	pH <sub>water</sub>	CEC cmol <sub>c</sub> kg <sup>-1</sup>	Sand	Silt	Clay
				%		
<u>Coastal Plain</u>						
CT	9	6.1 a	5.6 a	67.3	23.6	9.1
CTO	9	5.9 a	6.4 a	70.2	20.3	9.4
NT	9	5.9 a	6.0 a	64.7	24.6	10.7
S	9	4.8 b	4.2 b	74.2	17.2	8.6
FmrCTO	15	5.7	5.9	71.9	22.9	5.2
Forest	30	4.8	9.8	58.5	19.3	22.2
<u>Mountain</u>						
CT	13	5.9 b	6.9 b	60.8	19.8	19.4
CTO	13	6.3 a	7.4 b	53.0	23.2	23.8
CTS	13	6.1 ab	6.7 b	57.6	20.2	22.1
NTO	13	6.9 a	10.5 a	52.1	26.6	21.3
NTS	13	6.0 b	7.6 b	50.9	26.9	22.2
Forest	30	4.7	7.0	44.2	31.1	24.6
<u>Piedmont</u>						
MBP	24	5.4 b	4.6 b	50.7	26.9	22.4
NT	24	5.9 a	6.0 a	56.9	26.1	17.1
SCP	24	6.1 a	5.9 a	58.1	18.3	23.6
FmrNT	20	6.3	7.8	66.1	15.9	17.7
Forest	30	5.2	6.3	66.5	19.7	13.8

†Coastal Plain systems/ CT: Conventional tillage (CT) under Best Management Practices (BMP); NT: no-till under BMP; CTO: organic system under CT; S: old field succession ecosystem under fallow; FmrCTO, farmer-managed organic farm under conventional tillage; Forest: natural forest system adjacent to experiments site. Mountain systems/ CT: Conventional tillage (CT) receiving no inputs; CTO: CT receiving organic amendments; CTS: CT receiving synthetic amendments; NTO: no-till receiving organic amendments; NTS: no-till receiving synthetic amendments; Forest: natural forest system adjacent to experiments site. Piedmont systems/ MBP: moldboard plow; NT: no-till system; SCP: spring chisel plow; FmrNT: farmer-managed no-till system; Forest: natural forest system adjacent to experiments site.

Values with similar or no letters indicate no significant difference between tillage systems,  $p < 0.05$ .

Values for forests and farmer-managed systems are means of duplicated samples but were not included in statistical analysis for experiment sites; but are included for non-statistical comparisons only.

Table 4. Masses of soil and various pools of C and N per area in equivalent soil mass.

System†	Soil mass Mg ha <sup>-1</sup>	T_add cm	WSC††	HWEC††	Mineralizable	Mineralizable
					C	N
					Mg ha <sup>-1</sup>	
<u>Coastal Plain</u>						
CT	1304.5 b‡	0.03 ab	0.15 b	0.51 b	2.04 c	11.97
CTO	1230.8 b	0.63 a	0.20 b	0.67 ab	1.04 d	12.23
NT	1500.8 a	-1.30 b	0.18 b	0.57 b	3.34 b	15.64
S	1410.5 a	-0.73 b	0.30 a	0.72 a	4.73 a	14.35
FmrCTO	1265.0	0.31	0.15	0.54	0.57	10.17
Forest	1036.6	2.61	1.29	2.75	7.77	33.47
<u>Mountain</u>						
CT	1322.9 a	0.05 b	0.18 b	0.31 b	0.66 b	8.76
CTO	1167.5 b	1.33 a	Nd	nd	0.52 b	6.51
CTS	1175.0 b	1.25 a	0.17 b	0.31 b	0.33 b	11.26
NTO	1152.5 b	1.48 a	0.28 a	0.76 a	0.48 b	4.83
NTS	1132.5 b	1.68 a	0.28 a	0.47 b	1.23 a	5.04
Forest	1040.8	3.11	0.43	1.46	0.88	4.89

‡Values with similar or no letters indicate no significant difference between tillage systems,  $p < 0.05$ ; nd: not determined.

Table 4. Continued.

System†	Soil mass	T_add	WSC††	HWEC††	Mineralizable C	Mineralizable N
	Mg ha <sup>-1</sup>	cm	Mg ha <sup>-1</sup>			
	<u>Piedmont</u>					
MBP	1313.6‡	0.05	0.12 b	0.29 b	0.27	2.94
NT	1412.5	-0.68	0.21 a	0.69 a	0.61	11.96
SCP	1252.1	0.53	0.15 b	0.35 b	0.57	3.05
FmrNT	1326.6	-0.10	0.35	0.98	1.23	14.13
Forest	1174.8	1.19	0.43	1.58	1.03	21.88

‡Values with similar or no letters indicate no significant difference between tillage systems,  $p < 0.05$ ; nd: not determined.

T\_add: Additional soil thickness required to attain the equivalent soil mass.

†Coastal Plain systems/ CT: Conventional tillage (CT) under Best Management Practices (BMP); NT: no-till under BMP; CTO: organic system under CT; S: old field succession ecosystem under fallow; FmrCTO, farmer-managed organic farm under conventional tillage; Forest: natural forest system adjacent to experiments site. Mountain systems/ CT: Conventional tillage (CT) receiving no inputs; CTO: CT receiving organic amendments; CTS: CT receiving synthetic amendments; NTO: no-till receiving organic amendments; NTS: no-till receiving synthetic amendments; Forest: natural forest system adjacent to experiments site. Piedmont systems/ MBP: moldboard plow; NT: no-till system; SCP: spring chisel plow; FmrNT: farmer-managed no-till system; Forest: natural forest system adjacent to experiments site.

Values for forests and farmer-managed systems are means of duplicate samples but were not included in statistical analysis for experiment sites; they were included for non-statistical comparisons only.

††WSC, HWEC: water soluble and hot water extractable C, respectively.

Table 5. Soil C and C pools, expressed as concentration and equivalent mass, for different tillage systems in the Coastal Plain, Mountain, and Piedmont.

System	SOC†	SON	HWEC	MBC	MBN	SOC	SON	HWEC	MBC	MBN
	Concentration basis (Mg kg <sup>-1</sup> soil)					Equivalent mass basis (Mg ha <sup>-1</sup> in equivalent soil mass)				
	<u>Coastal Plain</u>									
CT	9.47 b	0.67	0.39 c	0.16	0.02 b	12.35 b	0.87	0.51 b	0.21	0.03
CTO	10.17 ab	0.83	0.51 ab	0.19	0.03 a	13.26 ab	1.08	0.67 ab	0.25	0.04
NT	9.87 b	0.80	0.44 b	0.21	0.02 b	12.87 b	1.04	0.57 b	0.28	0.03
S	10.73 a	0.83	0.56 a	0.16	0.03 b	14.00 a	1.08	0.72 a	0.20	0.03
	<u>Mountain</u>									
CT	8.43 b	0.78 bc	0.23 b	0.14 c	0.03 c	11.15 b	1.02 b	0.31 b	0.18 c	0.04 c
CTO	9.25 b	0.80 bc	nd	0.27 c	0.06 b	12.24 b	1.06 b	nd	0.37 b	0.08 b
CTS	8.63 b	0.73 c	0.23 b	0.17 c	0.03 c	11.41 b	0.96 b	0.31 b	0.22 c	0.04 c
NTO	15.85 a	1.48 a	0.58 a	0.49 a	0.09 a	20.97 a	1.95 a	0.76 a	0.65 a	0.12 a
NTS	11.03 b	0.95 b	0.36 b	0.10 b	0.02 c	14.59 b	1.26 b	0.47 b	0.13 c	0.03 c
	<u>Piedmont</u>									
MBP	5.20 b	0.53 c	0.22 b	0.13	0.02 b	6.83 b	0.69 c	0.29 b	0.16 b	0.02
NT	10.60 a	0.98 a	0.53 a	0.13	0.04 a	13.92 a	1.28 a	0.69 a	0.17 b	0.04
SCP	6.68 b	0.65 b	0.27 b	0.17	0.02 b	8.77 b	0.86 b	0.35 b	0.22 a	0.03

†SOC, SON: Soil organic C and N, respectively; HWEC: hot water extractable C



Table 7. Stratification ratios of C and N pools under different tillage systems.

System	Ratio depth 1 (0-10:10-20 cm)				Ratio depth 2 (0-10:20-30 cm)			
	SOC	SON	MBC	MBN	SOC	SON	MBC	MBN
<u>Coastal Plain</u>								
CT	1.7	1.4	1.3	2.8	2.6	2.6	0.9	5.3
CTO	1.5	1.6	1.5	3.2	2.6	2.8	1.3	3.5
NT	1.7	1.7	1.4	2.6	3.0	2.6	1.2	4.4
S	2.0	1.8	1.0	1.1	3.0	2.9	1.4	3.8
FmrCTO	1.0	1.1	1.6	1.6	1.2	1.4	1.5	1.8
Forest	2.6	2.6	1.7	2.9	3.6	4.1	1.8	4.2
<u>Mountain</u>								
CT	1.0 c	1.0 c	1.1 b	1.1 b	1.1 c	1.1 c	1.0 c	1.3 b
CTO	1.1 c	1.0 c	1.4 b	1.6 aB	1.2 c	1.1 c	1.6 b	2.4 bA
CTS	1.0 c	1.0 c	1.2 b	1.5 ab	1.0 c	1.0 c	1.2 c	1.8 b
NTO	2.1 aB	2.0 aB	1.1 b	1.0 b	2.9 aA	2.6aA	1.0 c	1.0 b
NTS	1.5 bB	1.5 bB	2.1 aB	3.0 aB	1.7 bA	1.8 bA	3.8 aA	6.0 aA
Forest	3.7	2.9	1.9	3.3	7.2	4.4	1.8	4.0
<u>Piedmont</u>								
MBP	1.0	1.1 b	0.9	0.9	1.0 b	1.1 c	0.8	1.1
NT	1.9 B	1.9 aB	1.5	2.3	3.0 aA	2.8 aA	1.6	3.1
SCP	1.3 B	1.3 ab	1.3	1.9	2.2 aA	1.8 b	1.5	1.7
FmrNT	2.8	2.4	1.5	2.6	4.1	4.3	1.5	3.2
Forest	2.0	2.2	1.1	2.4	2.9	3.5	1.5	3.1

†Different upper or lower case letters indicate significant difference at  $p < 0.05$ . Lower case letters (within columns) represent comparisons between tillage systems for a ratio depth (0–10:10–20 cm or 0–10:20–30 cm); and upper case letters (across rows) are comparisons between ratio depths within a tillage system. Ratio depth 1 (0-10:10-20 cm) is the upper SR depth ratio, while ratio depth 2 (0-10:20-30 cm) is the lower SR depth ratio.

Coastal Plain systems / CT, NT: conventional tillage and no-till practices, respectively; CTO: Organic-amended CT; S: successional system; FmrCTO, farmer-managed organic-amended CT. Mountain systems / CT: CT with no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic- amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; SCP: spring chisel plow; NT: no-till; FmrNT: farmer-managed no-till system. All locations/ Forest: natural forest system adjacent to experiments site.

Values for forests and farmer-managed systems are means of duplicated samples but were not included in statistical analysis for experiment sites; they were included for non-statistical comparisons only.

Table 8. Differential stratification ratios of soil organic C and N (SOC and SON) and microbial biomass C and N (MBC and MBN) under different tillage systems.

System <sup>††</sup>	SOC	SON	MBC	MBN
<u>Coastal Plain</u>				
CT	0.8	1.2	- 0.4	2.5
CTO	1.1	1.1	- 0.2	0.2
NT	1.3	0.8	- 0.2	1.8
S	1.0	1.1	0.4	2.7
FmrCTO	0.2	0.3	- 0.1	0.2
Forest	0.9	1.5	0.2	1.3
<u>Mountain</u>				
CT	0.1 b <sup>†</sup>	0.1 c	0.0 b	0.2 b
CTO	0.1 b	0.1 c	0.2 b	0.8 b
CTS	0.0 b	0.0 c	0.0 b	0.3 b
NTO	0.7 a	0.6 a	1.8 a	3.0 a
NTS	0.2 b	0.3 b	- 0.1 b	0.0 b
Forest	3.5	1.5	0.0	0.7
<u>Piedmont</u>				
MBP	0.0	0.1	- 0.2	0.2
NT	1.1	0.8	0.1	0.7
SCP	1.0	0.5	0.2	- 0.3
FmrNT	1.2	1.8	0.0	0.6
Forest	0.9	1.3	0.4	0.7

Values with similar or no letters indicate no significant differences at  $p < 0.05$  for comparisons between the lower and higher depth ratios in tillage systems.

<sup>††</sup> Coastal Plain systems / CT, NT: conventional tillage and no-till practices, respectively; CTO: Organic-amended CT; S: successional system; FmrCTO, farmer-managed organic-amended CT. Mountain systems / CT: CT with no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic- amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; SCP: spring chisel plow; NT: no-till; FmrNT: farmer-managed no-till system. All locations/ Forest: natural forest system adjacent to experiments site.

Values for forests and farmer-managed systems are means of duplicated samples but were not included in statistical analysis for experiment sites.

Table 9. Correlations between SOC and soil properties affecting SOC sequestration in the Coastal Plain, Mountain, and Piedmont.

	SOC	HWEC	Ncrys_Fe	Clay	MWD	SOCSR1	SOCSR2	DSRSOC	HM
<u>Coastal Plain</u>									
Cqrate	1.00***	0.62*	0.17	0.66*	0.39	0.02	0.05	0.02	0.23
SOC		0.62*	0.17	0.66*	0.39	0.02	0.05	0.02	0.22
HWEC			-0.24	0.09	-0.21	0.25	0.25	0.08	0.27
Ncrys_Fe				0.55	0.84***	-0.12	-0.07	0.06	0.06
Clay					0.66*	0.19	0.24	0.10	0.25
MWD						-0.29	0.04	0.25	-0.14
<u>Mountain</u>									
Cqrate	1.00***	0.96***	-0.20	-0.06	0.60*	0.63**	0.66**	0.58*	-0.55
SOC		0.96***	-0.20	-0.06	0.60*	0.63**	0.66**	0.58*	-0.55
HWEC			-0.26	-0.02	0.61*	0.75***	0.81***	0.75***	-0.59*
Ncrys_Fe				0.48	0.19	-0.51*	-0.44	-0.21	0.66*
Clay					0.02	-0.10	0.04	0.15	-0.19
MWD						0.19	0.28	0.41	0.01

Cqrate: C sequestration rate; SOC: soil organic C; HWEC: hot water extractable C; NcrysFe: non-crystalline Fe (hydr)oxides; Clay: percent clay content; MWD: mean weight diameter; HM: humic matter; SOCSR1 (and SOCSR2): stratification ratios for SOC in the 0-10:10-20 cm and 0-10:0-20-30 cm soil depth ratios, respectively; DSRSOC: difference between SOCSR2 and SOCSR1. \*, \*\*, \*\*\* show significant correlations at p = 0.05, 0.01, and 0.001, respectively.

Table 9. Continued.

	SOC	HWEC	Ncrys_Fe	Clay	MWD	SOCSR1	SOCSR2	DSRSOC	HM
				<u>Piedmont</u>					
Cqrate	1.00***	0.94***	-0.56	-0.10	0.91***	0.91***	0.80***	0.63*	0.62
SOC		0.94***	-0.57	-0.10	0.91***	0.91***	0.80***	0.62*	0.62*
HWEC			-0.60	-0.31	0.86***	0.85***	0.71**	0.51	0.80**
Ncrys_Fe				-0.24	-0.23	-0.66*	-0.61*	-0.52	-0.52
Clay					-0.25	-0.09	0.08	0.19	-0.39
MWD						0.82	0.64	0.48	0.52

Cqrate: C sequestration rate; SOC: soil organic C; HWEC: hot water extractable C; NcrysFe: non-crystalline Fe (hydr)oxides; Clay: percent clay content; MWD: mean weight diameter; HM: humic matter; SOCSR1 (and SOCSR2): stratification ratios for SOC in the 0-10:10-20 cm and 0-10:0-20-30 cm soil depth ratios, respectively; DSRSOC: difference between SOCSR2 and SOCSR1.

\*, \*\*, \*\*\* show significant correlations at p = 0.05, 0.01, and 0.001, respectively.

## CHAPTER III

### TILLAGE EFFECTS ON THE DISTRIBUTION OF ORGANIC CARBON AND NITROGEN IN SOIL PARTICLE-SIZE FRACTIONS

#### Abstract

The non-labile organic matter pool associated with clay particles is a vital reservoir for long-term C sequestration. This study assessed tillage impacts on C and N distribution in particle size fractions in the 0 to 10 cm depth in three long-term experiments under no-till (NT) and conventional tillage (CT). Silt+clay from a Wickham sandy loam (fine-loamy, mixed, semi active, thermic Typic Hapludult), Delanco fine-sandy loam (fine-loamy, mixed, mesic, Aquic Hapludult), and Wedowee sandy clay loam (fine, kaolinitic, thermic Typic Kanhapludult) in Coastal Plain, Mountain, and Piedmont locations, respectively, was fractionated according to particle size and analyzed for C and N. Under NT and CT, the silt+coarse clay proportion was 3-12x greater than fine clay but C and N concentrations and enrichment factors (EC and EN) were 1.1-3x greater in fine clay than silt+coarse clay across locations. Silt+coarse clay and fine clay C and N concentrations were 1.1 and 1.6x greater under NT than CT, while EC and EN were 1.3 and 1.1-1.2x greater under CT than NT in the Mountain and Piedmont locations, respectively; while EC was 1.1x greater under CT than NT in the Coastal Plain. The results suggest that NT potentially increases C sequestration by increasing the fine clay associated C concentration. Tillage practices did not enrich C in

particle-size fractions. The physical fraction procedure used in this study can differentiate organic C and N distributions in particle-size fractions in diverse agroecosystems.

## Introduction

Soil OM (SOM) comprises a complex mixture of organic substances in association with inorganic soil components (Guggenberger et al., 1994), resulting in a series of size fractions or pools (Schimel et al., 1985; Rosell et al., 2001) with turnover times that define the pools (Elliott et al., 1996; Rosell et al., 2001). Particle-size fractionation suggests that particle-size associated organic C and N pools differ in structure and function (Christensen, 1992, 2001). For instance, SOM recovered in primary particle-size fractions > 0.05-mm was noted to be of plant origin (Balesdent, 1996), while that in clay sizes was attributed to microbial biomass (Guggenberger et al., 1994). Organic C and N associated with sand is enriched in plant residues, while that associated with clay and silt are rich in microbial derived products and both plant and microbial residues (Guggenberger et al. 1995). Particle-size fractionation has, therefore, been used to examine the response of organic C and N dynamics to the impacts of management practices (Tiessen and Stewart 1983).

Particle-size fractions differentiate between labile or active and non-labile or intermediate and refractory pools of SOM (von Lützow et al. 2007). While the labile pools readily supply nutrients but are quickly depleted (Rosell et al., 2001), the non-labile pool associated with clay particles is an important reservoir for long-term C sequestration (Kiem et al., 2002). Soil incorporation of organic matter via tillage results in increased contact of incorporated organic C with the mineral components (Balesdent et al., 2000). While both plant- and microbial-derived organic substances influence the composition of OM in the

organomineral complex, the degree of decomposition from plant-derived substances follows the order: sand-sized > silt-sized > clay-sized complexes. In contrast, microbial derived OM increases with decreasing particle size, so that clay-complexed OM contain more microbial- than plant-derived substances (Guggenberger et al., 1994 and 1995).

The objective of this study was to use physical fractionation techniques to quantify the differences in the distribution of organic C and N in silt+coarse clay and fine clay fractions in the surface 10 cm of three long-term experiments under diverse tillage systems, located in the Coastal Plain, Mountain, and Piedmont physiographic regions of North Carolina. It was hypothesized that in soils under diverse tillage systems; organic C and N distribution will be greater in the fine clay (< 0.0002-mm diameter) than silt+coarse clay (0.053-0.0002-mm diameter) particle-size fractions, irrespective of tillage system, but that tillage will preferentially affect the distribution.

## **Materials and Methods**

### **Soils and soil sampling**

Soil samples were collected from long-term field experiments at the Center for Environmental Farming Systems (CEFS) in the Coastal Plain; Mountain Horticultural Crops Research Station (MHCRS) in the Mountain; and Upper Piedmont Research Station (UPRS) in the Piedmont. The experimental designs studied consisted of 4, 5, and 3 treatments in 3, 4, and 4 replications at the CEFS, MHCRS, and UPRS experimental sites, respectively. Samples

were also collected from forests adjacent to each experimental site and from farmer-managed fields in the Coastal Plain and Piedmont. Sampling at CEFS was around previously geo-referenced points. At other locations, sampling was from 3-6 randomly assigned and demarcated sampling locations selected within defined soil-landscape positions in each replication or farm. Samples were taken within plant rows from the 0 to 10 cm soil depth using a 7.5-cm diameter by 30-cm deep long soil probe at all locations except CEFS, where a 2.5-cm diameter by 30-cm deep long soil probe was used. Three samples were taken around each sampling location, composited, placed into plastic bags, and transported to the laboratory. In the laboratory, the field moist samples were divided into two: one for air-drying and then determination of physico-chemical properties and another for wet-sieving and microbiological determination. The samples for wet-sieving and microbiological determination were stored at 0-4°C until processed.

#### **Particle size fractionation (separation of non aggregate-occluded particle size fractions)**

In this study, the particle-size fractions that were outside of aggregates were called the non-aggregate occluded particle size fractions. A modification of the methods of Laird et al. (1991, 1994) was used to separate silt+coarse clay (0.053-0.0002-mm diameter) and fine clay (< 0.0002-mm diameter) particle-size fractions from material that passed through the 0.053-mm sieve screen by a modification of the wet-sieving method of Yoder (1936). Briefly, the <0.053-mm diameter material was mixed and quantitatively transferred into pre-

weighed 250-ml polypropylene centrifuge bottles using distilled, deionized water to a pre-determined depth based on Stoke's Law. The suspension was centrifuged at 90 relative centrifugal force (RCF) for 3 min 55 s on a GSA fixed angle rotor (6 × 250 ml bottles) Sorvall RC 5B Superspeed Centrifuge, collecting the supernatant in 4-L plastic beakers, re-suspending the mainly silt material at the bottom of the bottles by adding distilled deionized water close to the pre-determined mark, dispersed by sonicating for 90-100 s at 12-15% intensity (or 66-85 W) of the output power of a 1.3 cm diameter probe-type ultrasonic unit (Sonic Dismembrator probe Model 550 from Fisher Scientific), and centrifuged again. The tip of the sonicator probe was immersed to a depth of ~ 2 to 3 cm from the bottom of the centrifuge bottle. The sonication-centrifugation-decantation steps were repeated for a total of 4 to 8 times or until a clear supernatant was obtained. Then the mainly silt material at the bottom of the bottle was quantitatively transferred into pre-weighed plastic cups, weighed, and freeze-dried.

The supernatant suspensions containing clay in the 4-L plastic beakers were stirred and poured again into 250-ml bottles, centrifuged as above, but this time at 2600 RCF for 13 min 48 s, and the supernatants decanted into other 4-L beakers. The suspension-sonication-centrifugation-decantation procedures were repeated 3 more times, after which the coarse clay at the bottom of each bottle was quantitatively transferred into pre-weighed plastic sample cups, weighed, freeze-dried, weighed again after freeze-drying, combined with the silt fraction (the silt+coarse clay fraction), and stored at room temperature.

Solid sodium chloride (NaCl) was added (at approximately  $60 \text{ g L}^{-1}$  suspension) to the fine clay suspension to flocculate the fine clay. After flocculation, a hooked capillary tube was used to siphon off the clear supernatant solution and to reduce the volume of water. The concentrated clay was rinsed into a dialysis membrane (molecular weight of 12,000-14,000 Daltons; thickness of 0.0008 inches) and dialyzed in a bucket of distilled deionized water, changing the water 3 to 4 times a day for 2 to 3 days until the samples were free of NaCl salt (indicated when a 20 to 25 mL sample of distilled water from the dialysis bucket remained clear after adding 1 to 2 drops of  $\text{AgNO}_3$ ). When dialysis was complete, fine clay in the dialysis tubing was quantitatively transferred, using distilled water, into a tared centrifuge bottle, centrifuged at 16250 RCF for 1 h, discarding the supernatant and quantitatively transferring the residue at the bottom to pre-weighed cups. The residue was weighed, freeze-dried, re-weighed after freeze drying, and stored in 20-mL vials. Freeze-dried fine clay and silt+coarse clay particle size fractions were finely ground, separately, and stored at room temperature until analysis for total C and N by direct combustion in a Perkin–Elmer 2400 CHN analyzer. Particle size analysis was according to the hydrometer method of Gee and Bauder (1986).

**Determination of mineral associated and particulate organic matter (separation of aggregate-occluded particle size fractions)**

The silt+clay particle-size fractions contained in each aggregate size class were called

the aggregate- occluded particle size fractions in that size class. These comprised the mineral-associated organic matter (MAOM) or the <0.053 mm diameter materials, and the particulate organic matter or the >0.053 mm diameter particles. Determination of MAOM was determined according to Mikha and Rice (2004). Briefly, 3 g (oven dry equivalent) of intact macro- or micro-aggregate size class was placed in a 150-mL plastic sample cup and 15 mL of 5 g L<sup>-1</sup> (0.5% w/v) sodium hexametaphosphate (SMP) added. Samples were covered, left to stand overnight (16 h), and dispersed in the SMP by shaking on a gyrating shaker at 350 rpm for 4 h. The dispersed samples were passed through a 0.053-mm sieve and the dispersed OM collected in an Al tray. A flush of distilled water from a water bottle was used to wash over the sieve several times until most or all dispersed OM was washed into the Al tray. Sand (and particulate organic C and N associated with macro- and micro-aggregates, MPOC/N and mPOC/N, respectively) remaining on sieves were transferred into Al cans, dried in an oven at 105°C for 24 h, and weighed. The material that passed through the sieve, containing the mineral associated C and N in macro- and micro-aggregates (MMAC/N and mMAC/N), was transferred into pre-weighed Al cans and placed in a forced air oven at 50°C. Depending on the amount of water in the slurry, it took between 24 to 72 h to dry the samples. Once dried, the samples were weighed, ground to a fine powder in a mortar and pestle and stored at room temperature. Macro- and micro-aggregate associated POC and PON in material that was retained on the sieve was estimated as the difference between C or N in material that passed through the 0.053-mm sieve screen and C or N in

non-dispersed aggregates (Cambardella and Elliott, 1992; Chan, 2001; Al-Kaisi and Grote, 2007). Analysis for total C and N in bulk soil was also by direct combustion in a Perkin–Elmer 2400 CHN analyzer. Soils in this study were generally at  $\text{pH} \leq 6.5$  and low in carbonates. Therefore, total C was assumed to be equal to organic C. Selected physical and chemical properties of the soils studied are shown in Table 1.

### Calculations and statistical analysis

So that differences due to bulk soil contents of organic C and N were eliminated, and thereby allow for region-wide comparison of organic C and N concentrations of particle size fractions separated from different soils, enrichment factors for C (EC) and N (EN), which express the ratio of the concentration of C (or N) in a particle size fraction to the concentration in bulk soil, were calculated (Christensen, 2001) as follows:

$$1) \text{ EC (or EN)}_{\text{fraction}} = \text{g C(or N) kg}^{-1}_{\text{fraction}} / \text{g SOC(or SON) kg}^{-1} \text{ bulk soil},$$

where:  $\text{EC}_{\text{fraction}}$  (or EN) is the enrichment factor for C or N in a particle-size fraction;  $\text{g C (or N) kg}^{-1}_{\text{fraction}}$  is the concentration of C (or N) in fraction ( $\text{g C or N kg}^{-1}$  fraction); and SOC (or N) is the concentration of C or N in bulk soil ( $\text{g C or N kg}^{-1}$  bulk soil). Since the material retained on the sieve in the determination of MAC and MAN contained some POM (Cambardella and Elliott, 1993; Manna et al., 2007), even though the POM is not involved in

stabilizing organo-mineral complexes (Christensen, 1986), EC and EN were calculated for the POM.

Tillage and region effects were evaluated by using the mixed procedure (Proc Mixed) model of the Statistical Analytical Software (SAS Inst., Cary, NC) to compare treatment means and particle size fractions. Pearson's correlation coefficients of the correlation matrix of C and N measured in particle size fractions were determined.

## **Results and Discussion**

### **Distribution of primary particle-size fractions**

Figure 1 shows that the distribution of the aggregate size classes (macro- and micro-aggregates and silt+clay) within locations did not differ significantly. Percent recoveries of samples relative to bulk soil fractionated ranged between 87 and 92% (data not shown). Particle-size fractions (silt, coarse clay, fine clay, and silt+coarse clay) outside of aggregates were not affected by tillage treatments in the Coastal Plain and Mountain. In contrast, the percentage coarse clay under no-till (NT) in the Piedmont was half that under spring chisel plow (SCP) or moldboard plow (MBP) (Fig. 2). Distribution of other particle-size fractions did not differ in this location. Averaged across systems, between 3-5% of the total soil mass fractionated from the conventional tillage systems in the three locations was comprised of silt+coarse; 3-6% of coarse clay; and 1-3% of fine clay. Under minimal or no disturbance systems (no-till and successional), average soil mass contributed by silt fractions in the three

locations was between 3 and 4%; whereas coarse clay and fine clay respectively contributed 3 to 4 and 1 to 2% of the soil mass.

Tillage effects on the distribution of sand size particle-size fractions within macro- (MPOM) and micro-aggregates (mPOM) were not significant in all locations (Fig. 3). The proportions of MPOM in macroaggregates ranged from 81 to 94% for the Coastal Plain location, 59 to 67% in the Mountain location, and 75 to 79% in the Piedmont location. Corresponding proportions for mPOM were 68 to 89%, 46 to 74, and 81 to 89% in the Coastal Plain, Mountain, and Piedmont locations, respectively. Tillage also had no effect on the distribution of silt+clay size fractions (mineral associated organic matter, or MAOM) in macro- (MMAOM) and micro-aggregates (mMMAOM) in the Mountain and Piedmont locations. Aggregate size effects were observed in the Piedmont location, however, where the proportions of MMAOM were 1.3 and 1.4 times greater than mMMAOM in the SCP and NT systems, respectively (Fig. 3). In the Coastal Plain location, the distribution of silt+clay size fractions in aggregates differed significantly among tillage systems; being of the order  $NT \sim CT = CTO = S$  for MMAOM and  $CTO \sim CT = NT > S$  for mMMAOM. The mMMAOM proportion was greater than MMAOM.

Kiem et al. (2002) found no significant within-location differences in the distribution of particle-size fractions in three soils with similar climatic features but under different management systems (fertilizer or organic amended versus unmanured or unfertilized) for over 40 years. The soils in the authors' study were two Luvisols and a Chernozem (or two

Alfisols and a Mollisol in the US Soil Taxonomy System). Puget et al. (2000) also observed no significant differences in the distributions of primary particles in aggregates from two silty soils (Inceptisols; Eutric Cambisols in the FAO classification) in France. According to the aggregate hierarchy of Tisdall and Oades (1982), binding agents in the < 0.002-mm diameter range are not impacted by management practices (Stott et al., 1999). The absence of statistical evidence for tillage effects on the distributions of silt, coarse clay and fine clay fractions (outside aggregates) in the Coastal Plain and Mountain locations (Fig. 2), and in the distribution of silt+coarse clay and fine clay fractions within aggregates in the Mountain and Piedmont locations (Fig. 3), supports the findings of these authors.

There are possible explanations for the significant differences observed in the distributions of coarse clay size fractions in the Piedmont (Fig. 2) or in the distribution of silt+clay fractions within aggregates (mineral associated organic matter in the Coastal Plain (Fig. 3). Gruver (2007) and Muruganadam (2007) observed higher clay contents in the surface depths (0-7.5 and 0-10 cm, respectively) under MBP relative to SCP and NT systems in this location. Gruver (2007) attributed this occurrence to soil inversion (differential mixing of clay-rich B horizon with sandier A horizon soil) during tillage operations. Results from the present study also suggest that erosion could be involved. Biomass removal under CT, as opposed to leaving crop residues in place under NT to provide mulch and protection for surface soil, results in bare soils that are susceptible to soil erosive forces which transport soil and expose the finer textured soil below. The topography of the Piedmont

region is rolling to hilly (Daniels et al., 1999); and long-term erosion under conventional tillage under such circumstances can have significant consequences on clay content in the surface soil. A possible explanation for the differences in distribution of silt+coarse clay in macro- or micro-aggregates may be the possibility of incomplete dispersion of aggregates; e.g. incomplete dispersion of macroaggregates from the other tillage systems relative to the NT samples, or of microaggregates from the other tillage systems relative to those from the CTO samples, during sonication. These results suggest that silt and clay contents do not make major contributions to aggregate stability in the surface soil depth studied in these locations. According to the aggregate hierarchy of Tisdall and Oades (1982), binding agents in the clay (< 0.002-mm diameter) range are not impacted by management practices (Stott et al., 1999).

### **Carbon and nitrogen composition of particle-size fractions**

Tillage had no significant effects on C concentrations in particle-size fractions in the Coastal Plain and Mountain locations; but C concentrations were greater in the fine clay than silt+coarse clay particle-size fractions at each location (Fig. 4). In the Piedmont, there were significant effects due to tillage and aggregate size. The C concentration in the silt+coarse clay fraction was 2 times greater under NT than under MBP but not different from the C concentration under SCP; whereas the C concentration in the fine clay fraction was 2 times greater under NT than either MBP or SCP. The C concentrations under the NT

system were greater in micro- than macro-aggregates. In , There were no significant interaction effects at any location. The N concentrations were impacted by tillage and particle size effects in the Coastal Plain and Piedmont locations (Fig. 5). In the Coastal Plain, tillage had no effect on N concentrations in the silt+ coarse clay fraction; but N concentration in the fine clay fraction was of the order  $CTO = S \sim NT = CT$ . In the Piedmont, N concentration in the silt+coarse clay fraction was of the order  $NT \sim SCP = MBP$ ; whereas the order in the fine clay fraction was of the order  $NT > SCP = MBP$ . In both locations, N concentrations under the different tillage systems were greater in the fine clay than silt+coarse clay fraction (Fig. 5). Although tillage effects were not significant in the Mountain, N concentrations were greater in fine clay than silt+coarse clay fractions; and significant tillage  $\times$  particle size interaction occurred, suggesting that tillage effects depended on particle-size fraction.

The lower contents of C associated with the fine clay fraction under the SCP and MBP systems relative to the undisturbed NT system in the Piedmont, where cultivation has been used for 24 years (2 and 3 times longer than in the Mountain and Coastal Plain locations, respectively), suggest that long-term cultivation affects the soil clay fraction. Tillage practices under the SCP and MBP reduced the C associated with the fine clay fraction. Tillage and other management practices in arable soils generally influence particle-size fractions to a lesser extent than initial management practices in permanent vegetation (Christensen, 2001). It is thus possible that the relatively short duration of management in

the Coastal Plain and Mountain experiment sites may be responsible for the general lack of significant effects on the C contents, and N contents to a greater extent, associated with silt+coarse clay and the fine clay fractions.

The greater *concentrations* of C and N observed in the fine clay fraction relative to the coarser fraction (silt+coarse clay) in this study agree with Christensen (2001); who observed greater C concentrations in clay-sized than either silt- or sand-sized particles in temperate arable soils. Organic C associated with the fine clay fraction has been described as containing more microbial than plant derived substances and to play a greater role in organic matter stabilization than does the silt+coarse clay fraction (Guggenberger et al., 1994, 1995; Christensen, 2001). It is, therefore, possible that more humification occurred in the fine clay than silt+coarse clay particles in these soils. These results are also in accord with other findings (Elliott et al., 1991; Stemmer et al., 1998; Kiem et al., 2002); where increasing C concentrations were observed with decreasing particle size. The present study observed that the region-wide average proportion of silt+coarse clay was 5.8 times greater than the proportions of fine clay (Fig. 2); whereas concentration of C or N was 1.9 times greater in the fine clay than silt+coarse clay fraction (Fig. 4). These results suggest that the fine clay particle-size fractions bind with organic matter more effectively than coarse particle-size fractions in these soils.

The distribution of total soil organic C (SOC) and N (SON) pools among aggregate and particle size fractions in this study are shown in Table 2. The proportions of SOC and SON

derived from the particle size fractions decreased with decreasing particle size; i.e. from silt+coarse clay to fine clay. These results suggest that only very small proportions of the organic C and N in the surface depth studied were stored in the fine particle-size fraction over the durations of the experiments. There were significant correlations between the SOC (or SON) concentrations and the proportions of silt+coarse clay and fine clay in the Mountain location; but none was observed in the Coastal Plain and Piedmont locations (Table 3). Correlations between the SOC (or SON) and the C (or N) concentrations in size fractions were significant in the Mountain location; whereas the correlations were not significant in the Coastal Plain and Piedmont locations. Significant correlations among SOC and SON and the proportions (of SOC and SON) coming from C and N in both particle size fractions were also observed in the Mountain location; whereas significant correlations between SOC and the proportions from fine clay C and silt+coarse clay N were observed in the Piedmont. No significant correlations were observed for C and N pools in the Coastal Plain location (Table 3). Christensen (2001) suggests that actual amounts of organic matter in soils do not show direct relationships with the contents of silt+clay fractions or with the distribution of SOC or SON in these fractions. Furthermore, soil type and management are important factors affecting the relationship between silt, clay, and SOC concentrations (Lorenz et al., 2008).

### **Concentrations of C and N in particle-size fractions within aggregates**

Figures 6 and 7 show the concentrations of C and N in silt+clay- (mineral associated C and N; MAC and MAN, respectively) and sand-sized (particulate organic C and N; POC and PON, respectively) particles associated with macro- (Ma) and micro- (Mi) aggregates in the three locations studied. Tillage effects on MAC were significant in all locations. There were also significant effects due to particle-size fraction in the Piedmont and to the interaction between tillage and particle-size fraction in the Mountain and Piedmont locations. In the Coastal Plain, MAC associated with macroaggregates was of the order  $S = CTO > NT = CT$ ; and the order for MAC associated with microaggregates was similar:  $S = CTO \sim NT = CT$  (Fig. 6). In the Mountain location, MAC associated with macro- and micro-aggregates were of the orders  $NTO = NTS > CTO = CTS = CT$  and  $NTO > CTO = NTS = CTS = CT$ , respectively. The MAC content was greater in macro- than micro-aggregates under NTS (Fig. 6). In the Piedmont, the orders for MAC associated with macro- and micro-aggregates were  $NT > SCP = MBP$  and  $NT > SCP > MBP$ , respectively. The concentration of MAC associated with aggregates was greater in macro- than micro-aggregates under the NT system in the Piedmont (Fig. 6). Tillage effects on the concentrations of MAN were similar to the effects on MAC; i.e. the concentrations of MAN in both macro- and micro-aggregates were generally greater under undisturbed (NT or S) than disturbed or tilled systems in all locations (Fig. 7).

There were no significant effects of tillage on C and N in sand-sized particles (POC and PON) associated with macro- and micro-aggregates in the Coastal Plain location; but

there were significant effects in the Mountain and Piedmont locations (Figs. 6 and 7). In the Mountain location, tillage effects on POC in macroaggregates were of the order NTO ~ NTS ~ CTO ~ CTS = CT; whereas effects on POC in microaggregates were of the order NTO > CTS = NTS = CTO ~ = CT. In the Piedmont, the order for POC in macroaggregates was NT > SCP = MBP; POC in microaggregates did not differ between tillage systems. As with MAC and MAN, tillage effects on PON were similar to those on POC. However, PON in macroaggregates did not differ between tillage systems in the Piedmont.

The generally greater concentrations of MAC/N and POC/N associated with macro- and micro-aggregates under NT relative to CT may be related to mechanisms that result in physical protection of organic matter. Aggregate disruption is much greater under CT, but relatively inconsequential under NT, since tillage exposes soil aggregates to microbial attack and OM loss. Other authors (Bayer et al., 2006; Cambardella and Elliot, 1992) have reported greater values for both MAC/N and POC/N in surface soil depths under NT than CT. Increased soil aggregation under NT reduces microbial biomass accessibility to OM and, therefore, enhances physical protection of the OM fractions (Bayer et al., 2006). In a separate but integral component of the assessment of the soils in this study, it was observed that NT systems increased water stable aggregation and OM content of soils relative to CT systems.

### **Enrichment of particle-size fractions**

Tillage did not impact enrichment factors for C (EC) associated with particle-size fractions outside aggregates (silt+coarse clay and fine clay fractions not contained in macro- and micro-aggregates) in all locations studied. There were also no tillage × particle-size interaction effects in all locations but there were significant particle size effects in the Coastal Plain and Mountain locations, where C enrichment was greater in fine clay than the silt+clay fraction in most of the tillage systems (Fig. 8). In all tillage systems combined, the EC factors were 1.3 to 2.7 times higher in fine clay than silt+coarse clay fractions. There were significant effects due to tillage and particle-size fraction on EN in the Mountain location, with values in the silt+coarse clay fraction of the order  $CTS \sim CT \sim NTS = NTO = CTO$ ; and the EN in the fine clay of the order  $CTS \sim CTO = CT \sim NTS = NTO$ . The EN values were 1.1 to 2.8 times greater in the fine clay fraction than silt+coarse clay fraction in all tillage systems in this location. Considering all locations together, the EN values were either equal to or 1.1 to 1.4 times greater than the EC values in the silt+coarse clay or the fine clay fraction. Although tillage effects on EC and EN were generally not significant (Figs. 8 and 9), greater EC and EN values for fine clay relative to silt+coarse clay suggests that C and N were more enriched in the fine clay than the silt+coarse clay fraction.

There were no tillage effects on EC in particle-size fractions contained in aggregates in all locations (Fig. 10). However, EC in sand-sized particles (POC) was greater in micro- than macro-aggregates under the disturbed or tilled systems (CT, CTO, and CTS) in the

Mountain location (Fig. 10); and MAC was greater in macro- than micro-aggregates under MBP but was lower in macro- than micro-aggregates under NT and SCP in the Piedmont location. There were also no tillage or particle-size effects on C pools in the Coastal Plain location. Tillage effects on EN in particle-size fractions in macro- and micro-aggregates did not differ in the Coastal Plain and Mountain locations; but there were significant differences in the Piedmont location (Fig. 11). However, there were particle-size effects in the Mountain location where the EN in PON was generally greater in micro- than macro-aggregates (Fig. 11). The EN was significantly impacted in the Piedmont location; where the EN in MAN in macro- and micro-aggregates was greater under NT than MBP (Fig. 11).

Enrichment factors for both C and N under undisturbed (untilled) and disturbed (tilled) systems were greater in the fine clay than silt+coarse clay by 2-3, 2, and 1.1-1.4 times in the Coastal Plain, Mountain, and Piedmont locations, respectively. Averaged by location, both EC and EN for combined particle-size fractions (fine clay plus silt+coarse clay) were 1.3 and 1.1-1.2 times greater under CT than NT in the Mountain and Piedmont locations, respectively; but EC was 1.1 times greater under CT than NT. The EC and EN values under both NT and CT in all locations were also generally greater in finer fractions (MAC and MAN) than in the sand-sized fractions (POC and PON) in both macro- and micro-aggregates. Thus C and N enrichments were greater in the finer particle-size fractions than the coarse fractions, suggesting that greater quantities of C and N are potentially sequestered in fine clay or mineral associated organic matter than silt+coarse clay or

particulate organic matter size fractions in these soils, irrespective of tillage system (Christensen, 2001). Generally, EN values for both coarse and fine fractions were greater than the corresponding EC values at all locations.

### **Carbon-to-nitrogen ratios**

Wider C/N ratios were observed in the sand-sized (particulate organic matter in macro- and micro-aggregates; POM<sub>Ma</sub> and POM<sub>Mi</sub>) than silt+clay-sized (mineral associated organic matter in macro- and micro-aggregates; MAOM<sub>Ma</sub> and MAOM<sub>Mi</sub>) fractions (Table 4). The C/N ratios in the particulate organic matter (sand-sized fractions) were higher than the C/N ratios in the silt+coarse clay or the fine clay fractions (not contained in aggregates), suggesting the presence of partially decomposed organic matter in the sand-sized fractions.

### **Conclusions**

This study assessed tillage effects on soil C and N distribution in silt+coarse clay and fine clay fractions isolated from the surface 10 cm in three long-term experiments in the Coastal Plain, Mountain, and Piedmont. Tillage did not have significant effects on the C and N concentrations in particle size fractions in the Coastal Plain and Mountain locations; but there were significant impacts in the Piedmont where tillage operations had been in place for 24 years, 2-3 times longer than were employed in the Coastal Plain and Mountain locations. The proportion of soil that comprised the silt+coarse clay fraction was greater

than the proportion that was fine clay; but the fine clay-size fraction was quantitatively more effective in sequestering SOC than the silt+coarse clay fraction, irrespective of tillage system. No-till systems, alone or in combination with organic amendments, generally contained more fine clay or silt+coarse clay associated C and N associated with particle-size fractions in or outside aggregates. Therefore, no-till systems receiving organic amendments can potentially increase C sequestration by increasing the concentration of C associated with the fine clay particle-size fractions. Over the duration of the experiments, tillage practices have not enriched the C contents of both silt+coarse clay and fine clay fractions. The physical fraction procedure used in this study can differentiate the distribution of organic C and N in particle-size fractions in under diverse tillage systems.

## References

- Al-Kaisi, M. M. and J. B. Grote. 2007. Cropping systems effects on improving soil carbon stocks of exposed subsoil. *Soil Sci. Soc. Am. J.* 71(4): 1381-1388.
- Angers, D.A, A. Peasant, and J. Vigneux. 1992. Early cropping-induced changes in soil aggregation, organic matter, and microbial biomass. *Soil Sci. Soc. Am. J.* 56: 115-19.
- Balesdent, J. 1996. The significance of organic separates to carbon dynamics and its modeling in some cultivated soils. *European J. Soil Sci.* 47: 485-493.
- Balesdent, J., C. Chenu, M. Balabane. 2000. Relationship of soil organic matter dynamics to physical protection and tillage. *Soil and Till. Res.* 53: 215-230.
- Bayer, C., J. Mielniczuk, E. Giasson, L. Martin-Neto, and A. Pavinato. 2006. Tillage Effects on Particulate and Mineral-Associated Organic Matter in Two Tropical Brazilian Soils, *Communications in Soil Science and Plant Analysis* 37 (3): 389-400.
- Bossuyt, H., J. Six, and P. F. Hendrix. 2002. Aggregate-Protected Carbon in No-tillage and Conventional Tillage Agroecosystems. Using Carbon-14 Labeled Plant Residue. *Soil Sci. Soc. Am. J.* 66: 1965–1973.
- Cambardella, C.A., and E.T. Elliott. 1992. Particulate organic matter across a grassland cultivation sequence. *Soil Sci. Soc. Am. J.* 56: 777–783.
- Cambardella, C.A. and E. T. Elliott. 1993. Carbon and nitrogen distribution in aggregates from cultivated and native grassland soils. *Soil Sci. Soc. Am. J.* 57:1071-1076.
- Chan, K.Y. 2001. Soil particulate organic carbon under different land use and management. *Soil Use and Management* 17: 217-221.
- Christensen, B.T. 1986. Straw incorporation and soil organic matter in macro-aggregates and particle size separates. *J. Soil Sci.* 37: 125-135.
- Christensen, B.T., 1992. Physical fractionation of soil and organic matter in primary particle size and density separates. *Adv. Soil Sci.* 20: 1–90.
- Christensen, B.T. 2001. Physical fractionation of soil and structural and functional complexity in organic matter turnover. *European J. Soil Sci.* 52: 345-353.

- Daniels, R.B., S.W. Buol, H.J. Kleiss, and C.A. Ditzler. 1999. Soil Systems in North Carolina. Tech. Bull. 314. North Carolina State University. Soil Sci. Dept. Raleigh, NC 27695-7619.
- Elliott, E.T., C.A. Palm, D.E. Reuss, and C.A. Monz. 1991. Organic matter in soil aggregates from a tropical chronosequence: correction for sand and light fraction. *Agriculture, Ecosystems and Environment* 34:443-451.
- Elliott, E.T., K. Paustian, and S. D. Frey. 1996. Modeling the measurable or measuring the modelable: a hierarchical approach to isolating meaningful soil organic matter fractionations. In: Powlson, D.S., Smith, P., Smith, J.U. (Eds.), *Evaluation of Soil Organic Matter Models Using Existing Long-term Datasets*, NATO ASI Series. Springer Verlag, Berlin, pp. 161–179.
- Gee, G.W. and J.W. Bauder. 1986. Particle size analysis. pp. 383-441. In: A. Klute (ed.), *Methods of Soil Analysis, Part 1*, 2<sup>nd</sup> Edition. ASA and SSSA Publ., Madison, WI, USA.
- Gruver, Joel Brookes. 2007. *Impact of Management and Texture on Soil Organic Matter Fractions* (Doctoral dissertation). North Carolina State University, Raleigh, NC. 312 p.
- Guggenberger, G., B.T. Christensen, and W. Zech. 1994. Land-use effects on the composition of organic matter in particle-size separates of soil: I. Lignin and carbohydrate signature. *European J. Soil Sci.* 45: 449-458.
- Guggenberger, G., W. Zech, L. Haumaier, and B. Christensen. 1995. Land-use effects on the composition of organic matter in particle-size separates of soils: II. CPMAS and solution <sup>13</sup>C NMR analysis. *European J. Soil Sci.* 46: 147-158.
- Kiem, R., H. Knicker, and I. Kögel-Knabner. 2002. Refractory organic carbon in particle size fractions of arable soils I: distribution of refractory carbon between the size fractions. *Organic Geochemistry* 33:1683-1697.
- Laird, D. A., P. Barak, E.A. Nater, and R.H. Howdy. 1991. Chemistry of smectitic and illitic phases in interstratified soil smectite. *Soil Sci. Soc. Am. J.* 55: 1499-1504.
- Laird, D. A., P. Y. Yen, W. C. Koskinen, T. R. Steinhelmer, and R. H. Dowdy. 1994. Sorption of atrazine on soil clay components. *Environ. Sci. Technol.* 28: 1054-1061.
- Lorenz, K., R. Lal, and M. J. Shipitalo. 2008. Chemical stabilization of organic carbon pools in particle size fractions in no-till and meadow soils. *Biol Fertil Soils* 44: 1043–1051.

- Manna, M.C., A. Swarup, R.H. Wanjari, B. Mishra, and D.K. Shahi. 2007. Long-term fertilization, manure and liming effects on soil organic matter and crop yields. *Soil and Till. Res.* 94: 397-409.
- Mikha, M. M. and C. W. Rice. 2004. Tillage and manure effects on soil and aggregate-associated carbon and nitrogen. *Soil Sci. Soc. Am. J.* 68: 809-816.
- Muruganadam, S. 2007. Soil aggregate-associated microbial community structure and nitrogen transformations in three different tillage systems. Dissertation. North Carolina State University. 138 p.
- Piovanelli, C., C. Gamba, G. Brandi, S. Simonini, and E. Batistoni. 2006. Tillage choices affect biochemical properties in the soil profile. *Soil Tillage Res.* 90: 84–92.
- Plante AF, R. T. Conant, C. E. Stewart, K. Paustian, and J. Six. 2006. Impact of soil texture on the distribution of soil organic matter in physical and chemical fractions. *Soil Sci Soc Am J* 70:287–296.
- Puget, P., C. Chenu, and J. Balesdent. 2000. Dynamics of soil organic matter associated with particle size fractions of water stable aggregates. *European J. Soil Sci.* 51: 595-605.
- Rosell, R.A., J.C. Gasparoni, and J.A. Galantini. 2001. Soil organic matter evaluation. *In* Assessment methods for soil carbon. Lal, R., J.M. Kimble, R.F. Follet, B.A. Stewart (eds.) CRC Press Boca Raton, FL 33431. pp. 311-322.
- Schimel, D.S., D.C. Coleman, and K.A. Horton. 1985. Soil organic matter dynamics in paired rangeland and cropland toposequences in North Dakota. *Geoderma* 36: 201-214.
- Stemmer, Michael, Martin H. Gerzabek, and Ellen Kandeler. 1998. Organic matter and enzyme activity in particle size fractions of soils obtained after low-energy sonication. *Soil Biol. Biochem.* 30(1): 9-17.
- Stott, D.E., A.C. Kennedy, and C.A. Cambardella. 1999. Impact of soil organisms and organic matter on soil structure. *In* Lal, Rattan (ed.) CRC Press, Inc. 1999. Pp. 57-73.
- Tisdall, J.M., and J.M. Oades. 1982. Organic matter and water stable aggregates in soils. *J. Soil Sci.* 33:141–163.
- von Lützow M, I. Kögel-Knabner, K. Ekschmitt, H. Flessa, G. Guggenberger, E. Matzner, and B. Marschner. 2007. SOM fractionation methods: relevance to functional pools and

to stabilization mechanisms. *Soil Biol. Biochem.* 39: 2183–2207.

Yoder, R.E. 1936. A direct method of aggregate analysis of soils and a study of the physical nature of erosion losses. *J. Am. Soc. Agron.* 28: 337-351.

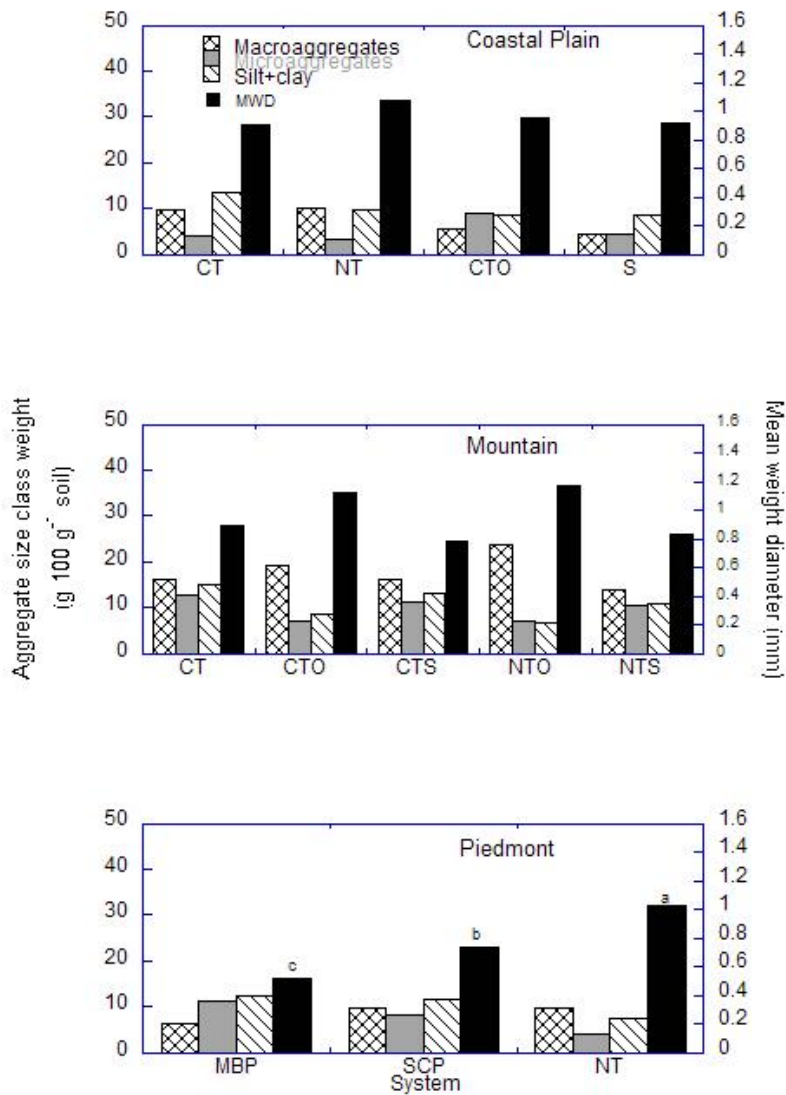


Fig. 1. Aggregate size class distribution and mean weight diameter of wet-sieved samples from three long-term experimental sites. Columns with the same or no letters indicate no significant difference between tillage systems ( $p < 0.05$ ). Coastal Plain systems/ CT: Conventional tillage (CT); NT: no-till; CTO: organic-amended CT; S: successional system. Mountain systems/ CT: CT receiving no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic-amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; NT: no-till system; SCP: spring chisel plow.

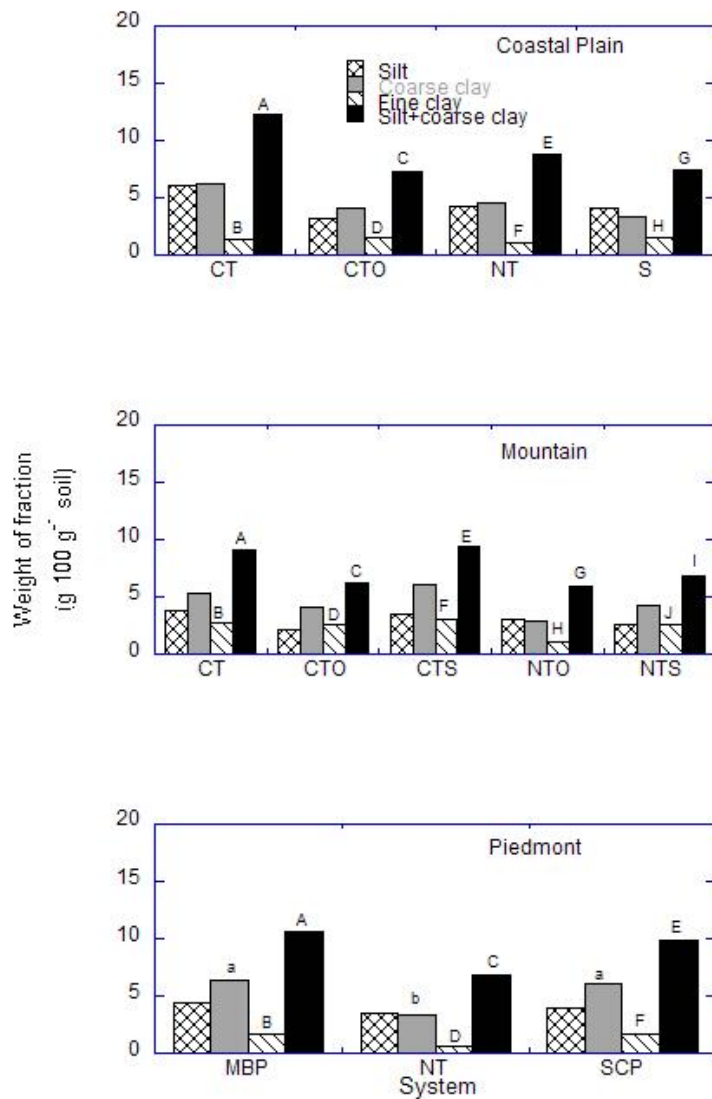


Fig. 2. Particle-size fraction distribution (not including sand particles) outside of macro- and micro-aggregates. Columns with similar or no lower or upper case letters show no significant differences between tillage systems or between fine clay and silt+coarse clay proportions, respectively ( $p < 0.05$ ). Coastal Plain systems/ CT: Conventional tillage (CT); NT: no-till; CTO: organic-amended CT; S: successional system. Mountain systems/ CT: CT receiving no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic-amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; NT: no-till system; SCP: spring chisel plow.

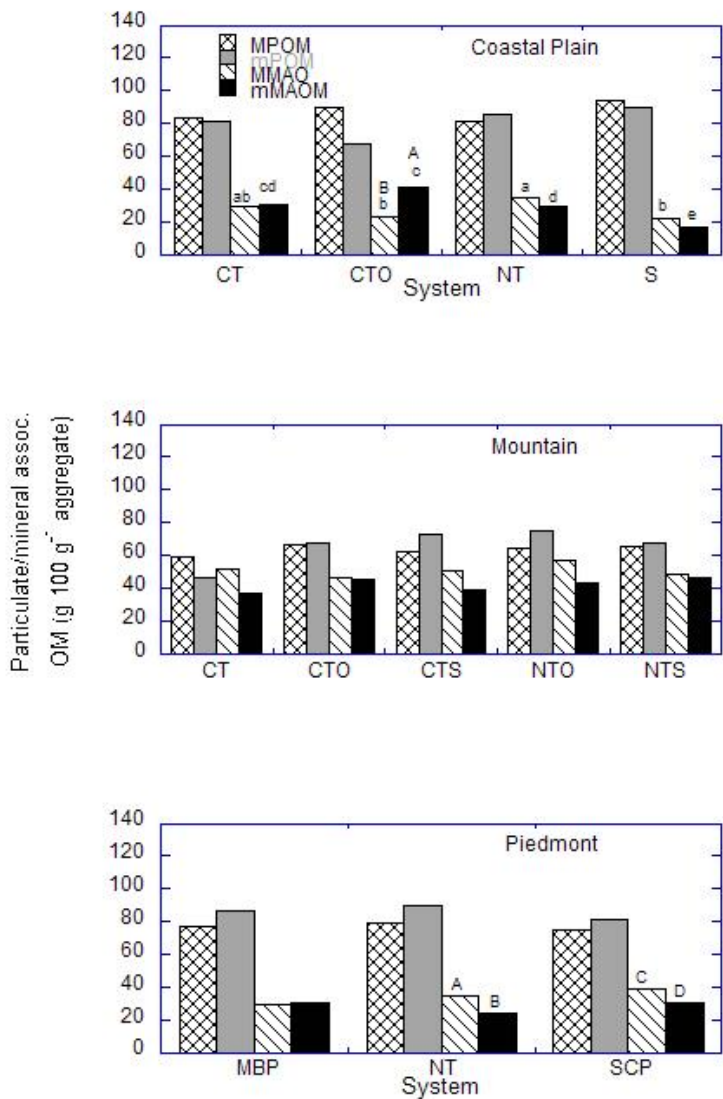


Fig. 3. Distribution of particulate and mineral associated organic matter. Columns with similar or no lower or upper case letters show no significant differences between tillage systems or between particle-size fractions, respectively ( $p < 0.05$ ). MPOM, mPOM: sand size particles within macro- and micro-aggregates, respectively; MMAOM, mMAOM: silt+clay size particles within macro- and micro-aggregates, respectively.

The sum of the proportions for each size fraction (e.g. MPOM + MMAOM, or mPOM + mMAOM) was slightly greater, or less, than 100% in some fractions. Such slight differences are likely due to variabilities in methodology and/or inherent soil properties.

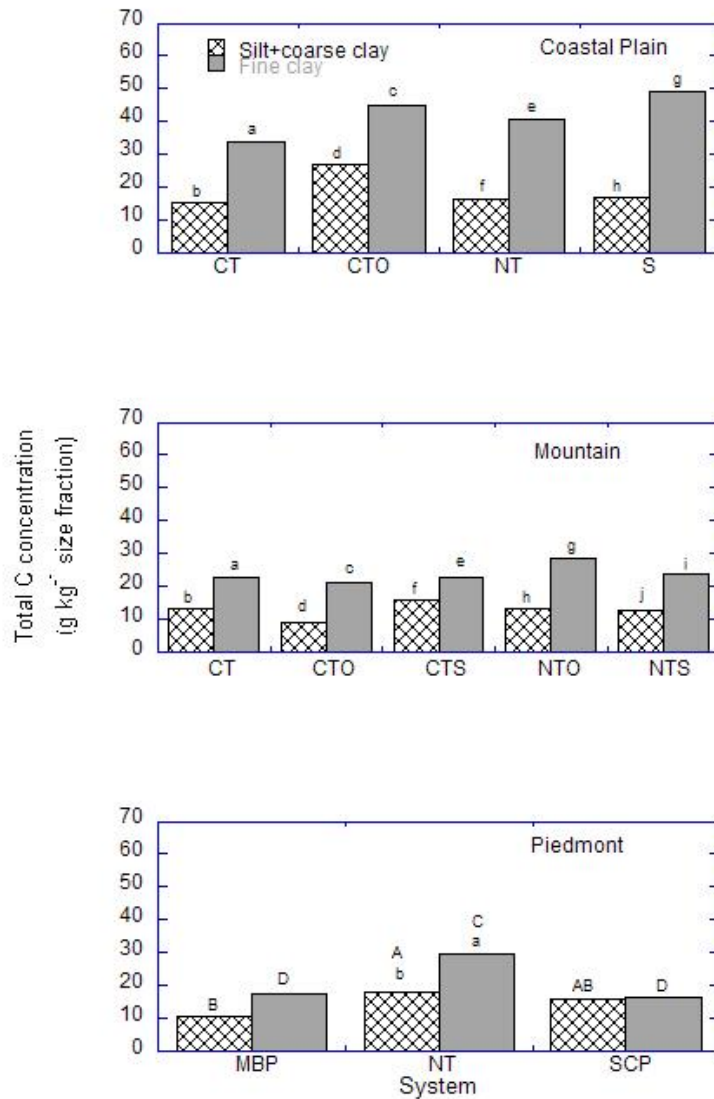


Fig. 4. Carbon concentrations in particle-size fractions in Coastal Plain, Mountain, and Piedmont locations. Columns with similar or no lower or uppercase letters indicate no significant differences between particle-size fractions or between tillage systems, respectively ( $p < 0.05$ ). Coastal Plain systems/ CT: Conventional tillage (CT); NT: no-till; CTO: organic-amended CT; S: successional system. Mountain systems/ CT: CT receiving no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic-amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; NT: no-till system; SCP: spring chisel plow.

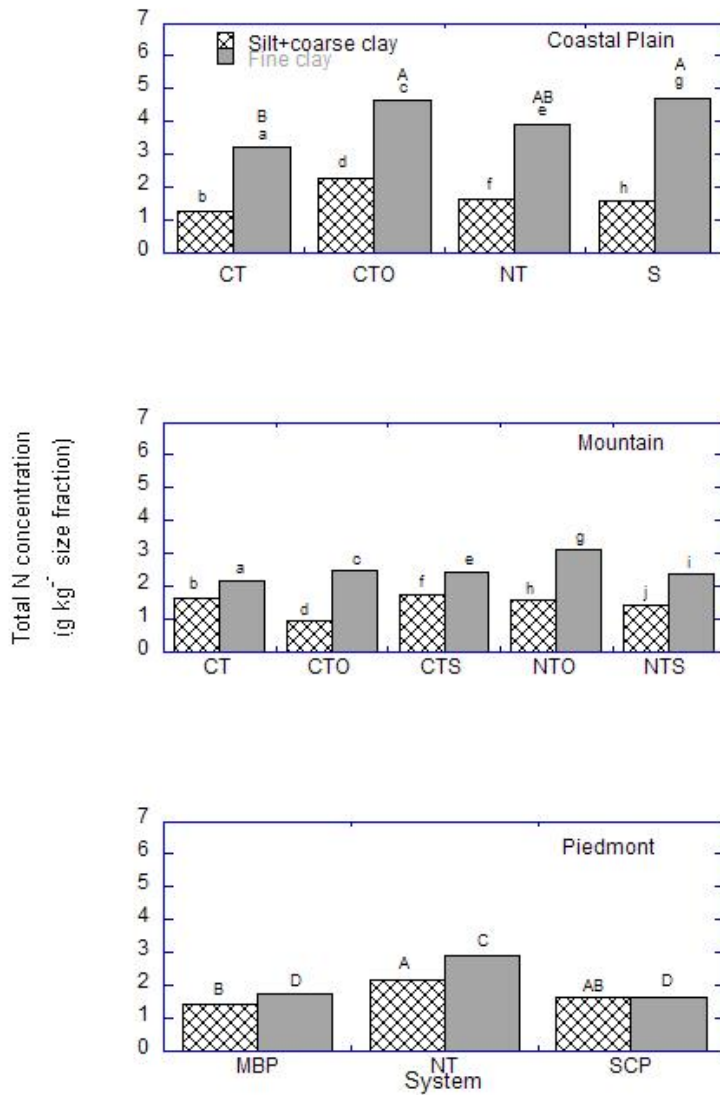


Fig. 5. Nitrogen concentrations in particle-size fractions in Coastal Plain, Mountain, and Piedmont locations. Columns with similar or no lower or uppercase letters indicate no significant differences between particle-size fractions or between tillage systems, respectively ( $p < 0.05$ ). Coastal Plain systems/ CT: Conventional tillage (CT); NT: no-till; CTO: organic-amended CT; S: successional system. Mountain systems/ CT: CT receiving no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic-amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; NT: no-till system; SCP: spring chisel plow.

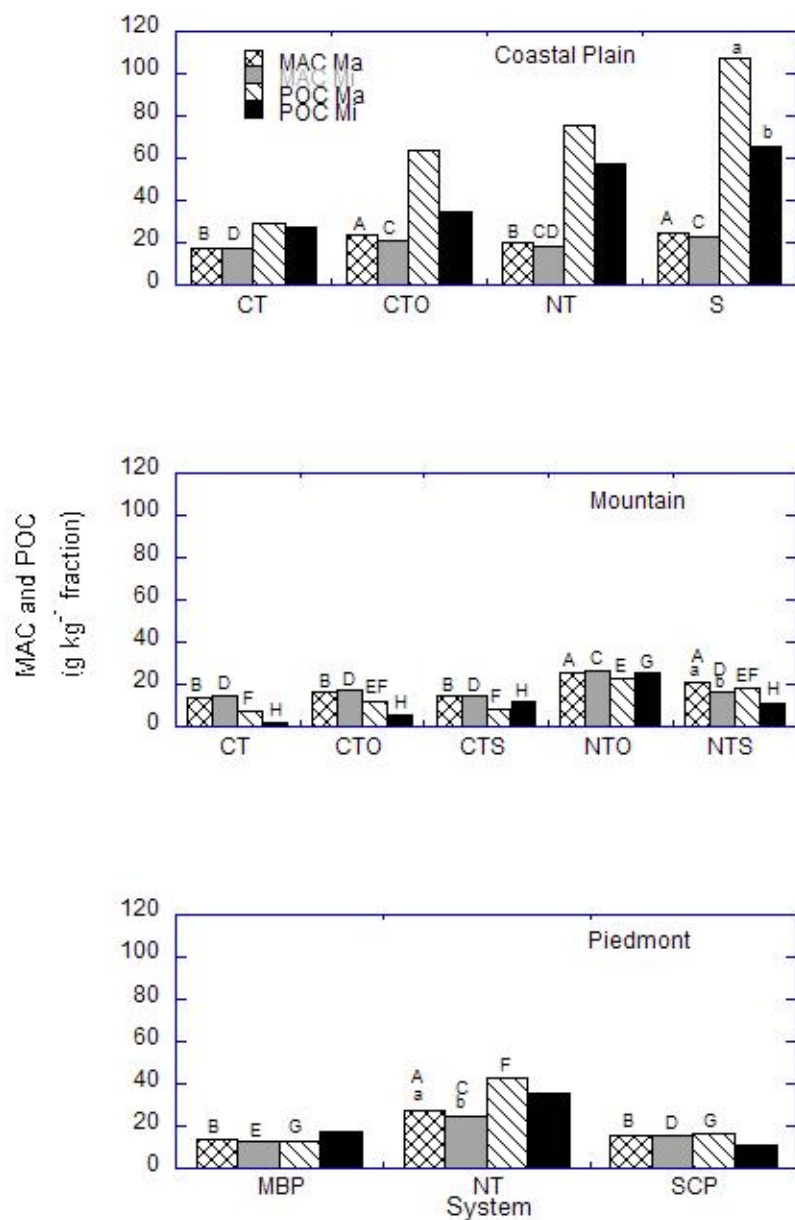


Fig. 6. C concentrations in silt+clay- (mineral associated C; MAC) and sand-sized (particulate organic C; POC) particles associated with macro- (Ma) and micro- (Mi) aggregates in Coastal Plain, Mountain, and Piedmont locations. Columns with similar or no upper/lowercase letters indicate no significant differences between tillage systems/particle-size fractions, respectively ( $p < 0.05$ ).

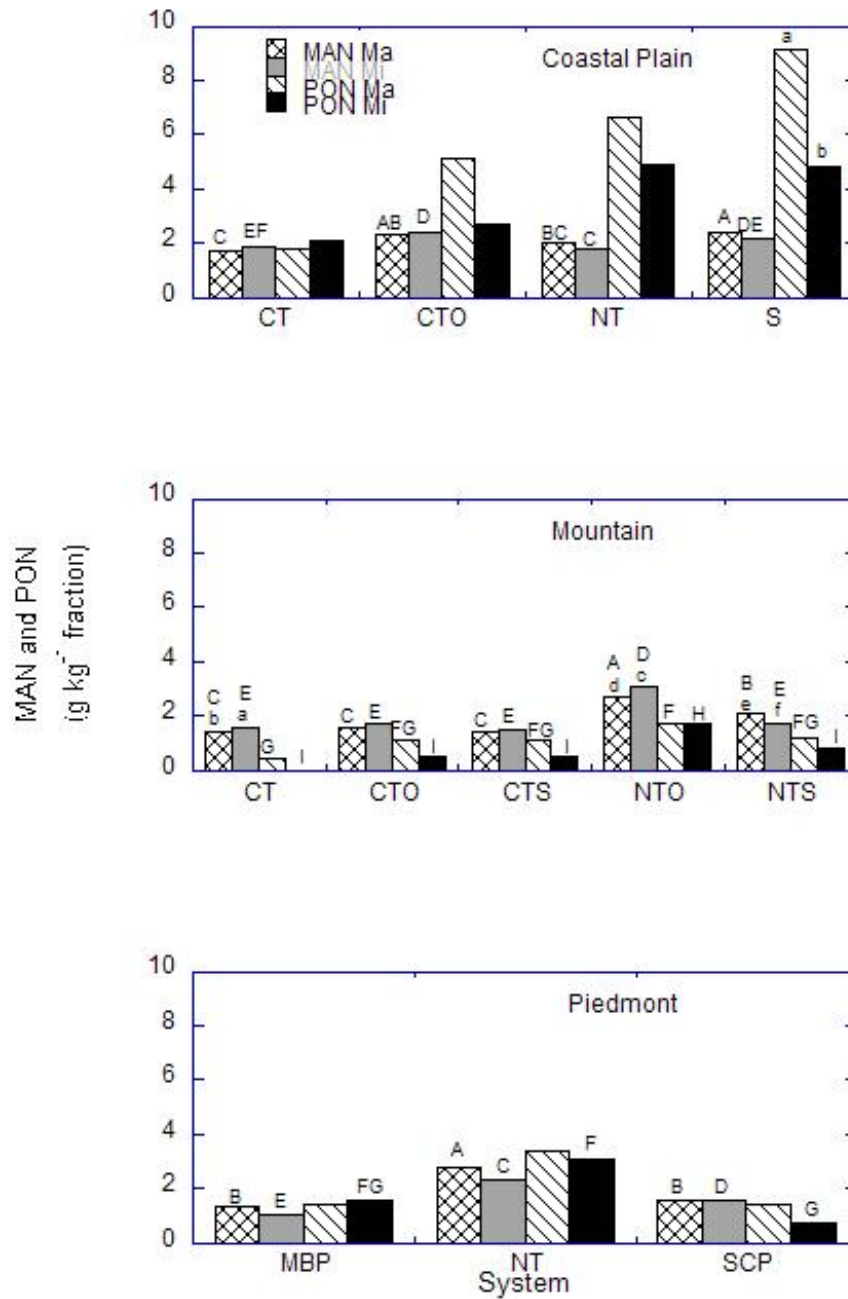


Fig. 7. N concentrations in silt+clay- (mineral associated N; MAN) and sand-sized (particulate organic N; PON) particles associated with macro- (Ma) and micro- (Mi) aggregates in Coastal Plain, Mountain, and Piedmont locations. Columns with similar or no upper/lowercase letters indicate no significant differences between tillage systems/particle-size fractions, respectively ( $p < 0.05$ ).

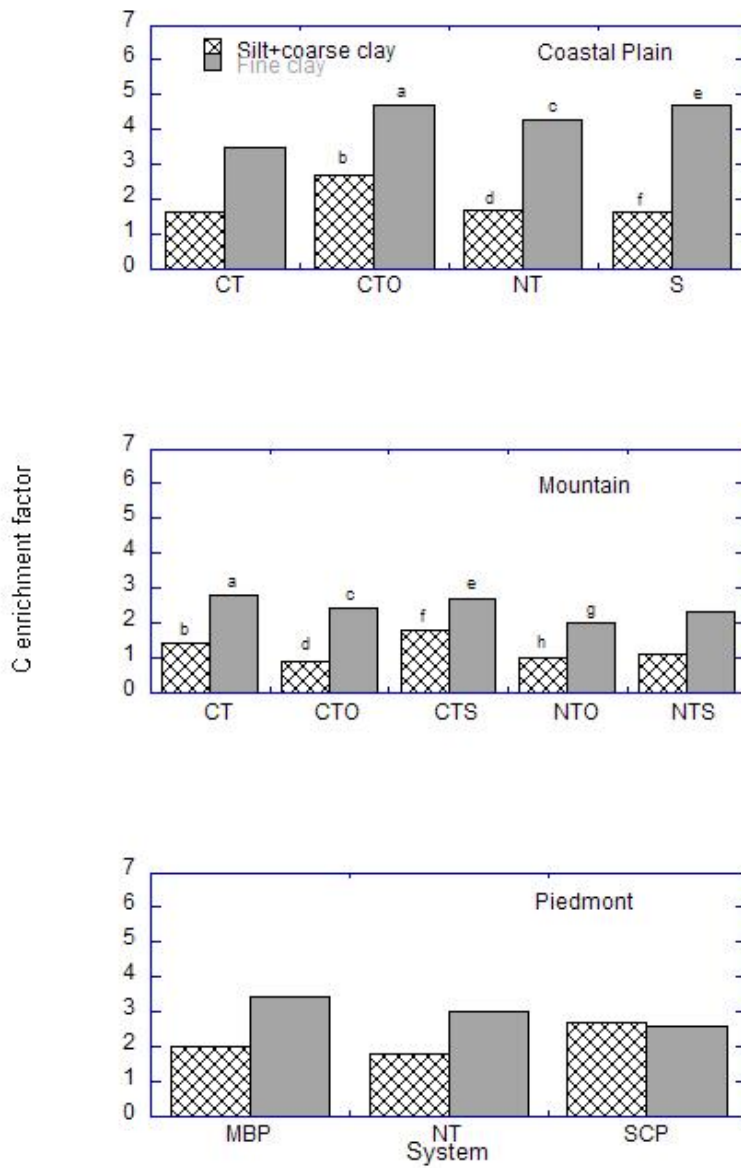


Fig. 8. Enrichment factors for C in Coastal Plain, Mountain, and Piedmont locations. Columns with similar or no letters indicate no significant differences between particle-size fractions ( $p < 0.05$ ). Coastal Plain systems/ CT: Conventional tillage (CT); NT: no-till; CTO: organic-amended CT; S: successional system. Mountain systems/ CT: CT receiving no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic-amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; NT: no-till system; SCP: spring chisel plow.

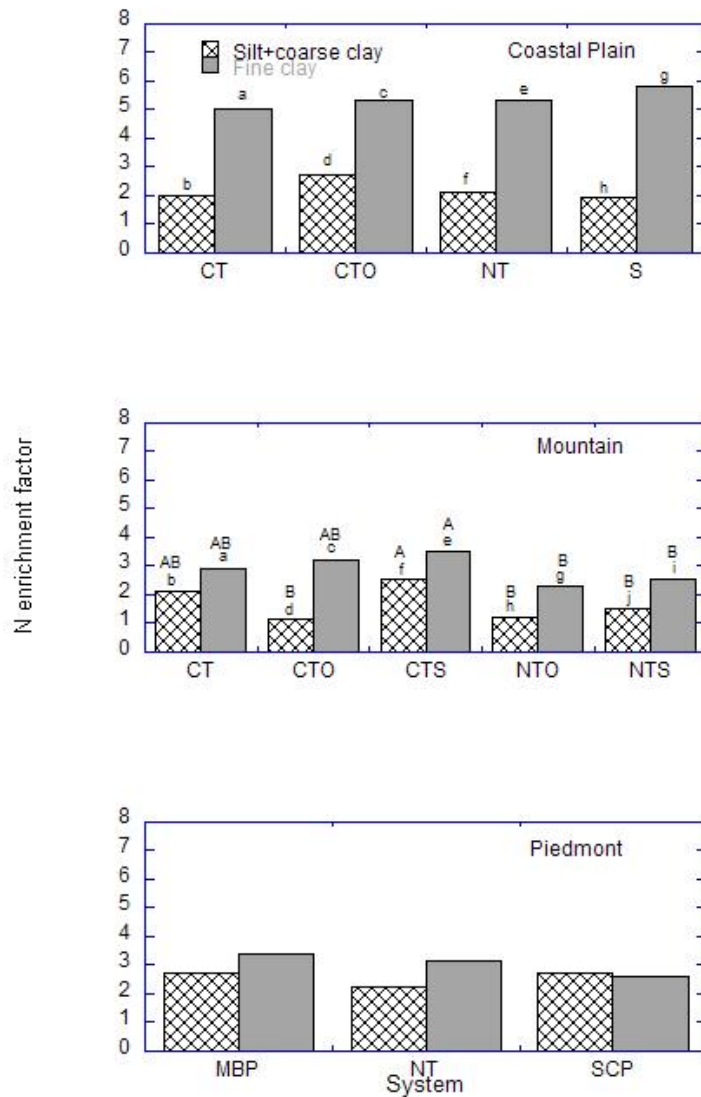


Fig. 9. Enrichment factors for N in Coastal Plain, Mountain, and Piedmont locations. Columns with similar or no uppercase letters indicate no significant differences between tillage systems; or with similar or no lowercase letters indicate significant differences between particle-size fractions ( $p < 0.05$ ). Coastal Plain systems/ CT: Conventional tillage (CT); NT: no-till; CTO: organic-amended CT; S: successional system. Mountain systems/ CT: CT receiving no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic-amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; NT: no-till system; SCP: spring chisel plow.

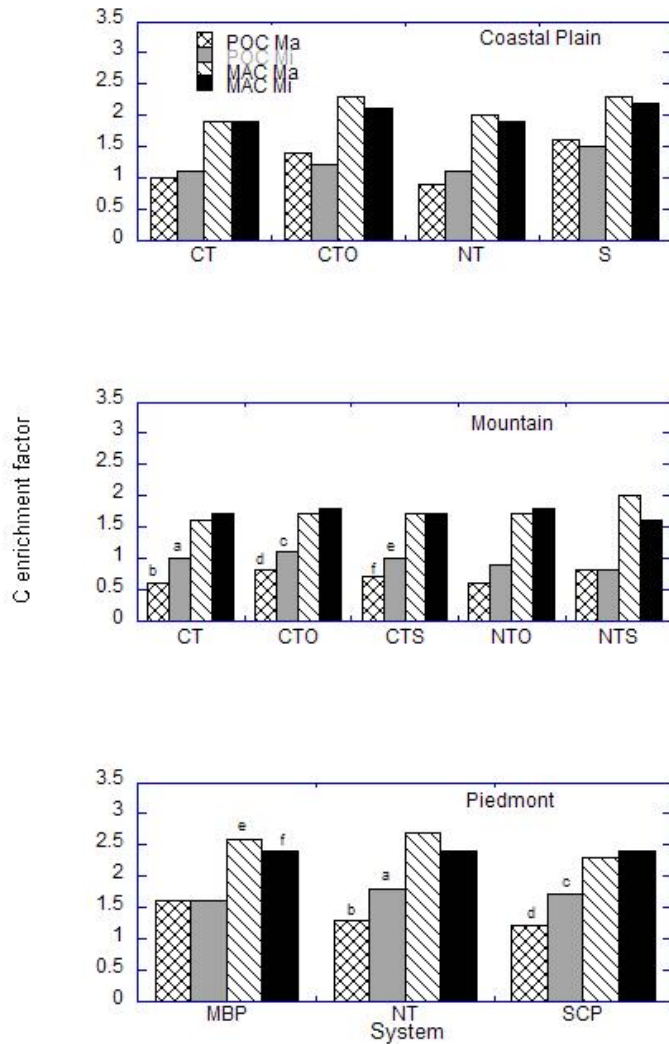


Fig. 10. Enrichment factors for C in particle size fractions contained in aggregates. Columns with similar or no upper/lower case letters indicate no significant differences between tillage systems/particle-size fractions, respectively ( $p < 0.05$ ). Coastal Plain systems/ CT: Conventional tillage (CT); NT: no-till; CTO: organic-amended CT; S: successional system. Mountain systems/ CT: CT receiving no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic-amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; NT: no-till system; SCP: spring chisel plow.

PON Ma/MAN Ma (or PON Ma/MAN Mi): mineral associated (or particulate organic) N in macro- and micro-aggregates, respectively.

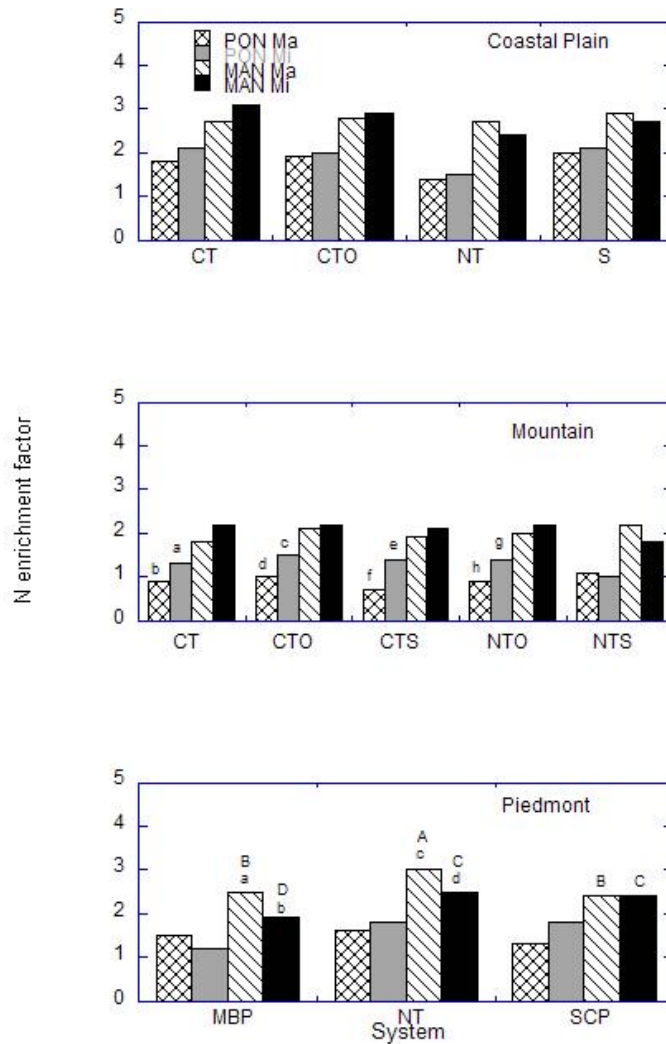


Fig. 11. Enrichment factors for N in particle size fractions contained in aggregates. Columns with similar or no upper/lower case letters indicate no significant differences between tillage systems/particle-size fractions, respectively ( $p < 0.05$ ). Coastal Plain systems/ CT: Conventional tillage (CT); NT: no-till; CTO: organic-amended CT; S: successional system. Mountain systems/ CT: CT receiving no inputs; CTO: organic-amended CT; CTS: synthetic-amended CT; NTO: organic-amended NT; NTS: synthetic-amended NT. Piedmont systems/ MBP: moldboard plow; NT: no-till system; SCP: spring chisel plow.

PON Ma/MAN Ma (or PON Ma/MAN Mi): mineral associated (or particulate organic) N in macro- and micro-aggregates, respectively.

Table 1. Tillage systems and selected chemical and physical properties for the 0 to 10 cm depth in soils studied.

System†	pH	CEC††	SOC	SON	Sand	Silt	Clay	Db
		cmol kg <sup>-1</sup>	..... g kg <sup>-1</sup> .....	..... g kg <sup>-1</sup> .....	..... % .....	Mg m <sup>-3</sup>		
<u>Coastal Plain</u>								
CTO	5.9	6.4	10.17	0.83	70.2	20.3	9.4	1.23
CT	6.1	5.6	9.47	0.67	67.3	23.6	9.1	1.30
NT	5.9	6.0	9.87	0.80	64.7	24.6	10.7	1.50
S	4.8	4.2	10.73	0.83	74.2	17.2	8.6	1.41
<u>Mountain</u>								
CTS	6.1	6.7	8.63	0.73	57.6	20.2	22.1	1.18
CTO	6.3	7.4	9.25	0.80	53.0	23.2	23.8	1.17
NTS	6.0	7.6	11.03	0.95	50.9	26.9	22.2	1.13
NTO	6.9	10.5	15.85	1.48	52.1	26.6	21.3	1.15
CT	5.9	6.9	8.43	0.78	60.8	19.8	19.4	1.32
<u>Piedmont</u>								
NT	5.9	6.0	10.60	0.98	56.9	26.1	17.1	1.41
MBP	5.4	4.6	5.20	0.53	50.7	26.9	22.4	1.31
SCP	6.1	5.9	6.68	0.65	58.1	18.3	23.6	1.25

†Coastal Plain systems/ CT, NT: conventional tillage and no-till practices, respectively; CTO: CT with organic amendments; S: successional vegetation re-growth. Mountain systems/ CT: CT with no inputs; CTO: organic-amended CT system; CTS: synthetic-amended CT system; NTO: organic-amended NT system; NTS: synthetic-amended NT system. Piedmont systems/ MBP: moldboard plow; SCP: spring chisel plow; NT: no-till.

††CEC: cation exchange capacity; SOC/N: soil organic C or N; Db: bulk density.

Table 2. Distribution of SOC and SON among aggregates and particle-size fractions.

System	% SOC contribution				% SON contribution					
	Ma†	Mi	S+CC	FC	Total	Ma	Mi	S+CC	FC	Total
<u>Coastal Plain</u>										
CT	47 b	17	19	4	87	50	23	23	6	101
CTO	60 ab	26	19	5	109	56	26	19	5	105
NT	63 a	19	15	4	100	64	21	19	5	108
S	51 ab	19	11	7	88	61	22	14	8	105
<u>Mountain</u>										
CT	51	27	17	7	102	42	26	24	7	100
CTO	67	19	6	6	98	66	19	8	8	101
CTS	60	37	18	8	123	68	44	25	10	147
NTO	55	10	6	2	73	55	12	7	2	77
NTS	53	38	8	6	105	52	38	10	7	107
<u>Piedmont</u>										
MBP	27	41	22	5	95	27	38	29	5	100
NT	63	24	12	1	100	60	22	15	1	99
SCP	41	32	28	4	105	42	29	26	4	101

†Ma: macroaggregates; Mi: microaggregates; S+CC: silt+coarse clay; FC: fine clay.

Note: The totals for each row (i.e. Ma + Mi + S+CC + FC) are slightly greater or less than 100% in the table above. These differences are likely due to variabilities in methodology and/or inherent soil properties.

Table 3. Main correlations among soil organic C and N with the C and N contents and proportions of SOC and SON contributed by particle-size fractions.

	SON	SCCC	FCC	SCCN	FCN	SCCprpn	FCprpn	SCCC/SOC	FCC/SOC	SCCN/SON	FCN/SON
<u>Coastal Plain</u>											
SOC	0.88***	0.09	- 0.09	0.24	- 0.11	0.24	0.27	0.04	-0.36	0.04	-0.44
SON		0.13	- 0.20	0.34	- 0.16	0.05	0.05	- 0.11	-0.42	- 0.20	-0.55
<u>Mountain</u>											
SOC	0.98***	0.58*	0.81***	0.49*	0.80***	- 0.56*	- 0.60**	- 0.45	-0.71***	- 0.51*	-0.72***
SON		0.47*	0.76***	0.41	0.77***	- 0.50*	- 0.58**	- 0.46	-0.70**	- 0.52*	-0.73***
<u>Piedmont</u>											
SOC	0.99***	0.25	0.51	0.50	0.48	- 0.50	- 0.26	- 0.57	-0.61*	- 0.67*	-0.48
SON		0.32	0.54	0.56	0.51	- 0.57	- 0.37	- 0.52	-0.68*	- 0.66*	-0.58

\*, \*\*, \*\*\*: Significant correlations at p = 0.05, 0.01, and 0.001, respectively.

SOC, SON: soil organic C and N, respectively; SCCC (or SCCN), FCC (or FCN): C (or N) associated with silt+coarse clay and fine clay fractions, respectively; SCCprpn, FCprpn: proportion of silt+coarse clay and fine clay particle-size fractions in mass of silt and clay material (outside aggregates) fractionated, respectively; SCCC/SOC, FCC/SOC, SCCN/SON, FCN/SON: proportions of SOC (or SON) contributed by C (or N) associated with silt+coarse clay and fine clay fractions, respectively.

Table 4. C/N ratios for organic matter associated with particle-size fractions within and outside aggregates from the 0 to 10 cm soil depth at three locations.

System†	SCC††	FC	POM <sub>Ma</sub>	POM <sub>Mi</sub>	MAOM <sub>Ma</sub>	MAOM <sub>Mi</sub>
<u>Coastal Plain</u>						
CT	12	10	18	13	10 aA	9 bB
NT	10	10	13	13	10 ab	10 ab
CTO	12	10	12	6	10 aA	8 bB
S	11	10	11	21	10 ab	10 a
<u>Mountain</u>						
CT	8B	10 A	30 ab	-	9 ab	9 a
CTO	9	9	15 ab	6	10 ab	9 a
CTS	8	9	7 b	10	10 a	10 a
NTO	8	9	13 ab	15	9 b	8 b
NTS	9	10	19 ab	16	10 ab	10 a
<u>Piedmont</u>						
MBP	7B	10 A	9	1	10 aB	12 aA
SCP	9	10	11	29	9 b	10 c
NT	8 B	10 A	13	12	10 a	11 b

†Coastal Plain systems/ CT, NT: conventional tillage and no-till practices, respectively; CTO: CT with organic amendments; S: successional vegetation re-growth. Mountain systems/ CT: CT with no inputs; CTO: organic-amended CT system; CTS: synthetic-amended CT system; NTO: organic- amended NT system; NTS: synthetic-amended NT system. Piedmont systems/ MBP: moldboard plow; SCP: spring chisel plow; NT: no-till.

††SCC: silt+coarse clay fraction; FC: fine clay fraction; MAOM<sub>Ma</sub>, MAOM<sub>Mi</sub>/ POM<sub>Ma</sub>, POM<sub>Mi</sub>: mineral associated (or particulate) organic matter in macro-/micro-aggregates, respectively. Values with similar or no lower- or uppercase letters indicate no significant differences between tillage systems (under columns) or between particle-size fractions (across rows) ( $p < 0.05$ ).

## CHAPTER IV

### MULTIVARIATE ANALYSIS OF SOIL FACTORS INFLUENCING AGGREGATE STABILITY AND ORGANIC CARBON CONTENT IN DIVERSE AGROECOSYSTEMS

#### Abstract

Many factors control water stable aggregation (WSA) and soil organic matter content (SOM) in diverse agroecosystems. This study used principal component analysis (PCA) to assess soil factors affecting WSA and SOM in the 0 to 10 cm depth in a Wickham sandy loam (fine-loamy, mixed, semi active, thermic Typic Hapludult), Delanco fine-sandy loam (fine-loamy, mixed, mesic, Aquic Hapludult), and Wedowee sandy clay loam (fine, kaolinitic, thermic Typic Kanhapludult); representing North Carolina Coastal Plain, Mountain, and Piedmont locations, respectively. Variables defining principal components (PCs) were used in multiple linear regressions (MLR) to predict SOC and MWD, an index of WSA. Four and three PCs together explained 82 and 70% of total variations among 11 variables examined in PCAs on MWD and SOC, respectively. Significant relationships existed between MWD and non-crystalline Fe (hydr)oxides, hot water extractable C (HWEC), and SOC and bulk density in the Coastal Plain, Mountain, and Piedmont, respectively; whereas SOC related with clay in the Coastal Plain, humic matter in the Piedmont, and HWEC in all locations. The HWEC, which measures potentially bioavailable C, was 1.6 and 1.4x greater in the Coastal Plain than Mountain and Piedmont, respectively; and MWD was 1.3 and 1.4x

greater in the Coastal Plain and Mountain, respectively, than Piedmont. The SOC ranged from 10.1 to 13.7 Mg ha<sup>-1</sup> but did not differ among locations. These results indicate that organic amendments can enhance MWD and SOC in these soils; and illustrate the usefulness of PCs in estimating WSA and SOC in diverse agroecosystems.

## Introduction

Soil aggregation and SOM content affect vital properties and functions of the soil. Aggregates protect SOM through physical protection and chemical and biochemical stabilization (Christensen, 1996). Soil organic matter influences nutrient cycling, water retention, C sequestration, energy availability to soil microorganisms, and environmental quality maintenance (Mitchell et al., 2000; Olk and Gregorich, 2006). Soil properties such as bulk density, cation exchange capacity (CEC), aggregate stability, and biological activity (Mitchell et al., 2000) are also influenced by SOM. The interaction of SOM with soil mineral components is a determining factor of aggregation and other properties (Ellert and Bettany, 1995). Chemical stabilization results from chemical or physicochemical binding between SOM and soil minerals. Key factors in chemical stabilization of organic C and N include clay content and type (Feller and Beare, 1997). Physical protection occurs when aggregates form physical barriers between microbes and enzymes and their substrates, and thus control food web interactions and microbial turnover (Elliott and Coleman, 1988). Soil organic matter is protected in free microaggregates (< 50  $\mu\text{m}$ ) (Balesdent et al., 2000) and in microaggregates within macroaggregates (Six et al., 2000b). Thus formation of microaggregates within macroaggregates and, therefore C stabilization within these microaggregates, is reduced by rapid turnover of macroaggregates (Six et al., 2000b). Biochemical stabilization is due to the chemical composition of soil organic materials (e.g. recalcitrant compounds such as lignin and polyphenols) and occurs through processes such

as condensation and complexation of decomposition residues in soil. Thus the inherent or acquired resistance to biochemical decomposition of this pool of SOM makes it stable (Six et al., 2002).

Many studies (Chaney and Swift, 1984; Albiach et al., 2001; Duiker et al., 2003) have reported significant relationships between aggregation and SOM, while other authors (Retzer & Russell, 1941; Guidi et al., 1988) found no relationships. Other factors that have been reported to influence aggregation and SOM content include clay content (Horn et al., 1994; Kay, 1997), aluminum (Al) and iron (Fe) (hydr)oxides contents (Despande et al., 1968; Arias et al., 1996; Duiker et al., 2003; Igwe and Stahr, 2004; Barthès et al., 2008), soil bulk density (Idowu, 2003; Celik, 2005), exchangeable ions, clay mineralogy, and soil profile depth (Kay, 1997). Soil texture and structure (Lal, 2004) are the main soil factors affecting SOM dynamics. Climate and management factors affect both aggregate stability and SOC content (Kay, 1997; Lal, 2004).

Different investigators (Ovalles and Collins, 1988; Nwadialo and Mbagwu, 1991; Mbagwu et al., 1994; Barthès et al., 2008) have used principal component analysis (PCA), a multivariate analysis technique used to reduce large amounts of variables to a few major components, to study relationships among variables, including aggregate stability. Using this technique, the few major components retained after conducting the analysis are uncorrelated and generally account for the bulk of the variation among the variables examined. Knowledge of the few principal components can be used, without loss of vital information and in place of the many properties, since they account for the bulk of the total

variation among the many properties. Information about the application of PCA to describe the principal soil components that account for the bulk of the variations in soil aggregate stability and SOM content for soils of the southeastern US coastal plain, mountains, and piedmont regions is not common. Such information is, however, useful for approaching management-related issues of ecosystem sustainability, soil quality and productivity, and biogeochemical cycles for C and related nutrients in these soils.

The objective of this study was to evaluate soil factors that characterize soil aggregate stability and SOM in diverse agroecosystems in the three physiographic regions studied, using the PCA technique. The model of aggregate formation proposed by Edwards and Bremner (1967) suggested that polyvalent cation bridges bind clay to clay (clay-pc-clay), organic matter to organic matter (OM-pc-OM), or clay to organic matter (clay-pc-OM) complexes to form organo-mineral micro aggregates. Since organically-bound Al and Fe (hydr)oxides are extracted along with non-crystalline Al and Fe (hydr)oxides (Jackson et al., 1986; Skjemstad et al., 1989), it was hypothesized in this study that non-crystalline (hydr)oxides of Al and Fe were contributing factors to soil aggregation and SOM stabilization in the soils studied.

## **Materials and Methods**

### **Soil sampling and analysis**

This study was conducted in experimental plots at the Farming Systems Unit of the

Center for Environmental Farming Systems (CEFS) near Goldsboro in Wayne County (Coastal Plain); the Mountain Horticultural Crops Research Station (MHCRS) near Mills River in Henderson County (Mountain); and the Upper Piedmont Research Station (UPRS) near Reidsville in Rockingham County (Piedmont) in the state of North Carolina. The experimental plots at the CEFS Farming Systems Unit, MHCRS, and UPRS were initiated in 1999, 1995, and 1984, respectively. Soil samples were collected from the 0 to 10 cm depth of diverse tillage systems representing these locations. Three samples were taken from either previously geo-referenced points (at CEFS) or around 3-6 randomly assigned and demarcated sampling points selected within defined landscape positions in each treatment replication (in the experimental plots at MHCRS and UPRS). The experimental design studied at the CEFS site consisted of four treatments in three replications: NT (no-till with Best Management Practices (BMP)); CT (conventional tillage with BMP); Org (organic farming system under CT); and S (succession or forest re-growth system). At MHCRS, there were four replications of five treatments: CTS (conventional tillage with synthetic amendments); CTO (conventional tillage with organic amendments); NTS (no-till with synthetic amendments); NTO (no-till with organic amendments); and CT (conventional tillage with no inputs; neither synthetic nor organic amendments). However, the CTO treatment was not included in this study. At UPRS, three treatments in four replications were studied: NT (no-till), SCP (spring chisel plow), and MBP (moldboard plow).

Aggregate stability was measured on moist soil samples using the method of Yoder

(1936) as modified by Haynes (1993). Briefly, four nests of three 12.5-cm diameter sieves, each nest consisting of a No. 10 sieve (2-mm diameter), No. 60 (0.250-mm diameter), and No. 270 (0.053-mm diameter) were mounted on a shaking device, with the largest sieve on top and the smallest at the bottom. Thirty-five g samples (oven dry equivalent) of field-moist soil (4-8mm aggregates size) previously stored at 4°C were placed on the largest size of each nest of sieves. After small roots and other obvious plant residues were removed, each nest of sieves was slowly lowered into distilled water in the Plexiglas cylinder, making necessary adjustments to the water volume in the cylinders, so that soil samples on the topmost sieves were just covered with water when the lift mechanism on the sieving device was at the highest point of oscillation. The device was left in this position for 5 min to allow pre-wetting. Then the samples were sieved by a raising and lowering action of samples in and out of the water through a distance of about 3.5 cm at a rate of 34 oscillations per min for 15 min, after which the device was stopped and the sieves raised out of the water to allow draining to occur. The contents remaining on each sieve were backwashed separately into small, aluminum foil trays and poured into labeled, pre-weighed, wide-mouth 120-mL plastic sample cups. The samples were separately weighed and freeze dried. The percentage of soil remaining on each sieve was calculated after excluding the fraction > 4 mm. The respective values obtained were designated as the water stable aggregate size distribution, in g aggregate size class per kg bulk soil, between 2.0 to 4.0, 0.250 to 2.0, and 0.053 to 0.250 mm. Aggregate stability was expressed as mean weight diameter (MWD),

which values were calculated as follows:

$$\text{MWD} = \sum(\text{proportion of sample retained on sieve} \times \text{size class midpoint or mean diameter of the size fraction})$$

The size class midpoints (mean intersieve diameters) were calculated as follows:  $((4.0+2.0)/2)$ ,  $((2+0.250)/2)$ ,  $((0.250+0.053)/2)$ , and  $((0.053+0)/2)$ , or 3.0, 1.125, 0.152, and 0.027 mm, respectively. Mean weight diameter was used to express aggregate stability because of its high degree of sensitivity (Conaway and Edward Strickling, 1962) and because of the relative ease of using a single value in comparing different soils and management practices.

Oven dry equivalent masses of air-dry whole soil and of freeze-dried samples of aggregate size materials were finely ground and their respective total C concentrations were determined by direct combustion in a Perkin–Elmer 2400 CHN analyzer. To obtain sand-free aggregate materials, eliminate the influence of texture and enhance across-region comparisons on aggregates, samples of aggregates were dispersed in 0.5% (w/v) sodium hexametaphosphate (SMP). Sand corrected weights of aggregates were calculated according to Wick et al. (2009) using the following relationship:

$$\text{Sand corrected weight} = \text{aggregate weight} - ((\text{weight of sand} \div \text{weight of SMP-dispersed soil sample}) \times \text{aggregate weight})$$

Sand-free C in aggregate size classes were calculated according to Denef et al. (2001) using the relationship:

Sand-free C concentration in fraction = C concentration in fraction/(1 – sand proportion in fraction).

Aluminum (Al) and iron (Fe) (hydr)oxides were determined using the citrate-ascorbate (CA) method of Reyes and Torres (1997) as described by Six et al. (2000b); and free Al and Fe were determined using the dithionite/citrate extraction method according to Blakenmore et al. (1987). The acid ammonium oxalate (AOD) method is effective for extracting non-crystalline as well as organically-bound Fe and Al (hydr)oxides (Jackson et al., 1986; Skjemstad et al., 1989) and has, therefore, been used in most studies examining these forms of Fe and Al in soil (Six et al., 2000b). The citrate-ascorbate (CA) method extracts similar amounts of non-crystalline components extracted by the AOD method but is a simpler method, has higher selectivity for these components, and does not contain toxic chemicals (Reyes and Torrent, 1997; Six et al., 2000). Hence it was used in this study. Dithionite/citrate extractable Fe and Al (hydr)oxides contain all (whether crystalline, poorly crystalline or organically bound) (hydr)oxides of Fe and Al (Skjemstad et al., 1989). To determine noncrystalline Fe and Al (hydr)oxides in bulk soil samples, 40 mL of pre-mixed 0.2 M sodium citrate–0.05 M sodium ascorbate solution (pH 6) was added to each of 200 mg (oven-dry weight) air-dry soil samples previously weighed into 50-mL polypropylene centrifuge tubes. The contents were placed on a gyrating shaker and shaken at 200 rpm. After 16 h shaking, the suspensions were centrifuged at about 26900 relative centrifugal force (rcf) for 15 min and the supernatant was decanted and analyzed for noncrystalline Fe

(noncrys-Fe) and Al (noncrys-Al) by inductively coupled plasma spectrometry. One gram sodium dithionite and 40 mL of 0.75 M sodium citrate were added to the residues in the tubes. The tubes were again shaken for 16 h and the suspensions centrifuged at 26900 rcf for 15 min. The supernatants were decanted and analyzed for “free” Fe (free-Fe) and Al (free-Al) (Blakenmore et al., 1987). The methods of Ghani et al. (2003) and Chan and Heenan (1999) were used to determine hot water extractable C (HWEC). Soils in this study were generally at pH 6.5 or less and low in carbonates. Therefore, total C was assumed equal to organic C.

Particle size distribution in the samples was determined according to the method Gee and Bauder (1986). Determination of CEC soil pH, and exchangeable bases in bulk soil were performed by the North Carolina Department of Agriculture and Consumer Services (NCDA&CS). At NCDA&CS, CEC (expressed as milliequivalents per 100 g soil; meq 100 g<sup>-1</sup>) is reported as the summation of extractable K, Ca, Mg, Na, and acidity. A buffer procedure is used to directly determine the meq of extractable acidity; while an extraction procedure is used to determine the meq of K, Ca, Na, and Mg (Mehlich, 1978). The chloroform fumigation extraction method, according to Vance et al. (1987), was used to determine soil microbial biomass C (BMC) on moist bulk soil samples without the subtraction of controls. Field moist samples (aggregates or bulk soil) that have been previously stored at 4°C were used in the extraction for MBC. Table 1 describes the tillage systems sampled and selected physical and chemical properties for samples from each study location. Soil properties were

separately determined on samples collected from different tillage systems in the three locations studied.

### **Principal component and statistical analyses**

Basically, PCA is an exploratory technique which returns new variables, called principal components (PCs) or axes, summarizing the information contained in a set of variables that were originally entered for analysis. The new variables will be fewer than the initial set of variables and will be linear combinations of the initial variables. In this study, two separate PCAs were performed: one for SOC and the other for MWD. There are 10 variables for each PCA: bulk density (Db), microbial biomass carbon (MBC), non-crystalline aluminum (ncrys\_Al), non-crystalline iron (ncrys\_Fe), humic matter (HM), hot water extractable carbon (HWEC), cation exchange capacity (CEC), soil pH, percent sand content (Sand), and percent clay content (Clay). However, MWD was added to these 10 variables, so that there are 11 variables in the PCA for SOC. Similarly, SOC was included in the PCA for MWD, making 11 variables. The intention was to perform a PCA on 11 variables (a combination of physical, chemical, and microbiological soil properties) to determine whether a smaller number of variables (the PCs) can adequately account for most of the variation among the initial 11 variables. The resulting PCs will then be used as the predictor variables (to predict MWD or SOC) in the respective multiple regression analyses. All of these factors are reported elsewhere in the literature to be involved in WSA and/or C

sequestration.

The SAS software was used in the application of the PCA procedure in this study. Since PCA is a variable reduction procedure, a correlation matrix was first developed to identify all variables that were significantly correlated and, therefore, possibly measuring the same construct and introducing some redundancy in those variables. The second step involved the computation of Eigen values, which are the variances of the principal components (PC). Of the 11 variables examined in this study, the first few components that were thought to meaningfully explain most of the variation among the variables examined were retained for interpretation and for use in subsequent regression analysis. The Mineigen or Kaiser criterion (Kaiser, 1960), wherein the number of components with Eigen values  $> 1$  is used to explain correlations among the variables examined, was used to determine the number of components to retain in the PCA. According to the principles of PCA, the first PC will explain most of the variation among the variables examined. The second PC will explain the majority of the variations that the first PC did not explain. Similarly, subsequent PCs will explain the majority of the variations that previous PCs did not explain. Subsequent PCs will not correlate with previous PCs but each PC may correlate with variables with which other PCs do not correlate. Thirdly, PC loadings (or eigen vectors), which are correlations of the measured variables and the PCs, are produced in a new correlation matrix. Varimax rotation was then applied to the PC loadings so as to make the PC loadings orthogonal or uncorrelated. Varimax rotation enhances PC loadings on

variables; e.g. can maximize PC loadings that are already high and lowers those that are already low (Hali and Rao, 1992). In this way, Varimax rotation can make interpretation of the PCA easier. Considering the general guidelines for significance of component loadings as outlined in Hair et al. (1987) regarding the ratio of observations to variables (about 3:1 in this study), an arbitrary cut-off point of 0.50 was used as criterion for declaring significance of PC loadings for interpretation. That is to say that a PC loading was considered as significant if its absolute value was  $\geq 0.50$ . Use of cut-off values are also discussed in Stevens (1986) and Peres-Neto et al. (2003). Further, any variable on which more than one PC loading occurred following the imposition of the 0.50 cut-off criterion was not included in the interpretation of the PC loadings, since such variables will not give a “pure” measurement of any single component (O'Rourke et al., 2005). Finally, all “surviving” or remaining variables with high loadings on the respective PCs were reviewed in order to determine common relationships between these variables or what each of the retained components measured. This step involved identification of the variables with high loadings for each component, and determination of underlying or common relationships between these variables.

Using the SAS software (Proc Reg), multiple linear regression equations were developed, via stepwise regression, in order to obtain quantitative relationships between MWD (or SOC) and variables on which the PCs loaded (referred to in this study as the component defining variables or CDVs); in order to identify which variables can adequately

predict MWD or SOC. The CDVs were used as independent variables while MWD and SOC were dependent variables. The Proc Mixed procedure was used to determine overall location effects on the CDVs. Finally, Pearson moments correlation coefficients between the component defining variables and SOC and MWD were determined according to location. In this study, MWD was used to express water stable aggregation and SOC to characterize soil organic C concentration.

### **Results and Discussion**

Location effects on SOC and MWD, the two variables on which the PCAs were run, are shown in Fig. 1. The figure shows that SOC did not differ among locations but that MWD was significantly greater in the Coastal Plain and Mountain than the Piedmont.

The correlation matrices of the PCAs for MWD and SOC (Table 2) show variables with significant correlations in each PCA. Since PCA is a variable reduction procedure, several pairs or sets of these significantly correlated variables are possibly measuring the same constructs and are, therefore, introducing redundancies in the full set of variables. For instance, in the correlation matrix of the PCA on MWD, Db showed positive correlations with HM, HWEC, and sand; and positive correlations with CEC, pH, clay, and non-crystalline Fe (hydr)oxides. Soil organic C showed positive correlations with HM, HWEC, and CEC; while MBC showed no significant correlations with any other variable in the correlation matrix. In the correlation matrix of the PCA on SOC, non-crystalline Al and Fe (hydr)oxides showed

significant positive correlations with each other and with clay content; while Fe (hydr)oxides showed significant negative correlation with sand content. There were significant positive correlations between MWD and HWECE and between humic matter (HM) and Db. Soil pH was negatively correlated with Db but positively correlated with CEC and non-crystalline Fe (hydr)oxides. Microbial biomass C did not significantly correlate with any other variable in the correlation matrix. The final results of the PCAs will, therefore, be expected to remove the redundancies among the complete sets of variables in both PCAs and produce fewer variables in each case.

Based on the Mineigen criterion, four orthogonal PCs explained the variations among the 11 soil variables examined in the PCA for MWD. The first, second, third, and fourth PCs respectively explained 38, 21, 13, and 10% of the total variation among the variables. Together, the four PCs explained 82% of the total variation (Table 3). In the PCA for SOC, three orthogonal PCs together explained 70% of the total variation among the 11 soil variables examined. The first PC explained 39% of the total variance, the second 20%, and the third 11%. Thus only the first four and three PCs were retained for interpretation of the results of the PCAs on MWD and SOC, respectively.

Principal component loadings on variables after Varimax rotation are shown in Table 4. Principal component loadings are correlations between PCs and the variables that load on them; and they show the relative importance of each examined variable within a PC. Following the criteria for declaring significance of PC loadings (namely, that a PC loading

was considered significant if its absolute value was  $\geq 0.50$ ; and that any variable on which more than one PC loading occurred following the imposition of the 0.50 cut-off criterion was not included in the interpretation of the PC loadings), the underlined PC loadings in Table 4 were declared as significant. In the PCA on MWD, the remaining or “surviving” variables that made up the significant PC loadings under the first PC were CEC, pH, sand, and Db. The other surviving variables were non-crystalline Al and Fe (hydr)oxides under the second PC; SOC and HWEC under the third; and MBC under the fourth. In the PCA on SOC, the “surviving” variables were clay, sand, and non-crystalline Al and Fe (hydr)oxides under the first PC; MWD, HWEC, and MBC under the second; and HM, Db, and pH under the third. Examination of these surviving variables, also called in this study the PC defining variables (PCDVs) suggests that the “surviving” variables under each PC in the two PCAs are related to MWD and SOC (Table 4).

Mbagwu et al. (1994) used the variables with the highest PC loadings to interpret the results of a PCA performed on MWD in some Italian soils. The authors suggested that these variables would provide the best relationship between MWD and the initial set of variables examined in the PCA. Applying the concept to the present study (Table 4), it is observed that CEC, non-crystalline Al, SOC, and MBC control MWD; while non-crystalline Al, MWD, and HM control SOC across the locations studied. However, biplots are also a useful tool in PCA and since they allow for visual examination of patterns in data matrices (Gabriel, 1971), they can provide more information as well as permit comparison of patterns in

different locations. To enhance the interpretation of the results of the PCAs in the present study using biplots, MWD and SOC values have been added as references to the biplots, resulting in three-dimensional plots (Figs. 2 and 3). Each biplot consists of a vector for each row and a vector for each column in each matrix of PC loadings (Gabriel, 1971). Examination of the biplot presented in Figs. 2 and 3 suggest the presence of some clustering or patterns in the PCs.

### **Interpretation of PCs based on values for MWD**

In the biplot with MWD as reference (Fig. 2), there seems to be a general change in direction or “slope” of the points (each representing positions or coordinates for PC1, PC2, and MWD) from positive or high to negative or low values along PC1. Greater values of MWD in the no-till system with organic amendments (NTO), in the Mountain location seem to occur in a band at the furthestmost end (where PC1 = 2.12) and on the positive portion of the PC1 axis (Fig. 1). Values of MWD at these points range from about 1.06 to more than 1.38 Mg ha<sup>-1</sup>. This band is followed by the band of points from the no-till system with synthetic amendments (NTS); this next band occurs around the point where PC1 = 0.78. The corresponding values for MWD at these points range from about 1.04 to 1.06 Mg ha<sup>-1</sup>. The last band of points is for those from the conventional tillage systems with synthetic amendments (CTS) and with no inputs (CT). This band occurs almost at the positive-negative boundary and into the negative portion of PC1. With regard to PC2, practices in the

Mountain location generally tend to occur on the positive side.

The patterns observed in tillage systems in the Mountain location in the biplot suggest that among the variables defining PC1 (CEC, pH, sand, and Db), some have positive, while others have negative, impacts on MWD; whereas both variables defining PC2 (non-crystalline Al and Fe (hydr)oxides) have positive influences on MWD. The CEC and pH were both significantly greater in the Mountain than either Coastal Plain or Piedmont location (Table 5). Levy and Miller (1997) studied the aggregate stability in the 0- to 10 cm depth of soils from the Piedmont, Mountain, and Coastal Plain regions of Georgia; and related stability in these soils to the high CEC of “2:1 type clay impurities” which have been reported to favor aggregation. The CEC in the soils in the authors’ study ranged from 1.9 to 11.3  $\text{cmol}_c \text{ kg}^{-1}$ . The ranges of CEC values for the soils in the present were 4.2 to 6.4, 6.9 to 10.5, and 4.6 to 6.1 for the Coastal Plain, Mountain, and Piedmont, respectively (Table 1); and seem to be of similar range to that of Levy and Miller (1997). Weed and Nelson (1962) observed the occurrence of “chlorite-like intergrade” minerals in the surface 10-cm depths of soils occurring in the locations in the present study. The CEC in the soils studied by Uribe and Cox (1988) in their study of the effects of CEC and HM on potassium availability indices in seventeen soils from the Coastal Plain, Piedmont, and Mountain regions of North Carolina ranged from 2.2 to 12.9  $\text{cmol}_c \text{ kg}^{-1}$ . Many studies (Chaney and Swift, 1984; Albiach et al., 2001; Duiker et al., 2003) have reported significant links between aggregation and SOC. This study also found a significant correlation ( $r = 0.59$ ) between SOC and MWD in this

location; and ( $r = 0.52$ ) between MWD and CEC (Table 6). Therefore, the effect of CEC on MWD in these soils may also be via the relationship between CEC and SOC in soils with low SOM. Investigating the nature of the exchangeable hydrogen in SOM in 18 soils from the Atlantic Coastal Plain, Kamprath and Welch (1962) observed a high correlation ( $r = 0.89$ ) between SOM and CEC; reporting that 55% of the total CEC of the soil at pH 7 came from SOM. The authors suggested that in low SOM status soils with low clay content, the little SOM present can still be an important source of CEC. Evangelou and Blevins (1985), studying soil-solution exchange phase interactions in NT with annual N fertilizer amendments, NT with no N fertilizer amendments, and CT with no N fertilizer amendments in a field experiment on a Maury silt loam (Typic Paleudalfs) at the Kentucky Agricultural Experiment Station Farm at Lexington, Kentucky, observed that CEC increased with SOM build-up under NT. Soil CEC is influenced by pH, generally increasing as soil pH increases; this suggesting a positive influence of pH.

The variables that can negatively influence MWD in the Mountain location are sand and Db, since these have negative loadings on PC1 (Table 4). However, the influence of these two variables will be discussed under the Coastal Plain and Mountain locations, since sand content is significantly greater in the Coastal Plain than in either Mountain or Piedmont location; and Db is significantly greater in the Coastal Plain and Piedmont locations than in the Mountain location (Table 5).

The positive influences of non-crystalline Al and Fe (hydr)oxides on MWD is related

to the role of these minerals in linking clay particles and organic molecules together to form microaggregates. Aluminum and Fe (hydr)oxides are involved in bridging the generally negatively charged clay surfaces and anionic groups of polymeric organic matter in soil, such as in the OM-(Al, Fe)-clay or clay-(Al, Fe)-clay or OM-(Al, Fe)-OM complexes (Edwards and Bremner, 1967). Several studies (Deshpande, 1964; Hsu, 1989; Arias et al., 1996) have suggested that the Al (hydr)oxides are more effective than Fe (hydr)oxides in aggregate formation, since Fe (hydr)oxides do not generally occur as cementing but discrete crystals in soil. However, Lutz (1936) suggested the involvement of Fe (hydr)oxides as cementing agents in soil aggregation in soils from locations in the regions of this study. Mbagwu also (1999) suggested that “any cementing agent” can enhance soil aggregation. Duiker et al. (2003) found that non-crystalline Fe (hydr)oxide may stabilize soil more effectively than free Fe and that non-crystalline Fe (hydr)oxides may be more influential than SOC on water stable aggregation in low SOM status soils. This study did not, however, observe any significant correlations between MWD and Al and Fe (hydr)oxides (Table 6).

The practices in the Coastal Plain location are positioned along the mid-point of PC1 (from the positive end around the 0.78 point for PC1) to the furthestmost negative point (where PC1 = -1.89). In this location, best management practices under no-till (NT1) and under conventional tillage (CT1), as well as the organic farming system under conventional tillage (CTO) appear to be “mixed” together, with the CTO points are even difficult to spot in the figure. These points form a band along PC1, where PC1 ranges from -0.55 to +0.78. The

successional system (S) occurs further down the negative portion, from just about  $-0.55$  to the end of the axis ( $PC1 = -1.89$ ). Along  $PC2$ , all the systems in this location generally occur on the negative portion of  $PC2$ . Tillage practices in the Piedmont generally tend to occur along the negative side of  $PC1$  but on the positive side of  $PC2$ ; except the MBP system which occurs clearly on the negative side of  $PC2$ .

The “mixing” together of points or bands suggest a combination of interaction or counteraction of the influences of the variables that define  $PC1$  and  $PC2$ . Sand content was significantly greater in this location than in the Mountain or Piedmont (Table 5); and there are reports (Kemper and Koch, 1966) that discrete sand grains may act as aggregates, in this way contributing to the proportion of water stable aggregates. The potential contribution by sand to aggregate stability may explain the necessity for sand correction in aggregate stability experiments (Seybold and Herrick, 2001; Márquez et al., 2004). In the present study, no sand corrections were made for soil masses. However, in other related studies (e.g. Chapter1: Tillage effects on water stable aggregates and aggregate associated microbial biomass carbon and nitrogen), proportions of sand-corrected aggregates in bulk soil were always lower than non sand-corrected aggregates. Seybold and Herrick (2001) also measured water stable aggregation in soils (surface 7.6 cm) from different regions in the United States and reported lower quantities of sand-corrected aggregates in bulk soil than in non sand-corrected aggregates. The negative correlation returned in the  $PC$  loadings (Table 4) suggests that MWD has a negative relationship with sand content. A significant

negative correlation ( $r = -0.60$ ) between sand and MWD was also observed in this location (Table 6).

Because of the generally positive relationship between SOM content and aggregate stability (e.g. Chaney and Swift, 1984; Albiach et al., 2001; Duiker et al., 2003), a negative relationship between Db and MWD may be generally expected. Idowu (2003) found significant negative linear correlations between aggregate stability index and Db in the 0 to 10 cm depth of an Alfisol in Nigeria. Celik (2005), studying the effects of different land uses (cultivated, pasture, and forest) on SOM and physical properties in the 0 to 20 cm depth of a Typic Haploxeroll in Turkey, observed an increase in bulk density and attributed this to a decline in MWD. The present study did not find any significant correlations between Db and MWD, with the exception of a significant correlation between Db and SOC ( $r = 0.66$ ) in the Piedmont location (Table 6). The occurrence of the band of points (for all systems in this location) on the same side of the PC2 axis, suggests that the variables that describe PC2 (i.e. non-crystalline Al and Fe (hydr)oxides) affect the systems in similar way. Although the masses of non-crystalline Al and Fe (hydr)oxides are significantly greater in the Mountain and Piedmont locations than the Coastal plain (Table 5), a significant correlation was observed between MWD and non-crystalline Fe (Hydr)oxides in the Coastal Plain (Table 7). The involvement of non-crystalline Fe (Hydr)oxides in bridging clay and organic matter molecules to form aggregates has already been mentioned.

Because tillage practices in the Piedmont generally tend to occur along the negative

side of PC1 but on the positive side of PC2, it is speculated that a decrease in the amounts of one or more of the variables defining PC1 and/or an increase in one or both factors defining PC2 results in increased MWD in the location. Similarly, an increase in the amounts of variables under PC1 or increase in the amounts of the variables under PC2 will decrease MWD in the location. Since machinery is often used on these plots as well as on the plots at the other sites, one option would be to reduce trafficking and thereby reduce Db. The Db at the Piedmont and Coastal Plains did not differ but both were significantly greater than in the Mountain (Table 5).

#### **Interpretation of PCs based on values for SOC**

In the biplot for SOC (Fig. 3), tillage systems from both the Mountain and Piedmont locations occur on the positive portion of the PC1 axis while the systems in the Coastal Plain occur on the negative end. Considering that the contents of both (hydr)oxides are significantly greater in the Mountain and Piedmont than Coastal Plain, the involvement of non-crystalline Al and Fe (hydr)oxides in SOC accumulation in the Mountain and Piedmont locations is speculated. Several authors (Boudot, 1992; Sollins et al., 1996) have reported on the involvement of Al and Fe (hydr)oxides in SOM stabilization. Strong complexes of Al and Fe (hydr)oxides are formed as a result of complexation reactions that occur between the surface hydroxyl groups of the (hydr)oxides and the acid functional groups of organic matter. Several proposed mechanisms explaining the roles of Al and Fe (hydr)oxides in SOM

accumulations, including the formation of Al-organic matter complexes that protect organic matter from microbial decomposition or that reduce mineralization rates are discussed in the literature (Boudot, 1992; Sollins et al. 1996; Percival et al., 2000). Both Al and Fe (hydr)oxides showed significant correlations with SOC (Table 6).

The occurrence of tillage systems in the Coastal Plain location (NT1, CTO, CT1, and S) along the negative end of PC1 suggests the negative effect on SOC of one of the variables that define PC1. This variable is likely the sand content, with a PC loading of  $-0.60$  (Table 4). Soil organic C content is generally expected to be considerably lower in sandy than fine or medium textured soils (Burke et al., 1989). Lower SOC contents have been reported in sand separates relative to silt or clay separates (Christensen, 2001; Kiem et al., 2002). Sand content was greater in this location than in either the Mountain or Piedmont location; while sand content did not differ in the latter locations (Table 5). In this location, the pattern also shows a direction or “slope” that changes from high (where SOC values are between 15.51 to 20.53  $\text{Mg ha}^{-1}$ ) under NT1 through moderate under S (SOC value about 15.51  $\text{Mg ha}^{-1}$ ) to low (SOC values below 15.51  $\text{Mg ha}^{-1}$ ) under CTO and CT1. In the Mountain location, the slope changes mainly from high under NTO through moderate under NTS to low under CTS. In the Piedmont, the pattern of change is from a high under NT (SOC values around 18  $\text{Mg ha}^{-1}$ ) to low under SCP and MBP (SOC values below 10.5  $\text{Mg ha}^{-1}$ ). These patterns of greater SOC under NT systems and lower SOC under CT systems suggest that no-till or less disturbed systems enhance SOC than cultivated or more disturbed systems. The SOC values for NTO,

NTS, CTS, and CT (in the Mountain) and MBP, SCP and NT (in the Piedmont) occur along the positive side of PC1, suggesting positive influences of clay and non-crystalline Al and Fe (hydr)oxides on SOC; these variables show positive correlations with PC1 (Table 4).

Another pattern observed is that greater SOC values for NTO system (Mountain location) seem to occur mainly along the positive side of PC2 while the other systems in the location are along the negative portion of the (PC2) axis. This pattern suggests that relative to other tillage systems in the location, no-till plus organic amendments increase SOC via enhancement of one or more of the variables defined by PC2 (MWD, HWEC, and MBC). The SCP and MBP systems in the Piedmont form a band on the negative side of PC2, below the  $-0.11$  point on the PC2 line; and show SOC values of  $10.50 \text{ Mg ha}^{-1}$  or less. The points for the no-till system (NT) form a band on the positive side of the (PC2) axis and have SOC values approaching  $20 \text{ Mg ha}^{-1}$ . These patterns suggest that the variables describing PC2 will not exhibit a positive influence on SOC under SCP and MBP but will favorably impact SOC under no-till. The tillage systems in the Coastal Plain all occur in the lower positive and upper negative regions on PC2, suggesting that the effects from variables under PC1 are more dominant in impacting SOC in this location. Considering the significantly low clay content in this location, relative to the Mountain or Piedmont, it is speculated that clay content plays an important part in accumulating SOC in the location.

## Multiple regression analysis

Results of multiple linear regression analysis of MWD and SOC on the PC defining variables are shown in Table 7. The model tests show that significant general relationships existed between MWD (and SOC) and the PC defining variables in each location. Together, the variables selected in the stepwise regression explained 75, 40, and 97% of the variations in MWD in the Coastal Plain, Mountain, and Piedmont locations, respectively. The corresponding values for the variations in SOC were 83, 97, and 99%, respectively. In the Coastal Plain, non-crystalline Fe (hydr)oxides showed a significant relationship with MWD (p values for pair tests,  $P_r > |t| = 0.0011$ ); and the regression equation showed that MWD is directly related with non-crystalline Fe (hydr)oxides. On the other hand, SOC in this location showed a significant relationship with clay content and HWEC. Several authors (Hassink, 1994; Sollins et al., 1996; Percival et al., 2000) have reported poor correlation between SOC and clay content. Oades' (1988) suggested exercising caution in interpreting significant correlations between the two, since it is difficult to distinguish which factors, among those that correlate with clay content, are causative. Since there is a significant correlation between clay content and both SOC and MWD (Table 6), it is suggested that an enhancement of MWD via clay content will in turn enhance SOC, since SOC and MWD are significantly correlated. The highly significant correlation ( $r = 0.84$ ) between MWD and non-crystalline Fe (hydr)oxides (Table 6) may also explain the presence of non-crystalline Fe(hydr)oxides in the equation predicting MWD in the Coastal Plain location. In the

Mountain, significant relationships between HWEC and both MWD and SOC were observed. In the Piedmont, SOC and Db showed significant relationships with MWD; whereby MWD can be enhanced by increasing SOC and decreasing Db. This has important implications for the traffic in the plots at this location. Hot water extractable C and humic matter showed significant relationships with SOC in the location. The regression model suggested a direct relationship between SOC and HWEC but a negative relationship with HM.

### **Variables distinguishing between tillage effects on across locations**

Because PC1 and PC2 together accounted for 59 and 60% of the total variations in the PCA on MWD and SOC, respectively (Table 3), only these two (PCs) are discussed in more detail. However, Figs. 4 and 5 will be briefly mentioned with reference to HWEC, which showed positive loadings on PC3 and PC2 in the PCA on MWD and SOC, respectively (Table 4). Among the C pools (SOC, MBC, HWEC, and HM) measured in this study, HWEC is the only pool that showed significant differences among locations; its mass did not differ in the Mountain and Piedmont but was significantly greater in the Coastal Plain than in either Mountain or Piedmont (Table 5). This study speculates that the shifts along PC3 in Fig. 4 and PC2 in Fig. 5 are describing loadings by HWEC on these PCs. In Fig. 5, organics-amended (NTO) and/or undisturbed (NT, NTS, NT1) systems are seen to load on the positive side of PC3, the axis on which HWEC and SOC load; while disturbed systems (MBP, SCP, CTS, CT1) are generally loading on the negative side of this axis. Similarly in Fig. 5, the organics-

amended and undisturbed systems generally load on the positive side of PC2, while disturbed systems are generally loading on the negative side of this axis. In Figs. 6 and 7, HWEC is the reference variable in both biplots. A comparison of the two figures with Figs. 4 and 5 shows similar positions for all tillage systems for corresponding MWD and HWEC (Figs. 4 and 6) or SOC and HWEC (Figs. 5 and 7). Hot water extractable C is an indicator of potentially bio-available materials (Zsolnay and Gorlitz, 1994; Ghani et al., 2003) and has been found to strongly correlate with micro-aggregate characteristics (Puget et al., 1999). Among several soil biochemical and biological measurements made by Ghani et al. (2003), HWEC was described as “the most consistent measurement able to differentiate between treatments within and across ecosystems”. The significant correlations (Table 6) and relationships (Table 7) of this C pool with SOC in all three locations support this statement. Except in the Coastal Plain location, where it did not correlate significantly with MWD and where aggregate formation may be favored by non-crystalline Fe (hydr)oxides, HWEC showed significant correlations with MWD in both Mountain and Piedmont locations (Table 6). It also correlated with humic matter in the Coastal Plain and Piedmont locations.

### **Conclusions**

The present study used a PCA method with a varimax rotation to extract the main component variables, out of 11 soil properties examined, that are key indicators of WSA and SOM in the 0 to 10-cm depth of different agroecosystems. Only one or two soil

properties showed significant relationships with MWD or SOC in each location. The index of potentially bioavailable C, measured in hot water, showed a consistent significant relationship with SOC in all three locations studied. The hypothesis that non-crystalline (hydr)oxides of Al and Fe were contributing factors to soil aggregation and SOM stabilization in the soils studied was supported by the results of the PCA. However, the hypothesis was supported in the Coastal Plain only, where multiple regression analysis indicated that non-crystalline Fe (hydr)oxides were significantly related to MWD. It is concluded that the addition of bioavailable materials (e.g. compost, plant and animal residues, and cover crops) can enhance both MWD and SOC in the locations studied. Further examination into the extent to which the non-crystalline forms of these (hydr)oxides contribute to soil aggregation and SOC dynamics would be beneficial.

## References

- Albiach, R., R. Canet, F. Pomares, F. Ingelmo. 2001. Organic matter components and aggregate stability after the application of different amendments to a horticultural soil. *Bioresource Technology* 76: 125-129.
- Alderfer, R.B. and F.G. Merkle. 1941. The measurement of structural stability and permeability and the influence of soil treatments upon these properties. *Soil science* 51 (1): 201 -212.
- Angers, D.A. and C. Chenu. 1998. Dynamics of soil aggregation and C sequestration. In: Lal, R., Kimble, J.M., Follett, R.F., Stewart, B.A. (Eds.), *Soil Processes and the Carbon Cycle*. Advances in Soil Science. CRC Press, Boca Raton, FL, pp. 199–206.
- Arca, M.N. and S.B. Weed. 1966. Soil aggregation and porosity in relation to contents of free iron oxides and clay. *Soil Sci.* 101: 164-170.
- Arias, M., M. T. Barral, and F. D.az-Fierros. 1996. Effects of associations between humic acids and iron or aluminum on the flocculation and aggregation of kaolin and quartz. *European Journal of Soil Science* 47: 335-343.
- Baldock, J.A. and P.N. Nelson. 2000. Soil organic matter. In M.E. Sumner et al. (Eds.) *Handbook of Soil Science*. CRC Press, Boca Raton, FL. U.S.A. p. B25-B84.
- Balesdent, J., C. Chenu, M. Balabane. 2000. Relationship of soil organic matter dynamics to physical protection and tillage. *Soil and Till. Res.* 53: 215-230.
- Barnhisel, Richard I and Paul M. Bertsch. 1989. Chlorite and hydroxyl-interlayered vermiculite and smectite. *In* Dixon, J.B. and S.B. Weed (ed.) *Minerals in Soil Environments*. SSSA Book Series No.1. ASA-SSSA, Madison, WI. pp. 729-788.
- Barthès, Bernard G., Ernest Kouakoua, Marie-Christine Larré-Larrouy, Tantely M. Razafimbelo, Edgar F. de Luca, Anastase Azontonde, Carmen S.V.J. Neves, Pedro L. de Freitas, and Christian L. Feller. 2008. Texture and sesquioxide effects on water-stable aggregates and organic matter in some tropical soils *Geoderma* 143: 14–25.
- Blakemore, L.C., R.L. Searle, and B.K. Daly. 1987. *Methods for chemical analysis of soils*. N.Z. Soil Bur. Sci. Rep. 80. New Zealand Soil Bureau, Lower Hutt.

- Blanco-Canqui, Humberto, Rattan Lal, and Roque Lemus. 2005. Soil aggregate properties and organic carbon for switchgrass and traditional agricultural systems in the southeastern United States. *Soil Sci.* 170: 998-1012.
- Boudot, J.-P. 1992. Relative efficiency of complexed aluminum, non-crystalline Al hydroxide, allophane, and imogolite in retarding the biodegradation of citric acid. *Geoderma* 52:29–39.
- Bryant, J.C., T.W. Bendixen, , and C.S. Slater. 1948. The measurement of the water stability of soils. *Soil science* 65 (4): 341 -345.
- Burke, I. C. , C. M. Yonker, W. J. Parton, C. V. Cole, K. Flach, and D. S. Schimel.1989. Texture, climate, and cultivation effects on soil organic matter content in U.S. grassland soils. *Soil Sci. Soc. Am. J.* 53:800-805.
- Cabria, F. N.; Bianchini, M. R.; Mediavilla, M. C. 2005. Free iron oxides associated to organic carbon in soils aggregates in Balcarce County / Oxidos de hierro libres asociados a carbono organico en agregados de suelos del partido de Balcarce. *Ciencia del Suelo* 23 (1): 23-29.
- Caracava, F., A. Lax, and A. Albaladejo. 2001. Soil aggregate stability and organic matter in clay and fine silt fractions in urban refuse and refuse-amended semi arid soils. *Soil Sci. Soc. Am. J.* 65:1235–1238.
- Celik, I. 2005. Land use effects on organic matter and physical properties of soil in a southern Mediterranean highland of Turkey. *Soil & Tillage.* 83: 270- 277.
- Chaney, K. and R.S. Swift. 1984. The influence of organic matter on aggregate stability in some British soils. *Journal of soil Science* 35: 223-230.
- Chesters, G., O. J. Attoe, and O. N. Allen. 1957. Soil aggregation in relation to various soil constituents. *Soil Sci Soc Am J.* 21: 272–277.
- Chotte, J.L., J.N. Ladd, and M. Amato. 1998. Sites of microbial assimilation, and turnover of soluble and particulate <sup>14</sup>C-labelled substrates decomposing in a clay soil. *Soil Biol. Biochem.* 30: 205–218.
- Christensen, B.T. 1985. Carbon and nitrogen in particle size fractions isolated from Danish arable soils by ultrasonic dispersion and gravity-sedimentation. *Acta Agric. Scand.* 35: 175-187.

- Christensen, B.T. 1992. Physical fractionation of soil and organic matter in primary particle size and density separates. *Adv. Soil Sci.* 20: 1-90.
- Christensen, B.T. 1996. Carbon in primary and secondary organomineral complexes. *In* Carter, Martin R. and B.A. Stewart (eds.) *Structure and organic matter storage in agricultural soils*. CRC Press, Inc.
- Christensen, B.T. 2001. Physical fractionation of soil and structural and functional complexity in organic matter turnover. *Eur. J. Soil Sci.* 52:345–353.
- Conaway, Andrew W. Jr. and E. Strickling. 1962. A Comparison of Selected Methods for Expressing Soil Aggregate Stability. *Soil Sci Soc Am J* 26:426-430.
- Coughlan, K.J., W.E. Fox, and J.D. Hughes. 1973. A study of the mechanisms of aggregation in a Krasnozern soil. *Aust. J. Soil Res.* 11:65–73.
- Denef, K., Six, J., Bossuyt, H., Frey, S.D., Elliott, E.T., Merckx, R., Paustian, K., 2001. Influence of dry–wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. *Soil Biol. & Biochem.* 33: 1599–1611.
- Deshpande, T.L., D.J. Greenland, and J.P. Quirk. 1964. Role of iron oxides in the bonding of soil particles. *Nature.* 201 (4914): 107-108.
- Deshpande, T.L., D.J. Greenland, and J.P. Quirk. 1968. Changes in soil properties associated with the removal of iron and aluminium oxides. *J. Soil Sci.* 19, 108-22.
- Duiker, Sjoerd W., Fred E. Rhoton, José Torrent, Neil E. Smeck, and Rattan Lal. 2003. Iron (hydr)oxide crystallinity effects on soil aggregation. *Soil Sci. Soc. Am. J.* 67: 606-611.
- Edwards, A.P. and J.M. Bremner. 1967. Microaggregates in soils. *J. Soil Sci.* 18: 64-73.
- Ellert, B.H. and J.R. Bettany. 1995. Calculation of organic matter and nutrients stored in soils under contrasting management regimes. *Can. J. Soil Sci.* 75: 529-538.
- Elliott, E.T. and D.C. Coleman. 1988. Let the soil work for us. *Ecol. Bullet.* 39: 23-32.
- Evangelou, V.P. and R.L. Blevins. 1985. Soil-Solution Phase Interactions of Basic Cations in Long-Term Tillage Systems. *Soil Sci. Soc. Am. J.* 49: 357-362.
- Feller, C., Beare, M.H., 1997. Physical control of soil organic matter dynamics in the tropics. *Geoderma* 79, 69–116.

- Filho, C. Castro, A. Lourenço, M. de F. Guimarães, and I. C. B. Fonseca. 2002. Aggregate stability under different soil management systems in a red latosol in the state of Parana, Brazil. *Soil Tillage and Research*. 65: 45-51.
- Gabriel, K. R. 1971. The biplot graphic display of matrices with application to principal component analysis. *Biometrika* 58 (3): 453-467.
- Gee, G.W. and J.W. Bauder. 1986. Particle size analysis. pp. 383-441. In: A. Klute (ed.), *Methods of Soil Analysis, Part 1, 2<sup>nd</sup> Edition*. ASA and SSSA Publ., Madison, WI, USA.
- Ghani, A, M. Dexter, K.W. Perrott. 2003. Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. *Soil Biology & Biochemistry* 35 1231–1243.
- Guidi, G., Pera, A., Giovannetti, M., Poggio, G., Bertoldi, M., 1988. Variations of soil structure and microbial population in a compost amended soil. *Plant Soil* 106, 113-119.
- Hair, Joseph F., Jr., Rolph E. Anderson, and Ronald L. Tatham. 1987. *Multivariate data analysis with readings*. 2<sup>nd</sup> ed. Macmillan Publishing Company, New York ; Collier Macmillan Publishers, London. pp 233-292.
- Halli, Shivalingappa S. and K. Vaninadha Rao. 1992. Causal relationships in population analysis. *In Advanced techniques of population analysis*. pp 63-100. Plenum Press, New York. 1992. 226 p.
- Hassink, J., L.A. Bouman, K.B. Zwart, J. Bloem, and L. Brussard. 1993. Relationships between soil texture, physical protection of organic matter, soil biota, and C and N mineralization in grassland soils. *Geoderma* 57: 105-102.
- Haynes, R.J. 1993. Effect of sample pretreatment on aggregate stability measured by wet sieving or turbidimetry on soils of different cropping history. *J. Soil Sci.* 44: 261-270.
- Heinonen, R., 1956. Soil aggregation in relation to texture and organic matter. *Abstract in Soils and Fertilizers* 19: 43.
- Horn, R.; Taubner, H.; Wuttke, M.; Baumgart, T. 1994. Soil physical properties related to soil structure. *Soil Tillage Res.* 30: 187–216.
- Hsu, Pa Ho. 1989. Aluminum hydroxides and oxyhydroxides. In Dixon, J.B. and S.B. Weed

- (ed.) Minerals in Soil Environments. SSSA Book Series No.1. ASA-SSSA, Madison, WI. pp. 331-378.
- Idowu, O.J. 2003. Relationship between aggregate stability and selected soil properties in humid tropic environment. *Communications in Soil Science and Plant Analysis*. 34: 695–708.
- Igwe, Charles A. and K. Stahr. 2004. Water-stable aggregates of flooded Inceptisols from south-eastern Nigeria in relation to mineralogy and chemical properties. *Aust. J. Soil Res.* 42: 171-179.
- Jackson, M.L., C.H. Lim, and L.W. Zelany. 1986. Oxides, hydroxides, and aluminosilicates. pp. 101-150. *In* A. Klute (ed.) *Methods of Soil Analysis, Part 1. Physical and mineralogical methods*. ASA, SSSA, Madison, WI.
- Jastrow, J.D. and R.M. Miller. 1998. Soil aggregate stabilization and carbon sequestration: Feedbacks through organomineral associations. *In* *Soil Processes and the carbon cycle*. Lal, Rattan, M. Kimble, Ronald F. Follet, and Bobby A. Stewart (eds.) CRC Press LLC. pp. 207-223.
- Kaiser, H. F. 1960. The application of electronic computers to factor analysis. *Educational and Psychological Measurement*. 20: 141-151.
- Kamprath E.J. and C.D. Welch. 1962. Retention and cation exchange properties of organic matter in Coastal Plain soils. *Soil Sci. Soc. Am. Proc.* 26: 263-265.
- Kay, B.D. 1997. Soil structure and organic carbon: A review. *In* *Soil Processes and the Carbon Cycle. Advances in Soil Science*. pp 169-198. Lal, Rattan, John M. Kimble, Ronald F. Follet, and Bobby A. Stewart (eds.) CRC Press, Boca Raton, FL.
- Kemper, W.D., Koch, E.J., 1966. Aggregate stability of soils from western United States and Canada. *USDA-ARS Tech. Bull.*, vol. 1355. U.S. Govt. Print. Office, Washington, DC.
- Lal, R. 2004. Soil carbon sequestration impacts on global climate change and food security. *Science* 304: 1623-1627.
- Levy, G.J. and J.R. Torrento. 1995. Clay dispersion and macroaggregate stability as affected by exchangeable potassium and sodium. *Soil sci.* 160 (5): 352-358.
- Levy, G. L., and W. P. Miller. 1997. Aggregate stability of some southeastern U.S. soils. *Soil*

- Science Society of America Journal 61: 1176-1182.
- Lutz, J.F. 1936. The relation of free iron in the soil to aggregation. *Soil Sci. Soc. Am. Proc.* 1: 43-45.
- Márquez, C.O., V.J. Garcia, C.A. Cambardella, R.C. Schultz, and T.M. Isenhardt. 2004. Aggregate-size stability distribution and soil stability. *Soil Sci. Soc. Am. J.* 68: 725-735.
- Mazurak, A.P. 1950. Effect of gaseous phase on water stable synthetic aggregates. *Soil science* 69 (2) 135-148.
- Mbagwu, J.S.C., W.I.E. Chukwu, and P. Bazzoffi. 1994. A multivariate analysis of intrinsic soil components influencing the mean weight diameter of water-stable aggregates. Internal Report IC/94/105. International Atomic Energy Agency and United Nations Educational Scientific and Cultural Organization. International Center for Theoretical Physics.
- Mehlich, A. 1978. New extractant for soil test evaluation of phosphorus, potassium, magnesium, calcium, sodium, manganese and zinc. *Commun. in Soil Sci. and Plant Anal.* 9 (6): 477-492.
- Mitchell, Jeff, Mark Gaskell, Richard Smith, Calvin Fouche, and Steven T. Koike. 2000. Soil management and soil quality for organic crops. Vegetable Research and Information Center, California. Publication 7248 (at <http://anrcatalog.ucdavis.edu/pdf/7248.pdf>).
- Nwadialo, B.E. and J.S.C. Mbagwu. 1991. An analysis of soil components active in microaggregate stability, *Soil Technology* 4: 343-350.
- Oades, J.M. 1988. The retention of organic matter in soils. *Biogeochem.* 5:35–70.
- Oades, J.M. 1990. Associations of colloids in soil aggregates, *In* De Doodt, M.F., Hayes M.H.B. and Herbillon, A. (eds.), *Soil Colloids and Their Associations in Aggregates*, NATO ASI Series, Series B: Physics Vol. 215, Plenum Press, New York, pp. 463-483.
- Olk, Daniel C. and Edward G. Gregorich 2006. Overview of the Symposium Proceedings, “Meaningful Pools in Determining Soil Carbon and Nitrogen Dynamics” *Soil Sci. Soc. Am. J.* 70:967–974.

- O'Rourke, Norm, Larry Hatcher, and Edward J. Stepanski. 2005. A step-by-step approach to using SAS for univariate & multivariate statistics. Published by Wiley-Interscience, New York; Cary, NC SAS. 2<sup>nd</sup> ed. pp. 429-481.
- Ovalles, F.A. and M.E. Collins. 1988. Variability of north west Florida soils by principal component analysis. *Soil Sci. Soc. Am. J.* 52: 1430-1435.
- Percival, H.J., Parfitt, R.L., Scott, N.A., 2000. Factors controlling soil carbon levels in New Zealand grasslands: is clay content important? *Soil Sci. Soc. Am. J.* 64: 1623– 1630.
- Peres-Neto, Pedro, Donald A. Jackson, and Keith M. Somers. 2003. Giving meaningful interpretation to ordination axes: Assessing loading significance in principal component analysis. *Ecology.* 84 (9): 2347–2363.
- Puget, P., D.A. Angers, and C. Chenu. 1999. Nature of carbohydrates associated with water-stable aggregates of two cultivated soils. *Soil Biol. & Biochem.* 31: 55–63.
- Puri, A.N. and B.R. Puri. 1939. Physical characteristics of soils II Expressing mechanical analysis and state of aggregation of soils by single values. *Soil Sci.* 47 (1): 77 -81.
- Rasmussen, Craig, Margaret S. Torn, and Randal J. Southard. 2005. Mineral assemblage and aggregates control carbon dynamics in a California conifer forest. *Soil Sci. Soc. Am. J.* 69:1711–1721.
- Raykov, Tenko and George A. Marcoulides. 2008. Principal component analysis. *In* An introduction to applied multivariate analysis. Taylor and Francis Group, Routledge, New York. 2008. pp. 211-240.
- Retzer, J.L. and M.B. Russell. 1941. Differences in the aggregation of a prairie and a gray-brown podzolic soil. *Source: Soil science* 52 (1): 47-58.
- Reyes, I., and J. Torrent. 1998. Citrate–Ascorbate as a highly selective extractant of poorly crystalline iron oxides. *Soil Sci. Soc. Am. J.* 61: 1647–1654.
- Russell, M.B. and C.L. Feng. 1947. Characterization of the stability of soil aggregates. *Soil science* 63 (4): 299 -304.
- Seybold, C.A. and J.E. Herrick. 2001. Aggregate stability kit for soil quality assessments. *Catena* 44: 37–45.

- Six, J., K. Paustian, E. T. Elliott, and C. Combrink. 2000a. Soil Structure and organic matter: I. Distribution of aggregate-size classes and aggregate-associated carbon. *Soil Sci. Soc. Am. J.* 64:681–689.
- Six, J., E.T. Elliott, and K. Paustian. 2000b. Soil structure and soil organic matter: II. A normalized stability index and the effect of mineralogy. *Soil Sci. Soc. Am. J.* 64: 1042-1049.
- Six, J., R. T. Conant, E. A. Paul & K. Paustian. 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. *Plant and Soil* 241: 155–176.
- Six, J., H. Bossuyt, S. Degryze, and K. Denef. 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. *Soil & Tillage Research* 79: 7–31.
- Skjemstad, J. O., H. V. A. Bushby, and R. W Hansen. 1989. Extractable Fe in the surface horizons of a range of soils from Queensland. *Aust. J. Soil Res.*, 28, 259-66.
- Sollins, P., P. Homann, and B.A. Caldwell. 1996. Stabilization and destabilization of soil organic matter: Mechanisms and controls. *Geoderma* 74: 65–105.
- Stevens, J. 1986. *Applied multivariate statistics for the social sciences*. Hillsdale, NJ: Lawrence Erlbaum Associates.
- Tedeschi, A., and R. Dell’Aquila. 2005. Effects of irrigation with saline waters, at different concentrations, on soil physical and chemical characteristics. *Agric. Water Manage.* 77:308–322.
- Tisdall, J.M., and J.M. Oades. 1982. Organic matter and water stable aggregates in soils. *J. Soil Sci.* 33:141–163.
- Tiulin, A.F. 1932. Considerations of the genesis of soil structure and on methods for its determination. *Trans. 1<sup>st</sup> Comm. Intern. Soc. Soil Sci., Soviet Section (Moscow)*. Vol. A: 111-132.
- Uribe, E. and F.R. Cox. 1988. Soil Properties Affecting the Availability of Potassium in Highly Weathered Soils. *Soil Sci. Soc. Am. J.* 52:148-152.
- Van Bavel, C.H.M. 1950. Mean weight diameter of soil aggregates as a statistical index of aggregation. *Soil Sci. Soc. Am. Proc.* 14: 20-23.

- Vance, E.D., P.C. Brookes, and D.S. Jenkinson. 1987. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19(6): 703-707.
- Wick, Abbey F., Lachlan J. Ingram, and Peter D. Stahl. 2009. Aggregate and organic matter dynamics in reclaimed soils as indicated by stable carbon isotopes *Soil Biol. and Biochem.* 41: 201–209.
- Yoder, R.E. 1936. A direct method of aggregate analysis of soils and a study of the physical nature of erosion losses. *J. Am. Soc. Agron.* 28: 337-351.
- Youker, R.E., and J.L. McGuiness. 1957. A short method of obtaining mean weight diameter values of aggregate analyses of soils. *Soil Sci.* 83:291–294.
- Zhang, X.C. and L.D. Norton. 2002. Effect of exchangeable Mg in hydraulic conductivity, disaggregation and clay dispersion in disturbed soils. *J. Hydrology.* 260: 194-205.
- Zsolnay, A., Gorlitz, H., 1994. Water-extractable organic matter in arable soils: effects of drought and long-term fertilization. *Soil Biol. Biochem.* 26, 1257–1261.

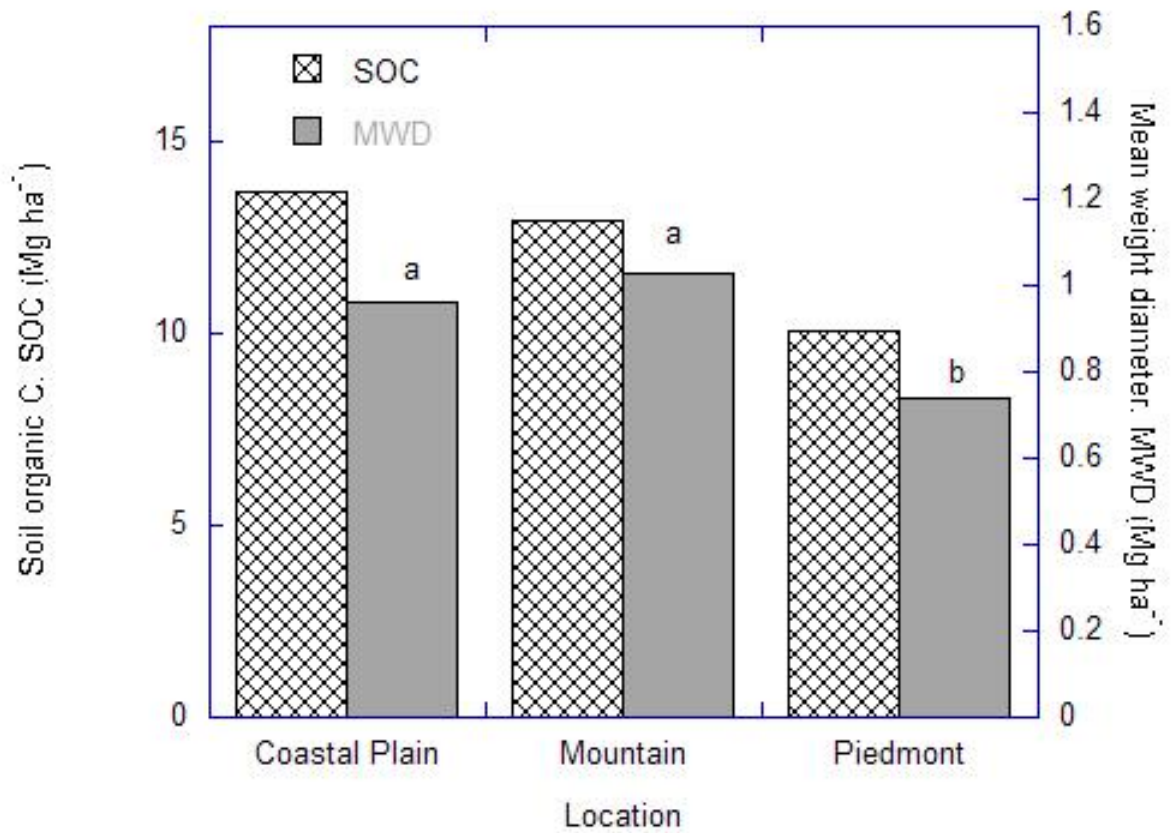


Fig. 1 Distribution of soil organic C (SOC) and mean weight diameter (MWD) in the Coastal Plain, Mountain, and Piedmont locations. Locations with similar or no letters indicate no significant differences,  $p < 0.05$ .

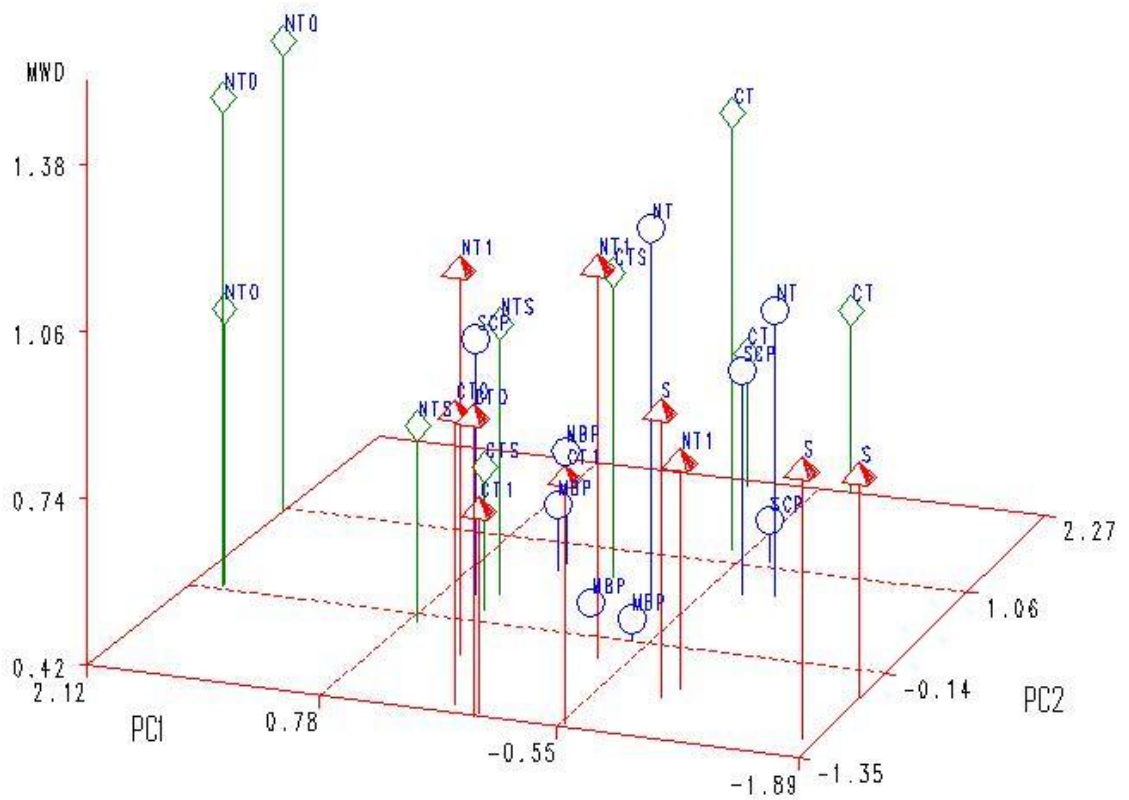


Fig. 2. Biplot of the first and second PCs (PC1 and PC2) showing their relationships to MWD. To distinguish between no-till systems in the Coastal Plain and Piedmont locations in this figure, the lettering codes NT1 and NT have been used to denote no-till in the Coastal Plain and Piedmont locations, respectively. Similarly, CT1 and CT refer to conventional tillage systems in the Coastal Plain and Mountain locations, respectively. Coastal Plain-- CTO: CEFS station managed organic farming system under conventional tillage; CT1: conventional tillage (CT) system with best management practices (BMP); NT1: no-till system with BMP; S: successional or forest re-growth farming system (no-till); Mountain -- CTS: conventional tillage (CT) with chemical amendments; NTS: no-till (NT) with chemical amendments; NTO: NT with organics amendments; CT: CT with neither chemical nor organic amendments; Piedmont – NT: UPRS managed no-till system; MBP: mould board plow; SCP: spring chisel plow.

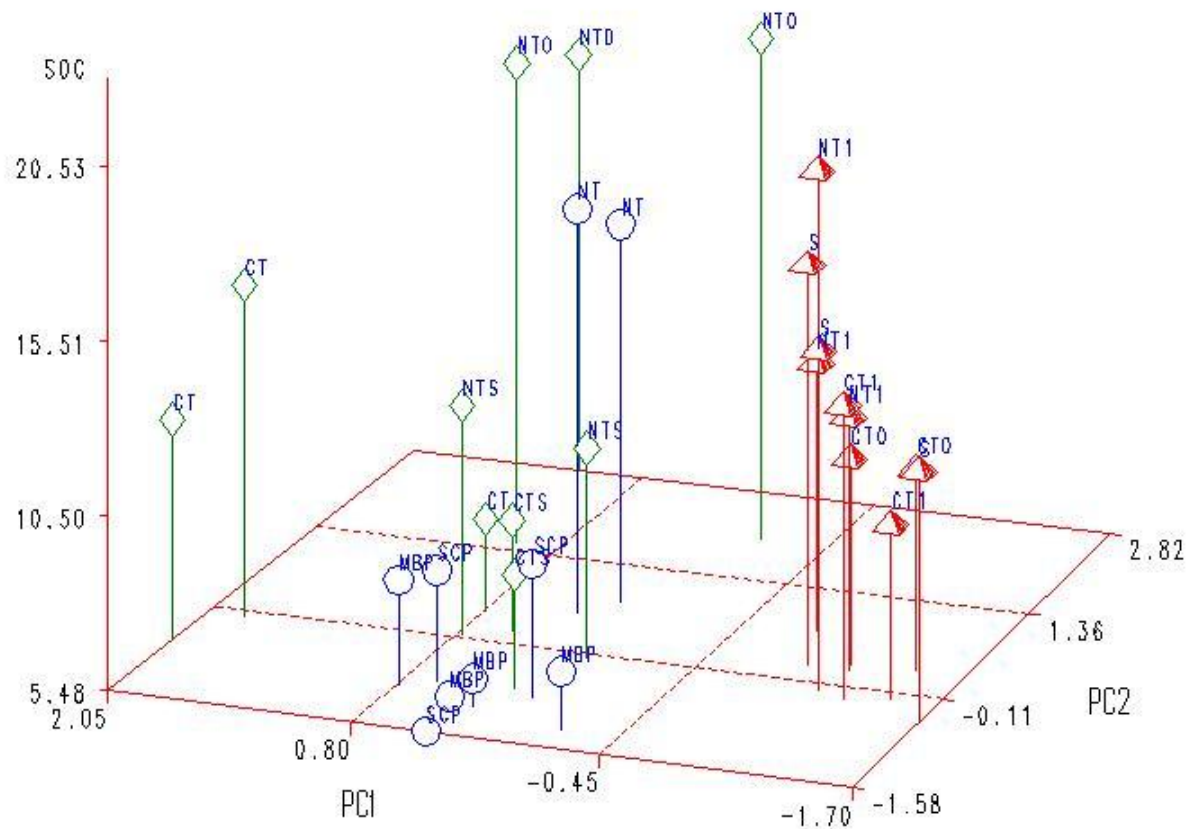


Fig. 3. Biplot of the first and second PCs (PC1 and PC2) showing their relationships to SOC. To distinguish between no-till systems in the Coastal Plain and Piedmont locations in this figure, the lettering codes NT1 and NT have been used to denote no-till in the Coastal Plain and Piedmont locations, respectively. Similarly, CT1 and CT refer to conventional tillage systems in the Coastal Plain and Mountain locations, respectively. Coastal Plain-- CTO: CEFS station managed organic farming system under conventional tillage; CT1: conventional tillage (CT) system with best management practices (BMP); NT1: no-till system with BMP; S: successional or forest re-growth farming system (no-till); Mountain -- CTS: conventional tillage (CT) with chemical amendments; NTS: no-till (NT) with chemical amendments; NTO: NT with organics amendments; CT: CT with neither chemical nor organic amendments; Piedmont – NT: UPRS managed no-till system; MBP: mould board plow; SCP: spring chisel plow.

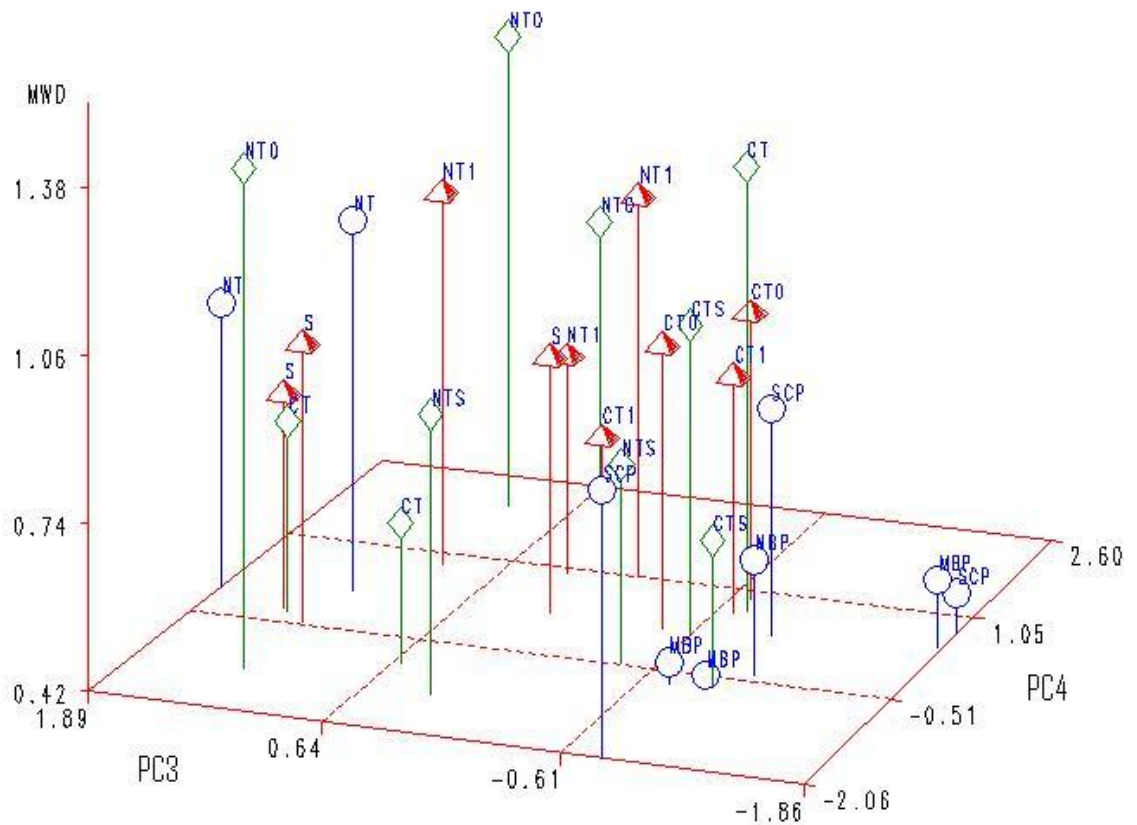


Fig. 4. Biplot of the third and fourth PCs (PC3 and PC4) showing their relationships to MWD. To distinguish between no-till systems in the Coastal Plain and Piedmont locations in this figure, the lettering codes NT1 and NT have been used to denote no-till in the Coastal Plain and Piedmont locations, respectively. Similarly, CT1 and CT refer to conventional tillage systems in the Coastal Plain and Mountain locations, respectively. Coastal Plain-- CTO: CEFS station managed organic farming system under conventional tillage; CT1: conventional tillage (CT) system with best management practices (BMP); NT1: no-till system with BMP; S: successional or forest re-growth farming system (no-till); Mountain -- CTS: conventional tillage (CT) with chemical amendments; NTS: no-till (NT) with chemical amendments; NTO: NT with organics amendments; CT: CT with neither chemical nor organic amendments; Piedmont – NT: UPRS managed no-till system; MBP: mould board plow; SCP: spring chisel plow.

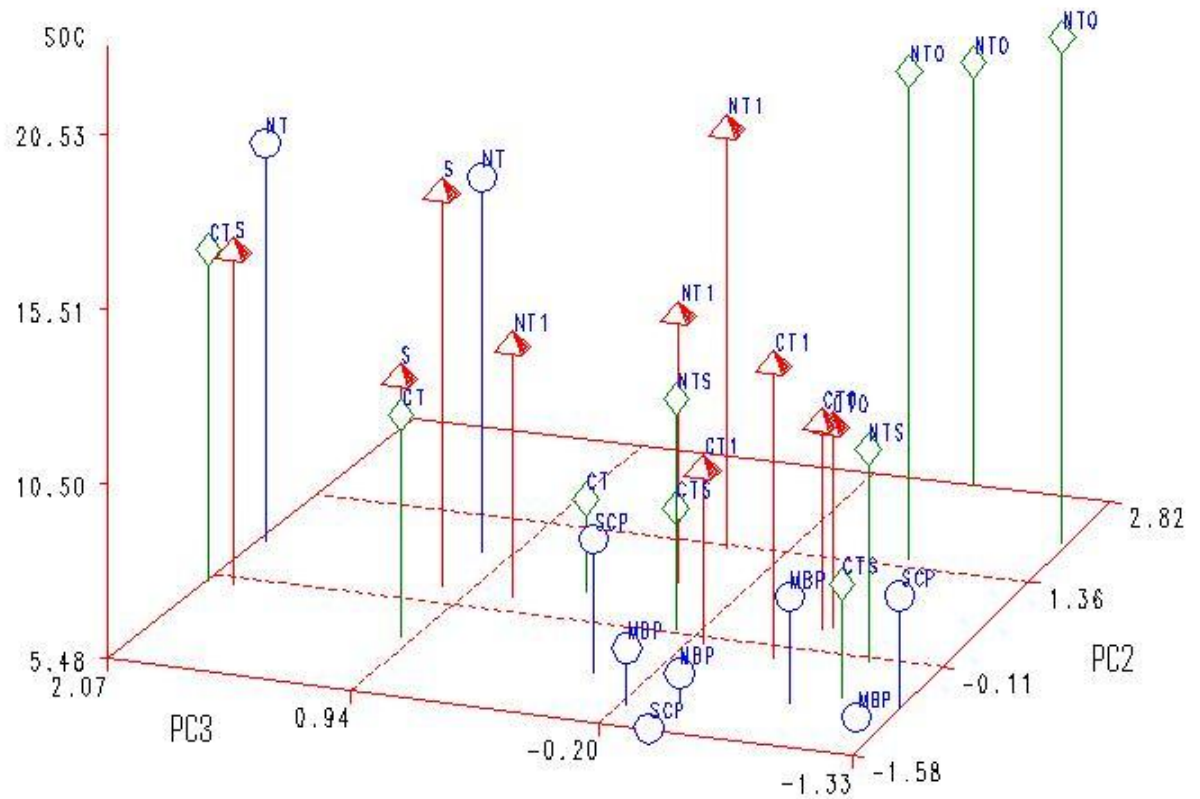


Fig. 5. Biplot of the second and third PCs (PC2 and PC3) showing their relationships to SOC. To distinguish between no-till systems in the Coastal Plain and Piedmont locations in this figure, the lettering codes NT1 and NT have been used to denote no-till in the Coastal Plain and Piedmont locations, respectively. Similarly, CT1 and CT refer to conventional tillage systems in the Coastal Plain and Mountain locations, respectively. Coastal Plain-- CTO: CEFS station managed organic farming system under conventional tillage; CT1: conventional tillage (CT) system with best management practices (BMP); NT1: no-till system with BMP; S: successional or forest re-growth farming system (no-till); Mountain -- CTS: conventional tillage (CT) with chemical amendments; NTS: no-till (NT) with chemical amendments; NTO: NT with organics amendments; CT: CT with neither chemical nor organic amendments; Piedmont – NT: UPRS managed no-till system; MBP: mould board plow; SCP: spring chisel plow.

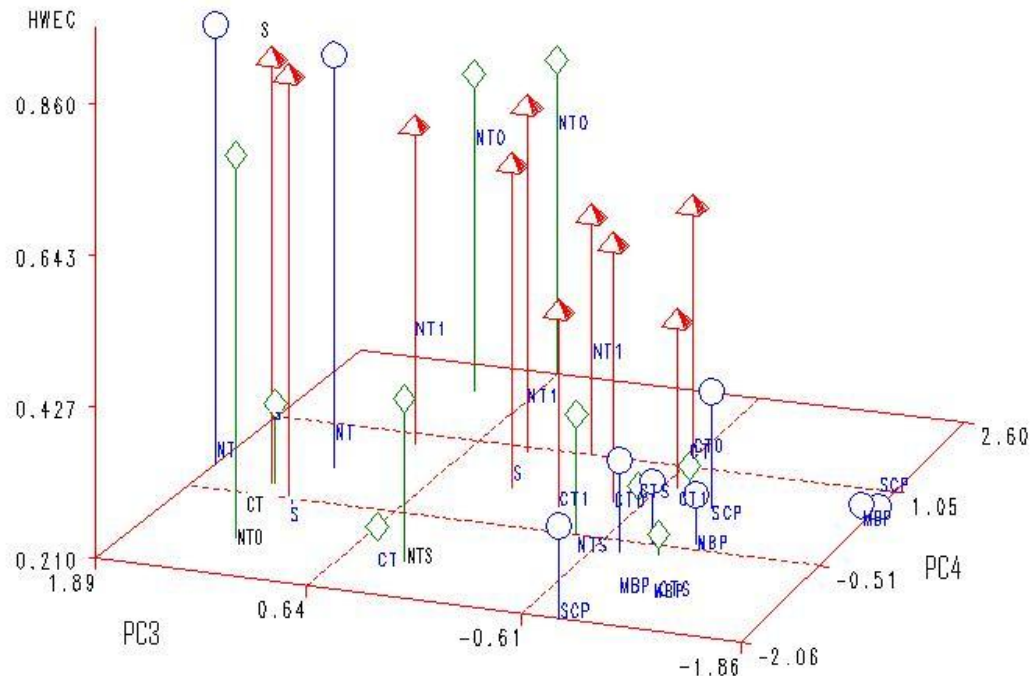


Fig. 6. Biplot of the third and fourth PCs (PC3 and PC4) showing their relationships to HWEC. To distinguish between no-till systems in the Coastal Plain and Piedmont locations in this figure, the lettering codes NT1 and NT have been used to denote no-till in the Coastal Plain and Piedmont locations, respectively. Similarly, CT1 and CT refer to conventional tillage systems in the Coastal Plain and Mountain locations, respectively. Coastal Plain-- CTO: CEFS station managed organic farming system under conventional tillage; CT1: conventional tillage (CT) system with best management practices (BMP); NT1: no-till system with BMP; S: successional or forest re-growth farming system (no-till); Mountain -- CTS: conventional tillage (CT) with chemical amendments; NTS: no-till (NT) with chemical amendments; NTO: NT with organics amendments; CT: CT with neither chemical nor organic amendments; Piedmont – NT: UPRS managed no-till system; MBP: mould board plow; SCP: spring chisel plow.

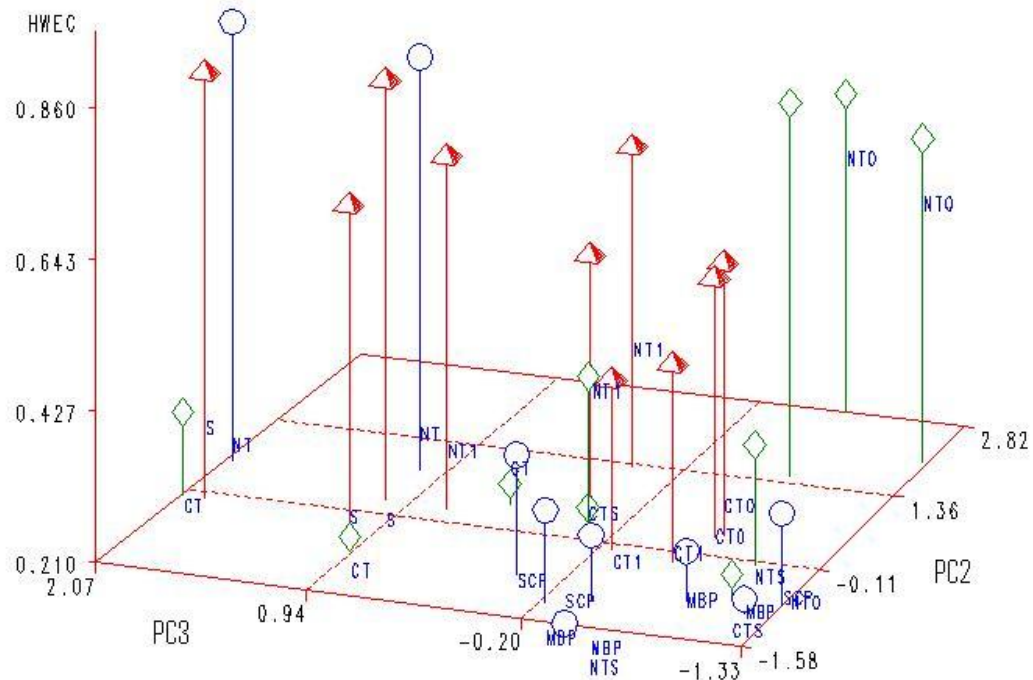


Fig. 7. Biplot of the second and third PCs (PC2 and PC3) showing their relationships to HWEC. To distinguish between no-till systems in the Coastal Plain and Piedmont locations in this figure, the lettering codes NT1 and NT have been used to denote no-till in the Coastal Plain and Piedmont locations, respectively. Similarly, CT1 and CT refer to conventional tillage systems in the Coastal Plain and Mountain locations, respectively. Coastal Plain-- CTO: CEFS station managed organic farming system under conventional tillage; CT1: conventional tillage (CT) system with best management practices (BMP); NT1: no-till system with BMP; S: successional or forest re-growth farming system (no-till); Mountain -- CTS: conventional tillage (CT) with chemical amendments; NTS: no-till (NT) with chemical amendments; NTO: NT with organics amendments; CT: CT with neither chemical nor organic amendments; Piedmont – NT: UPRS managed no-till system; MBP: mould board plow; SCP: spring chisel plow.

Table 1. Tillage systems and selected soil physical and chemical properties for the 0 to 10-cm depth of the experimental locations†.

System	MWD	SOC	MBC	ncrys_Al	ncrys_Fe	Free_Al	Free_Fe	HM	HWEC	CEC	pH	Sand	Clay	Db
	mm	Mg ha <sup>-1</sup>								Cmol <sub>c</sub> kg <sup>-1</sup>		%		Mg m <sup>-3</sup>
<u>Coastal Plain</u>														
CT	0.91	12.3 c	0.21	0.04	0.11	0.37	0.84 b	58.3 b	0.50 b	5.6 bc	6.1 a	67.3	9.1	1.31 b
NT	1.07	14.8 ab	0.32	0.05	0.22	0.47	1.52 a	59 b	0.66 a	6.0 ab	5.9 a	64.7	10.7	1.50 a
CTO	0.95	12.4 bc	0.24	0.04	0.15	0.31	0.77 b	40.5 b	0.63 b	6.4 a	5.9 a	70.2	9.4	1.23 b
S	0.92	15.1 a	0.22	0.04	0.10	0.48	0.81 b	93.4 a	0.79 a	4.2 c	4.8 b	74.2	8.6	1.41 a
<u>Mountain</u>														
CT	0.99	11.0 b	0.19	0.16	0.48	0.83 a	2.64 a	92.9	0.31 b	6.9 b	5.9 b	60.8	19.4	1.32 a
CTS	0.84	10.1 b	0.20	0.13	0.38	0.47 b	1.95 b	53.9	0.28 b	6.7 b	6.1 b	57.6	22.1	1.18 b
NTO	1.24	18.3 a	0.29	0.09	0.33	0.47 b	1.53 c	40.6	0.67 a	10.5 a	6.9 a	52.1	21.3	1.15 b
NTS	1.09	12.4 b	0.11	0.09	0.36	0.51 b	1.58 c	59.4	0.41 b	7.6 b	6.0 b	50.9	22.2	1.13 b
<u>Piedmont</u>														
MBP	0.52 c	6.8 b	0.16	0.11	0.34	0.39	1.05	32.9 b	0.29 b	5.94 a	6.1 a	58.1	23.6	1.32
NT	1.02 a	15.1 a	0.18	0.08	0.22	0.49	0.75	65.4 a	0.74 a	6.05 a	5.9 a	56.9	17.1	1.41
SCP	0.74 b	8.3 b	0.21	0.12	0.35	0.35	0.81	28.4 b	0.33 b	4.58 b	5.4 b	50.7	22.4	1.25

†Values are means of 3, 4, and 4 replications at CEFS, MHCRS, and UPRS in the Coastal Plain, Mountain, and Piedmont, respectively.

††Coastal Plain/ CTO: CEFS station managed organic farming system under conventional tillage; CT: conventional tillage (CT) system with best management practices (BMP); NT: no-till system with BMP; S: successional or forest re-growth farming system (no-till); Mountain/ CTS: CT with chemical amendments; NTS: NT with chemical amendments; NTO: NT with organics amendments; CT: CT with neither chemical nor organic amendments; Piedmont/ NT: UPRS managed no-till system; MBP: mould board plow; SCP: spring chisel plow.

Table 2. Correlation matrices of the PCA for MWD and SOC.

Correlation matrix of the PCA on mean weight diameter										
	SOC	MBC	ncrys_Al	ncrys_Fe	HM	HWEC	CEC	pH	Sand	Clay
Db										
SOC	0.15	0.15	-0.24	-0.41**	0.39*	0.45**	-0.52**	-0.49**	0.50**	-0.48***
MBC		0.34	-0.23	-0.25	0.39*	0.82***	0.43**	0.19	0.02	-0.19
ncrys_Al			0.01	0.07	0.01	0.29	0.08	0.09	0.11	-0.24
ncrys_Fe				0.82***	0.11	-0.48**	0.20	0.17	-0.29	0.54**
HM					-0.26	-0.55***	0.39*	0.31*	-0.41*	0.58***
HWEC						0.32	-0.11	-0.32	0.35*	-0.33*
CEC							0.06	-0.13	0.28	-0.44**
pH								0.75***	-0.41*	0.43*
Sand									-0.28	0.32
										-0.84***

Correlation matrix of the PCA on soil organic C										
	MWD	MBC	ncrys_Al	ncrys_Fe	HM	HWEC	CEC	pH	Sand	Clay
Db										
MWD	0.02	0.09	-0.29	-0.45**	0.40*	0.45**	-0.47**	-0.45**	0.50**	-0.49***
MBC		0.33	-0.18	0.00	0.10	0.55***	0.53**	0.08	-0.01	-0.18
ncrys_Al			-0.09	0.00	-0.05	0.32	0.23	0.21	0.07	-0.19
ncrys_Fe				0.80***	0.06	-0.47**	0.32	0.26	-0.35	0.65**
HM					-0.32	-0.55***	0.49*	0.39*	-0.46*	0.67***
HWEC						0.35	-0.08	-0.31	0.34*	-0.30*
CEC							0.08	-0.14	0.29	-0.48**
pH								0.71***	-0.41*	0.42*
Sand									-0.25	0.29
										-0.83***

Db: bulk density; SOC: soil organic C; MBC: microbial biomass C; ncrys\_Al (or ncrys\_Fe): non-crystalline Al (or Fe) (hydr)oxides; HM: humic matter; HWEC: hot water extractable C; CEC: cation exchange capacity; Sand: percent sand content; Clay: percent clay content; MWD: mean weight diameter. \*, \*\*, \*\*\* show significant correlations at p = 0.05, 0.01, and 0.001, respectively.

Table 3. Eigen values of the correlation matrices of the PCAs for MWD and SOC showing orthogonal components explaining the variations among 11 soil variables examined in the PCAs.

<u>Eigen values of the correlation matrix of the</u>					<u>Eigen values of the correlation matrix of the</u>				
<u>PCA for MWD†</u>					<u>PCA for SOC†</u>				
PC #	Eigenvalue	Difference	Proportion	Cumulative	PC #	Eigenvalue	Difference	Proportion	Cumulative
1	4.22	1.91	0.38	0.38	1	4.31	2.07	0.39	0.39
2	2.31	0.92	0.21	0.59	2	2.24	1.01	0.20	0.60
3	1.39	0.33	0.13	0.72	3	1.23	0.28	0.11	0.71
4	1.06	0.21	0.10	0.82	4	0.94	0.17	0.09	0.79
5	0.85	0.39	0.08	0.89	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.
11	0.03		0.00	1.00	11	0.03		0.00	1.00

†The eigenvalues indicate the number of axes or PCs  $\geq 1.00$  and that explain the majority of the variability among the full set of variables examined in each PCA. Thus in the table above, the eigenvalues indicate that the first four and three axes or PCs were  $\geq 1.00$  and accounted for most of the variability among the 11 soil variables examined in the PCA on MWD and SOC, respectively. Therefore, only these axes or PCs were retained for interpretation of the results of the two PCAs.

For any two adjacent eigenvalues, the column labeled “Difference” is obtained by subtracting the subsequent eigenvalue from the previous eigenvalue. For example, in the PCA on MWD:  $4.22 - 2.31 = 1.91$ .

The column labeled “Proportion” is the proportion of the total variation accounted for by a PC: i.e.

Proportion = Eigenvalue for the component in consideration/total eigenvalues of the correlation matrix.

Values in the column labeled “Cumulative” are obtained by summing the proportions of the variance explained by each PC and subsequent ones.

Table 4. Principal component loadings after Varimax rotation†.

	<u>PC loadings for PCA on MWD</u>				<u>PC loadings for PCA on SOC</u>			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	
CEC	<u>0.84</u>	0.19	0.33	0.03	ncrys_Al	<u>0.91</u>	-0.14	0.14
pH	<u>0.82</u>	0.08	-0.01	0.20	ncrys_Fe	<u>0.85</u>	0.01	-0.23
Clay	0.54	0.52	-0.13	-0.52	Clay	<u>0.82</u>	-0.14	-0.32
Sand	<u>-0.61</u>	-0.29	0.05	0.49	Sand	<u>-0.60</u>	-0.03	0.44
Db	<u>-0.70</u>	-0.17	0.30	0.20	MWD	-0.04	<u>0.83</u>	0.11
ncrys_Al	0.06	0.96	-0.04	-0.03	CEC	0.55	0.72	-0.25
ncrys_Fe	0.34	0.84	-0.24	0.07	HWEC	-0.47	<u>0.63</u>	0.39
SOC	0.26	-0.21	<u>0.89</u>	0.21	MBC	-0.14	<u>0.61</u>	-0.07
HWEC	-0.06	-0.49	<u>0.76</u>	0.22	HM	0.02	0.10	<u>0.89</u>
HM	-0.52	0.20	0.70	-0.05	Db	-0.44	-0.04	<u>0.66</u>
MBC	0.10	0.09	0.17	<u>0.84</u>	pH	0.37	0.44	<u>-0.50</u>

†Principal component loadings show the relative importance of each variable within a PC. Significant PC loadings are underlined.

Table. 5. Selected soil physical and chemical properties for the 0 to 10-cm depth of soils at the experimental sites studied.†

Location	Sand %	Clay	Db Mg m <sup>-3</sup>	HM	HWEC	MBC	ncrys_Al Mg ha <sup>-1</sup>	ncrys_Fe	Free_Al	Free_Fe	CEC Cmol <sub>c</sub> kg <sup>-1</sup>	pH
Coastal Plain	69.11 a	9.47 b	1.36 a	64.26	0.65 a	0.25	0.04 b	0.15 c	0.41 b	0.93 b	5.57 b	5.7 b
Mountain	55.36 b	21.26 a	1.20 b	60.87	0.41 b	0.19	0.12 a	0.38 a	0.56 a	1.89 a	7.94 a	6.2 a
Piedmont	55.70 b	21.03 a	1.35 a	51.04	0.45 b	0.18	0.10 a	0.31 b	0.45 b	0.93 b	5.21 b	5.6 b

†Values are means of 3, 4, and 4 replications at CEFS (Coastal Plain), MHCRS (Mountain), and UPRS (Piedmont), respectively. Locations with similar or no letters indicate no significant differences,  $p < 0.05$ .

Table 6. Pearson correlations among selected soil properties at the experimental sites.

	MWD	SOC	MBC	ncrys_Al	ncrys_Fe	HM	HWEC	CEC	pH	Sand	Clay	Free_Al	Free_Fe
	<u>Coastal Plain</u>												
Db	0.22	0.38	0.46	0.34	0.28	0.42	0.37	-0.36	-0.29	-0.05	0.07	0.64*	0.72*
MWD		0.49	0.46	-0.25	0.84***	-0.27	-0.10	0.63*	0.25	-0.60*	0.66*	0.32	0.76*
SOC			0.39	-0.09	0.32	0.50	0.65*	0.33	-0.26	-0.55	0.67*	0.63	0.49
MBC				0.11	0.47	-0.02	0.18	0.23	0.07	-0.43	0.62*	0.26	0.69*
ncrys_Al					-0.08	0.05	0.21	-0.15	0.16	0.20	-0.28	0.18	.
ncrys_Fe						-0.43	-0.11	0.55	0.37	-0.56	0.55	-0.04	0.78**
HM							0.78**	-0.62	-0.82**	0.11	0.03	0.42	-0.14
HWEC								-0.27	-0.72**	0.08	0.10	0.49	-0.04
CEC									0.68*	-0.68*	0.66*	-0.05	0.35
pH										-0.39	0.22	-0.38	0.26
Sand											-0.92***	-0.01	-0.69*
Clay												0.14	0.71*
Free_Al													0.18

Db: bulk density; SOC: soil organic C; MBC: microbial biomass C; ncrys\_Al (or ncrys\_Fe): non-crystalline Al (or Fe) (hydr)oxides; HM: humic matter; HWEC: hot water extractable C; CEC: cation exchange capacity; Sand: sand content; Clay: clay content; MWD: mean weight diameter.

\*, \*\*, \*\*\* show significant correlations at p = 0.05, 0.01, and 0.001, respectively.





Table 7. Multiple linear regression (MLR) equations of mean weight diameter (MWD) and soil organic C (SOC) on PC defining variables.

Location	Variable	DF	Parameter Estimate	Standard Error	t Value	Pr >  t	Standardized Estimate		R-Square	Adj R-Sq
<b>Pr &gt; F</b>										
<u>MWD</u>										
Coastal Plain	Intercept	1	0.72	0.05	13.98	<.0001	0.00	0.0011	0.75	0.72
	ncrys_Fe	1	1.57	0.32	4.93	0.0011	0.87			
MLR equation†: MWD = 0.87 × ncrys_Fe										
Mountain	Intercept	1	0.64	0.13	5.16	0.0002	0.00	0.0152	0.40	0.35
	HWEC	1	0.81	0.29	2.83	0.0152	0.63			
MLR equation†: MWD = 0.63 × HWEC										
Piedmont	Intercept	1	2.13	0.30	7.21	0.0008	0.00	0.0003	0.97	0.96
	CEC	1	-0.06	0.02	-2.56	0.0510	-0.22			
	Db	1	-1.38	0.23	-6.07	0.0018	-0.61			
	SOC	1	0.08	0.01	12.5	<.0001	1.36			
MLR equation†: MWD = (1.36 × SOC) – (0.61 × Db)										
<u>SOC</u>										
Coastal Plain	Intercept	1	0.06	2.36	0.02	0.9810	0.00	0.0019	0.83	0.78
	Clay	1	0.58	0.16	3.59	0.0088	0.57			
	HWEC	1	12.58	3.24	3.89	0.0060	0.61			
MLR equation†: SOC = (0.57 × Clay) + (0.61 × HWEC)										
Mountain	Intercept	1	1.21	1.22	0.99	0.3568	0.00	<.0001	0.97	0.96
	HWEC	1	23.92	1.64	14.55	<.0001	1.02			
	HM	1	0.03	0.01	2.29	0.056	0.16			
MLR equation†: SOC = 1.02 × HWEC										
Piedmont	Intercept	1	1.94	0.44	4.45	0.0043	0.00	<.0001	0.99	0.98
	HWEC	1	23.03	2.05	11.23	<.0001	1.26			
	HM	1	-0.05	0.02	-2.73	0.0343	-0.31			
MLR equation†: SOC = (1.26 × HWEC) – (0.31 × HM)										

†Variables that do not show statistical evidence for relationship with MWD or SOC (i.e. (Pr > |t|) ≥ 0.05) were omitted in the regression equations above.