Effects of Biosolids on Carbon Sequestration and Nitrogen Cycling

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Abstract

Land application of biosolids has been demonstrated to improve nutrient availability (mainly N and P) and improve organic matter in soils, but the effects of biosolids on C sequestration and N cycling in the Mid-Atlantic region is not well understood. The objectives were: 1) to investigate soil C sequestration at sites with a long-term history of biosolids either in repeated application or single large application; 2) to characterize and compare soil C chemistry using advanced ^{13}C nuclear magnetic resonance (NMR) and C (1s) near edge x-ray absorption fine structure (NEXAFS) spectroscopic techniques; and 3) to compare biosolids types and tillage practices on short-term N availability in the Coastal Plain soils. Biosolids led to C accumulation in the soil surface (< 15 cm) after long-time application in both Piedmont and Coastal Plain soils. The C saturation phenomenon occurred in Coastal Plain soils, thus additional soil C accumulation was not achieved by increasing C inputs from biosolids to the Coastal Plain. Soil organic C from profiles in the field sites was not different at depths below the plow layer (15-60 cm). The quantitative NMR analyses concluded that O-alkyl C was the dominant form in the particulate organic matter (POM), followed by aromatic C, alkyl C, COO/N-C=O, aromatic C-O, OCH_3 / NCH and ketones and aldehydes. The aliphatic C and aromatic C were enriched but the O-alkyl C was decreased in the biosolids-amended soils. The changes indicated that the biosolids-derived soil C was more decomposed and, thus, more stable than the control. The NEXAFS spectra showed that O-alkyl C was the dominant form in the POM extracted from biosolids-amended soils, followed by aromatic C, alkyl

C, carboxylic C and phenolic C groups. These results were similar to those from NMR analysis. The regression and correlation analyses of C functional groups in the POM between NEXAFS and NMR indicated that both techniques had good sensitivity for the characterization of C from biosolids-amended soils. To evaluate short-term biosolids N availability, a three-year field study to investigate the effects of lime-stabilized (LS) and anaerobically digested (AD) biosolids on N availability in a corn-soybean rotation under conventional tillage and no-tillage practices was set up in 2009-2011. Results showed that both LS and AD biosolids increased spring soil nitrate N, plant tissue N at silking, post-season corn stalk nitrate N, grain yield, and soil total N by the end of the growing season. The same factors used to calculate plant available N for incorporated biosolids can be used on biosolids applied to no-till systems in coarse-textured soils. All these results indicated that the application of biosolids affects the long-term quantification and qualification of soil organic C and also improve short-term N availability in the Mid-Atlantic region.

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Attributions

Chapter 3: The Effects of Long-term Application of Organic Residuals on Carbon Sequestration

Chapter 3 was submitted to Soil Science Society of America Journal and currently is under review.

Gregory K. Evanylo, PhD (Crop and Soil Environmental Sciences, Virginia Tech): Dr. Evanylo was a co-author for the manuscript and a principal investigator for the grant supporting the research.

Chapter 4: Carbon Dynamics from Long-term Application of Organic Residuals Described by ^{13}C Solid-state Nuclear Magnetic Resonance

Gregory K. Evanylo, PhD (Crop and Soil Environmental Sciences, Virginia Tech): Dr. Evanylo was a co-author for the manuscript and a principal investigator for the grant supporting the research.

Kang Xia, PhD (Crop and Soil Environmental Sciences, Virginia Tech): Dr. Xia was a collaborator for the X-ray absorption research and a co-author for the manuscript.

Na Chen (Chemistry and Biochemistry, Old Dominion University): Ms. Chen is a PhD candidate who helped conduct the NMR experiment on the samples.

Jingdong Mao, PhD (Chemistry and Biochemistry, Old Dominion University): Dr. Mao was a collaborator for the NMR research and a co-author for the manuscript.

Chapter 5: Carbon (1s) K-edge Near Edge X-ray Absorption Fine Structure (NEXAFS) Spectroscopy for Carbon Dynamics from Long-term Application of Organic Residual

Gregory K. Evanylo, PhD (Crop and Soil Environmental Sciences, Virginia Tech): Dr. Evanylo was a co-author for the manuscript and a principal investigator for the grant supporting the research.

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Chapter 6: Effects of Biosolids Treatment Processes on N Cycling and C Accumulation under Various Tillage Practices

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1. Introduction

1.1. Background

Biosolids are nutrient-rich organic materials from municipal wastewater treatment plants. When treated and processed, sewage sludge becomes biosolids which can be safely recycled and applied as fertilizer to sustainably improve and maintain productive soils and stimulate plant growth (USEPA, 1994). Biosolids are composed of inorganic constituents, such as macro- and micro-nutrients and non-nutrient trace elements, organic compounds, and microorganisms including pathogens (Evanylo, 2003). About 60 percent of biosolids in the United States was used for land application in 2002 (Goodman and Goodman, 2006). In Virginia, incineration and landfilling comprise 35% and 15%, respectively, of biosolids disposal methods, with nearly all of the remaining 50% land applied (Evanylo, 2003).

The purpose of application of biosolids is to replace commercial fertilizers, supply essential nutrients (N, P, K and other trace elements), improve soil properties, increase crop production, and improve soil organic carbon (C) (Lal, 2002; Kimberley *et al.*, 2004). Land application of appropriately treated biosolids has been demonstrated to be a safe and effective means for recovery of plant nutrients while simultaneously improving soil structure, soil water retention, root penetration and microbiological properties (Wallace *et al.*, 2009; Rojas-Oropeza *et al.*, 2010; Zerzghi *et al.*, 2010; Kwon and Xia, 2011). The application of biosolids is regulated under 40 CFR Part 503 which limits loading rates for biosolids (USEPA, 1993). The method of biosolids application, the processing type of biosolids (e.g., anaerobic digestions, lime stabilization, etc.), and the environmental properties (esp., temperature, moisture, and aeration) control biosolids plant available nitrogen (PAN) in amended soil (Adamsen and Sabey, 1987; Cripps *et al.*, 1992; Quemada *et al.*, 1998).Accurate prediction of biosolids PAN is very important for agriculture, as changes of soil air and water quality as well as resource use efficiency can affect biosolids PAN (Evanylo and Alley, 1997; Gilmour and Skinner, 1999).

There is evidence that application of biosolids leads to an increase in soil organic C content (Bulluck III *et al.*, 2002; Spargo *et al.*, 2006; Franzluebbers, 2010). The macroscopic scales at which most soil organic C measurements are made have provided useful information on the effects of land application of biosolids on soil C sequestration; however, the evaluation cannot provide specific information about the exact chemical species of C in biosolids-amended soils. Without understanding such direct information from soils, the mechanistic foundation for soil C stabilization remains unsolidified. Consequently, it is difficult to predict long-term effects of biosolids on soil C because of our limited understanding of the actual dynamics of C present in soils.

Recent microscopic analyses have begun to address the issue of C characterization, and may eventually provide a basis for building more robust models of larger scale C cycling. Solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy has been recognized as a popular analytical method for determining the chemical composition of soil C (Preston, 1996). Another newly developed technique is the synchrotron-based C (1s) near-edge X-ray absorption fine structure (NEXAFS) spectroscopy, which can be used to identify and fingerprint the complex structural characteristics of soil C, as it may overcome some of the aforementioned limitations of other techniques, e.g., iron interference in NMR (Sollins *et al.*, 2007). These two techniques have been demonstrated to be powerful and non-destructive techniques for the chemical characterization of soil C from biosolids-amended soil.

The evaluation of long-term application of biosolids on soil organic matter stability at both macroscopic and microscopic scales is needed, especially in the Mid-Atlantic region. Additionally, the effects of biosolids use in agricultural systems on nutrient dynamics, plant response, and environmental impacts need more investigations. More reliable predictions, especially for local soil and climatic conditions, of the overall nutrient value, the N-supplying capacity, and crop yield response of various biosolids types are required. Research is needed to better determine potential benefits and assess associated local and global environmental benefits from the land-application of biosolids.

1.2. Objective

The overall goal of this research was to quantify and qualify the effects of biosolids application on long-term soil C sequestration and short-term N cycling. The specific objectives were: 1) to investigate soil C sequestration at sites with a long-term history of biosolids either in repeated application or single large application; 2) to characterize and compare the soil C chemistry using advanced ^{13}C NMR and C (1s) NEXAFS spectroscopic techniques; and 3) to compare biosolids treatments (i.e., lime-stabilized and anaerobically digested biosolids) and tillage practices (i.e., conventional tillage and no-tillage) on short-term N availability in a corn-soybean rotation in the Mid-Atlantic Coastal Plain.

1.3. References

Adamsen, F. J. and Sabey, B. R. 1987. Ammonia volatilization from liquid digested sewage sludge as affected by placement in soil. Soil Sci. Soc. Am. J. 51: 1080-1082.

Bulluck III, L. R., Brosius, M., Evanylo, G. K. and Ristaino, J. B. 2002. Organic and synthetic fertility amendments influence soil microbial, physical and chemical properties on organic and conventional farms. Applied Soil Ecology 19: 147-160.

Cripps, R. W., Winfree, S. K. and Reagan, J. L. 1992. Effects of sewage sludge application method on corn production. Comm. Soil Sci. Plant Anal. 23: 1705-1715.

Evanylo, G. K. 1999. Agricultural Land Application of Biosolids in Virginia: Production and Characteristics of Biosolids, Virginia Cooperative Extension.

Evanylo, G. K. 2003. Land application of biosolids for agricultural purposes in Virginia. Virginia Cooperative Extension: 452-330.

Evanylo, G. K. and Alley, M. M. 1997. Presidedress soil nitrogen test for corn in Virginia. Communications in Soil Science and Plant Analysis 28: 1285 - 1301.

Franzluebbers, A. J. 2010. Achieving soil organic carbon sequestration with conservation agricultural systems in the southeastern United States. Soil Sci. Soc. Am. J. 74: 347-357.

Gilmour, J. T. and Skinner, V. 1999. Predicting plant available nitrogen in land-applied biosolids. J Environ Qual 28: 1122-1126.

Goodman, J. R. and Goodman, B. P. 2006. Beneficial or biohazard? How the media frame biosolids. Public understanding of science 15: 359-375.

Kimberley, M. O., Wang, H., Wilks, P. J., Fisher, C. R. and Magesan, G. N. 2004. Economic analysis of growth response from a pine plantation forest applied with biosolids. Forest Ecology and Management 189: 345-351.

Kwon, J.-W. and Xia, K. 2011. Fate of triclosan and triclocarban in soil columns with and without biosolids surface application. Environmental Toxicology and Chemistry 31: 262-269.

Lal, R. 2002. Soil carbon sequestration in China through agricultural intensification, and restoration of degraded and desertified ecosystems. Land Degradation & Development 13: 469-478.

Poulsen, T. G. and Hansen, J. A. 2009. Assessing the impacts of changes in treatment technology on energy and greenhouse gas balances for organic waste and wastewater treatment using historical data. Waste Management Research 27: 861-870.

Preston, C. M. 1996. Applications of NMR to soil organic matter analysis: History and prospects. Soil Science 161: 144-166.

Quemada, M., Lasa, B., Lamsfus, C. and Aparicio-Tejo, P. M. 1998. Ammonia volatilization from surface or incorporated biosolids by the addition of dicyandiamide. J. Environ. Qual. 27: 980.

Rojas-Oropeza, M., Dendooven, L., Garza-Avendaño, L., Souza, V., Philippot, L. and Cabirol, N. 2010. Effects of biosolids application on nitrogen dynamics and microbial structure in a saline–sodic soil of the former Lake Texcoco (Mexico). Bioresource Technology 101: 2491-2498.

Sollins, P., Swanston, C. and Kramer, M. 2007. Stabilization and destabilization of soil organic matter-a new focus. Biogeochemistry 85: 1-7.

Spargo, J. T., Evanylo, G. K. and Alley, M. M. 2006. Repeated compost application effects on phosphorus runoff in the Virginia Piedmont. J. Environ. Qual. 35: 2342-2351.

USEPA 1993. 40 CFR part 503 Standards for the use and disposal of sewage sludge subpart B Land Application. 503.10 to 503.18. Print., U. S. G. Washington, DC.

USEPA 1994. A plain English guide to the EPA Part 503 biosolids rule US EPA/832/r-93/003. United States Environmental Protection Agency Office of Wastewater Management, Washington, DC.

Wallace, B. M., Krzic, M., Forge, T. A., Broersma, K. and Newman, R. F. 2009. Biosolids increase soil aggregation and protection of soil carbon five years after application on a crested wheatgrass pasture. J. Environ. Qual. 38: 291-298.

Zerzghi, H., Brooks, J. P., Gerba, C. P. and Pepper, I. L. 2010. Influence of long-term land application of Class B biosolids on soil bacterial diversity. Journal of Applied Microbiology 109: 698-706.

2. Literature Review

2.1. Biosolids Application

Biosolids are good resources for improving soil structure, soil water retention, nutrient availability, and microbiological properties (Epstein, 1975; Banerjee *et al.*, 1997; Khalilian *et al.*, 1998; Novak and Watts, 2004; Tsadilas *et al.*, 2005; Garland *et al.*, 2010). Biosolids can be more effective amendments than commonly employed inorganic fertilizers because biosolids supplement soil organic matter (Jjemba, 2002; Sánchez-Monedero *et al.*, 2004; Sarkar *et al.*, 2005).

Studies have addressed the capacity of biosolids to improve nutrient availability and crop yields (Sabey and Hart, 1975; Hernández *et al.*, 1991; Cortellini *et al.*, 1996; Wong *et al.*, 1998; Feldkirchner *et al.*, 2003). Banuelos *et al.* (2004) reported that application of biosolids significantly increased the total dry matter yields of canola by 1.5x to 3.8x compared with the unfertilized control. Harrison *et al.* (1994) evaluated a single heavy application of biosolids on a coarse-textured soil and reported that biosolids-amended samples had higher C (139 vs. 67 mg g^{-1}), N (12 vs. 3.4 mg g^{-1}), P (14 vs. 2.2 mg g^{-1}) and S (2.5 vs. 0.4 mg g^{-1}) contents in 0-7 cm mineral soil compared with adjacent unamended soil horizons. Warman and Termeer (2005) demonstrated that biosolids produced equivalent corn yields but provided higher nutrient contents (soil N, P, and K) than the commercial fertilizer in a two-year experiment on a silt loam soil.

2.2. Carbon Sequestration

The increase in atmospheric concentration of CO_2 has been identified as a source for global warming (Lal, 2002). Soil C sequestration can reduce agriculture's contribution to CO_2 emissions (Post and Kwon, 2000; Halvorson *et al.*, 2002; Lal, 2004). Use of biosolids as a soil amendment has been demonstrated to increase soil C and, thus, can be used for C sequestration (Brown and Leonard, 2004; Sukkariyah *et al.*, 2005).

Biosolids typically contain from 50 to 70% organic matter, and their continued application over several years will gradually increase soil organic C (SOC) (Stehouwer, 1999). There is evidence that long-term application of biosolids leads to an increase in the SOC content (Bulluck III *et al.*, 2002; Spargo *et al.*, 2006; Franzluebbers, 2010). Lindsay and Logan (1998) reported that soil organic C in a Miamian silt loam in Ohio increased linearly with biosolids application, and 4 yr after application there was three times as much C in the highest rate of biosolids plots as in the control plots. Tian *et al.* (2009) reported that the SOC sequestration rate was 1.73 Mg C ha^{-1} yr^{-1} in biosolids-amended fields after 34-yr continuous applications in Illinois. Cogger *et al.* (2001) evaluated biosolids applied on a sandy loam in western Washington and they increased SOC levels by 2 to 5 g kg^{-1} after seven annual applications. Granato *et al.* (2004) reported that a ten-yr cumulative application of 543 Mg ha^{-1} biosolids (1974 to 1984) to a land-reclamation site in Fulton County, IL increased SOC to 5.1% and then decreased to 3.8% following cessation of biosolids applications (1985-1997). Spargo *et al.* (2008) reported that a history of biosolids application resulted in an increase of 4.19 ± 1.93 Mg C ha^{-1} in the surface 15 cm of soils in the Coastal Plain of Virginia. Evanylo *et al.* (2006) reported that long-term application of biosolids had a relatively positive influence on soil organic matter, and that organic matter increased linearly with biosolids rate.

2.2.1. Soil organic carbon decomposition

Soil organic C mineralization rates increase with biosolids application rate and decline over time (Boyle and Paul, 1989; Wong *et al.*, 1998). Organic C decomposition is, in part, dependent on the C to N ratio in the biosolids (Gilmour and Skinner, 1999). Some studies have shown that N fertilization can have a positive effect on soil C decomposition (Khan *et al.*, 2007), while others have concluded that N fertilization can decrease soil C decomposition (Alvarez, 2005). A relationship established between N and C in compost indicated that 85% of the initial total N of the compost was available for microbial degradation and that 70% of the available C was lost as CO_2 during the immobilization process (Barrington *et al.*, 2002). The organic C decomposition rate is constant for some biosolids, while other biosolids exhibit a fast decomposition stage followed by slow decomposition (Gilmour *et al.*, 2003). The two stage process involves labile organic matter in the fast stage and recalcitrant organic matter during the slow decomposition stage (Parnaudeau *et al.*, 2004).

Some experiments show little or no increase in SOC content with biosolids application (Diacono and Montemurro, 2010). Sukkariyah *et al.* (2007) investigated a Coastal Plain Bojac sandy loam after application of biosolids for 15 years and found no increase of SOC to a depth of 25 cm in biosolids-amended soils. Soil organic C may reach an upper limit and reveal an asymptotic relationship with respect to C input (Six *et al.*, 2002). As a soil approaches a saturation limit, the soil retains less C stocks with more C inputs (Kong *et al.*, 2005). There is evidence that biosolids can increase the mobilization of low-weight soil organic C fractions (<14 000 Da) while at the same time limiting the mobility of organic colloids (Han and Thompson, 1999). Soil organic C can be physically protected by fine particle size fractions, i.e., silt and clay, by forming organo-mineral complexes (Hassink, 1997; Stewart *et al.*, 2012). Coarse-textured soils, e.g., Coastal Plain top soils, appear to have more limitations to the soil C accumulation than fine textured soils, e.g., Piedmont soils (Stewart *et al.*, 2008).

2.2.2. Soil organic carbon speciation

Land application of biosolids affects not only the quantity but also the quality of soil organic C through their influence on the decomposition and humification processes (Wu *et al.*, 2000; Butler *et al.*, 2001; Haynes *et al.*, 2009). Generally, the quantity of soil C can be determined via dry combustion analysis at high temperatures in a furnace with collection and detection of evolved CO_2 (Tiessen and Moir, 1993). For C characterization, spectroscopic methods, i.e., X-ray absorption and NMR, are widely used to indicate the structural C forms in the soil samples (Skjemstad *et al.*, 1996; Mahieu *et al.*, 1999; Schumacher *et al.*, 2005; Wan *et al.*, 2007).

Solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy offers the possibility of direct chemical characterization of soil organic C (Preston, 1996; Cox *et al.*, 2000). NMR spectroscopy measures the characteristic energy absorbed and re-emitted by atomic nuclei that are placed in a static magnetic field and subjected to an oscillatory magnetic field of known radio-frequency (Mehring and Physicist, 1976; Schmidt-Rohr and Spiess, 1994). It allows the determination of the chemical composition of C functional groups by visualizing various chemical shifts of C structures with the magnetic relaxation frequency (Wilson, 1987). The development of cross-polarization magic angle spinning (CP/MAS) ^{13}C NMR technique allows high resolution solid-state ^{13}C NMR spectra of soil organic matter to be acquired (Hatcher *et al.*, 1980; Wilson *et al.*, 1981;

Preston *et al.*, 1994). This technique greatly improves the sensitivity of NMR with non-destructive nature that eliminates C loss during analysis (Braun *et al.*, 1996). However, CP/MAS detects C-H spins selectively and reduces efficiency for unprotonated C, mobile components or regions with short relaxation time, causing the results to be semi-quantitative (Stover and Frechet, 1989; Preston, 2001). Direct polarization magic angle spinning (DP/MAS) technique (also known as Bloch decay) is an alternative for demining chemical structures of organic C (Waugh *et al.*, 1968; Cory and Ritchey, 1988). DP/MAS NMR does not require the proximity of protons, and relies on relaxation processes through the C nuclei (Schmidt *et al.*, 2001). Each type of ^{13}C nuclei provides the same strength of signal, therefore providing complete and unbiased average chemical structures (Piccolo and Conte, 1998). DP/MAS NMR technique provides quantitative structural information by detecting C-C spins and avoids problems associated with CP/MAS (Mao *et al.*, 2008).

The ^{13}C NMR technology has been applied widely to plant litter, organic wastes, whole soil, and extracted soil fractions, i.e., particle size fractions and humic acid fractions (Traina *et al.*, 1990; Kinchesh *et al.*, 1995; Mathers and Xu, 2003; Smernik *et al.*, 2003; Conte *et al.*, 2006; Helfrich *et al.*, 2006; Mathers *et al.*, 2007). For soils containing < 5% organic C, it may be necessary to use various physical methods to concentrate the organic C (Vereecken *et al.*, 1989). Particle size and density fractionation schemes have been used successfully to acquire highly concentrated C from the organic materials associated in soils (Christensen, 1992; Barrios *et al.*, 1996; Gee and Or, 2002). Many researchers have acquired well resolved ^{13}C NMR spectra on the chemical structures of particulate organic matter (Golchin *et al.*, 1994; Preston *et al.*, 1994;

Golchin *et al.*, 1995; Roscoe *et al.*, 2001; Kölbl and Kögel-Knabner, 2004). ^{13}C NMR technology has proven to be a good approach to qualify soil C decomposition, assess soil C stability and evaluate C structural information from organic residual-amended soils (Schulten and Leinweber, 1991; Leifeld *et al.*, 2002; Adani *et al.*, 2006; Mao *et al.*, 2008).

There are two disadvantages which can hamper the usefulness of ^{13}C NMR spectroscopy. Firstly, ^{13}C has a very low natural abundance (only 1.1% C in the soil exists as^{13}C) (Mahieu *et al.*, 1999); thus, soil containing C content lower than 5% makes the acquisition of spectra difficult unless long scan periods (>24 hrs) are obtained (Kögel-Knabner, 2000). Secondly, the relative signal intensities observed in NMR spectra acquired for samples containing paramagnetic minerals, i.e., iron and manganese oxides, may not reflect the actual distribution of C structures because of a selective interference by the paramagnetic minerals (Baldock *et al.*, 1992; Skjemstad *et al.*, 1994).

The disadvantages of the NMR techniques have motivated the development of alternative advanced spectroscopic methods for characterization of soil C structures. Recent investigations using synchrotron-based C (1s) near-edge X-ray absorption fine structure (NEXAFS) spectroscopy, also known as x-ray absorption near-edge structure (XANES), have indicated that it is a powerful technique to identify and fingerprint the complex structural characteristics of C at the molecular level (Robertson and O’reilly, 1987; Ade *et al.*, 1992; Jokic *et al.*, 2003; Sollins *et al.*, 2007). It uses the intense, tunable, polarized X-ray beams generated by a synchrotron light source to probe the electronic states of a sample (Watts *et al.*, 2006). The synchrotron light source probes the X-ray absorption cross section of a sample through inner-shell excitation processes (Koningsberger and Prins, 1987). At photon energy close to an atomic absorption edge, inner shell electrons are excited to an unoccupied energy level, creating resonance peaks in the absorption spectra (Heymann *et al.*, 2011). The spectra features of NEXAFS reflect the molecular structure of the dominant absorbing atoms and provide the contrast mechanism for high-resolution imaging with chemical sensitivity (Ade *et al.*, 1992; Imamura *et al.*, 1994; Bergmann *et al.*, 2003).

Carbon (1s) NEXAFS has been effectively employed in the past to study soil colloids (Schumacher *et al.*, 2005), soil aggregates (Wan *et al.*, 2007; Solomon *et al.*, 2012), humic substances (Solomon *et al.*, 2005; Sedlmair *et al.*, 2009), and black carbon (Haberstroh *et al.*, 2006; Heymann *et al.*, 2011). The NEXAFS technique can scan and identify the chemical structure of a sample in couple of minutes rapidly providing element-specific information. It can detect N, O, and S species in whole soils and particle size fractions simultaneously without additional sample preparation (Morra *et al.*, 1997; Turner *et al.*, 1997; Leinweber *et al.*, 2007). The NEXAFS has the potential to evaluate the influence of anthropogenic changes on the nature and distribution of the elements, i.e., C, N, O and S, and to follow the dynamics in terrestrial ecosystems (Mullins *et al.*, 1993; Solomon *et al.*, 2003; Prietzel *et al.*, 2007).

2.3. Nitrogen Cycling

2.3.1. Biosolids plant available nitrogen

Land application of biosolids is an inexpensive fertilization method that permits recycling of plant nutrients (Christie *et al.*, 2001). Accurate prediction of N availability from biosolids is key to recommending application rates that will benefit crops and minimize the risk N loss. The inorganic N pool, commonly nitrate-N and ammonium-N, are both directly plant available. The ammonium-N can be lost into atmosphere through ammonia volatilization. The organic N pool, which is the largest pool of N in biosolids, must be transformed into plant-available forms via mineralization. Typically, more than half of first-year organic-N mineralization occurs within the first 3 to 6 weeks after biosolids application (Mendoza *et al.*, 2006), and little of the organic N fraction in the biosolids remains available for further mineralization following 48 weeks of incubation (Epstein *et al.*, 1978). Total biosolids plant available nitrogen (PAN) is the sum of the portion of the initial ammonium that does not volatilize plus the amount of organic N that is mineralized in a given time period.

Biosolids PAN varies among biosolids processing treatments as do N forms (Gilmour *et al.*, 2003). Biosolids PAN released during field experiments was linearly related to biosolids C/N ratio, organic N, or total N (Gilmour and Skinner, 1999). PAN from the same biosolids applied to soils may vary under different tillage practices, i.e., conventional tillage and no-tillage. Soil temperature and moisture also influence the release of biosolids PAN (Pu *et al.*, 2012). Mineralization and transformation processes of biosolids N are accelerated by warmer temperatures (Terry *et al.*, 1981; Wang *et al.*, 1997), and can be affected by soil texture (Lindemann and Cardenas, 1984; Wang *et al.*, 2003). Ammonia volatilization rate was similarly related to biosolids content and clay content (Fine *et al.*, 1989), although volatilization can be nearly entirely reduced by incorporating the biosolids. Ammonia volatilization losses may be higher in coarse-textured Coastal Plain soils due to greater aeration (Stewart *et al.*, 2012). In addition, incorporated biosolids may mineralize faster in coarse-textured soils due to greater aeration (Sukkariyah *et al.*, 2007),

2.3.2. Biosolids processing treatment effects on biosolids PAN

Anaerobic digestion is a biological treatment process which decomposes organic matter into gases and water. It reduces pathogen levels and odor, and in the process, decreases organic matter content (Evanylo, 1999). In the lime stabilization process, lime (CaO) is added into biosolids to raise the pH to 12 or higher (Oleszkiewicz and Mavinic, 2002). The process is designed to inactivate viruses, bacteria and pathogens, and it also reduces the solubility of several heavy metals (Jamal *et al.*, 2011). The treatment process used to produce biosolids can influence their pH and the amount of organic matter, plant nutrients (especially nitrogen and phosphorus) (Maguire *et al.*, 2001), and the levels of pathogens they contain (Krogmann *et al.*, 1998).

Generally, the proportions of ammonium and organic N in biosolids vary with the stabilization process (e.g., digestion, stabilization). Ammonia volatilization can be greater in lime-stabilized than anaerobically digested biosolids (25-75% vs. 15-50%) (USEPA, 1995). In laboratory studies, Terry *et al.* (1978) found that 10 to 35% of NH_4-N was volatilized from anaerobically digested biosolids, and Ryan and Keeney (1975) measured volatilization losses of 10 to 70% of NH_4-N.

Biosolids N mineralization rates can be affected by biosolids treatment processes. Cogger *et al.* (2004) reported that the surface-applied biosolids PAN for the first year was 37 ± 5% averaged across all biosolids with different treatment processes, including anaerobic digestion and lime stabilization. The USEPA (1983) recommended a mineralization rate of 20% in the first year after application for anaerobically digested biosolids and 30% for lime stabilized biosolids. Now the USEPA (1995) has encouraged states to develop their biosolids N mineralization factors; thus Virginia has modified the

N mineralization factor to 30% for both anaerobically digested and lime-stabilized biosolids (Gilmour *et al.*, 2003). Biosolids N mineralization rate is positively affected by total N content of the soil and negatively affected by the C/N ratios of the biosolids (Barbarika *et al.*, 1985). With higher rates of biosolids application, the aerobic conditions increase mineralization rates of organic N (Ryan *et al.*, 1973). However, this conclusion is different from that of Garau *et al.* (1986) who reported that the potentially mineralizable N was lower for anaerobically digested biosolids.

2.3.3. Tillage effects on biosolids PAN

The potential benefits of land-applied biosolids under conventional tillage are well documented. Zebarth *et al.* (2000) reported that cumulative recovery of N from incorporated biosolids in harvested forage averaged 11% and the fertilizer N equivalency of the biosolids N (ratio of recovery of biosolids N to urea N) was estimated at 41%. Barbarick and Ippolito (2000) found that tilling 1 Mg of biosolids into the top 8 inches of soil to winter wheat-fallow rotation provided an overall equivalency of about 8 kg N fertilizer. Fernandes *et al.* (2005) found that incorporated biosolids rates (0, 1x, 2x, 4x and 8x agronomic N rates) increased the concentration of soil C and N. Particularly, the soil N contents at the highest rate of biosolids (8x agronomic N rate) increased by 59% and 66% in the layers from 0 to 10 cm and from 10 to 20 cm, respectively.

Surface-applied biosolids increase the concentration of macronutrients, improve surface soil water retention, and reduce run-off (Maguire *et al.*, 2000; Moffet *et al.*, 2005). Although surface application of biosolids has become the primary distribution technique (Harmel *et al.*, 1995; White *et al.*, 1997; Cogger *et al.*, 1998; Joshua *et al.*, 1998; Brown *et al.*, 2003; Hahm and Wester, 2004), most reports are focused on dry-land, forage and range land (Hahm and Wester, 2004; Jurado-Guerra *et al.*, 2006; Jin *et al.*, 2011; Koenig *et al.*, 2011). Very few studies report biosolids application under row-crops managed using no-tillage in humid climates similar to the Coastal Plain of the Mid-Atlantic region.

The method of biosolids application can have a significant influence on ammonia volatilization. King (1973) concluded NH_3 losses were significantly higher for surface applied vs. incorporated biosolids. Beauchamp *et al.* (1978) reported that 60% of NH_4-N in surface-applied biosolids was lost through volatilization. Adamsen and Sabey (1987) observed that incorporated biosolids lost 0.35 % of NH_4-N, while surface-applied biosolids lost 40.3% during the first 14 d of incubation. Quemada *et al.* (1998) reported that surface-applied biosolids lost 20% of NH_4-N, and incorporated biosolids lost less than 5%. Liquid biosolids can retain more NH_4-N than dried biosolids, as liquid biosolids can be injected into soils, reducing NH_4-N volatilization (Jacobs and McCreary, 2003). NH_4-N in surface-applied biosolids lost due to ammonia volatilization poses a significant ecological threat when it leads to N enrichment of natural systems (e.g., soil, plant, and surface water) and also reduces nutrient value of biosolids (Ryan and Keeney, 1975; Terry *et al.*, 1978; Sommers *et al.*, 1981).

The method of biosolids application can affect organic N mineralization rate, reducing biosolids PAN (Magdoff and Amadon, 1980; Cripps *et al.*, 1992; Cartron and Weil, 1998). Organic N mineralization in biosolids-amended soils can be predicted using laboratory incubations (Rowell *et al.*, 2001; Gilmour *et al.*, 2003). Stanford and Smith (1972) found that mineralization of organic N from biosolids applied to soil followed first order kinetics. Surface-applied biosolids enhanced the initial steps of biosolids decomposition, leading to a faster N mineralization and an increase in soil inorganic N accumulation (Hseu and Huang, 2005). Terry *et al.* (1979) reported that surface application increased decomposition of biosolids as compared to incorporation into the soil. Quemada *et al.* (1998) reported that surface-applied biosolids produced a 1.5x increase in N mineralization compared with incorporated biosolids. These results differ from the studies of King (1973) who reported that NO_3-N accumulation was 22% of the applied N when biosolids were applied to the soil surface and 38% when biosolids were incorporated with the soil.

Biosolids PAN can also be lost to groundwater and surface water if biosolids are surface-applied at rates that supply more N than crops can utilize (Oberle and Keeney, 1994; Evanylo, 2003; Esteller *et al.,* 2009). However, more studies show that leaching nitrate-N from excessive application of biosolids in Coastal Plain is not an environmental issue (Smith, 1996). Daniels *et al.* (2002) investigated surface-applied bioslids to the Coastal Plain soils from King William County, Virginia at various rates (0x, 1x, 2x, 3x, 4x, and 5x biosoldis agronomic rate) and found that the NO_3-N contamination of ground-water from surface-applied high biosolids loading rates was not significant. Daniels *et al.* (2003) conducted similar experiments on biosolids with loading rates (0x, 1x, 3x, 5x, and 7x biosolids agronomic rate) surface-applied to Coastal Plain soils from Charles City County, Virginia, and found that mass leaching losses of NO_3-N ranged from 6 to 60 kg ha^{-1}, which was from 0.7 to 3.1% of total-N applied.

Overall, biosolids have been demonstrated to supply plant available nutrients, improve soil properties, and increase organic matter. Application of biosolids can increase soil organic C concentration and result in C sequestration, but the speciation of organic C from biosolids-amended soils is not well understood. Advanced spectroscopic techniques have been developed to characterize the C functional groups, providing potentials and opportunities to investigate the chemistry of C from biosolids-amended soils. Additionally, the purpose of application of biosolids is to replace commercial fertilizers and provide nutrient availability. Tillage management and biosolids processing treatments can both affect biosolids plant available nitrogen, summed by inorganic N and mineralized organic N. Predictions of biosolids PAN will be influenced by ammonia volatilization, organic N mineralization rate, and leaching losses. The short-term N availability of biosolids needs to be clarified in the coarse textured soils in Mid-Atlantic region. These influences of biosolids need further assessment in order to develop recommendations that benefit agronomic responses and reduce environmental impairment.

2.4. References

Adamsen, F. J. and Sabey, B. R. 1987. Ammonia volatilization from liquid digested sewage sludge as affected by placement in soil. Soil Sci. Soc. Am. J. 51: 1080-1082.

Adani, F., Genevini, P., Tambone, F. and Montoneri, E. 2006. Compost effect on soil humic acid: A NMR study. Chemosphere 65: 1414-1418.

Ade, H., Zhang, X., Cameron, S., Costello, C., Kirz, J. and Williams, S. 1992. Chemical contrast in X-ray microscopy and spatially resolved XANES Spectroscopy of organic specimens. Science 258: 972-975.

Alvarez, R. 2005. A review of nitrogen fertilizer and conservation tillage effects on soil organic carbon storage. Soil Use and Management 21: 38-52.

Baldock, J., Oades, J., Waters, A., Peng, X., Vassallo, A. and Wilson, M. 1992. Aspects of the chemical structure of soil organic materials as revealed by solid-state 13C NMR spectroscopy. Biogeochemistry 16: 1-42.

Banerjee, M. R., Burton, D. L. and Depoe, S. 1997. Impact of sewage sludge application on soil biological characteristics. Agriculture, Ecosystems & Environment 66: 241-249.

Banuelos, G. S., Sharmasarkar, S. and Pasakdee, S. 2004. Utilization of biosolids as a fertilizer for canola. Compost Science & Utilization 12: 61-68.

Barbarick, K. A. and Ippolito, J. A. 2000. Nitrogen fertilizer equivalency of sewage biosolids applied to dryland winter wheat. J. Environ. Qual. 29: 1345-1351.

Barbarika, A., Sikora, L. J. and Colacicco, D. 1985. Factors affecting the mineralization of nitrogen in sewage sludge applied to soils1. Soil Sci. Soc. Am. J. 49: 1403-1406.

Barrington, S., Choinière, D., Trigui, M. and Knight, W. 2002. Effect of carbon source on compost nitrogen and carbon losses. Bioresource Technology 83: 189-194.

Barrios, E., Buresh, R. and Sprent, J. 1996. Organic matter in soil particle size and density fractions from maize and legume cropping systems. Soil Biology and Biochemistry 28: 185-193.

Beauchamp, E. G., Kidd, G. E. and Thurtell, G. 1978. Ammonia volatilization from sewage sludge applied in the field. J. Environ. Qual. 7: 141-146.

Bergmann, U., Groenzin, H., Mullins, O. C., Glatzel, P., Fetzer, J. and Cramer, S. 2003. Carbon K-edge X-ray Raman spectroscopy supports simple, yet powerful description of aromatic hydrocarbons and asphaltenes. Chemical Physics Letters 369: 184-191.

Boyle, M. and Paul, E. A. 1989. Carbon and nitrogen mineralization kinetics in soil previously amended with sewage sludge. Soil Sci. Soc. Am. J. 53: 99-103.

Braun, S., Kalinowski, H. O. and Berger, S. 1996. 100 and more basic NMR experiments, VCH Weinheim. New York: 1-108.

Brown, S. and Leonard, P. 2004. Building carbon credits with biosolids recycling. BioCycle 45: 25-29.

Brown, S. L., Henry, C. L., Chaney, R., Compton, H. and DeVolder, P. S. 2003. Using municipal biosolids in combination with other residuals to restore metal-contaminated mining areas. Plant and Soil 249: 203-215.

Bulluck III, L. R., Brosius, M., Evanylo, G. K. and Ristaino, J. B. 2002. Organic and synthetic fertility amendments influence soil microbial, physical and chemical properties on organic and conventional farms. Applied Soil Ecology 19: 147-160.

Butler, T., Sikora, L., Steinhilber, P. and Douglass, L. 2001. Compost age and sample storage effects on maturity indicators of biosolids compost. J. Environ. Qual. 30: 2141-2148.

Cartron, J. M. and Weil, R. R. 1998. Seasonal trends in soil nitrogen from injected or surface-incorporated sewage sludge applied to corn. Comm. Soil Sci. Plant Anal. 29: 121-139.

Christensen, B. 1992. Physical fractionation of soil and organic matter in primary particle size and density separates. Advances in Soil Sciences. 20: 1.

Christie, P., Easson, D. L., Picton, J. R. and Love, S. C. P. 2001. Agronomic value of alkaline-stabilized sewage biosolids for spring barley. Agron J 93: 144-151.

Cogger, C. G., Bary, A. I., Fransen, S. C. and Sullivan, D. M. 2001. Seven years of biosolids versus inorganic nitrogen applications to tall fescue. J. Environ. Qual. 30: 2188-2194.

Cogger, C. G., Bary, A. I., Sullivan, D. M. and Myhre, E. A. 2004. Biosolids processing effects on first- and second-year available nitrogen. Soil Sci. Soc. Am. J. 68: 162-167.

Cogger, C. G., Sullivan, D. M., Bary, A. I. and Fransen, S. C. 1998. Nitrogen recovery from heat-dried and dewatered biosolids applied to forage grasses. J. Environ. Qual. 28: 754-759.

Conte, P., Spaccini, R. and Piccolo, A. 2006. Advanced CPMAS 13C NMR techniques for molecular characterization of size-separated fractions from a soil humic acid. Analytical and Bioanalytical Chemistry 386: 382-390.

Cortellini, L., Toderi, G., Baldoni, G. and Nassisi, A. 1996. Effects on the content of organic matter, nitrogen, phosphorus and heavy metals in soil and plants after application of compost and sewage sludge. The Science of Composting. Blackie Academic & Professional, Chapman & Hall, London: 457-468.

Cory, D. and Ritchey, W. 1988. Suppression of signals from the probe in Bloch decay spectra. Journal of Magnetic Resonance 80: 128-132.

Cox, P. M., Betts, R. A., Jones, C. D., Spall, S. A. and Totterdell, I. J. 2000. Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. Nature 408: 184-187.

Cripps, R. W., Winfree, S. K. and Reagan, J. L. 1992. Effects of sewage sludge application method on corn production. Comm. Soil Sci. Plant Anal. 23: 1705-1715.

Daniels, W. L., Evanylo, G. K., Nagle, S. M. and Schmidt, J. M. 2003. Effects of Biosolids Loading Rate on Nitrate Leaching Potentials in Sand and Gravel Mine Reclamation in Virginia. Proceedings of the Water Environment Federation 2003: 271-278.

Daniels, W. L., Nagle, S. G., Whittecar, R. and Evanylo, G. K. 2002. Effects of biosolids application on ground water nitrate-N levels in sand and gravel mine reclamation in Virginia. National Meeting of the American Society of Mining and Reclamation. Lexington, KY, ASMR.

Diacono, M. and Montemurro, F. 2010. Long-term effects of organic amendments on soil fertility. A review. Agronomy for Sustainable Development 30: 401-422.

Epstein, E. 1975. Effect of sewage sludge on some soil physical properties. J. Environ. Qual. 4: 139-142.

Epstein, E., Keane, D., Meisinger, J. and Legg, J. 1978. Mineralization of nitrogen from sewage sludge and sludge compost. J. Environ. Qual. 7: 217-221.

Esteller, M. V., Martínez-Valdés, H., Garrido, S. and Uribe, Q. 2009. Nitrate and phosphate leaching in a Phaeozem soil treated with biosolids, composted biosolids and inorganic fertilizers. Waste Management 29: 1936-1944.

Evanylo, G., Sukkariyah, B., Eborall, M. A. and Zelazny, L. 2006. Bioavailability of heavy metals in biosolids-amended soils. Communications in Soil Science and Plant Analysis 37: 2157-2170.

Evanylo, G. K. 1999. Agricultural Land Application of Biosolids in Virginia: Production and Characteristics of Biosolids, Virginia Cooperative Extension.

Evanylo, G. K. 2003. Effects of biosolids application timing and soil texture on nitrogen availability for corn. Communications in Soil Science and Plant Analysis 34: 125-143.

Feldkirchner, D. C., Wang, C., Gower, S. T., Kruger, E. L. and Ferris, J. 2003. Effects of nutrient and paper mill biosolids amendments on the growth and nutrient status of hardwood forests. Forest Ecology and Management 177: 95-116.

Fernandes, S. A. P., Bettiol, W., Cerri, C. C. and Camargo, P. 2005. Sewage sludge effects on gas fluxes at the soil–atmosphere interface, on soil δ13C and on total soil carbon and nitrogen. Geoderma 125: 49-57.

Fine, P., Mingelgrin, U. and Feigin, A. 1989. Incubation studies of the fate of organic nitrogen in soils amended with activated sludge. Soil Sci. Soc. Am. J. 53: 444-450.

Franzluebbers, A. J. 2010. Achieving soil organic carbon sequestration with conservation agricultural systems in the southeastern United States. Soil Sci. Soc. Am. J. 74: 347-357.

Garau, M. A., Felipó, M. T. and de Villa, M. C. R. 1986. Nitrogen mineralization of sewage sludges in soils1. J. Environ. Qual. 15: 225-228.

Garland, J. L., Mackowiak, C. L. and Zabaloy, M. C. 2010. Organic waste amendment effects on soil microbial activity in a corn–rye rotation: Application of a new approach to community-level physiological profiling. Appl. Soil Ecol. 44: 262-269.

Gee, G. W. and Or, D. 2002. 2.4 Particle-size analysis. Methods of Soil Analysis: Physical Methods 5: 255.

Gilmour, J. T., Cogger, C. G., Jacobs, L. W., Evanylo, G. K. and Sullivan, D. M. 2003. Decomposition and plant-available nitrogen in biosolids. J. Environ. Qual. 32: 1498-1507.

Gilmour, J. T. and Skinner, V. 1999. Predicting plant available nitrogen in land-applied biosolids. J. Environ. Qual. 28: 1122-1126.

Golchin, A., Oades, J., Skjemstad, J. and Clarke, P. 1994. Study of free and occluded particulate organic matter in soils by solid state ^{13}C CP/MAS NMR spectroscopy and scanning electron microscopy. Soil Research 32: 285-309.

Golchin, A., Oades, J., Skjemstad, J. and Clarke, P. 1995. Structural and dynamic properties of soil organic-matter as reflected by ^{13}C natural-abundance, pyrolysis mass-spectrometry and solid-state ^{13}C NMR-spectroscopy in density fractions of an oxisol under forest and pasture. Soil Research 33: 59-76.

Granato, T. C., Pietz, R. I., Knafl, G. J., Carl R. Carlson, J., Tata, P. and Lue-Hing, C. 2004. Trace element concentrations in soil, corn leaves, and grain after cessation of biosolids applications. J. Environ. Qual. 33: 2078-2089.

Haberstroh, P. R., Brandes, J. A., Gélinas, Y., Dickens, A. F., Wirick, S. and Cody, G. 2006. Chemical composition of the graphitic black carbon fraction in riverine and marine sediments at sub-micron scales using carbon X-ray spectromicroscopy. Geochimica et Cosmochimica Acta 70: 1483-1494.

Hahm, J. M. and Wester, D. B. 2004. Effects of surface-applied biosolids on grass seedling emergence in the Chihuahuan desert. Journal of Arid Environments 58: 19-42.

Halvorson, A. D., Wienhold, B. J. and Black, A. L. 2002. Tillage, nitrogen, and cropping system effects on soil carbon sequestration contribution from USDA-ARS. Soil Sci. Soc. Am. J. 66: 906-912.

Han, N. and Thompson, M. L. 1999. Soluble organic carbon in a biosolids-amended Mollisol. J. Environ. Qual. 28: 652-658.

Harmel, R. D., Zartman, R. E., Mouron, C., Wester, D. B. and Sosebee, R. E. 1995. Modeling ammonia volatilization from biosolids applied to semiarid rangeland. Soil Sci. Soc. Am. J. 61: 1794-1798.

Harrison, R., Xue, D., Henry, C. and Cole, D. W. 1994. Long-term effects of heavy applications of biosolids on organic matter and nutrient content of a coarse-textured forest soil. Forest Ecology and Management 66: 165-177.

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.

Hatcher, P. G., VanderHart, D. L. and Earl, W. L. 1980. Use of solid-state ^{13}C NMR in structural studies of humic acids and humin from Holocene sediments. Organic Geochemistry 2: 87-92.

Haynes, R., Murtaza, G. and Naidu, R. 2009. Inorganic and organic constituents and contaminants of biosolids: implications for land application. Advances in Agronomy 104: 165-267.

Helfrich, M., Ludwig, B., Buurman, P. and Flessa, H. 2006. Effect of land use on the composition of soil organic matter in density and aggregate fractions as revealed by solid-state ^{13}C NMR spectroscopy. Geoderma 136: 331-341.

Hernández, T., Moreno, J. I. and Costa, F. 1991. Influence of sewage sludge application on crop yields and heavy metal availability. Soil Science and Plant Nutrition 37: 201-210.

Heymann, K., Lehmann, J., Solomon, D., Schmidt, M. W. I. and Regier, T. 2011. C 1s K-edge near edge X-ray absorption fine structure (NEXAFS) spectroscopy for characterizing functional group chemistry of black carbon. Organic Geochemistry 42: 1055-1064.

Hseu, Z.-Y. and Huang, C.-C. 2005. Nitrogen mineralization potentials in three tropical soils treated with biosolids. Chemosphere 59: 447-454.

Imamura, M., Shimada, H., Matsubayashi, N., Yumura, M., Uchida, K., Oshima, S., Kuriki, Y., Yoshimura, Y., Sato, T. and Nishijima, A. 1994. C K-edge X-ray absorption near-edge structure of carbon nanotubes. Japanese Journal of Applied Physics 33: 1016-1018.

Jacobs, L. W. and McCreary, D. S. 2003. Utilizing biosolids on agricultural land, Michigan State University Extension.

Jamal, A., Norieh, N. and Farzadkia, M. 2011. Comparison of aerobic and lime stabilization methods for evaluation of sewage sludge reuse. Journal of Environmental Science & Technology 4: 182-190.

Jin, V. L., Johnson, M.-V. V., Haney, R. L. and Arnold, J. G. 2011. Potential carbon and nitrogen mineralization in soils from a perennial forage production system amended with class B biosolids. Agriculture, Ecosystems & Environment 141: 461-465.

Jjemba, P. K. 2002. The potential impact of veterinary and human therapeutic agents in manure and biosolids on plants grown on arable land: a review. Agriculture, Ecosystems & Environment 93: 267-278.

Jokic, A., Cutler, J. N., Ponomarenko, E., van der Kamp, G. and Anderson, D. W. 2003. Organic carbon and sulphur compounds in wetland soils: insights on structure and transformation processes using K-edge XANES and NMR spectroscopy. Geochimica et Cosmochimica Acta 67: 2585-2597.

Joshua, W. D., Michalk, D. L., Curtis, I. H., Salt, M. and Osborne, G. J. 1998. The potential for contamination of soil and surface waters from sewage sludge (biosolids) in a sheep grazing study, Australia. Geoderma 84: 135-156.

Jurado-Guerra, P., Wester, D. B. and Fish, E. B. 2006. Soil nitrate nitrogen dynamics after biosolids application in a Tobosagrass desert grassland. J. Environ. Qual. 35: 641-650.

Khalilian, A., Sullivan, M. J., Mueller, J. D., Wolack, F. J., Williamson, R. E. and Lippert, R. M. 1998. Composted municipal solid waste applications impacts on cotton yield and soil properties. Composting in the Southeast. 193-202.

Khan, S. A., Mulvaney, R. L., Ellsworth, T. R. and Boast, C. W. 2007. The myth of nitrogen fertilization for soil carbon sequestration. J. Environ. Qual. 36: 1821-1832.

Kinchesh, P., Powlson, D. S. and Randall, E. W. 1995. 13C NMR studies of organic matter in whole soils: I. Quantitation possibilities. European Journal of Soil Science 46: 125-138.

King, L. D. 1973. Mineralization and gaseous loss of nitrogen in soil-applied liquid sewage sludge. J. Environ. Qual. 2: 356-358.

Koenig, R. T., Cogger, C. G. and Bary, A. I. 2011. Dryland winter wheat yield, grain protein, and soil nitrogen responses to fertilizer and biosolids applications. Applied and Environmental Soil Science 2011.

Kögel-Knabner, I. 2000. Analytical approaches for characterizing soil organic matter. Organic Geochemistry 31: 609-625.

Kölbl, A. and Kögel-Knabner, I. 2004. Content and composition of free and occluded particulate organic matter in a differently textured arable Cambisol as revealed by solid-state ^{13}C NMR spectroscopy. Journal of Plant Nutrition and Soil Science 167: 45-53.

Kong, A. Y. Y., Six, J., Bryant, D. C., Denison, R. F. and van Kessel, C. 2005. The relationship between carbon input, aggregation, and soil organic carbon stabilization in sustainable cropping systems. Soil Sci. Soc. Am. J. 69: 1078-1085.

Koningsberger, D. C. and Prins, R. 1987. X-ray absorption: Principles, applications, techniques of EXAFS, SEXAFS and XANES. New York, NY, John Wiley and Sons Inc.

Krogmann, U., Qu, M., Boyles, L. S. and Martel, C. J. 1998. Biosolids and sludge management. Water Environment Research: 557-580.

Lal, R. 2002. Soil carbon sequestration in China through agricultural intensification, and restoration of degraded and desertified ecosystems. Land Degradation & Development 13: 469-478.

Lal, R. 2004. Soil carbon sequestration impacts on global climate change and food security. Science 304: 1623-1627.

Leifeld, J., Siebert, S. and Kögel-Knabner, I. 2002. Changes in the chemical composition of soil organic matter after application of compost. European Journal of Soil Science 53: 299-309.

Leinweber, P., Kruse, J., Walley, F. L., Gillespie, A., Eckhardt, K.-U., Blyth, R. I. R. and Regier, T. 2007. Nitrogen K-edge XANES - an overview of reference compounds used to identify `unknown' organic nitrogen in environmental samples. Journal of Synchrotron Radiation 14: 500-511.

Lindemann, W. C. and Cardenas, M. 1984. Nitrogen mineralization potential and nitrogen transformations of sludge-amended soil. Soil Sci. Soc. Am. J. 48: 1072-1077.

Lindsay, B. J. and Logan, T. J. 1998. Field response of soil physical properties to sewage sludge. J. Environ. Qual. 27: 534-542.

Magdoff, F. R. and Amadon, J. F. 1980. Nitrogen availability from sewage sludge. J. Environ. Qual. 9: 451-455.

Maguire, R. O., Sims, J. T. and Coale, F. J. 2000. Phosphorus solubility in biosolids-amended farm soils in the Mid-Atlantic region of the USA. J. Environ. Qual. 29: 1225-1233.

Maguire, R. O., Sims, J. T., Dentel, S. K., Coale, F. J. and Mah, J. T. 2001. Relationships between biosolids treatment process and soil phosphorus availability. J. Environ. Qual. 30: 1023-1033.

Mahieu, N., Randall, E. and Powlson, D. 1999a. Statistical analysis of published carbon-^{13}CPMAS NMR spectra of soil organic matter. Soil Sci. Soc. Am. J. 63: 307-319.

Mahieu, N., Randall, E. W. and Powlson, D. S. 1999b. Statistical analysis of published carbon-^{13} CPMAS NMR spectra of soil organic matter. Soil Sci. Soc. Am. J. 63: 307-319.

Mao, J., Olk, D. C., Fang, X., He, Z. and Schmidt-Rohr, K. 2008. Influence of animal manure application on the chemical structures of soil organic matter as investigated by advanced solid-state NMR and FT-IR spectroscopy. Geoderma 146: 353-362.

Mathers, N. J., Jalota, R. K., Dalal, R. C. and Boyd, S. E. 2007. 13C-NMR analysis of decomposing litter and fine roots in the semi-arid Mulga Lands of southern Queensland. Soil Biology and Biochemistry 39: 993-1006.

Mathers, N. J. and Xu, Z. 2003. Solid-state ^{13}C NMR spectroscopy: characterization of soil organic matter under two contrasting residue management regimes in a 2-year-old pine plantation of subtropical Australia. Geoderma 114: 19-31.

Mehring, M. and Physicist, G. 1976. High resolution NMR spectroscopy in solids, Springer-Verlag Berlin. Volume 11: 1-246.

Mendoza, C., Assadian, N. W. and Lindemann, W. 2006. The fate of nitrogen in a moderately alkaline and calcareous soil amended with biosolids and urea. Chemosphere 63: 1933-1941.

Moffet, C. A., Zartman, R. E., Wester, D. B. and Sosebee, R. E. 2005. Surface biosolids application: Effects on infiltration, erosion, and soil organic carbon in Chihuahuan desert grasslands and shrublands. J. Environ. Qual. 34: 299-311.

Morra, M. J., Fendorf, S. E. and Brown, P. D. 1997. Speciation of sulfur in humic and fulvic acids using X-ray absorption near-edge structure (XANES) spectroscopy. Geochimica et Cosmochimica Acta 61: 683-688.

Mullins, O. C., Mitra-Kirtley, S., Van Elp, J. and Cramer, S. P. 1993. Molecular structure of nitrogen in coal from XANES spectroscopy. Appl. Spectrosc. 47: 1268-1275.

Novak, J. M. and Watts, D. W. 2004. Increasing the phosphorus sorption capacity of southeastern coastal plain soils using water treatment residuals. Soil Sci. 169: 206-214.

Oberle, S. L. and Keeney, D. R. 1994. Interactions of sewage sludge with soil-crop-water systems. Sewage Sludge: Land Utilization and the Environment. Clapp, C. E., Larson, W. E. and Dowdy, R. H. Madison, WI, ASA, CSSA, SSSA: *pp.* 17-20.

Oleszkiewicz, J. and Mavinic, D. 2002. Wastewater biosolids: an overview of processing, treatment, and management. Journal of Environmental Engineering and Science 1: 75-88.

Parnaudeau, V., Nicolardot, B. and Pagès, J. 2004. Relevance of organic matter fractions as predictors of wastewater sludge mineralization in soil. J. Environ. Qual. 33: 1885-1894.

Post, W. M. and Kwon, K. C. 2000. Soil carbon sequestration and land-use change: processes and potential. Global Change Biology 6: 317-327.

Preston, C. M. 1996. Applications of NMR to soil organic matter analysis: History and prospects. Soil Science 161: 144-166.

Preston, C. M. 2001. Carbon ^{13} solid-state NMR of soil organic matter - using the technique effectively. Canadian Journal of Soil Science 81: 255-270.

Preston, C. M., Newman, R. H. and Rother, P. 1994. Using ^{13}C CPMAS NMR to assess effects of cultivation on the organic matter of particle size fractions in a grassland soil. Soil Science 157: 26-35.

Prietzel, J., Thieme, J., Salomé, M. and Knicker, H. 2007. Sulfur K-edge XANES spectroscopy reveals differences in sulfur speciation of bulk soils, humic acid, fulvic acid, and particle size separates. Soil Biology and Biochemistry 39: 877-890.

Pu, G., Bell, M., Barry, G. and Want, P. 2012. Estimating mineralisation of organic nitrogen from biosolids and other organic wastes applied to soils in subtropical Australia. Soil Research 50: 91.

Quemada, M., Lasa, B., Lamsfus, C. and Aparicio-Tejo, P. M. 1998. Ammonia volatilization from surface or incorporated biosolids by the addition of dicyandiamide. J. Environ. Qual. 27: 980-983.

Robertson, J. and O’reilly, E. 1987. Electronic and atomic structure of amorphous carbon. Physical Review B 35: 2946.

Roscoe, R., Buurman, P., Velthorst, E. and Vasconcellos, C. 2001. Soil organic matter dynamics in density and particle size fractions as revealed by the ^{13}C ^{12}C isotopic ratio in a Cerrado's oxisol. Geoderma 104: 185-202.

Rowell, D. M., Prescott, C. E. and Preston, C. M. 2001. Decomposition and nitrogen mineralization from biosolids and other organic materials: relationship with initial chemistry. J. Environ. Qual. 30: 1401-1410.

Ryan, J. A. and Keeney, D. R. 1975. Ammonia volatilization from surface-applied wastewater sludge. Water Poll. Control Fed. J. 47: 386-393.

Ryan, J. A., Keeney, D. R. and Walsh, L. M. 1973. Nitrogen transformations and availability of an anaerobically digested sewage sludge in soil1. J. Environ. Qual. 2: 489-492.

Sabey, B. R. and Hart, W. E. 1975. Land application of sewage sludge: I. Effect on growth and chemical composition of plants. J. Environ. Qual. 4: 252-256.

Sánchez-Monedero, M. A., Mondini, C., de Nobili, M., Leita, L. and Roig, A. 2004. Land application of biosolids. Soil response to different stabilization degree of the treated organic matter. Waste Management 24: 325-332.

Sarkar, D., Ferguson, M., Datta, R. and Birnbaum, S. 2005. Bioremediation of petroleum hydrocarbons in contaminated soils: comparison of biosolids addition, carbon supplementation, and monitored natural attenuation. Environmental Pollution 136: 187-195.

Schmidt-Rohr, K. and Spiess, H. W. 1994. Multidimensional solid-state NMR and polymers, Academic Press London.

Schmidt, M., Skjemstad, J., Gehrt, E. and Kögel ‐ Knabner, I. 2001. Charred organic carbon in German chernozemic soils. European Journal of Soil Science 50: 351-365.

Schulten, H. R. and Leinweber, P. 1991. Influence of long-term fertilization with farmyard manure on soil organic matter: Characteristics of particle-size fractions. Biology and Fertility of Soils 12: 81-88.

Schumacher, M., Christl, I., Scheinost, A. C., Jacobsen, C. and Kretzschmar, R. 2005. Chemical heterogeneity of organic soil colloids investigated by scanning transmission X-ray microscopy and C-1s NEXAFS microspectroscopy. Environmental Science & Technology 39: 9094-9100.

Sedlmair, J., Geber, S.-C., Peth, C., Mann, K. and Thieme, J. 2009. NEXAFS spectroscopy with a laser plasma x-ray source on soil samples. Journal of Physics: Conference Series 186: 012034.

Six, J., Conant, R. T., Paul, E. A. and Paustian, K. 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant and Soil 241: 155-176.

Skjemstad, J., Clarke, P., Taylor, J., Oades, J. and Newman, R. 1994. The removal of magnetic materials from surface soils-a solid state ^{13}C CP/MAS NMR study. Soil Research 32: 1215-1229.

Skjemstad, J. O., Clarke, P., Taylor, J., Oades, J. M. and McClure, S. 1996. The chemistry and nature of protected carbon in soil. Soil Research 34: 251-271.

Smernik, R. J., Oliver, I. W. and Merrington, G. 2003. Characterization of sewage sludge organic matter using solid-state carbon-13 nuclear magnetic resonance spectroscopy. J. Environ. Qual. 32: 1516-1522.

Smith, S. R. 1996. The agricultural recycling of sewage sludge and the environment. CABI Wallingford, UK.

Sollins, P., Swanston, C. and Kramer, M. 2007. Stabilization and destabilization of soil organic matter—a new focus. Biogeochemistry 85: 1-7.

Solomon, D., Lehmann, J., Harden, J., Wang, J., Kinyangi, J., Heymann, K., Karunakaran, C., Lu, Y., Wirick, S. and Jacobsen, C. 2012. Micro- and nano-environments of carbon sequestration: Multi-element STXM–NEXAFS spectromicroscopy assessment of microbial carbon and mineral associations. Chemical Geology.

Solomon, D., Lehmann, J., Kinyangi, J., Liang, B. and Schäfer, T. 2005. Carbon K-edge NEXAFS and FTIR-ATR spectroscopic investigation of organic carbon speciation in soils. Soil Sci. Soc. Am. J. 69: 107-119.

Solomon, D., Lehmann, J. and Martínez, C. E. 2003. Sulfur K-edge XANES spectroscopy as a tool for understanding sulfur dynamics in soil organic matter. Soil Sci. Soc. Am. J. 67: 1721-1731.

Sommers, L., Parker, C. and Meyers, G. 1981. Volatilization, plant uptake and mineralization of nitrogen in soils treated with sewage sludge, Indiana Water Resources Research Center. 133.

Spargo, J. T., Alley, M. M., Follett, R. F. and Wallace, J. V. 2008. Soil carbon sequestration with continuous no-till management of grain cropping systems in the Virginia coastal plain. Soil Till. Res. 100: 133-140.

Spargo, J. T., Evanylo, G. K. and Alley, M. M. 2006. Repeated compost application effects on phosphorus runoff in the Virginia Piedmont. J. Environ. Qual. 35: 2342-2351.

Stanford, G. and Smith, S. J. 1972. Nitrogen mineralization potentials of soils. Soil Sci. Soc. Am. J. 36: 465-472.

Stehouwer, R. 1999. Land application of sewage sludge in Pennsylvania: Use of biosolids in crop production. Coop. Ext. Fact Sheet UC163. Pennsylvania State Univ., University Park.

Stewart, C. E., Follett, R. F., Wallace, J. and Pruessner, E. G. 2012. Impact of biosolids and tillage on soil organic matter fractions: Implications of carbon saturation for conservation management in the Virginia Coastal Plain. Soil Sci. Soc. Am. J. 76: 1257-1267.

Stewart, C. E., Plante, A. F., Paustian, K., Conant, R. T. and Six, J. 2008. Soil carbon saturation: linking concept and measurable carbon pools. Soil Sci. Soc. Am. J. 72: 379-392.

Stover, H. D. H. and Frechet, J. M. J. 1989. Direct polarization carbon-13 and proton magic angle spinning NMR in the characterization of solvent-swollen gels. Macromolecules 22: 1574-1576.

Sukkariyah, B., Evanylo, G. and Zelazny, L. 2007. Distribution of copper, zinc, and phosphorus in Coastal Plain Soils receiving repeated liquid biosolids applications. J. Environ. Qual. 36: 1618-1626.

Sukkariyah, B. F., Evanylo, G., Zelazny, L. and Chaney, R. L. 2005. Cadmium, copper, nickel, and zinc availability in a biosolids-amended Piedmont soil years after application. J. Environ. Qual. 34: 2255-2262.

Terry, R. E., Nelson, D. W. and Sommers, L. E. 1979. Decomposition of anaerobically digested sewage sludge as affected by soil environmental conditions. J. Environ. Qual. 8: 342-347.

Terry, R. E., Nelson, D. W. and Sommers, L. E. 1981. Nitrogen transformations in sewage sludge—amended soils as affected by soil environmental factors. Soil Sci. Soc. Am. J. 45: 506-513.

Terry, R. E., Nelson, D. W., Sommers, L. E. and Meyer, G. J. 1978. Ammonia volatilization from wastewater sludge applied to soils. Water Poll. Control Fed. J. 50: 2657-2665.

Tian, G., Granato, T. C., Cox, A. E., Pietz, R. I., Carlson, C. R. and Abedin, Z. 2009. Soil carbon sequestration resulting from long-term application of biosolids for land reclamation. J. Environ. Qual. 38: 61-74.

Tiessen, H. and Moir, J. 1993. Total and organic carbon. Soil Sampling Methods of Analysis, M. R, Carter, Ed., Canadian Society of Soil Science: 187-199.

Traina, S. J., Novak, J. and Smeck, N. E. 1990. An ultraviolet absorbance method of estimating the percent aromatic carbon content of humic acids. J. Environ. Qual. 19: 151-153.

Tsadilas, C. D., Mitsios, I. K. and Golia, E. 2005. Influence of biosolids application on some soil physical properties. Communications in Soil Science and Plant Analysis 36: 709-716.

Turner, J. A., Thomas, K. M. and Russell, A. E. 1997. The identification of oxygen functional groups in carbonaceous materials by oxygen K-edge XANES. Carbon 35: 983-992.

USEPA 1983. Process Design Manual for Land Application of Municipal Sludge. USEPA-625/1-83-016. Washington, DC., U.S. Environmental Protection Agency.

USEPA 1995a. Process design for agricultural utilization. In Process design manual—Land application of sewage sludge and domestic septage. EPA/625/R-95/001. Washington, DC., U.S. Environmental Protection Agency: 6:1-6:48.

USEPA 1995b. Process design manual: Land application of sewage sludge and domestic septage. USEPA Rep. 625/R-95/001. USEPA. Washington, D.C., U.S. Gov. Print. Office.

Vereecken, H., Maes, J., Feyen, J. and Darius, P. 1989. Estimating the soil moisture retention characteristic from texture, bulk density, and carbon content. Soil Science 148: 389.

Wan, J., Tyliszczak, T. and Tokunaga, T. K. 2007. Organic carbon distribution, speciation, and elemental correlations within soil microaggregates: Applications of STXM and NEXAFS spectroscopy. Geochimica et Cosmochimica Acta 71: 5439-5449.

Wang, H., Kimberley, M. O. and Schlegelmilch, M. 1997. Biosolids-derived nitrogen mineralization and transformation in forest soils. J. Environ. Qual. 32: 1851-1856.

Wang, H., Kimberley, M. O. and Schlegelmilch, M. 2003. Biosolids-derived nitrogen mineralization and transformation in forest soils. J. Environ. Qual. 32: 1851-1856.

Warman, P. R. and Termeer, W. C. 2005. Evaluation of sewage sludge, septic waste and sludge compost applications to corn and forage: yields and N, P and K content of crops and soils. Bioresource Technology 96: 955-961.

Watts, B., Thomsen, L. and Dastoor, P. C. 2006. Methods in carbon K-edge NEXAFS: Experiment and analysis. Journal of Electron Spectroscopy and Related Phenomena 151: 105-120.

Waugh, J., Huber, L. and Haeberlen, U. 1968. Approach to high-resolution NMR in solids. Physical Review Letters 20: 180-182.

White, C. S., Loftin, S. R. and Aguilar, R. 1997. Application of biosolids to degraded semiarid rangeland: Nine-year responses. J. Environ. Qual. 26: 1663-1671.

Wilson, M. A. 1987. NMR techniques and applications in geochemistry and soil chemistry. Oxford, Pergamon Press.

Wilson, M. A., Pugmire, R. J., Zilm, K. W., Goh, K. M., Heng, S. and Grant, D. M. 1981. Cross-polarization 13C-NMR spectroscopy with /`magic angle/' spinning characterizes organic matter in whole soils. Nature 294: 648-650.

Wong, J. W. C., Lai, K. M., Fang, M. and Ma, K. K. 1998. Effect of sewage sludge amendment on soil microbial activity and nutrient mineralization. Environment International 24: 935-943.

Wu, L., Ma, L. and Martinez, G. 2000. Comparison of methods for evaluating stability and maturity of biosolids compost. J. Environ. Qual. 29: 424-429.

Zebarth, B. J., McDougall, R., Neilsen, G. and Neilsen, D. 2000. Availability of nitrogen from municipal biosolids for dryland forage grass. Canadian Journal of Plant Science 80: 575-582.

3. The Effects of Long-term Application of Organic Residuals on Carbon Sequestration

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3.1. Abstract

Application of organic residual by-products (e.g. biosolids, composts) to soil may provide an effective method for sequestering considerable amounts of carbon (C), but the long term stability of such C is not well known. We investigated study sites in Virginia to determine the amounts of C remaining in soils several years following amending with biosolids and composts. The first study employed a Fauquier silty clay loam (*Fine, mixed, mesic Ultic Hapludalfs*) to which four treatments (control, poultry litter-yard waste compost, biosolids-woodchip compost, and poultry litter) were continuously applied during 2000-2004. The second study was conducted on a Davidson clay loam (*clayey, kaolinitic, thermic, Rhodic Paleudults*) to which six rates of aerobically digested biosolids (0, 42, 84, 126, 168, and 210 Mg ha^{-1}) were applied in 1984. The third study was on a Pamunkey sandy loam (*Fine-loamy, mixed, thermic Ultic, Hapludalfs*) to which five rates of anaerobically digested biosolids (0, 14, 42, 70, and 98 Mg ha^{-1}), with and without sawdust were applied in 1996. Total soil organic C concentration and bulk density were measured to calculate C accumulation. The organic residual-treated soils increased C in the surface soil depth (< 15 cm), ranging from 2 to 12 % of C across all three sites. Soil C movement was limited to a depth of 15 cm. Evidence of C saturation was revealed in the third study site. These results indicate that organic amendments applied over long time remain in soil and benefit C sequestration in Mid-Atlantic region.

3.2. Introduction

Increasing atmospheric carbon dioxide (CO_2) concentration has been identified as the main factor leading to global warming (IPCC, 1990). Carbon (C) sequestration may play an important role in mitigating carbon dioxide emission (Batjes, 1998; Lal, 2004). Organic residual by products, e.g., biosolids and composts, utilized as the replacement of synthetic fertilizers may offer an additional benefit as a practice for sequestering soil C (Lal, 2007). Land application of organic residuals improves soil aggregate stability, nutrient capacity, and microbial activity, while simultaneously storing soil C (Zebarth *et al.*, 1999; Foley and Cooperband, 2002; Kirchhoff *et al.*, 2004; Ros *et al.*, 2006).

There is much evidence in the literature that application of organic amendments leads to an increase in the soil organic C (SOC) content (Bulluck III *et al.*, 2002; Spargo *et al.*, 2008b; Franzluebbers, 2010) . For example, Lindsay and Logan (1998) reported that soil organic C in a Miamian silt loam in Ohio increased linearly with biosolids application, and 4 yr after application there was three times as much C in the highest rate of biosolids plots as in the control plots. Tian *et al.* (2009) reported that the SOC sequestration rate was 1.73 Mg C ha^{-1} yr^{-1} in biosolids-amended fields after 34 annual applications in Illinois. Spargo *et al.* (2008b) reported that a history of biosolids application resulted in an increase of 4.19 ± 1.93 Mg C ha^{-1} in the surface 15 cm of soils in the Coastal Plain of Virginia. Whalen *et al.* (2008) investigated composted cattle manure applied in a sandy-loam Humic Gleysol in Quebec at rates of 0, 5, 10 and 15 Mg ha^{-1} and found that after 5 years, compost-amended plots gained 1.35 to 2.02 Mg C ha^{-1} yr^{-1}.

Soil C accumulation depends on the C inputs and decomposition (Johnston *et al.*, 2009). In many cases there is a positive relationship between C inputs from the organic residual applied and the resulting SOC content (Fortuna *et al.*, 2003; Brown *et al.*, 2011). Mature compost leads to a higher SOC concentration than uncomposted materials do, even if comparable amounts of C inputs are evaluated from both amendments (Busby *et al.*, 2007). Pasture retains more crop residues and thus has greater potential to store natural SOC than crop land (Franzluebbers *et al.*, 2000; Post and Kwon, 2000).

There are several factors that determine organic C decomposition. Organic matter mineralization rates increase with amount of organic residuals applied and decline over time (Boyle and Paul, 1989; Logan *et al.*, 1997). Changes in soil structure associated with tillage can significantly affect organic matter decomposition rate (Krull *et al.*, 2003). Higher clay content and cooler temperature contribute to lower organic C decomposition rates (Causarano *et al.*, 2008), while the C decomposition rate is generally greater in sandy soils than in clayey soils, as a result of greater aeration in sandy soils as well as hot and wet conditions (Sukkariyah *et al.*, 2007).

Generally, soil C accumulation is modified by first-order kinetics (Boyle and Paul, 1989), which assumes that soil C stocks are linearly proportional to C inputs (Ågren and Bosatta, 1987). This linearity means that soil C accumulation efficiency (CAE) (i.e. ΔSOC/ΔC input) is constant and that equilibrium soil C stocks can increase continuously and without limit with C inputs increase (Stewart *et al.*, 2007). Some studies suggest a new concept, C saturation, which assumes that soil C stocks may reach an upper limit and reveal an asymptotic relationship with respect to C input (Six *et al.*, 2002). As a soil approaches a saturation limit, soils retain less C stocks with more added C (Kong *et al.*, 2005). Usually, SOC can be physically protected by silt and clay particles and soil aggregates, as the association between organic matter and minerals will retain soil C (Hassink, 1997; Stewart *et al.*, 2012). Coarse-textured soils, e.g., Coastal Plain soils, appear to have more limits to soil C accumulation than fine-textured soils, e.g., Piedmont soils (Stewart *et al.*, 2008).

Some experiments show little or no increase in SOC content with increase in C inputs (Campbell *et al.*, 1991). Sukkariyah *et al.* (2007) investigated a Coastal Plain Bojac sandy loam after application of 154 dry Mg ha^{-1} of biosolids for 15 years and found no increase in SOC to a depth of 25 cm in biosolids-amended soils. Most investigations rely on conceptual modeling of C saturation limits (Six *et al.*, 2002; Stewart *et al.*, 2007); thus, very few studies have reported evidence of C saturation from organic-amended soils, especially in the mid-Atlantic region.

Previous studies have largely reported on-farm observations (Shober *et al.*, 2003; Tian *et al.*, 2009), where the sampling designs may suffer from potentially uncontrolled conditions (Franzluebbers, 2010). Analysis of soil C accretion and/or decomposition in long term replicated field studies is desirable. Furthermore, there have been few studies that document vertical transport of applied C through soils >30 cm (Fontaine *et al.*, 2007). The objectives of this study were to: 1) to investigate C sequestration in three study sites across Virginia that received organic amendments over periods of long time, 2) to assess vertical soil C accumulation to a depth of 60 cm, and 3) to test the evidence of soil C saturation theory.

3.3. Materials and Methods

3.3.1. Experimental sites

<u>Study site I</u>

This experimental site was located at the Northern Piedmont Agricultural Research and Extension Center (NPAREC) on a Fauquier silty clay loam (*Fine, mixed, mesic Ultic Hapludalfs*) in Orange, VA. Eight treatments were established in the spring of 2000 to investigate the agronomic and environmental effects of compost use in the Virginia Piedmont (Table 3.1) (Bowden *et al.*, 2010). The soil properties prior to organic amendment application are shown in Table 3.2. The treatments were arranged in a randomized complete block design with four replicates. Each plot measured 3.6 m wide by 7.5 m long. The following four treatments were selected for reporting in this study as: unamended control (CTL); poultry litter (PL); poultry litter-yard waste compost (PYC); and biosolids-woodchip compost (BSC).

The PL was annually applied at rates estimated to meet crop N requirements. The compost treatments established in 2000 were biennial (2000, 2002) agronomic N rates of poultry litter-yard waste compost with (2001) and without supplemental inorganic fertilizer N. Then beginning in the spring of 2003, two of the four replicates of each of the two biennial compost treatments continued to receive annual (2003, 2004) applications of agronomic N rates of poultry litter-yard waste compost, and the other two replicates from the same treatments began to receive annual (2003, 2004) applications of agronomic N rates of a biosolids-woodchip compost. No organic amendment or inorganic fertilizer was applied beyond spring 2005. However, in August 2010, N fertilizer was applied to the entire site for tall fescue maintenance. Lime was applied in April 2004 to achieve a target pH of 7 to all plots except the BSC treatment, as lime was added during treatment of biosolids .

The poultry litter-yard waste compost (PYC) was a commercially-produced material (PYC; Panorama Farms, Earlysville, Virginia), whose poultry litter and yard waste feedstocks were combined at a ratio of 1:2 (v/v) and composted using turned windrow technology for 120 days. The biosolids-woodchip compost (BSC) was produced at the Rivanna Water and Sewer Authority (Charlottesville, Virginia) from anaerobically-digested biosolids dewatered with $Ca(OH)_2$ and composted with wood chips (1:2 ratio of biosolids and woodchips) for 21 days, including five consecutive days at 66°C, via static pile technology. The material was cured for an additional 10 days after screening through a 0.95 cm sieve to remove oversized woodchips. A commercially-processed, screened poultry litter (PL; Glen Hill Farm, Harrisonburg, Virginia) was used for the poultry litter treatment. Chemical properties of the organic residuals applied are summarized in Table 3.3. Soil amendments were hand-applied and incorporated within 24 h associated with seedbed preparation by roto-tilling.

The initial crops included pumpkin (*Cucurbita pepo* V. Magic Lantern) in 2000, sweet corn (*Zea mays* L. V. Silver Queen) in 2001, and bell pepper (*Capsicum annuum* V. Aristotle) in 2002. Following modification of the treatments in spring 2003, corn (*Zea mays* L. V. Pioneer 31G20) was grown in 2003 and 2004 and soybean (*Glycine max* V. Delta Pine 4933RR) was grown in 2005. Cereal rye (*Secale cereal* L.) was planted in all plots in the autumn of 2000-2005 as a winter cover crop. Tall fescue (*Festuca arundinacea* Schreb.) was planted in spring 2006 and has been maintained in all plots with twice annual bush hogging as the only management practice.

Study site II

This experimental site was also located at the NPAREC on a Davidson clay loam (*Clayey, kaolinitic, thermic, Rhodic Paleudults*). Six rates of aerobically digested biosolids (0, 42, 84, 126, 168, and 210 dry Mg ha^{-1}; the agronomic rate of biosolids was 42 Mg ha^{-1} for corn) were applied in single applications in the spring of 1984 (Table 3.1) (Rappaport *et al.*, 1988). Soil properties prior to organic amendment application are shown as Table 3.2. The treatments were arranged in a randomized complete block design with four replicates. Each plot consisted of a volume of soil 2.3 m long x 1.5 m wide x 0.9 m high isolated by plastic wrapping below ground and wooden boards above ground to prevent lateral movement of biosolids constituents. Treatments included a control (CTL), 42 dry Mg ha^{-1} biosolids (BS 1x), 84 dry Mg ha^{-1} biosolids (BS 2x), 126 dry Mg ha^{-1} biosolids (BS 3x), 168 dry Mg ha^{-1} biosolids (BS 4x), and 210 dry Mg ha^{-1} biosolids (BS 5x).

The aerobically digested biosolids were obtained from a Bristol, VA wastewater treatment plant having high industrial inputs. The chemical properties of the organic residuals applied are shown in Table 3.3. The biosolids were dewatered on sand beds, which allowed for maximum NH_3 volatilization and NO_3^- leaching losses; therefore, the biosolids contained negligible amounts of inorganic N (<8 mg kg^{-1}). The biosolids were incorporated into the soils to a depth of approximately 20 cm in spring 1984 prior to planting. The plots were rototilled every year to a depth of 15 cm from 1984-2005.

Corn (*Zea mays* L.) was grown each year from 1984-2000, except in 1994 when sorghum (*Sorghum bicolor* L. V. Dekalb 41Y) was planted instead of corn because of drought. Corn varieties were Pioneer 3192 in 1984–1990 and 1996–2000 and Pioneer

3136 in 1991–1995. Radish (*Raphanus sativus* L.) and Romaine lettuce (*Lactuca sativa* V. longifolia) cultivar Parris Island Cos replaced corn in the cropping system from 2001 to 2004. Winter barley (*Hordeum vulgare* L. V. Nomini) was grown in 2002, 2004 and 2005. The plots had been left fallow since 2005, with an occasional herbicide application to reduce weed pressure. Lime applications in 1989 and 1998 were made to raise the pH to 6.0.

Study site III

This experimental site was located on a Pamunkey sandy loam (*Fine-loamy, mixed, thermic Ultic Hapludalfs*) in Charles City County, VA. Five rates of anaerobically digested biosolids (BS) (0, 14, 42, 70, and 98 dry Mg ha^{-1}; the agronomic rate of biosolids was 14 Mg ha^{-1}), with and without sawdust (SD) to adjust the C:N ratio to 20:1, were applied in single applications in March 1996 (Table 3.1) (Daniels *et al.*, 2003). Soil properties prior to organic amendment application are summarized in Table 3.2. The treatments were arranged in a randomized complete block design with four replicates. Each plot was approximately 36 x 15 m in size. The entire area of each experimental block was approximately 3 ha. The following seven treatments were selected for reporting in this study: control (CTL), 14 dry Mg ha^{-1} biosolids (BS 1x), 42 dry Mg ha^{-1} biosolids (BS 3x), 70 dry Mg ha^{-1} biosolids (BS 5x), 98 dry Mg ha^{-1} biosolids (BS 7x), 42 dry Mg ha^{-1} biosolids+sawdust (BS 3x+SD), and 98 dry Mg ha^{-1} biosolids+sawdust (BS 7x+SD).

The anaerobically digested secondary biosolids was obtained from Chesterfield, VA. The chemical properties of the organic residuals applied are shown in Table 3.3. Biosolids N composition required a dry biosolids : sawdust ratio of 0.75:1 to attain the desired C:N ratio (20:1). The sawdust had a bulk C: N ratio of 198:1. The biosolids with and without sawdust were surface-applied to the soils in the spring of 1996 prior to planting.

A crop rotation of corn (*Zea mays* L.; planted April 1996), wheat (*Triticum aestivum;* planted November 1996), soybean (*Glycine max*; planted July 1997) and cotton (*Gossypium spp*.; planted in 1998) have been grown at the site since the establishment of the treatments. After the application of biosolids in 1996, this crop rotation has been managed as no-till system from 1997-2011 with only essential N, P, and K fertilizers applied to all the plots.

At each study site, nitrogen needs for crops were determined with the Virginia Agronomic Land Use Evaluation System (VALUES) (Simpson *et al.*, 1993). Phosphorus and potassium requirements were determined by Virginia Cooperative Extension soil testing results (Donohue and Heckendorn, 1994). Agronomic practices, seedbed preparation, pest control, and weeding control were performed according to Virginia Cooperative Extension recommendations (Virginia Cooperative Extension, 1992). The lime requirement was determined by Virginia Cooperative Extension soil testing results (Donohue and Heckendorn, 1994).

3.3.2. Soil sampling and analysis

Three soil cores measuring 5 cm in diameter were collected in plastic sleeves to a depth of 0.6 m using a Giddings hydraulic soil probe from selected treatments plots identified above. After removing the cores, bentonite was added to the holes to fill the lower 45 cm. The top 15 cm was filled with topsoil from the same plot. The intact cores were returned to the laboratory and sectioned into 0-7.5, 7.5-15, 15-30, 30-45, and 45-60 cm increments. Bulk density (BD) was determined on each core at each depth increment after oven drying (105^0C) a subsample to determine moisture content. For carbon analysis, soil samples were air-dried and ground to pass a 0.5 mm sieve for total SOC analysis by dry combustion in a Vario Max CNS macro elemental analyzer (Elementar, GER). Soil C stock by depth was calculated by the following equation (Guo and Gifford, 2002):

$$Cs = \rho_b \times Cc \times d \times 10^{-3}$$

where Cs is soil C stock (Mg ha^{-1}), ρ_b is soil bulk density (g cm^{-3}), Cc is total soil organic carbon concentration (g kg^{-1}) and d is soil sampling depth (cm).

Dry matter and C loading rates of the organic amendments were obtained directly from our initial application and composition data, from previously published articles on the research, and/or estimated from volatile solids data. Carbon accumulation (Mg ha^{-1}) was calculated by subtracting C stocks of control (Csc) from those of organic treatments (Csa). Then the soil C accumulation efficiency (CAE) was calculated by:

$$CAE = \frac{Csa - Csc}{Css} \times 100\%$$

where Csa is C stock of the amended soils in 2011, Csc is C stock of the control in 2011, Css is C application rate (Mg ha^{-1}) from organic amendment applied.

In addition, the total soil C accumulation over time (Mg ha^{-1} yr^{-1}) from 0-60 cm was calculated by summing up soil C stocks from each five depth increment and the relationship between total soil C accumulation and the applied C level over time (Mg ha^{-1} yr^{-1}) was examined by linear and asymptotic regression analyses.

3.3.3. Statistical analysis

A random complete block design with subsampling was used to statistically analyze the soil C via PROC GLM Statistical Analysis System 9.2 (SAS Institute, 2008). Analysis of Variance (ANOVA) and Least Significant Difference (LSD) measurement were applied at level of 0.05 to compare differences between treatment means. The relationship between total annual soil C accumulation (Mg ha^{-1} yr^{-1}) at 0-60 cm and annual applied C level (Mg ha^{-1} yr^{-1}) was examined with linear and asymptotic regression analyses using the PROC REG procedure of SAS (SAS Institute, 2008).

3.4. Results and Discussion

3.4.1. Soil organic carbon concentrations

The SOC concentrations with depth from the three study sites are shown as Figures 3 a-c. In each site, organic residual treatments maintained higher SOC concentrations over the control at the surface soil depths (<15 cm). As expected, deeper SOC concentration was lower than surface SOC concentration. The subsoil (>15 cm) SOC concentrations were not different among treatments.

Study site I

At surface soil depths (≤15 cm), PYC maintained the highest level of SOC among all treatments, followed by BSC. No difference in SOC concentrations were found between PL and CTL. There were no effects of treatments on SOC concentration below 15 cm. The mean SOC concentrations in the subsoil were 13 g kg^{-1} at 15-30 cm, 8.2 g kg^{-1} at 30-45 cm, and 4.7 g kg^{-1} at 45-60 cm.

Previous studies at this site (Spargo *et al.*, 2006) have shown that after cessation of organic amendment application (2004), SOC concentration at 0-15 cm depth was increased by both compost treatments, but not PL treatment. SOC concentration in the PYC treatment was higher than that in the BSC treatment, and was more than twice as high as CTL and PL treatments. This trend was consistent with our results in 2011 (Figure 3.1a). In addition, this site is located in Piedmont region where higher clay content may have reduced SOC decomposition, resulting in overall greater SOC concentration (Causarano *et al.*, 2008). Mechanisms for SOC stabilization include surface complexation with clay minerals and physical protection in micro-pores formed by clay aggregates (Blanco-Canqui and Lal, 2004). Furthermore, this site was vegetated with an undisturbed tall fescue sod between 2006 and 2011, which likely increased the SOC content. When cropland is replaced by deep rooted hayland / pasture, SOC tends to increase below 100 cm depth (Guo and Gifford, 2002). Franzluebbers *et al.* (2000) reported that tall fescue (20 years old) had greater SOC (31%) at a depth of 0-20 cm than adjacent cropland under conservation-tillage (24 years old) in the Southern Piedmont region of the USA.

Study site II

At 0-7.5 cm depth, SOC concentration increased with increasing application rates of biosolids (Figure 3.1b). At 7.5-15 cm depth, the SOC concentration increased in the order as: CTL=BS 1x=BS 2x<BS 3x=BS 4x<BS 5x. There were no effects of treatments on SOC concentration below 15 cm. The mean average SOC concentrations in the subsurface soil were 7.5 g kg^{-1} at 15-30 cm, 3.6 g kg^{-1} at 30-45 cm, and 2.9 g kg^{-1} at 45-60 cm depth.

Previous studies at this site (Sukkariyah *et al.*, 2005) have indicated that SOC concentration (Figure 3.4) at 0-15 cm increased from 13 g kg^{-1} in the control to 38 g kg^{-1}

at the high biosolids rate in 1984 soon after biosolids application. By 1992, SOC concentration from biosolids-treated soils had decreased by 3 to 15 g kg^{-1}, but no change in SOC occurred in 1995 and 2001. These data show that organic matter mineralization rate decreased with time as the organic matter stabilized. By 2011, SOC concentrations in biosolids-treated soils had decreased by 2 to 5 g kg^{-1}. These results demonstrate that the greatest decrease in SOC appears to occur during the first 1-2 years after biosolids application, with only small changes in the following decades (Boyle and Paul, 1989; Logan *et al.*, 1997; Sloan *et al.*, 1998).

Study site III

At the 0-7.5 cm depth, SOC concentration increased with increasing application rates of biosolids (Figure 3.1c). Similar differences occurred at 7.5-15 cm depth. There were no effects of treatments on SOC concentration below 15 cm. The mean average SOC concentrations in the subsoil were 6.7 g kg^{-1} at 15-30 cm, 3.2 g kg^{-1} at 30-45 cm, and 2.8 g kg^{-1} at 45-60 cm depth.

Spargo *et al.* (2008) reported that biosolids increased the SOC by 2.9 g kg^{-1} at 0-7.5 cm depth of Coastal Plain soils under continuous no-till management, which is consistent with our studies in 2011 (2.6 g kg^{-1} at 0-7.5 cm with BS 1x application). The Coastal Plain soils may have greater organic matter mineralization rates and, thus, a relatively lower SOC concentration given the warm and moist climate (Causarano *et al.*, 2008). The SOC concentration was slightly higher in BS+SD treatment plots than in corresponding BS treatment plots in the surface soil (<15 cm). Despite the statistical significance of the increase in SOC concentration with the organic amendments, it is questionable whether such small increases in SOC will indicate more soil C accumulation potentials in these Coastal Plain soils.

3.4.2. Soil carbon stocks

The soil C stocks from the three study sites are shown as Figure 3.2 a-c. In each of the study sites, soil C stocks were significantly different among organic residual treatments in the surface soils (0-15 cm). Subsoil C stocks were lower than topsoil C stocks. The results indicate that the C inputs from these organic amendments did not move down to subsoil depths (>15 cm) over long time.

Study site I

On the surface soil depth (<15 cm), soil C stocks response to treatment revealed the same trend as SOC concentration. The soil C stocks decreased in the following order as: CTL=PL<BSC<PYC. The mean soil C stocks in the subsoil were not affected by treatment and averaged 20 Mg ha^{-1} at 15-30 cm, 13 Mg ha^{-1} at 30-45 cm, and 6.7 Mg ha^{-1} at 45-60 cm depth.

The PL treatment maintained lower soil C stocks than PYC and BSC treatments, likely due to less C applied with the manure than the compost. In addition, compost C is more stable than manure C due to microbial decomposition during the composting processes (Hartz *et al.*, 2000; Eghball, 2002). Furthermore, the narrow C:N ratio (8:1) in the PL was more favorable for organic matter mineralization than the higher C:N ratio compost. Carbon decomposition of composts is much less affected by time and application rate than manure C decomposition (Busby *et al.*, 2007).

Study site II

Soil C stocks increased with increasing application rates of biosolids, at 0-7.5 cm as: CTL<BS1x<BS2x<BS 3x=BS 4x =BS 5x, and at 7.5-15 cm as: CTL =BS 1x<BS2x<BS3x<BS4x<BS5x. The mean average soil C stocks in the subsoils were 11 Mg ha^{-1} at 15-30 cm, 5.1 Mg ha^{-1} at 30-45 cm, and 4.1 Mg ha^{-1} at 45-60 cm depth.

Despite the minimal management on this site between 2005 and 2011, C inputs due to biosolids application at the surface soil (< 15 cm) were still stable and identifiable after 27 years after application. Possible mechanisms for persistence of elevated C storage in amended soils include formation of stable aggregates (Brown *et al.*, 2011). Biosolids did not increase C stocks in subsurface soil layers (15-60 cm). The C movement into the deep soil profile was relatively slow.

Study site III

The overall trends for the changes in mass of soil C with depth were similar to changes in C concentration. In the soil surface (<15 cm), soil C stocks increased in the order: CTL=BS 1x<BS 3x=BS 5x<BS 7x. There were no differences between BS and BS+SD treatments. The mean average soil C stocks in the subsoils were 11 Mg ha^{-1} at 15-30 cm, 5.6 Mg ha^{-1} at 30-45 cm, and 4.9 Mg ha^{-1} at 45-60 cm depth.

Although woody materials, e.g., hardwood chips, contain more recalcitrant fractions of C (e.g., lignin, tannin, cutin) or stable aromatic and long chain aliphatic compounds (Lorenz *et al.*, 2007), sawdust, despite coming from wood, appears to be comprised of fresh C that can readily degrade due to small particle size. The supply of fresh C from sawdust contains many energy-rich C compounds, which decomposes immediately after application and may accelerate the microbial decomposition (Fontaine *et al.*, 2004). The C:N ratio from biosolids and sawdust mixture was adjusted to 20:1, which is favorable for microbial biomass (Daniels *et al.*, 2001). Furthermore, the biosolids N inputs may have increased sawdust C decomposition/mineralization rates, as high nutrient inputs would increase microbial activity (Anderson and Domsch, 1989).

3.4.3. Soil carbon accumulation

Net soil carbon accumulation (Mg ha^{-1}) for each site as well as soil carbon accumulation efficiency (CAE) was calculated for all sites (Table 3.4). In general, soil C accumulation increased with increasing organic amendment application rates. The CAE tended to stay steady or approach an equilibrium in the compost-amended soil (Site I), compared with the biosolids-amended soil (Site III).

Study site I

Both applied C rates and C accumulation followed the order as: PL<BSC<PYC, at 0-7.5 cm and 7.5-15 cm depths (Table 3.4). Compost treatments, i.e., PYC and BYC, had 11% C accumulated at 0-7.5 cm depth and 7% C accumulated at the 7.5-15 cm depth. The PL treatment lost C compared to the control soil (-0.45 Mg ha^{-1}) at the 0-7.5 cm depth. This may be a result of induced microbial mineralization with the narrow C:N ratio residual (Epstein *et al.*, 1978; Petersen *et al.*, 2003). Although our previous analysis has shown that the organic amendment treatments produce no greater soil C accumulation in the subsurface soils, CAE in subsurface soils (> 15 cm) revealed higher values (i.e., 10.7%) occasionally. These results indicate that C movement downward might have occurred in some organic residual applied plots.

Previous data at this site (Spargo *et al.*, 2006) have shown that in 2005 after the cessation of organic amendment application, the soil C accumulation at 0-15 cm depth from PL treatment was 2.6 Mg ha^{-1}, which was approximately 25% of the applied C, and the CAE from PYC treatment attained 70% over 5 years. By contrast, almost no C accumulation from PL (0.3%) was detected in the soil at 0-15 cm depth in 2011, and only 17.6% of the C supplied by PYC and BSC treatments remained in the soil. These results demonstrate that with more time, more soil C was decomposed and thus CAE was decreased.

<u>Study site II</u>

Soil C accumulation increased linearly with increasing C inputs. At 0-7.5 cm depth, biosolids application rates accumulated C from 4.8% to 7.5%. At 7.5-15 cm, biosolids application rates accumulated C from 2.1% to 3.1%. Although plowing possibly burried surface-applied biosolids into a deeper layer, it seems likely that the additional disturbance increased the C decomposition rate and outweighd any benefits (Powlson *et al.*, 2011). Fontaine *et al.* (2007) suggested that increased movement of organic C to subsoil would provide additional energy to a substrate-limited microbial population in the subsoil and thus increase the decomposition of existing organic C and reduce SOC content.

Given that the ranges of C inputs for sites I and II, the topsoil C accumulation of site II was lower than that of site I. Site II was set up in 1984 with only a single application of biosolids, most biosolids C was decomposed and, thus, not accumulated over long-time. By contrast, a shorter time for mineralization to occur and the protective permanent fall fescue cover planted over site I contributed to higher C accumulation. The relatively constant CAE in a certain depth complies with the current first-order kinetics for decomposition processes, which predict linearity between C input level and SOC level at equilibrium (Stewart *et al.*, 2007). There was no indication of C saturation, likely due to the finer soil textures.

Study site III

The CAE for biosolids ranged from 6.3%-11.5% at 0-7.5 cm and 5.1%-8.6% at 7.5-15 cm. By contrast, the BS 3x+SD treatment accumulated 3.6% and 2.4% C, respectively, for the two depths; and the BS 7x+SD treatment accumulated 3.3% and 1.9% C, respectively, for the two depths. Surprisingly, the addition of sawdust with biosolids treatment failed to accumulate more C than biosolids alone treatment.

The total soil C accumulation to 60 cm (Mg ha^{-1} yr^{-1}) expressed as a function of C input level (Mg ha^{-1} yr^{-1}) for the three study sites is shown as Figure 3.3. Site I showed a higher slope of 0.2223, compared with Site II of 0.1189, indicating a greater soil C capacity. The soil (e.g. Piedmont) under a management regime (e.g., no-till or pasture) with a decreased inherent decomposition rate may therefore show a linear soil C accumulation response to C inputs to a certain limit. By contrast, the Coastal Plain with a coarser soil texture revealed an asymptotic relationship between soil C accumulation and C inputs. At the greater addition levels, the Coastal Plain soil appeared to be approaching limits due to little or no C accumulation with increased C inputs. Such evidence demonstrates that soil C saturation phenomenon may have occurred in the Coastal Plain soil (site III).

The conceptual model of C saturation implies that the further a soil is from saturation, the greater its capacity and efficiency to sequester added C, whereas a soil approaching saturation will accumulate a smaller amount of SOC at a slower rate and efficiency (Six *et al.*, 2002). At site III, the Coastal Plain soil attained a C saturation limit with lower C input than the Piedmont soil at site I and II (Figure 3.3), likely due to its higher potential for physical protection of organic matter (Hassink, 1997). According to the theory of C saturation, the Piedmont soil from site I and II did not receive enough C inputs to achieve saturation and test the hypothesis.

3.5. Conclusions

Long-term or single high application of organic amendments (i.e., compost and biosolids) in the Mid-Atlantic region of United States can increase SOC concentration and soil C stocks, providing evidence for C sequestration. Although the measured soil C stocks are fairly high for the Coastal Plain, additional SOC storage may be not achievable by increasing C inputs to the soil, due to possible C saturation limits at this site. Soil C did not accumulate in the soil profile below the surface, but one cannot discount the possibility that significant amounts of C applied in organic amendments may be transported to deeper soil depths.

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3.7. References

Ågren, G. I. and Bosatta, N. 1987. Theoretical analysis of the long-term dynamics of carbon and nitrogen in soils. Ecology 68: 1181-1189.

Anderson, T.-H. and Domsch, K. H. 1989. Ratios of microbial biomass carbon to total organic carbon in arable soils. Soil Biology and Biochemistry 21: 471-479.

Batjes, N. H. 1998. Mitigation of atmospheric CO_2 concentrations by increased carbon sequestration in the soil. Biology and Fertility of Soils 27: 230-235.

Blanco-Canqui, H. and Lal, R. 2004. Mechanisms of carbon sequestration in soil aggregates. Critical Reviews in Plant Sciences 23: 481-504.

Bowden, C. L., Evanylo, G. K., Zhang, X., Ervin, E. H. and Seiler, J. R. 2010. Soil carbon and physiological responses of corn and soybean to organic amendments. Compost Science & Utilization 18: 162-173.

Boyle, M. and Paul, E. A. 1989. Carbon and nitrogen mineralization kinetics in soil previously amended with sewage sludge. Soil Sci. Soc. Am. J. 53: 99-103.

Brown, S., Kurtz, K., Bary, A. and Cogger, C. 2011. Quantifying benefits associated with land application of organic residuals in Washington State. Environmental Science & Technology 45: 7451-7458.

Bulluck III, L. R., Brosius, M., Evanylo, G. K. and Ristaino, J. B. 2002. Organic and synthetic fertility amendments influence soil microbial, physical and chemical properties on organic and conventional farms. Applied Soil Ecology 19: 147-160.

Busby, R. R., Allen Torbert, H. and Gebhart, D. L. 2007. Carbon and nitrogen mineralization of non-composted and composted municipal solid waste in sandy soils. Soil Biology and Biochemistry 39: 1277-1283.

Campbell, C. A., Zentner, R. P., Bowren, K. E., Townley-Smith, L. and Schnitzer, M. 1991. Effect of crop rotations and fertilization on soil organic matter and some biochemical properties of a thick Black Chernozem. Canadian Journal of Soil Science 71: 377-387.

Causarano, H. J., Franzluebbers, A. J., Shaw, J. N., Reeves, D. W., Raper, R. L. and Wood, C. W. 2008. Soil organic carbon fractions and aggregation in the southern piedmont and coastal plain. Soil Sci. Soc. Am. J. 72: 221-230.

Daniels, W. L., Evanylo, G. K., Nagle, S. M. and Schmidt, J. M. 2001. Effects of biosolids loading rate and sawdust additions on row crop yield and nitrate leaching potentials in Virginia sand and gravel mine reclamation. National Meeting of the American Society for Surface Mining and Reclamation, Albuquerque, New Mexico, ASSMR. 399-406.

Daniels, W. L., Evanylo, G. K., Nagle, S. M. and Schmidt, J. M. 2003. Effects of biosolids loading rate on nitrate leaching potentials in sand and gravel mine reclamation in Virginia. Proceedings of the Water Environment Federation 2003: 271-278.

Donohue, S. J. and Heckendorn, S. E. 1994. Soil test recommendations for Virginia. Blacksburg, VA., Virginia Cooperative Extension Service Publication. 834.

Eghball, B. 2002. Soil properties as influenced by phosphorus- and nitrogen-based manure and compost applications. Agron. J. 94: 128-135.

Epstein, E., Keane, D. B., Meisinger, J. J. and Legg, J. O. 1978. Mineralization of nitrogen from sewage sludge and sludge compost. J. Environ. Qual. 7: 217-221.

Foley, B. J. and Cooperband, L. R. 2002. Paper mill residuals and compost effects on soil carbon and physical properties. J. Environ. Qual. 31: 2086-2095.

Fontaine, S., Bardoux, G., Abbadie, L. and Mariotti, A. 2004. Carbon input to soil may decrease soil carbon content. Ecology Letters 7: 314-320.

Fontaine, S., Barot, S., Barre, P., Bdioui, N., Mary, B. and Rumpel, C. 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450: 277-280.

Fortuna, A., Harwood, R. R. and Paul, E. A. 2003. The effects of compost and crop rotations on carbon turnover and the particulate organic matter fraction. Soil Science 168: 434-444.

Franzluebbers, A. J. 2010. Achieving soil organic carbon sequestration with conservation agricultural systems in the southeastern United States. Soil Sci. Soc. Am. J. 74: 347-357.

Franzluebbers, A. J., Stuedemann, J. A., Schomberg, H. H. and Wilkinson, S. R. 2000. Soil organic C and N pools under long-term pasture management in the Southern Piedmont USA. Soil Biology and Biochemistry 32: 469-478.

Guo, L. B. and Gifford, R. M. 2002. Soil carbon stocks and land use change: a meta analysis. Global Change Biology 8: 345-360.

Hartz, T. K., Mitchell, J. P. and Giannini, C. 2000. Nitrogen and carbon mineralization dynamics of manures and composts. HortScience 35: 209-212.

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.

IPCC 1990. Climate change: the IPCC impacts assessment. Canberra, Australia, Australian Govt. Pub. Service.

Johnston, A. E., Poulton, P. R. and Coleman, K. 2009. Chapter 1 soil organic matter: its importance in sustainable agriculture and carbon dioxide fluxes. Advances in Agronomy. Donald, L. S., Academic Press. Volume 101: *pp.* 1-57.

Kirchhoff, C., Malina, J., Barrett, M. and Cogburn, B. 2004. Composted animal manures and biosolids for use in erosion control and for water quality improvement. Proceedings of the Water Environment Federation 2004: 316-345.

Kong, A. Y. Y., Six, J., Bryant, D. C., Denison, R. F. and van Kessel, C. 2005. The relationship between carbon input, aggregation, and soil organic carbon stabilization in sustainable cropping systems. Soil Sci. Soc. Am. J. 69: 1078-1085.

Krull, E. S., Baldock, J. A. and Skjemstad, J. O. 2003. Importance of mechanisms and processes of the stabilisation of soil organic matter for modelling carbon turnover. Functional Plant Biology 30: 207-222.

Lal, R. 2004. Soil carbon sequestration impacts on global climate change and food security. Science 304: 1623-1627.

Lal, R. 2007. Carbon management in agricultural soils. Mitigation and Adaptation Strategies for Global Change 12: 303-322.

Lindsay, B. J. and Logan, T. J. 1998. Field response of soil physical properties to sewage sludge. J. Environ. Qual. 27: 534-542.

Logan, T. J., Lindsay, B. J., Goins, L. E. and Ryan, J. A. 1997. Field assessment of sludge metal bioavailability to crops: Sludge rate response. J. Environ. Qual. 16: 534-550.

Lorenz, K., Lal, R., Preston, C. M. and Nierop, K. G. J. 2007. Strengthening the soil organic carbon pool by increasing contributions from recalcitrant aliphatic bio(macro)molecules. Geoderma 142: 1-10.

O'Brien, B. J. and Stout, J. D. 1978. Movement and turnover of soil organic matter as indicated by carbon isotope measurements. Soil Biology and Biochemistry 10: 309-317.

Petersen, S. O., Petersen, J. and Rubæk, G. H. 2003. Dynamics and plant uptake of nitrogen and phosphorus in soil amended with sewage sludge. Applied Soil Ecology 24: 187-195.

Post, W. M. and Kwon, K. C. 2000. Soil carbon sequestration and land-use change: processes and potential. Global Change Biology 6: 317-327.

Powlson, D. S., Whitmore, A. P. and Goulding, K. W. T. 2011. Soil carbon sequestration to mitigate climate change: a critical re-examination to identify the true and the false. European Journal of Soil Science 62: 42-55.

Rappaport, B. D., Martens, D. C., Reneau, R. B. and Simpson, T. W. 1988. Metal availability in sludge-amended soils with elevated metal levels. J. Environ. Qual. 17: 42-47.

Ros, M., Klammer, S., Knapp, B., Aichberger, K. and Insam, H. 2006. Long-term effects of compost amendment of soil on functional and structural diversity and microbial activity. Soil Use and Management 22: 209-218.

SAS Institute 2008. SAS/STAT 9.2 User's Guide. Cary, NC, SAS Institute, Inc.

Shober, A. L., Stehouwer, R. C. and Macneal, K. E. 2003. On-farm assessment of biosolids effects on soil and crop tissue quality. J. Environ. Qual. 32: 1873-1880.

Simpson, T. W., Donohue, S. J., Hawkins, G. W., Monnett, M. M. and Baker, J. C. 1993. The development and implementation of the Virginia agronomic land use evaluation system (VALUES). Blacksburg, VA., Department of Crop and Soil Environmental Sciences, Virginia Tech.

Six, J., Conant, R. T., Paul, E. A. and Paustian, K. 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant and Soil 241: 155-176.

Sloan, J. J., Dowdy, R. H. and Dolan, M. S. 1998. Recovery of biosolids-applied heavy metals sixteen years after application. J. Environ. Qual. 27: 1312-1317.

Spargo, J. T., Alley, M. M., Follett, R. F. and Wallace, J. V. 2008. Soil carbon sequestration with continuous no-till management of grain cropping systems in the Virginia coastal plain. Soil Till. Res. 100: 133-140.

Spargo, J. T., Evanylo, G. K. and Alley, M. M. 2006. Repeated compost application effects on phosphorus runoff in the Virginia Piedmont. J. Environ. Qual. 35: 2342-2351.

Stewart, C., Paustian, K., Conant, R., Plante, A. and Six, J. 2007. Soil carbon saturation: concept, evidence and evaluation. Biogeochemistry 86: 19-31.

Stewart, C. E., Follett, R. F., Wallace, J. and Pruessner, E. G. 2012. Impact of biosolids and tillage on soil organic matter fractions: Implications of carbon saturation for conservation management in the Virginia Coastal Plain. Soil Sci. Soc. Am. J.: In progress.

Stewart, C. E., Plante, A. F., Paustian, K., Conant, R. T. and Six, J. 2008. Soil carbon saturation: linking concept and measurable carbon pools. Soil Sci. Soc. Am. J. 72: 379-392.

Sukkariyah, B., Evanylo, G. and Zelazny, L. 2007. Distribution of copper, zinc, and phosphorus in Coastal Plain soils receiving repeated liquid biosolids applications. J. Environ. Qual. 36: 1618-1626.

Sukkariyah, B. F., Evanylo, G. K., Zelazny, L. and Chaney, R. L. 2005. Cadmium, copper, nickel, and zinc availability in a biosolids-amended Piedmont soil years after application. J. Environ. Qual. 34: 2255-2262.

Tian, G., Granato, T. C., Cox, A. E., Pietz, R. I., Carlson, C. R. and Abedin, Z. 2009. Soil carbon sequestration resulting from long-term application of biosolids for land reclamation. J. Environ. Qual. 38: 61-74.

Virginia Cooperative Extension 1992. Pest management guide for field crops. Virginia Tech: Blacksburg, VA, VA Coop. Ext. Publ. No. 456-016: 1-276.

Whalen, J. K., Benslim, H., Jiao, Y. and Sey, B. K. 2008. Soil organic carbon and nitrogen pools as affected by compost applicationsto a sandy-loam soil in Québec. Canadian Journal of Soil Science 88: 443-450.

Zebarth, B. J., Neilsen, G. H., Hogue, E. and Neilsen, D. 1999. Influence of organic waste amendments on selected soil physical and chemical properties. Canadian Journal of Soil Science 79: 501-504.

Figure Captions

Figure 3.1a. Distribution of total soil organic C concentration (g kg^{-1}) with soil depth in the Fauquier silty clay loam 7 years after cessation of organic residuals application at study site I. Bars represent standard errors. Means for treatments with the same letter are not significantly different at $p \leq 0.05$.

Figure 3.1b. Distribution of total soil organic C concentration (g kg^{-1}) with soil depth in the Davidson clay loam 27 years after cessation of biosolids application at study site II. Bars represent standard errors. Means for treatments with the same letter are not significantly different at $p \leq 0.05$.

Figure 3.1c. Distribution of total soil organic C concentration (g kg^{-1}) with soil depth in the Pamunkey sandy loam 15 years after cessation of biosolids application at study site III. Bars represent standard errors. Means for treatments with the same letter are not significantly different at $p \leq 0.05$.

Figure 3.2a. Distribution of total soil C stocks (Mg ha^{-1}) with soil depth in the Fauquier silty clay loam 7 years after cessation of organic residuals application at study site I. Bars represent standard errors. Means for treatments with the same letter are not significantly different at $p \leq 0.05$.

Figure 3.2b. Distribution of total soil C stocks (Mg ha^{-1}) with soil depth in the Davidson clay loam 27 years after cessation of biosolids application at study site II. Bars represent standard errors. Means for treatments with the same letter are not significantly different at $p \leq 0.05$.

Figure 3.2c. Distribution of total soil C stocks (Mg ha^{-1}) with soil depth in the Pamunkey sandy loam 15 years after cessation of biosolids application at study site III. Bars represent standard errors. Means for treatments with the same letter are not significantly different at $p \leq 0.05$.

Figure 3.3. Averaged soil C accumulation per year (Mg ha^{-1} yr^{-1}) expressed as a function of averaged C input levels per year (Mg C ha^{-1} yr^{-1}) for the long-term experiments of organic amendment at 0-60 cm depth in the three study sites. Site I received annual application of composts and manure in 2000-2004; Site II received single application of biosolids in 1984; Site III received single application of biosolids in 1996.

Figure 3.4. Soil organic carbon concentration (g kg^{-1}) with biosolids application rate over time during 1984-2011 at study site II.

Table 3.1. The soil series and organic amendment treatments management in the three study sites across Virginia.

Study Site	County	Soil Series	Treatment	Application Frequency
Site I	Orange	Fauquier silty clay loam (*Fine, mixed, mesic Ultic Hapludalfs*)	Control, poultry litter, poultry litter-yard waste compost, and biosolids-woodchip compost; all were based on the agronomic N rate.	Continuous from 2000-2004
Site II	Orange	Davidson clay loam (*Clayey, kaolinitic, thermic, Rhodic Paleudult*)	Six rates of aerobically digested biosolids (0, 42, 84, 126, 168, and 210 dry Mg ha^{-1}); the agronomic rate of biosolids was 42 Mg ha^{-1}.	Single in 1984
Site III	Charles City	Pamunkey sandy loam (*Fine-loamy, mixed, thermic Ultic Hapludalfs*)	Five rates of anaerobically digested biosolids (0, 14, 42, 70, and 98 dry Mg ha^{-1}), with and without sawdust to adjust the C:N ratio; the agronomic rate of biosolids was 14 Mg ha^{-1}.	Single in 1996

Table 3.2. Soil properties and particle size fractions from the three study sites.

Study Site	pH	CEC	OM†	Sand	Silt	Clay
	--	cmol$_{(c)}$ kg^{-1}	g kg^{-1}			
Site I	6.3	--	30	215	524	261
Site II	6.3	12.5	18	153	471	376
Site III	--	--	--	292	572	136

†OM = organic matter.

Table 3.3. Selected chemical properties of the organic residuals applied to the three study sites.

Study Site	Treatment	C:N	C	TKN	NH$_4$-N	NO$_3$-N	Organic-N	Total P	K
			g kg^{-1}						
Site I	PL†	8	474	56.1	8.2	0.19	48	17.6	26.3
	BSC‡	10	239	26.0	3.2	0.88	22	15.6	1.1
	PYC§	20	379	19.3	0.2	0.73	18	4.2	6.8
Site II	BS¶	20	320	16	<0.008	--	16	31.7	1.1
Site III	BS	8	358	44.7	6.4	0.30	38	17	1.2
	SD#	198	475	2.4	--	--	2.4	--	--

†PL = poultry litter; ‡BSC = biosolids-woodchip compost; §PYC = poultry litter-yard waste compost; ¶BS = biosolids; #SD = sawdust.

Table 3.4. Soil C accumulation and applied C of organic amendments from the three study sites

Study Site	Organic amendment	Applied rate dry Mg ha^{-1}	C rate Mg ha^{-1}	Soil C accumulation Mg ha^{-1} (Soil C accumulation efficiency %)					
				0-7.5 cm	7.5-15 cm	15-30 cm	30-45 cm	45-60 cm	0-60 cm
Site I	CTL†	0	0	0	0	0	0	0	0
	PL‡	21.9	10.5	-0.45(-4.2)	0.47 (4.5)	-0.36(-2.9)	1.07 (10.2)	1.13 (10.7)	1.86 (18.3)
	BSC§	126	41.3	4.63 (11.2)	2.66 (6.4)	2.37 (5.7)	1.19 (2.9)	1.35 (3.3)	12.20 (29.5)
	PYC¶	202	76	7.88 (10.4)	5.44 (7.2)	0.39 (0.5)	0.89 (1.2)	1.44 (1.9)	16.04 (21.2)
Site II	CTL	0	0	0	0	0	0	0	0
	BS# 1x	42	13.4	0.74 (5.5)	0.28 (2.1)	0.06 (0.5)	0.06 (0.5)	0.53 (3.9)	1.67 (12.5)
	BS 2x	84	26.9	1.33 (5.0)	0.69 (2.6)	-0.32(-1.2)	-0.04(-0.1)	0.27 (1.0)	1.93 (7.3)
	BS 3x	126	40.3	3.03 (7.5)	1.26 (3.1)	0.38 (0.9)	0.23 (0.6)	0.04 (0.1)	4.94 (12.2)
	BS 4x	168	53.8	3.40 (6.3)	1.66 (3.1)	0.30 (0.6)	0.08 (0.2)	0.23 (0.4)	5.67 (10.6)
	BS 5x	210	77.2	3.72 (4.8)	2.33 (3.0)	1.25 (1.6)	1.00 (1.3)	0.97 (1.3)	9.27 (12)
Site III	CTL	0	0	0	0	0	0	0	0
	BS 1x	14	5	0.58 (11.5)	0.43 (8.6)	1.17 (23.3)	-0.39(-7.8)	-0.07(-1.4)	1.72 (34.2)
	BS 3x	42	15	1.49 (9.9)	0.97 (6.5)	0.34 (2.2)	0.24 (1.6)	0.40 (2.7)	3.44 (22.9)
	BS 5x	70	25.1	1.57 (6.3)	1.28 (5.1)	1.76 (7.0)	0.28 (1.1)	0.68 (2.7)	5.57 (22.2)
	BS 7x	98	35.1	3.29 (9.4)	2.10 (6.0)	0.04 (0.1)	0.19 (0.5)	0.57 (1.6)	6.19 (17.6)
	BS 3x+SD††	42+56(SD)	41.6	1.51 (3.6)	1.01 (2.4)	1.92 (4.6)	0.82 (2.0)	0.97 (2.3)	6.23 (14.9)
	BS 7x+SD	98+131(SD)	97.3	3.24 (3.3)	1.81 (1.9)	0.91 (0.9)	0.06 (0.1)	-0.01(0.0)	6.01 (6.2)

†CTL = control; ‡PL = poultry litter; §BSC = biosolids-woodchip compost; ¶PYC = poultry litter-yard waste compost; #BS = biosolids; ††SD = sawdust.

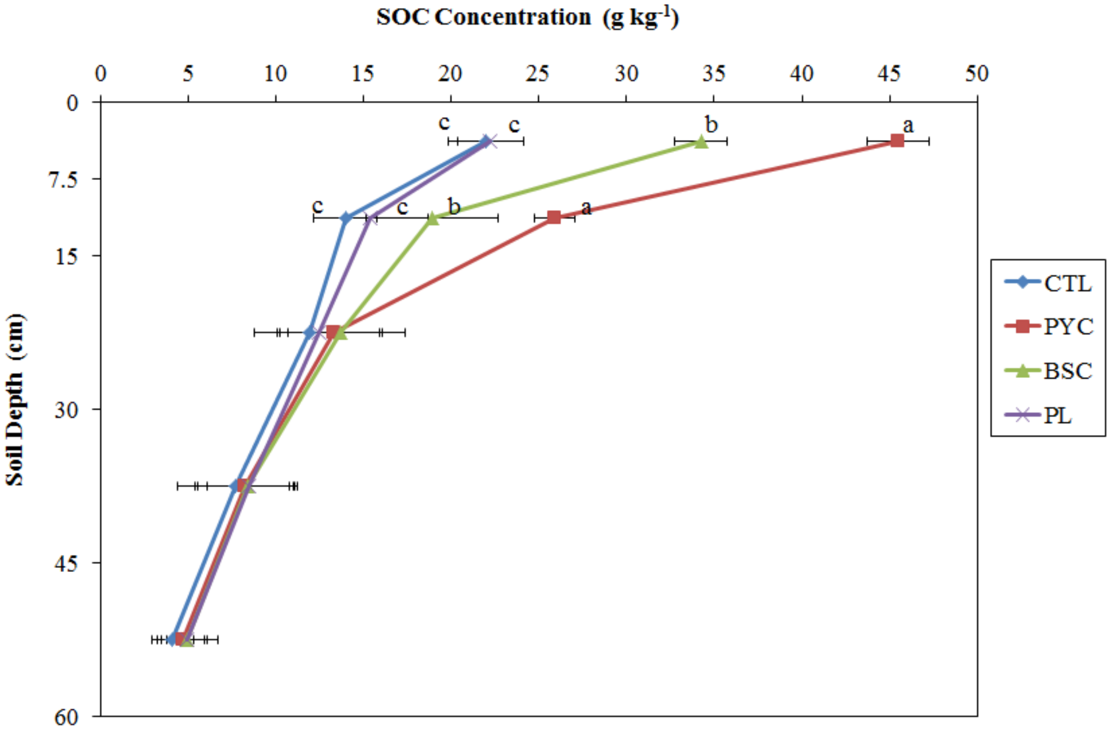
SOC Concentration (g kg^{-1})
0
5
10
15
20
25
30
35
40
45
50
Soil Depth (cm)
0
7.5
15
30
45
60
c
c
b
a
c
c
b
a
CTL
PYC
BSC
PL

Figure 3.1a

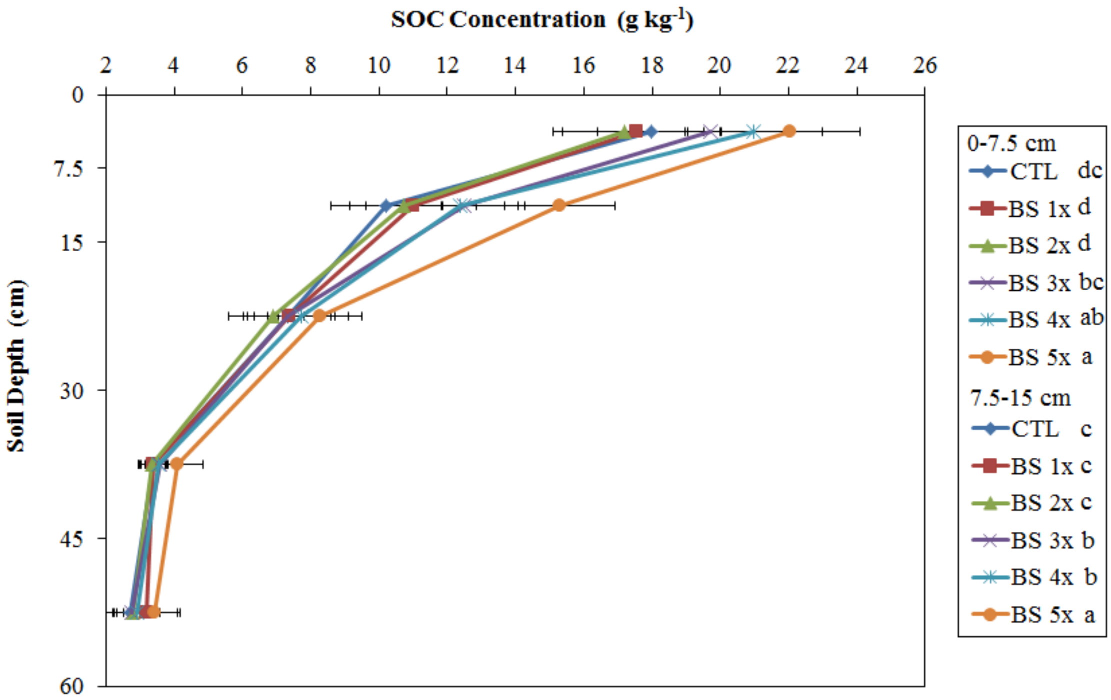
SOC Concentration (g kg-1)
2
4
6
8
10
12
14
16
18
20
22
24
26
0
7.5
15
30
45
60
Soil Depth (cm)
0-7.5 cm
CTL dc
BS 1x d
BS 2x d
BS 3x bc
BS 4x ab
BS 5x a
7.5-15 cm
CTL c
BS 1x c
BS 2x c
BS 3x b
BS 4x b
BS 5x a

Figure 3.1b

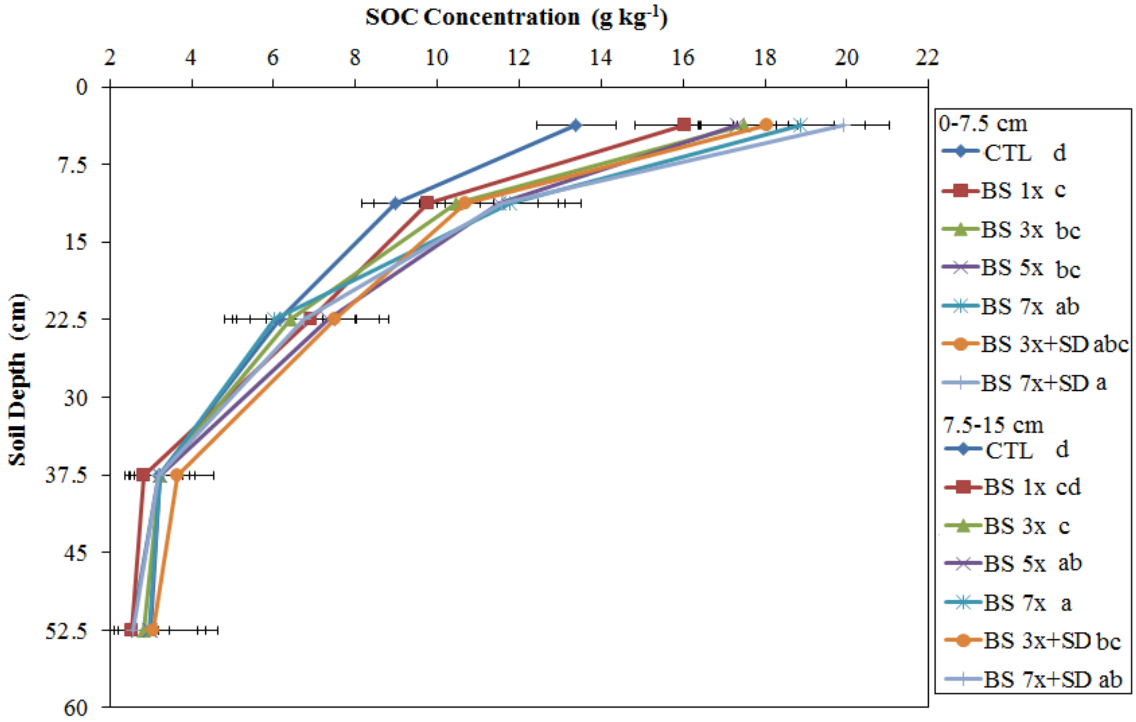
SOC Concentration (g kg-1)
2
4
6
8
10
12
14
16
18
20
22
Soil Depth (cm)
0
7.5
15
22.5
30
37.5
45
52.5
60
0-7.5 cm
CTL d
BS 1x c
BS 3x bc
BS 5x bc
BS 7x ab
BS 3x+SD abc
BS 7x+SD a
7.5-15 cm
CTL d
BS 1x cd
BS 3x c
BS 5x ab
BS 7x a
BS 3x+SD bc
BS 7x+SD ab

Figure 3.1c

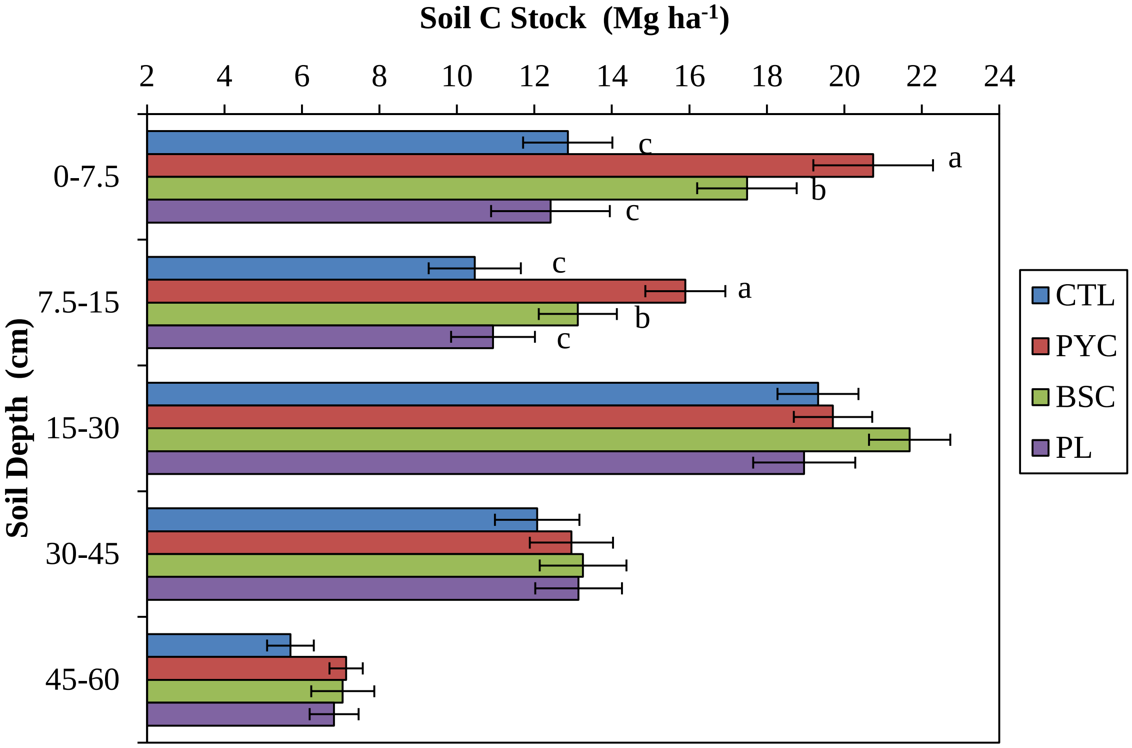
Soil C Stock (Mg ha-1)
2
4
6
8
10
12
14
16
18
20
22
24
Soil Depth (cm)
0-7.5
7.5-15
15-30
30-45
45-60
c
a
b
c
c
a
b
c
CTL
PYC
BSC
PL

Figure 3.2a

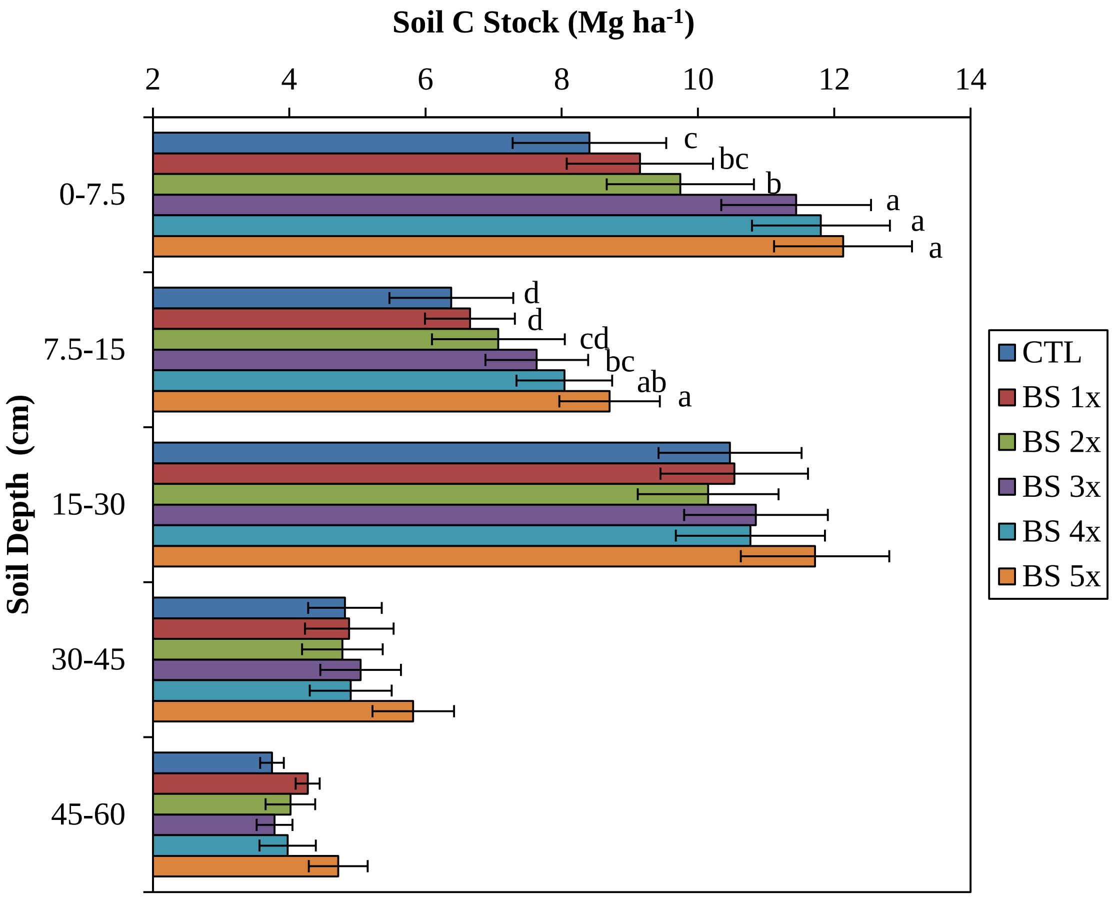
Soil C Stock (Mg ha^{-1})
2
4
6
8
10
12
14
Soil Depth (cm)
0-7.5
7.5-15
15-30
30-45
45-60
c
bc
b
a
a
a
d
d
cd
bc
ab
a
CTL
BS 1x
BS 2x
BS 3x
BS 4x
BS 5x

Figure 3.2b

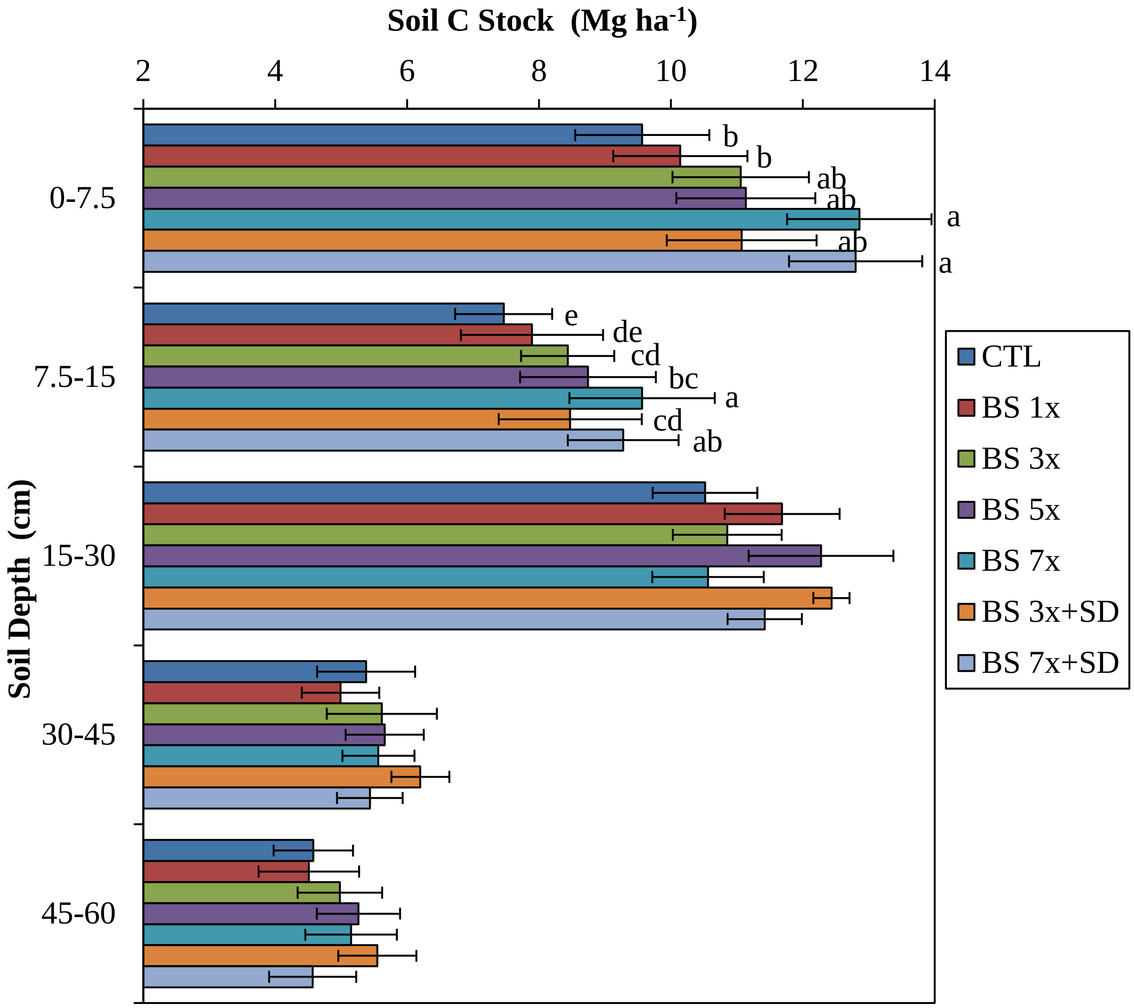
Soil C Stock (Mg ha-1)
2
4
6
8
10
12
14
Soil Depth (cm)
0-7.5
7.5-15
15-30
30-45
45-60
b
b
ab
ab
a
ab
a
e
de
cd
bc
a
cd
ab
CTL
BS 1x
BS 3x
BS 5x
BS 7x
BS 3x+SD
BS 7x+SD

Figure 3.2c

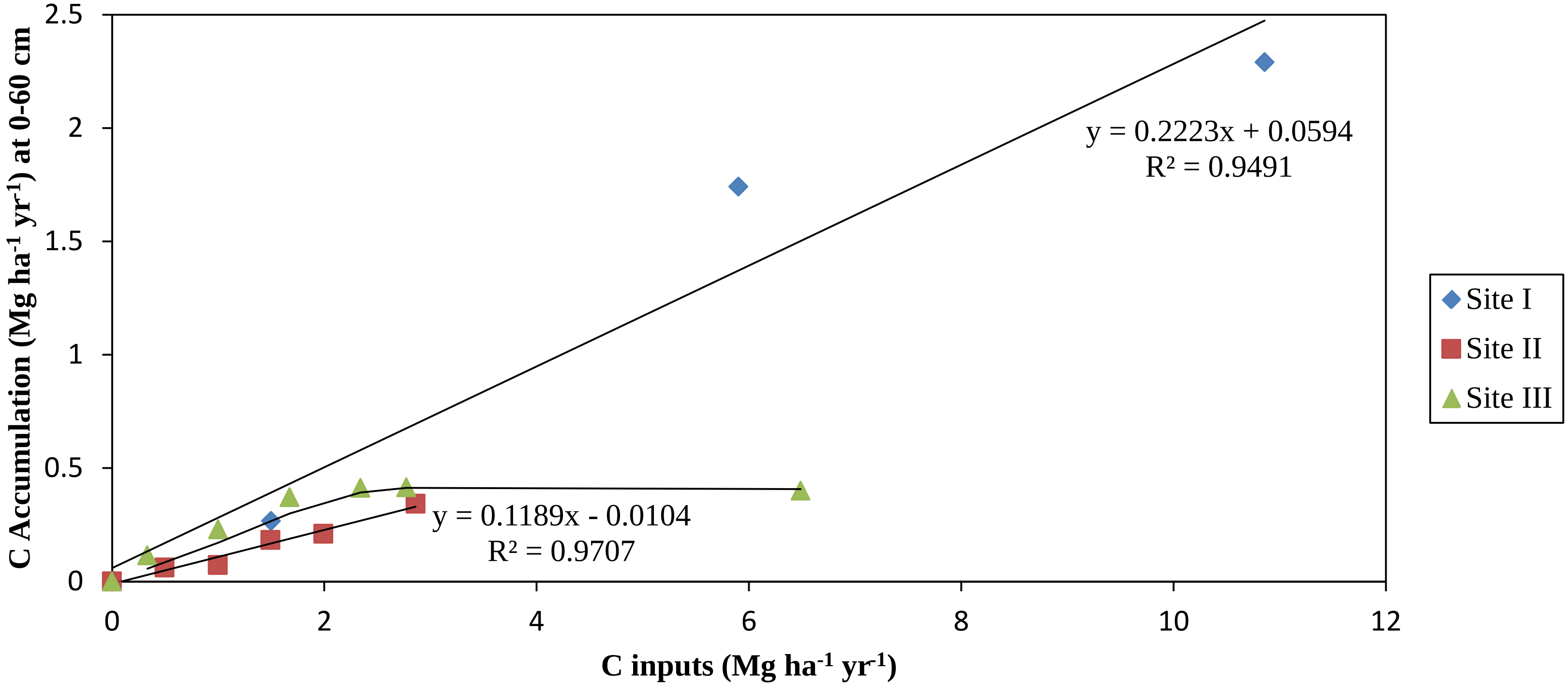
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10
12
C Accumulation (Mg ha^{-1} yr^{-1}) at 0-60 cm
C inputs (Mg ha^{-1} yr^{-1})
y = 0.2223x + 0.0594
R² = 0.9491
y = 0.1189x - 0.0104
R² = 0.9707
Site I
Site II
Site III

Figure 3.3

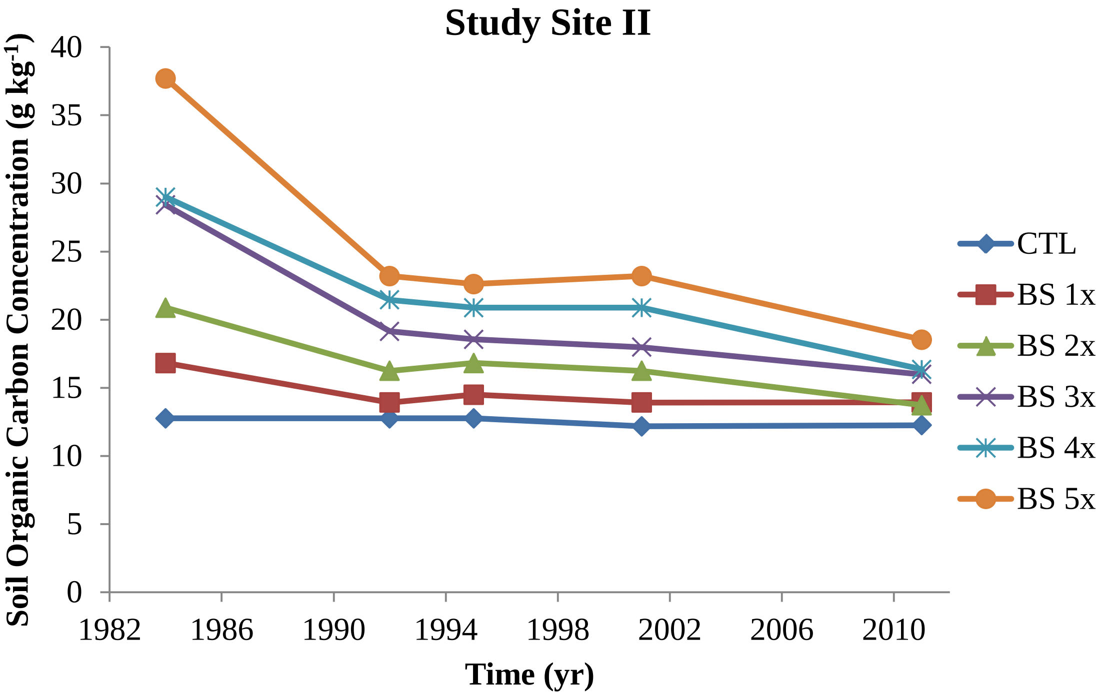
Study Site II
Soil Organic Carbon Concentration (g kg-1)
40
35
30
25
20
15
10
5
0
1982
1986
1990
1994
1998
2002
2006
2010
Time (yr)
CTL
BS 1x
BS 2x
BS 3x
BS 4x
BS 5x

Figure 3.4

4. Carbon Dynamics from Long-term Application of Organic Residuals Described by ^{13}C Solid-state Nuclear Magnetic Resonance

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4.1. Abstract

Land application of organic residuals (e.g. biosolids and composts) influences the quantity and quality of organic matter in agricultural soils. We investigated three field sites across Virginia to characterize soil C following addition of organic residuals either in single application or in repeated application during 1984-2004. Physical separation methods, i.e., particle size and density separation, were applied in order to extract particulate organic matter (POM). Solid state ^{13}C nuclear magnetic resonance (NMR) technique was used to investigate the chemistry of C functional groups in POM fractions from organic-residual amended soils. The direct polarization magic angle spinning (DP/MAS) ^{13}C NMR indicated the averaged presence of carbohydrates (28.4%), aromatic C-C (22.1%), alkyl C (19.7%), COO/N-C=O (10.8%), aromatic C-O (7.6%), OCH_3 / NCH (6.3 %), and ketones and aldehydes (5.1 %). The alkyl C / O-alkyl C ratio was within a range of 0.31-1.87, indicating a broad degree of soil organic C decomposition. These results suggest that the application of organic residuals can affect long-term soil C stability.

4.2. Introduction

The application of organic residuals, i.e., biosolids, manure and compost, to soil is increasing as both a replacement of nutrient strategy, i.e., nitrogen and phosphorus, and as a means of improving soil organic matter (SOM). Land application of organic residuals affects not only the quantity but also the quality of SOM through their influence on the organic matter decomposition and humification processes. The benefits of long-term application of organic residuals in soil carbon (C) sequestration have been well documented in the Mid-Atlantic region of the United States (Sukkariyah *et al.*, 2005; Spargo *et al.*, 2006; Franzluebbers, 2010; Stewart *et al.*, 2012). Our previous studies have justified the assumption that land application of organic residuals increase surface soil C concentration and C stocks over long term application (Chapter 3). However, the C chemistry in these organic residual-amended soils is not well known.

SOM is a heterogeneous mixture composed of living microbial biomass, fresh and partially decomposed plant, animal and microbial residues, and well-decomposed humic substances (Schnitzer and Khan, 1978). Particulate organic matter (POM) which is a relatively young C pool for SOM is thought to represent a transitional stage in the humification process (Christensen, 2001; Leifeld and Kögel-Knabner, 2005). Subdividing POM to further separate constituents differing in their degree of decomposition and similarity to plant residues may improve the resolution of differences in labile SOM characteristics (Marriott and Wander, 2006). Management practices, e.g., addition of organic residuals, can affect SOC pools and soil quality before net organic matter contents change (Wander *et al.*, 1994; Kumar and Goh, 1999; Sojka *et al.*, 2003; Lal, 2006). It has been suggested that enhanced protection of SOM by organic amendments results due to differences among the chemical composition of POM (Sollins *et al.*, 1999; Six *et al.*, 2002). Physical fractionation according to size and density of soil particles emphasizes the importance of interactions between organic and inorganic soil components in the turnover of organic matter (Christensen, 1992). Size and density-based separates are often subdivided to reveal POM's position within the soil matrix (Tiessen and Stewart, 1983).

Solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy has been recognized as a popular and powerful analytical method for determining the chemical composition of SOM (Preston, 1996). ^{13}C NMR spectroscopy allows the determination of the chemical composition of SOM by visualizing various chemical shifts of C structures with the magnetic relaxation frequency (Wilson, 1987). Generally, NMR spectrum reveals information of C functional groups, e.g. carbonyl, aromatic, O-alkyl, and alkyl, in the measured sample. During decomposition, the spectra of SOM change in characteristic ways, with alkyl C being lost initially, and O-alkyl C being increasing accordingly (Baldock *et al.*, 1997). Therefore, investigating dynamic C structures via NMR could provide essential information about the decomposition degrees of SOM.

The cross-polarization magic angle spinning (CP/MAS) ^{13}C NMR was first applied in studies of SOM in 1980s (Hatcher *et al.*, 1980; Wilson *et al.*, 1981; Preston *et al.*, 1994). This non-destructive technique greatly improves the sensitivity of NMR and eliminates C loss during analysis (Salati *et al.*, 2008). However, CP/MAS detects C-H spins selectively, thus causing the results to be semi-quantitative (Preston *et al.*; Stover and Frechet, 1989; Preston, 2001). The direct polarization magic angle spinning (DP/MAS) technique is an alternative for demining chemical structures of organic C. It provides quantitative structural information by detecting C-C spins and avoids problems associated with CP/MAS (Mao *et al.*, 2008).

^{13}C NMR technology has been applied widely to plant litter, organic wastes, whole soil, and the physical and chemical extracted soil fractions (Mahieu *et al.*, 1999; Mathers and Xu, 2003; Smernik *et al.*, 2003; Conte *et al.*, 2006; Helfrich *et al.*, 2006; Mathers *et al.*, 2007), permitting the acquisition of well resolved spectra which provide detailed information on the structure of POM (Golchin *et al.*, 1994; 1995; Stone *et al.*, 2001; Kölbl and Kögel-Knabner, 2004). The technique can also reveal the anthropogenic management effects on SOC, such as tillage management (Ding *et al.*, 2002). ^{13}C NMR technology has proven to be a good approach to characterize SOM decomposition, assess soil C stability and evaluate C structural information from organic residual-amended soils (Schulten and Leinweber, 1991; Leifeld *et al.*, 2002; Adani *et al.*, 2006; Mao *et al.*, 2008).

Few studies have reported soil organic C speciation from organic residual-amended soils using advanced ^{13}C NMR technique (Francioso *et al.*, 2000; Gerzabek *et al.*, 2001; Flavel and Murphy, 2006); thus, the main objective of this research was to characterize the soil C chemistry via advanced ^{13}C NMR techniques from field sites with various history of organic residuals across Virginia, US.

4.3. Materials and Methods

4.3.1. Experimental sites

Study site I

This experimental site was located at the Northern Piedmont Agricultural Research and Extension Center (NPAREC) on a Fauquier silty clay loam (*Fine, mixed,* *mesic Ultic Hapludalfs*) in Orange, VA. Eight treatments were established in the spring of 2000 to investigate the agronomic and environmental effects of compost use in the Virginia Piedmont (Table 4.1) (Bowden *et al.*, 2010). The soil properties prior to organic amendment application are shown in Table 4.2. The treatments were arranged in a randomized complete block design with four replicates. Each plot measured 3.6 m wide by 7.5 m long. The following four treatments were selected for reporting in this study as: unamended control (CTL); poultry litter (PL); poultry litter-yard waste compost (PYC); and biosolids-woodchip compost (BSC).

The PL was annually applied at rates estimated to meet crop N requirements. The compost treatments established in 2000 were biennial (2000, 2002) agronomic N rates of poultry litter-yard waste compost with (2001) and without supplemental inorganic fertilizer N. Then beginning in the spring of 2003, two of the four replicates of each of the two biennial compost treatments continued to receive annual (2003, 2004) applications of agronomic N rates of poultry litter-yard waste compost, and the other two replicates from the same treatments began to receive annual (2003, 2004) applications of agronomic N rates of a biosolids-woodchip compost. No organic amendment or inorganic fertilizer was applied beyond spring 2005. However, in August 2010, N fertilizer was applied to the entire site for tall fescue maintenance. Lime was applied in April 2004 to achieve a target pH of 7 to all plots except the BSC treatment, as lime was added during treatment of biosolids .

The poultry litter-yard waste compost (PYC) was a commercially-produced material (PYC; Panorama Farms, Earlysville, Virginia), whose poultry litter and yard waste feedstocks were combined at a ratio of 1:2 (v/v) and composted using turned windrow technology for 120 days. The biosolids-woodchip compost (BSC) was produced at the Rivanna Water and Sewer Authority (Charlottesville, Virginia) from anaerobically-digested biosolids dewatered with $Ca(OH)_2$ and composted with wood chips (1:2 ratio of biosolids and woodchips) for 21 days, including five consecutive days at 66°C, via static pile technology. The material was cured for an additional 10 days after screening through a 0.95 cm sieve to remove oversized woodchips. A commercially-processed, screened poultry litter (PL; Glen Hill Farm, Harrisonburg, Virginia) was used for the poultry litter treatment. Chemical properties of the organic residuals applied are summarized in Table 4.3. Soil amendments were hand-applied and incorporated within 24 h associated with seedbed preparation by roto-tilling.

The initial crops included pumpkin (*Cucurbita pepo* V. Magic Lantern) in 2000, sweet corn (*Zea mays* L. V. Silver Queen) in 2001, and bell pepper (*Capsicum annuum* V. Aristotle) in 2002. Following modification of the treatments in spring 2003, corn (*Zea mays* L. V. Pioneer 31G20) was grown in 2003 and 2004 and soybean (*Glycine max* V. Delta Pine 4933RR) was grown in 2005. Cereal rye (*Secale cereal* L.) was planted in all plots in the autumn of 2000-2005 as a winter cover crop. Tall fescue (*Festuca arundinacea* Schreb.) was planted in spring 2006 and has been maintained in all plots with twice annual bush hogging as the only management practice.

Study site II

This experimental site was also located at the NPAREC on a Davidson clay loam (*Clayey, kaolinitic, thermic, Rhodic Paleudults*). Six rates of aerobically digested biosolids (0, 42, 84, 126, 168, and 210 dry Mg ha^{-1}; the agronomic rate of biosolids was 42 Mg ha^{-1} for corn) were applied in single applications in the spring of 1984 (Table 4.1)

(Rappaport *et al.*, 1988). Soil properties prior to organic amendment application are shown as Table 4.2. The treatments were arranged in a randomized complete block design with four replicates. Each plot consisted of a volume of soil 2.3 m long x 1.5 m wide x 0.9 m high isolated by plastic wrapping below ground and wooden boards above ground to prevent lateral movement of biosolids constituents. Only one treatment as 210 dry Mg ha^{-1} biosolids (BS) was selected for reporting in this study.

The aerobically digested biosolids were obtained from a Bristol, VA wastewater treatment plant having high industrial inputs. The chemical properties of the organic residuals applied are shown in Table 4.3. The biosolids were dewatered on sand beds, which allowed for maximum NH_3 volatilization and NO_3^- leaching losses; therefore, the biosolids contained negligible amounts of inorganic N (<8 mg kg^{-1}). The biosolids were incorporated into the soils to a depth of approximately 20 cm in spring 1984 prior to planting. The plots were rototilled every year to a depth of 15 cm from 1984-2005.

Corn (*Zea mays* L.) was grown each year from 1984-2000, except in 1994 when sorghum (*Sorghum bicolor* L. V. Dekalb 41Y) was planted instead of corn because of drought. Corn varieties were Pioneer 3192 in 1984–1990 and 1996–2000 and Pioneer 3136 in 1991–1995. Radish (*Raphanus sativus* L.) and Romaine lettuce (*Lactuca sativa* V. longifolia) cultivar Parris Island Cos replaced corn in the cropping system from 2001 to 2004. Winter barley (*Hordeum vulgare* L. V. Nomini) was grown in 2002, 2004 and 2005. The plots had been left fallow since 2005, with an occasional herbicide application to reduce weed pressure. Lime applications in 1989 and 1998 were made to raise the pH to 6.0.

Study site III

This experimental site was located on a Pamunkey sandy loam (*Fine-loamy, mixed, thermic Ultic Hapludalfs*) in Charles City County, VA. Five rates of anaerobically digested biosolids (BS) (0, 14, 42, 70, and 98 dry Mg ha^{-1}; the agronomic rate of biosolids was 14 Mg ha^{-1} for corn), with and without sawdust (SD) to adjust the C:N ratio to 20:1, were applied in single applications in March 1996 (Table 4.1) (Daniels *et al.*, 2003). Soil properties prior to organic amendment application are summarized in Table 4.2. The treatments were arranged in a randomized complete block design with four replicates. Each plot was approximately 36 x 15 m in size. The entire area of each experimental block was approximately 3 ha. The following three treatments were selected for reporting in this study: Control (CON), 98 dry Mg ha^{-1} biosolids (BS) and 98 dry Mg ha^{-1} biosolids+sawdust (BS +SD).

The anaerobically digested secondary biosolids was obtained from Chesterfield, VA. The chemical properties of the organic residuals applied are shown in Table 4.3. Biosolids N composition required a dry biosolids : sawdust ratio of 0.75:1 to attain the desired C:N ratio (20:1). The sawdust had a bulk C: N ratio of 198:1. The biosolids with and without sawdust were surface-applied to the soils in the spring of 1996 prior to planting.

A crop rotation of corn (*Zea mays* L.; planted April 1996), wheat (*Triticum aestivum;* planted November 1996), soybean (*Glycine max*; planted July 1997) and cotton (*Gossypium spp*.; planted in 1998) have been grown at the site since the establishment of the treatments. After the application of biosolids in 1996, this crop rotation has been managed as no-till system from 1997-2011 with only essential N, P, and K fertilizers applied to all the plots.

At each study site, nitrogen needs for crops were determined with the Virginia Agronomic Land Use Evaluation System (VALUES) (Simpson *et al.*, 1993). Phosphorus and potassium requirements were determined by Virginia Cooperative Extension soil testing results (Donohue and Heckendorn, 1994). Agronomic practices, seedbed preparation, pest control, and weeding control were performed according to Virginia Cooperative Extension recommendations (Virginia Cooperative Extension, 1992). The lime requirement was determined by Virginia Cooperative Extension soil testing results (Donohue and Heckendorn, 1994).

4.3.2. Soil sampling and processing

Soil sampling

Three soil cores measuring 5 cm in diameter were collected at a depth of 0-7.5 cm from selected treatments plots identified above in 2011. Surface soil samples from 0-15 cm depth at the highest rate biosolids treatment were collected from site II in 1993 and in 2011. Nine treatments in the three field sites were selected for further analysis in this study: CON, PYC, BSC, and PL from site I; 1993 BS and 2011 BS from site II; CON, BS, and BS+SD from site III. Each soil core of 0-7.5 cm (0-15 cm for site II) including the three subsamples and the four replicates from these nine treatments were composited and processed for analysis.

Soil processing

For each treatment, 50 g of air-dried soil (≤2.0 mm) was dispersed in 250 mL of distilled water at 22 J mL^{-1} (Leifeld and Kögel-Knabner, 2005) with a 1.2 cm-diameter probe sonifier (Sonicator 3000, Misonix Inc., Farmingdale, NY). The suspension was passed through a 53-µm sieve and the filtrate was collected in a tall 1-L beaker for further separation. The residue on the sieve (sand plus POM) was transferred quantitatively to a 250-mL centrifuge tube and was stirred, soaked and centrifuged at 1949g for 20 min in 50 mL of sodium iodide (NaI) adjusted to a density of 1.8 g mL^{-1} with water. Then the light-weight POM was siphoned into a tared beaker and washed three times with DI water. The remaining sand fraction was washed, dried and weighed.

The filtrate (clay plus silt) was adjusted to a final volume of approximately 500 mL before it was dispersed at 450 J mL^{-1}. The clay fraction was separated by exhaustive sedimentation and decantation, and coagulated with 0.5M $MgCl_2$. The remaining sediment was the silt fraction. Both clay and silt fractions were freeze-dried and weighed to determine yield for each fraction.

The POM was washed twice with 1 M HF (2% v/v) and then twice with DI water and finally freeze-dried. The samples were processed in this manner to remove iron which can cause interference during ^{13}C NMR analysis (Keeler and Maciel, 2003).

A subsample of the freeze-dried POM and the remaining size-fractions, i.e., clay, silt, and sand, as well as the whole soils, were ground to pass a 0.5 mm sieve for total SOC concentration by dry combustion in a Vario Max CNS macro elemental analyzer (Elementar, GER).

4.3.3. NMR spectroscopy

The NMR experiments were performed on a Bruker Avance 400 spectrometer at 100 MHz for ^{13}C, using magic angle spinning (MAS) with 4-mm sample rotors in a double-resonance probe head.

Qualitative ^{13}C cross polarization/total sideband suppression (CP/TOSS) with dipolar dephasing NMR

Qualitative composition information was obtained with good sensitivity by ^{13}C CP/TOSS. The experiments were conducted at a spinning speed of 5 kHz and a CP time of 1 ms, with a ^{1}H 90º pulse-length of 4 μs and a recycle delay of 1 s. Four-pulse total suppression of sidebands (TOSS) (Dixon, 1982) was employed before detection, and two-pulse phase-modulated (TPPM) decoupling was applied for optimum resolution. The corresponding subspectrum with signals of nonprotonated carbons and carbons of mobile groups such as rotating CH_3 was obtained by ^{13}C CP/TOSS combined with 40-μs dipolar dephasing.

Quantitative ^{13}C direct polarization/magic angle spinning (DP/MAS) NMR

Quantitative ^{13}C DP/MAS NMR experiments were run at a spinning speed of 13 kHz. The 90º ^{13}C pulse-length was 4 s. Recycle delays were tested by the cross polarization/spin lattice relaxation time /total sideband suppression (CP/T_1-TOSS) technique to ensure that all carbon sites were fully relaxed by more than 95% (Mao *et al.*, 2000). This technique provides quantitative structural information. The details of this technique have been described elsewhere (Mao *et al.*, 2000).

^{13}C chemical-shift-anisotropy filter

In order to separate the signals of anomeric carbons (O-C-O) from those of aromatic carbons, both of which may resonate between 120 and 90 ppm, the aromatic-carbon signals were selectively suppressed by a five-pulse ^{13}C chemical-shift-anisotropy (CSA) filter with a CSA-filter time of 35 μs (Mao and Schmidt-Rohr, 2004).

Spectra processing

All spectra were zero-filled to 8192 data points and processed with a 50-Hz Lorentzian line broadening and a 0.010-s Gaussian broadening. Chemical shifts were externally calibrated to the methyl resonance of hexamethyl benzene with a peak at 17.36 ppm. The total signal intensity and the proportion of each type of C functional groups were determined by integration using the Mnova 8.0.2 NMR software package. It is important to note that the boundary assigned to each chemical shift region is only general and thought to be indicative of the major type of C functional groups present. Other techniques were used to reduce spinning side bands (SSB) by externally editing the spectral prior to integration.

4.4. Results and Discussion

4.4.1. Soil organic carbon concentration from size fractions

Carbon expressed on a basis of whole soil mass and carbon recovery rates in density fractions and in HF treatment at the three sites are shown in Table 4.3. The C recovery rate from size fractions was about 67-96.3%. The C concentration recovery rate from the 2% HF treatment was in a range of 60.1-101.1%. Treatments from site I revealed a relatively lower C recovery rate from HF treatment, compared with treatments from site II and site III. These soils from site I contained considerable proportions of iron minerals which were removed by the HF acids. Skjemstad *et al.* (1994) reported a recovery for C (83 to 92%) after treatment with 2% HF. Rumpel *et al.* (2006) reported a C recovery rate between 77%-93% from 2% HF treatment. The C recovery rates from 10% HF treatment are usually around 70%-90% (Schmidt *et al.*, 1997; Gonçalves *et al.*, 2003).

No significant difference in C loss was observed between treatments with different concentrations of HF (2% and 10%).

More POM C and HF-treated POM C were extracted from the compost and biosolids treated soils than from the control in Sites I and III, suggesting that organic amendments were capable of maintaining active soil C pool. The physical fractionation method used in combination with low-energy sonication allows researchers to follow changes in soil structure due to organic amendments on a long-term basis. Organic C present in particle-size fractions clearly responded to organic amendments in our study. Organic C in the silt-sized fraction contributed most to the whole soil, whereas the relative contribution of clay-sized and sand-sized C were relatively reduced (Gerzabek *et al.*, 2002). The organic residual-amended whole soils maintained higher total SOC concentration as well as higher soil C stocks than the control, indicating the capability of sequestering C after long-term application. Details of the C concentration and C stock of the whole soils at different depths from these three sites were analyzed and reported in previous study (Chapter 3).

Previous studies indicate that little organic matter is lost during HF treatment, which significantly improved NMR sensitivity and spectral resolution and decreased acquisition time and, hence, cost of NMR analysis (Smernik *et al.*, 2003). During the 2% HF treatments, some organic carbon loss had no significant effect on the organic chemistry of the samples (Skjemstad *et al.*, 1994; Schmidt *et al.*, 1997). For soils high in Fe, this procedure allows ^{13}C NMR spectra to be acquired that would otherwise be difficult to obtain. HF treatment of soil samples leads to SOM concentration and removal of paramagnetic substances and usually results in a significant improvement in the quality of the solid state ^{13}C NMR spectra.

4.4.2. Soil organic carbon speciation from NMR

Specific C functional groups by spectral editing techniques

Because ^{13}C CP/TOSS spectra of SOM are routinely broad, spectral-editing techniques such as dipolar dephasing and the ^{13}C CSA filter are required to identify specific functional groups. We selected the ^{13}C CP/TOSS spectra of the two samples as the representatives of all. ^{13}C CP/TOSS spectra Figure 4.1 (a) are used as reference spectra for the selective subspectra.

The spectra with CSA filter and dipolar dephasing are displayed in Figures 4.1 (b) (Nonprotonated and mobile aliphatic C). The spectra show the signals from nonprotonated carbons and carbons of mobile groups such as COO/N-C=O, aromatic C-O, nonprotonated aromatics, OC_qO, OCH_3, CCH_3 and mobile $(CH_2)_n$ groups. The samples showed signals of N-C=O/ COO around 173 ppm, aromatic C-O around 150 ppm, nonprotonated aromatic C-C around 130 ppm, OCH_3 between 50-60 ppm, slightly mobile CCH_2C around 30 ppm and CCH_3 around 22 ppm. The BS+SD sample revealed higher peaks of aromatic C-O around 150 ppm, nonprotonated aromatic C-C around 130 ppm, and OCH_3 between 50-60 ppm, suggesting higher proportions of phenolic C and aromatic C. By contrast, the 1993 BS from Site II displayed higher peaks of N-C=O/ COO around 173 ppm, slightly mobile CCH_2C around 30 ppm and CCH_3 around 22 ppm, indicating potentially more alkyl C.

Figures 4.1 (c) (sp^3-hybridized C) displays the spectra with only CH_n groups. The samples showed protonated aromatics between 110-140 ppm, OCHO around 105 ppm, OCH centered around 72 ppm, OCH_2 around 62 ppm, NCH between 50 -60 ppm, CCH around 40 ppm, CCH_2C around 30 ppm and CCH_3 around 22 ppm. The BS+SD sample revealed higher peaks of aromatics between 110-140 ppm, OCHO around 105 ppm, OCH centered around 72 ppm, OCH_2 around 62 ppm, NCH between 50 -60 ppm, suggesting a higher proportions of O-alkyl C. The anomeric peaks at 105 ppm are distinguished from aromatics by CSA filtering. Both samples contain appreciable anomerics, indicating the presence of sugar rings, although the BS + SD sample revealed a higher and clearer peak than the 1993 BS did. The 1993 BS sample show very small OC_qO signals above baselines, revealing that OCO groups are dominantly protonated in both samples. By contrast, the 1993 BS sample displayed higher peaks of CCH (~40 ppm), CCH_2C (~ 30 ppm) and CCH_3 (~ 22 ppm), which are assigned by CH_n groups.

In general, the BS+SD sample from Site III contained relatively more O-alkyl C, more aromatic C and less alkyl C than the 1993 BS sample. These results might indicate that the former sample is less decomposed or humified than the latter one.

Semi-quantitative ^{13}C CP/TOSS NMR of POM

Semi-quantitative ^{13}C CP/TOSS NMR spectra of POM from the three study sites are shown in Figure 4.2. Based on detailed structural information from the spectral editing techniques, the CP/TOSS spectra were integrated into eight chemical shift regions and the assignments were as follows: 0-45 ppm, nonpolar alkyl; 45-60 ppm, NCH and OCH_3; 60-94 ppm, O-alkyl C; 94-109 ppm, di-O-Alkyl C; 109-142 ppm, aromatic C-C; 142-163 ppm, aromatic C-O; 163-190 ppm, COO and N-C=O; and 190-220 ppm, ketone, quinone, or aldehyde C (Mao *et al.*, 2000). The relative intensities of C functional groups from CP/TOSS NMR are given in Table 4.4. The dominant composition of POM is carbohydrates (sum of O-alkyl and di-alkyl C), accounting for average 45.3 % of total SOC, followed by alkyl C groups (18.4%), aromatic C-C (14.2%), and OCH_3 / NCH 9.6 %. COO/N-C=O comprise 6.5%, aromatic C-O groups comprise 5.2%, and ketones and aldehydes comprise 0.8 %.

The spectra of POM from Site II showed relatively high intensity of alkyl C at 30 ppm, aromatic C at 130 ppm, and carboxylic C at 175 ppm compared with the other sites (Figure 4.2), indicating these two samples were more decomposed and humified than the other samples. The 1993 BS and 2011 BS samples were collected at a depth of 0-15 cm which contains more decomposed plant tissues and SOM. Ussiri and Johnson (2003) examined the spatial variations in the structure and chemistry of soil organic matter at the Hubbard Brook Experimental Forest in New Hampshire, and found that alkyl C, aromatic C and carbonyl C increased, while *O*-alkyl C decreased with soil depth in whole soils, humin, and humic acid.

The O-alkyl signal is an index of polysaccharides, such as cellulose, while the di-O-alkyl region provides a critical marker for the presence of condensed tannins. Tannins input from foliage, roots and bark is comparable to that of lignin (Preston, 2001). The nonpolar alkyl signal at 30 ppm originates from methylenic C in long-chain aliphatic compounds of varying origin, such as fatty acids, lipids, cutin acids, and other probably not yet identified aliphatic biopolymers. The OCH_3 and aromatic C-O signals are indicators of lignin residues. The NCH reveals the presence of proteins or peptides. The relative intensity of the peak at 130 ppm for aromatic C-C shows that the original lignin structure is altered and/or that material with such structures derived from other sources (e.g., charcoal, soot) is present in the soil. The signal at 175 ppm is derived from carboxyl and amide groups in various compounds (Kögel-Knabner, 1997).

The spectra can be treated as snapshots of the decomposition process. Organic matter entering the soil can be divided into four major groups of biomolecules, namely, polysaccharides (e.g., cellulose, hemicellulose, chitin), proteins, lipids/aliphatic materials (e.g., waxes, cutin, suberin), and lignins. Biomolecules, such as lipids and lignins, take longer to be degraded than polysaccharides, sugar, and proteins and can accumulate during the initial phase or organic residue decomposition. As the plant tissue decomposes to POM, the polysaccharide indicator (O-alkyl) declines, the lignin indicator (OCH_3) remains stable, and the lipid index (nonpolar alkyl) increases. At the same time, the abundance of carboxyl C roughly doubles, presumably a result of oxidative cleavage of ether groups as cellulose and lignin decompose (Fang *et al.*, 2010). Therefore, the relative amount of alkyl C increases during biodegradation, whereas the amount of O-alkyl C shows a relative decrease. The amount of aromatic components remains constant or shows various trends. Baldock and Preston (1995) suggested that the alkyl C intensity increased concomitantly with decreases in the O-alkyl C intensity. During decomposition of SOM, the relative O-alkyl C intensity decreases and the alkyl C intensity increases (Pîcek *et al.*, 2001). This information leads to the suggestion that the alkyl C to O-alkyl C ratio of a soil indicates the degree of SOM decomposition. Quideau *et al.* (2000) reported that there was a progressive decrease in O-alkyl C, and an increase in alkyl and carbonyl C from the litter to the fine silt and clay fractions due to the oxidative degradation of the litter material, with preferential decomposition of the cellulose and hemicellulose entities and selective preservation of recalcitrant waxes and resins.

Typically, CP NMR spectrum showed a much higher signal-to-noise (S/N) ratio and a considerably higher resolution than did DP NMR. The CP time is a compromise between the needs of quantitative reliability in a reasonable length of time. However, carbon forms with some mobility in the solid state may also be poorly detected by CP NMR. Keeler and Maciel (2003) suggested that the paramagnetic components, e.g., Fe (III) are likely to be largely responsible for the C discrepancy between CP/MAS and DP/MAS experiments. Our samples are Ultisols, which have accumulated iron oxide. Although 2% HF acid washes could remove most iron from soils, the remaining components still exist and affect NMR spectra.

We did not discuss the C functional groups proportions identified by CP/TOSS NMR in this study; instead, we relied on DP/MAS NMR technique to reveal a more confident and trustworthy results from SOC. However, plenty of studies focused on the comparison between CP/MAS and DP/MAS NMR techniques (Simpson and Hatcher, 2004). Schmidt *et al.* (1997) compared the spectra of six whole soils, some associated particle-size fractions, plant litter, and compost via both CP and DP NMR. The spectrum showed a very similar pattern and a similar relative intensity distribution but a possible loss of carbohydrates. Golchin *et al.* (1997) compared the CP and DP NMR of whole soil and three density fractions and showed that the proportions of aromatic and carbonyl C were higher, and the proportions of alkyl and O-alkyl were lower in the DP spectra.

Quantitative ^{13}C DP/MAS NMR of POM

Just as the CP/TOSS spectra, the DP/MAS spectra were also integrated into eight chemical shift regions and the assignments were as follows (Mao *et al.*, 2000): 0-45 ppm, nonpolar alkyl; 45-60 ppm, NCH and OCH_3; 60-94 ppm, O-alkyl C; 94-109 ppm, Di-O-

Alkyl C; 109-142 ppm, aromatic C-C; 142-163 ppm, aromatic C-O; 163-190 ppm, COO and N-C=O; and 190-220 ppm, ketone, quinone, or aldehyde C. The relative intensities of C functional groups are given in Table 4.5. The sidebands of sp^2-hybridized carbons are corrected based on the method provided by Mao and Schmidt-Rohr (2004) and Mao *et al.* (2000). The percentages of anomerics were obtained based on ^{13}C DP/MAS combined with the ^{13}C CSA filter and the aromatics were corrected by subtracting the overlapped anomerics.

The dominant component in POM is carbohydrates (sum of O-alkyl and di-alkyl C), accounting for average 28.4 % of total SOC, followed by aromatic C-C (22.1%), alkyl C groups (19.7%), and COO/N-C=O (10.8%). Aromatic C-O groups comprise 7.6%, OCH_3 / NCH 6.3 %, and ketones and aldehydes 5.1 %. Generally, the results of DP/MAS are consistent with those of CP/TOSS, although CP/TOSS is only a semi-quantitative technique. The contributions of the samples can be seen graphically as the relationship between the ratio of alkyl C to O-alkyl C and the C: N ratio (Figure 4.3), which have both been used as indicators for the degree of decomposition (Baldock *et al.*, 1997). All the samples clustered at relatively wide C/N ratio groups, but distributed variously at narrow alkyl C/O-alkyl C ratio groups. The alkyl C/O-alkyl C ratio is within the range of 0.31-1.87.

Study Site I

The alkyl C (0-45 ppm) decreased in order as: BSC (18.5%) > PYC (17.3%) > CON (13.0%)> PL (12.1%). The two compost-amended treatments maintained relatively higher POM alkyl C than CON and PL treatments. The increase in the alkyl C indicated greater decomposition with the addition of decomposable materials in the treatments

(Mathers and Xu, 2003). As decomposition proceeds, the relative concentration of the alkyl C should have increased (Baldock *et al.*, 1992b). All the treatments showed a major peak in the alkyl C region at 20-30 ppm from CP/TOSS NMR spectra (Figure 4.2). This signal can be assigned to methylene C (-CH_2-). Usually, surface waxes, lipids, cutins and resins contain this kind of C and is relative stable (Kögel-Knabner, 1997).

The O-alkyl C (60-109 ppm) decreases as: CON (40.8%) > PL (39.0%) > BSC (33.5%) > PYC (26.1%). This O-alkyl C region includes polysaccharides, alcohols, and ether-linked C. Although composts are reported to contain higher proportions of O-alkyl C prior to application, O-alkyl C decomposes faster than all the other types of C functional groups (Skjemstad *et al.*, 1994c; Schöning *et al.*, 2005). Leifeld *et al.* (2002) reported that polysaccharide C decreased 20-49% for the compost-amended soils and 9-20% for the controls after incubation for 18 months, which resulted in a decline of O–alkyl C for all soils. Additionally, the two compost-amended samples revealed lower O-alkyl C than manure-treated sample. Composting processes might have changed the C functional groups distribution in the organic residuals. Inbar *et al.* (1989) reported that the level of carbohydrates decreased while levels of alkyl C, aromatic C, and carboxyl groups increased during composting. Chen *et al.* (1989) investigated cattle manure at the start and mature stages of the composting process and reported an increase in aromaticity and carboxyl groups content and a decrease in carbohydrates during composting.

The alkyl C / O-alkyl C ratio decreases as: PYC (0.66) > BSC (0.55) > CON (0.32) > PL (0.31). There was more alkyl C and less O-alkyl C in compost-treated POM samples compared with PL and CON treatments. As decomposition is almost always associated with an increased content of alkyl C and a decreased content of O-alkyl C

(Pîcek *et al.*, 2001), the higher ratio revealed that the POM from the two compost-treated soils was more decomposed than CON and PL treatments. Baldock *et al.* (1997) showed that the ratio of alkyl C to O-alkyl C increases during the decomposition of composts due to decreasing proportions of O-alkyl C and an enrichment of alkyl C. These results indicated that the organic amendments might increase SOM decomposition and mineralization over time due to C inputs (Fontaine *et al.*, 2007), or that the addition of N strongly stimulated C mineralization in the composts (Paré *et al.*, 1998).

The aromatic C-C (109-142 ppm) decreased in the order PYC (23.6%) > BSC (22.3%) > CON (21.3%) > PL (20.8%). The overall aromatic C was higher for both compost-amended soil samples than CON and PL treated samples. Since aromatic C is very stable and can be accumulated during SOM humification processes, the higher aromatic C content in the compost-treated samples might have been generated during the composting processes. Samples taken from biosolids during the composting process showed that the residual OM contained an increasing level of aromatic structures (Chefetz *et al.*, 1996). Leifeld *et al.* (2002) found an increase in aromaticity and in carboxylic groups during composting of cattle manure and solid wastes, while O-alkyl carbon decreases. Chefetz *et al.* (1998) reported that the aromatic and phenolic C-containing groups increased by 23 and 16% during composting biosolids, respectively.

The organic residual-amended soil samples maintained higher aromatic C-O (142-163 ppm) than CON, especially PYC treatment (9.9%). This region covers carbonyl C in aromatic ring and partly phenolic C region. Composting degrades the phytotoxic compounds and produces phenolic C (Bustamante *et al.*, 2008), such as polyphenols, thus the two compost treatments might result in higher aromatic C-O in the SOM.

The COO/N-C=O (163-190 ppm) followed the order PYC (10.6%) > PL (9.6%) > BSC (8.6%) = CON (8.6%). The OCH_3/NCH (45-60 ppm) follows: BSC (7.45) > PYC (6.9%) > CON (6.0%) > PL (5.4%). The two groups often indicate carbohydrate residues (OCH and OCO) and peptides (NCH and N-C=O). These results indicated that the composts might contain higher proportions of carbohydrates and peptides than the control. Marche *et al.* (2003) investigated the organic composition of a paper mill sludge-hardwood sawdust composts and reported that the major components were lipids, sterols, lignin, N-compounds, and carbohydrates. However, the manure (PL) treatment which is a type of high N-containing waste did not exhibit higher proportions of these two kinds of C functional groups, although PL typically contains a high proportion of woody material in the form of wood shavings. Usually, N-containing compounds in organic residues are highly heterogenous, and proteinaceous materials are some of the first to be used by microbes (Paré *et al.*, 1998). Paul and Veen (1978) suggested that proteinaceous C (N-CH-) and carbohydrate C (CH_2-O-) were mineralized and assimilated faster than alkyl C during decomposition processes.

In addition, the presence of both OCH_3 and aromatic C-O signals are indicative of the existence of a small proportion of lignin residues, which is a cross-linked aromatic macromolecule in nature. During composting, the decomposition of the carbohydrates results in the accumulation of modified lignin. Lignin is known to be a recalcitrant compound and to resist microbial decomposition, potentially altering the decomposition rate of plant residues and the turnover time of POM (Fortuna *et al.*, 2003). Grandy and Neff (2008) established a model to describe that lignin presented a constraint to decomposition of plant litter and POM. The increase in aromaticity, in aromatic C and in phenolic C in the compost-treated samples reveals a preference by micro-organisms for easily biodegradable C compounds (Vinceslas-Akpa and Loquet, 1997).

The Ketone/aldehyde C (190-220 ppm) is decreasing as follows: PL (6.0%) > PYC (5.5%) > CON (3.7%) > BSC (2.4%). This region has traditionally been assigned to ketone, aldehyde, carboxylic, amide and ester C. Carboxylic C is related to humic acids, as it is a by-product of the humification processes. Little information was obtained from this region due to SSB overlaps with other regions, as well as chemical stabilities for these functional groups.

The alterations in the composition of organic matter during composting may partly account for the relative stability of C in soils, as indicated by larger C contents in compost-amended soils. During composting, easily degradable plant compounds such as carbohydrates and proteins are decomposed, and the more recalcitrant plant compounds, such as lignin, together with microbial products and non-identifiable aromatics remain. Among all the organic residual treatments, the PL treatment revealed highest proportions of O-alkyl C in the SOM. The PYC treatment resulted in higher alkyl C, lower O-alkyl C, and higher aromatic C, compared with BSC treatment. These differences of C functional groups might indicate various degrees of decomposition of SOC among the organic residuals. Lima *et al.* (2009) compared long-term applications of biosolids, manure and compost, and reported an increase in lignin in the SOM from soils amended with manure, an increase of protein in the SOM in soil amended with compost and a higher content of polysaccharides in the SOM from soils amended with biosolids.

Study site II

The alkyl C (0-45 ppm) accounts for contrasting proportions of SOC between the two samples (30.4 % for 1993 BS and 28.1% for 2011 BS). Compared with that from the other study sites, alkyl C from site II accounted for exceptionally higher proportions of SOC, as indicated by CP/TOSS NMR spectra (Figure 4.2). The two samples from site II were collected at a depth of 0-15 cm from surface soils, while the other samples were obtained by 0-7.5 cm depth. Evidence demonstrates a higher contribution of alkyl C in the deeper depth of the soils (Rumpel *et al.*, 2002; Fontaine *et al.*, 2007). Furthermore, Smith *et al.* (2008) reported that digestion process results in the specific organic domains rich in alkyl C. Aliphatic C accumulated in the biosolids has been reported to be resistant to the degradation (Schnaak *et al.*, 1997). Lipids, which constitute an important fraction of biosolids, can have an influence on the biodegradation -humification balance in soils (Réveillé *et al.*, 2003). Smernik *et al.* (2003) analyzed six anaerobically digested biosolids from wastewater treatment plants in Australia and reported that the alkyl C represents average 32 % of the whole biosolids.

The O-alkyl C (60-109 ppm) for the two samples are 16.3% for 1993 BS and 20.2% for 2011 BS, respectively. The alkyl C / O-alkyl C ratio is 1.87 for 1993 BS and 1.39 for 2011 BS, respectively. Since the alkyl C / O-alkyl C ratio of a soil indicates the degree of SOM decomposition, this result indicated that the C from 1993 BS was more decomposed and, thus, more stable than that from 2011 BS; however, the 2011 BS whole soil contained lower SOC than 1993 BS whole soil.

The aromatic C-C (109-142 ppm) is different between the two samples. The 1993 BS sample revealed a proportion of 18.6% aromatic C, while the 2011 BS treatment showed a value of 22.2% aromatic C. Long-term application of biosolids might have enhanced soil C humification processes and resulted in more aromatic C. Crop residues typically contain aromatic compounds, such as ferulic and p-coumaric acids, which occur as esters and ethers associated with plant cell wall structures (Montgomery, 2004). These results indicated that more crop residues might have been partly decomposed and accumulated as POM in the surface soils during 18 yrs of agricultural management.

The aromatic C-O (142-163 ppm) proportions are similar between 1993 BS (6.7%) and 2011 BS (6.4%). The COO/N-C=O (163-190 ppm) decreases in an order as: 1993 BS (13.7%) > 2011 BS (12.6%). The OCH_3/NCH (45-60 ppm) is equal in the two samples with a value of 5.7%. There were very few differences in these C functional groups between the two samples. The aromatic C-O (142-163 ppm) produced during biosolids processing, i.e., anaerobic digestion, might persist in soil and not degrade for a long time. Long-term application and decomposition of biosolids did not change the peptide, protein, or phenol proportions in the SOM. The biosolids N might have been decomposed and mineralized quickly during the initial period after application. Boyd *et al.* (1980) reported that the humic acids extracted from biosolids appeared to be more aliphatic, N-enriched polymer with decreased amounts of carboxyls and phenolic hydroxyls.

The NMR spectra reflect an increase of O-alkyl structures and the continual aromatization of the SOC but a decrease of alkyl components during 18 yrs of application. The enrichment of O-alkyl C and aromatic C in the POM might be resulted from the partly decomposition of crop residues. Newly accumulated POM from crop residues is less stable than the original SOC. However, the slight differences of C between the two samples might be insufficient to verify the assumptions that biosolids-amended soil C would be susceptible to decay and decompose after long-term of application..

Study site III

The alkyl C (0-45 ppm) decreases as: BS+SD (21.9%) > BS (18.5%) > CON (17.5%). During wastewater treatment, sludge contains bacterial metabolites that may enrich biosolids in alkyl C groups. Single high application of biosolids, as in this study, might add a high amount of alkyl C to the soil, thus leading to high alkyl C concentration in SOC. Leinweber *et al.* (1996) investigated the biosolids-treated soils from south of Berlin and reported enrichments of the biosolids-treated soils with aliphatic C, OCH_3 groups and amino acids.

The O-alkyl C (60-109 ppm) decreases as: CON (27.6%) =BS (27.5%) > BS+SD (24.0%). The O-alkyl C group mainly consists of cellulose and hemicelluloses, which are susceptible to microbial decomposition. Biosolids application might lead to the transformation of O-alkyl-dominant to alkyl-dominant which explains a low content of O-alkyl C in BS treatment. Particularly, the BS+SD treatment contained slightly lower O-alkyl C as a result of sawdust addition, it might be because that sawdust contained less O-alkyl C.

The alkyl C / O-alkyl C ratio is decreasing as: BS+SD (0.91) > BS (0.67) > CON (0.63). The BS+SD treatment maintained higher alkyl C / O-alkyl C ratio and thus indicated higher degree of SOM decomposition than the BS treatment did. Evidence has demonstrated that addition of labile C can increase SOC mineralization/decomposition and results in less C sequestration (Six *et al.*, 2002). The supply of labile C from sawdust contains many energy-rich C compounds, which may accelerate the microbial activity and induce a negative C balance (Fontaine *et al.*, 2004). Furthermore, the biosolids N inputs may have increased sawdust C decomposition/mineralization rates, as a higher N availability would have increased microbial activity (Anderson and Domsch, 1989).

The aromatic C-C (109-142 ppm) follows as: CON (24.1%) = BS+SD (24.1%) > BS (22.3%). The BS+SD treatment showed a higher aromatic C intensity than the BS treatment. Because aromatic C is related to humification processes, these results might indicate that C from sawdust is more humified into aromatic C (Melillo *et al.*, 1982). Although sawdust is reported to be highly resistant to microbial decomposition due to a high component of lignin (Mathers and Xu, 2003), our findings revealed that this C input from sawdust was more decomposed and did not accumulate in the active C pool. Tuomela *et al.* (2000) reported that lignin undergoes a gradual oxidative transformation process during biodegradation, thus introducing carboxylic groups into the molecule.

The aromatic C-O (142-163 ppm) proportions are the same among the three treatments with a percentage of 8%. The COO/N-C=O (163-190 ppm) follows: BS+SD (11.3%) = CON (11.3%) > BS (11.1%). The OCH_3/NCH (45-60 ppm) follows: BS+SD (6.7%) > CON (6.6%) > BS (6.5%). There are few differences in these functional groups among all three treatments. Biosolids addition has been demonstrated to be effective means for recovery of N (Magdoff and Amadon, 1980; Epstein, 2003). Apparently, the organic N from biosolids underwent a series of decomposition and mineralization processes through microbial activity and, thus, did not accumulate in the soils.

These results were consistent with the traditional view that the decomposition and humification of biosolids and sawdust in soils included a loss of carbohydrate, an increased aromatization, and a continual aliphatization of the SOC. Comparatively, the addition of sawdust into biosolids treatment imposed a higher degree of SOM decomposition with a higher alkyl C / O-alkyl C ratio. The supply of fresh C from sawdust contains many energy-rich C compounds which may accelerate the microbial decomposition and induce a negative C balance (Fontaine *et al.*, 2004). Our previous study (Chapter 3) on the same site reported that the BS+SD treatment maintained equal or lower C content than did the BS treatment although with larger C inputs.

4.5. Conclusions

Solid-state ^{13}C NMR spectroscopy proved to be a useful technique for obtaining information about the C chemical structure from the organic residual-amended soils. The particle size and density fractionation scheme employed concentrated soil organic materials into specific fractions successfully. Both CP/TOSS and DP/MAS ^{13}C NMR spectra showed that O-alkyl C functional groups were the dominant forms of organic C, followed by moderate amounts of aromatic C, alkyl C, COO/N-C=O, aromatic C-O, OCH_3 / NCH and ketones and aldehydes. The changes in chemical composition indicated that the organic residual-derived soil organic C was more decomposed than soil organic C found in unamended control treatments. The decomposition of SOM involved an initial loss of carbohydrate (hemicellulose and cellulose) followed by the slow transformation of the aromatic structures of lignin molecules and the accumulation of the highly recalcitrant nature of alkyl carbon. This information substantiates the grounds for the C stability of organic residual-amended soils and enhances our understanding of the bioavailability of these organic residuals.

4.6. Acknowledgements

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4.7. References

Adani, F., Genevini, P., Tambone, F. and Montoneri, E. 2006. Compost effect on soil humic acid: A NMR study. Chemosphere 65: 1414-1418.

Anderson, T.-H. and Domsch, K. H. 1989. Ratios of microbial biomass carbon to total organic carbon in arable soils. Soil Biology and Biochemistry 21: 471-479.

Baldock, J. A., Oades, J. M., Nelson, P., Skene, T., Golchin, A. and Clarke, P. 1997. Assessing the extent of decomposition of natural organic materials using solid-state 13C NMR spectroscopy. Australian Journal of Soil Research 35: 1061-1083.

Baldock, J. A., Oades, J. M., Waters, A. G., Peng, X., Vassallo, A. M. and Wilson, M. A. 1992. Aspects of the chemical structure of soil organic materials as revealed by solid-state ^{13}C NMR spectroscopy. Biogeochemistry 16: 1-42.

Baldock, J. A. and Preston, C. M. 1995. Chemistry of carbon decomposition processes in forests as revealed by solid-state carbon-13 nuclear magnetic resonance. Carbon forms and functions in forest soils. Madison, WI, Soil Science Society of America: *pp.* 89-117.

Bowden, C. L., Evanylo, G. K., Zhang, X., Ervin, E. H. and Seiler, J. R. 2010. Soil carbon and physiological responses of corn and soybean to organic amendments. Compost Science & Utilization 18: 162-173.

Boyd, S. A., Sommers, L. E. and Nelson, D. W. 1980. Changes in the humic acid fraction of soil resulting from sludge application. Soil Sci. Soc. Am. J. 44: 1179-1186.

Bustamante, M. A., Paredes, C., Marhuenda-Egea, F. C., Pérez-Espinosa, A., Bernal, M. P. and Moral, R. 2008. Co-composting of distillery wastes with animal manures:

Carbon and nitrogen transformations in the evaluation of compost stability. Chemosphere 72: 551-557.

Chefetz, B., Adani, F., Genevini, P., Tambone, F., Hadar, Y. and Chen, Y. 1998. Humic-acid transformation during composting of municipal solid waste. J. Environ. Qual. 27: 794-800.

Chefetz, B., Hatcher, P. G., Hadar, Y. and Chen, Y. 1996. Chemical and biological characterization of organic matter during composting of municipal solid waste. J. Environ. Qual. 25: 776-785.

Chen, Y., Inbar, Y., Hadar, Y. and Malcolm, R. L. 1989. Chemical properties and solid-state CPMAS 13C-NMR of composted organic matter. Science of The Total Environment 81–82: 201-208.

Christensen, B. 1992. Physical fractionation of soil and organic matter in primary particle size and density separates. Advances in Soil Sciences. 20: 1.

Christensen, B. T. 2001. Physical fractionation of soil and structural and functional complexity in organic matter turnover. European Journal of Soil Science 52: 345-353.

Conte, P., Spaccini, R. and Piccolo, A. 2006. Advanced CPMAS-^{13}C NMR techniques for molecular characterization of size-separated fractions from a soil humic acid. Analytical and Bioanalytical Chemistry 386: 382-390.

Daniels, W. L., Evanylo, G. K., Nagle, S. M. and Schmidt, J. M. 2003. Effects of biosolids loading rate on nitrate leaching potentials in sand and gravel mine reclamation in Virginia. Proceedings of the Water Environment Federation 2003: 271-278.

Ding, G., Novak, J. M., Amarasiriwardena, D., Hunt, P. G. and Xing, B. 2002. Soil organic matter characteristics as affected by tillage management. Soil Sci. Soc. Am. J. 66: 421-429.

Donohue, S. J. and Heckendorn, S. E. 1994. Soil test recommendations for Virginia. Blacksburg, VA., Virginia Cooperative Extension Service Publication. 834.

Epstein, E. 2003. Land application of sewage sludge and biosolids, CRC Press. Boca Raton, Florida.

Evanylo, G., Sherony, C., Spargo, J., Starner, D., Brosius, M. and Haering, K. 2008. Soil and water environmental effects of fertilizer-, manure-, and compost-based fertility practices in an organic vegetable cropping system. Agriculture, Ecosystems & Environment 127: 50-58.

Fang, X., Chua, T., Schmidt-Rohr, K. and Thompson, M. L. 2010. Quantitative ^{13}C NMR of whole and fractionated Iowa Mollisols for assessment of organic matter composition. Geochimica et Cosmochimica Acta 74: 584-598.

Flavel, T. C. and Murphy, D. V. 2006. Carbon and nitrogen mineralization rates after application of organic amendments to soil. J. Environ. Qual. 35: 183-193.

Fontaine, S., Bardoux, G., Abbadie, L. and Mariotti, A. 2004. Carbon input to soil may decrease soil carbon content. Ecology Letters 7: 314-320.

Fontaine, S., Barot, S., Barre, P., Bdioui, N., Mary, B. and Rumpel, C. 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450: 277-280.

Fortuna, A., Harwood, R. R. and Paul, E. A. 2003. The Effects of compost and crop rotations on carbon turnover and the particulate organic matter fraction. Soil Science 168: 434-444.

Francioso, O., Ciavatta, C., Sànchez-Cortés, S., Tugnoli, V., Sitti, L. and Gessa, C. 2000. Spectroscopic characterization of soil organic matter in long-term amendment trials. Soil Science 165: 495-504.

Franzluebbers, A. J. 2010. Achieving soil organic carbon sequestration with conservation agricultural systems in the southeastern United States. Soil Sci. Soc. Am. J. 74: 347-357.

Gerzabek, M. H., Haberhauer, G., Kandeler, E., Sessitsch, A. and Kirchmann, H. 2002. Response of organic matter pools and enzyme activities in particle size fractions to organic amendments in a long-term field experiment. Developments in Soil Science. A. Violante, P. M. H. J. M. B. and Gianfreda, L., Elsevier. 28: *pp.* 329-344.

Gerzabek, M. H., Haberhauer, G. and Kirchmann, H. 2001. Soil organic matter pools and carbon-13 natural abundances in particle-size fractions of a long-term agricultural field experiment receiving organic amendments. Soil Sci. Soc. Am. J. 65: 352-358.

Golchin, A., Baldock, J. A., Clarke, P., Higashi, T. and Oades, J. M. 1997. The effects of vegetation and burning on the chemical composition of soil organic matter of a volcanic ash soil as shown by 13C NMR spectroscopy. II. Density fractions. Geoderma 76: 175-192.

Golchin, A., Oades, J., Skjemstad, J. and Clarke, P. 1994. Study of free and occluded particulate organic matter in soils by solid state ^{13}C CP/MAS NMR spectroscopy and scanning electron microscopy. Soil Research 32: 285-309.

Golchin, A., Oades, J., Skjemstad, J. and Clarke, P. 1995. Structural and dynamic properties of soil organic-matter as reflected by ^{13}C natural-abundance, pyrolysis mass-spectrometry and solid-state ^{13}C NMR-spectroscopy in density fractions of an oxisol under forest and pasture. Soil Research 33: 59-76.

Gonçalves, C. N., Dalmolin, R. S. D., Dick, D. P., Knicker, H., Klamt, E. and Kögel-Knabner, I. 2003. The effect of 10% HF treatment on the resolution of CPMAS 13C NMR spectra and on the quality of organic matter in Ferralsols. Geoderma 116: 373-392.

Grandy, A. S. and Neff, J. C. 2008. Molecular C dynamics downstream: The biochemical decomposition sequence and its impact on soil organic matter structure and function. Science of The Total Environment 404: 297-307.

Hatcher, P. G., VanderHart, D. L. and Earl, W. L. 1980. Use of solid-state ^{13}C NMR in structural studies of humic acids and humin from Holocene sediments. Organic Geochemistry 2: 87-92.

Helfrich, M., Ludwig, B., Buurman, P. and Flessa, H. 2006. Effect of land use on the composition of soil organic matter in density and aggregate fractions as revealed by solid-state 13C NMR spectroscopy. Geoderma 136: 331-341.

Inbar, Y., Chen, Y. and Hadar, Y. 1989. Solid-state Carbon-13 Nuclear Magnetic Resonance and Infrared Spectroscopy of Composted Organic Matter. Soil Sci. Soc. Am. J. 53: 1695-1701.

Keeler, C. and Maciel, G. E. 2003. Quantitation in the Solid-State ^{13}C NMR Analysis of Soil and Organic Soil Fractions. Analytical Chemistry 75: 2421-2432.

Kögel-Knabner, I. 1997. ^{13}C and ^{15}N NMR spectroscopy as a tool in soil organic matter studies. Geoderma 80: 243-270.

Kölbl, A. and Kögel-Knabner, I. 2004. Content and composition of free and occluded particulate organic matter in a differently textured arable Cambisol as revealed by solid-state 13C NMR spectroscopy. Journal of Plant Nutrition and Soil Science 167: 45-53.

Kumar, K. and Goh, K. M. 1999. Crop residues and management practices: Effects on soil quality, soil nitrogen dynamics, crop yield, and nitrogen recovery. Advances in Agronomy. Donald, L. S., Academic Press. Volume 68: *pp.* 197-319.

Lal, R. 2006. Enhancing crop yields in the developing countries through restoration of the soil organic carbon pool in agricultural lands. Land Degradation & Development 17: 197-209.

Leifeld, J. and Kögel-Knabner, I. 2005. Soil organic matter fractions as early indicators for carbon stock changes under different land-use. Geoderma 124: 143-155.

Leifeld, J., Siebert, S. and Kögel-Knabner, I. 2002. Changes in the chemical composition of soil organic matter after application of compost. European Journal of Soil Science 53: 299-309.

Leinweber, P., Blumenstein, O. and Schulten, H. R. 1996. Organic matter composition in sewage farm soils: Investigations by 13C-NMR and pyrolysis-field ionization mass spectrometry. European Journal of Soil Science 47: 71-80.

Lima, D. L. D., Santos, S. M., Scherer, H. W., Schneider, R. J., Duarte, A. C., Santos, E. B. H. and Esteves, V. I. 2009. Effects of organic and inorganic amendments on soil organic matter properties. Geoderma 150: 38-45.

Magdoff, F. R. and Amadon, J. F. 1980. Nitrogen availability from sewage sludge. J. Environ. Qual. 9: 451-455.

Mahieu, N., Randall, E. W. and Powlson, D. S. 1999. Statistical analysis of published carbon-13 CPMAS NMR spectra of soil organic matter. Soil Sci. Soc. Am. J. 63: 307-319.

Mao, J., Hu, W.-G., Schmidt-Rohr, K., Davies, G., Ghabbour, E. A. and Xing, B. 2000. Quantitative characterization of humic substances by solid-state carbon-13 nuclear magnetic resonance. Soil Sci. Soc. Am. J. 64: 873-884.

Mao, J., Olk, D. C., Fang, X., He, Z. and Schmidt-Rohr, K. 2008. Influence of animal manure application on the chemical structures of soil organic matter as investigated by advanced solid-state NMR and FT-IR spectroscopy. Geoderma 146: 353-362.

Mao, J. and Schmidt-Rohr, K. 2004. Separation of aromatic-carbon ^{13}C NMR signals from di-oxygenated alkyl bands by a chemical-shift-anisotropy filter. Solid State Nuclear Magnetic Resonance 26: 36-45.

Marche, T., Schnitzer, M., Dinel, H., Paré, T., Champagne, P., Schulten, H. R. and Facey, G. 2003. Chemical changes during composting of a paper mill sludge–hardwood sawdust mixture. Geoderma 116: 345-356.

Marriott, E. E. and Wander, M. 2006. Qualitative and quantitative differences in particulate organic matter fractions in organic and conventional farming systems. Soil Biology and Biochemistry 38: 1527-1536.

Mathers, N. J., Jalota, R. K., Dalal, R. C. and Boyd, S. E. 2007. ^{13}C-NMR analysis of decomposing litter and fine roots in the semi-arid Mulga Lands of southern Queensland. Soil Biology and Biochemistry 39: 993-1006.

Mathers, N. J. and Xu, Z. 2003. Solid-state 13C NMR spectroscopy: characterization of soil organic matter under two contrasting residue management regimes in a 2-year-old pine plantation of subtropical Australia. Geoderma 114: 19-31.

Melillo, J. M., Aber, J. D. and Muratore, J. F. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63: 621-626.

Montgomery, R. 2004. Development of biobased products. Bioresource Technology 91: 1-29.

Paré, T., Dinel, H., Schnitzer, M. and Dumontet, S. 1998. Transformations of carbon and nitrogen during composting of animal manure and shredded paper. Biology and Fertility of Soils 26: 173-178.

Paul, E. A. and Veen, J. A. V. 1978. The use of tracers to determine the dynamic nature of organic matter. 11th Congress Int. Soc. Soil Science. Edmonton, Canada. 3: 61-102.

Pîcek, T., Hopkins, D. W., Haslam, S. F., Chudek, J. A., Webster, E. A. and Simek, M. 2001. The relationship between microbial carbon and the resource quality of soil carbon. J. Environ. Qual. 30: 147-150.

Preston, C. M. 1996. Applications of NMR to soil organic matter analysis: History and Prospects. Soil Science 161: 144-166.

Preston, C. M. 2001. Carbon-13 solid-state NMR of soil organic matter - using the technique effectively. Canadian Journal of Soil Science 81: 255-270.

Preston, C. M., Newman, R. H. and Rother, P. 1994. Using ^{13}C Cpmas NMR to assess effects of cultivation on the organic matter of particle size fractions in a grassland soil. Soil Science 157: 26-35.

Preston, C. M., Smernik, R. J., Powers, R. F., McColl, J. G. and McBeath, T. M. 2011. The decomposition of windrowed, chipped logging slash and tree seedling response: A plant growth and nuclear magnetic resonance spectroscopy study. Organic Geochemistry 42: 936-946.

Quideau, S. A., Anderson, M. A., Graham, R. C., Chadwick, O. A. and Trumbore, S. E. 2000. Soil organic matter processes: characterization by 13C NMR and 14C measurements. Forest Ecology and Management 138: 19-27.

Rappaport, B. D., Martens, D. C., Reneau, R. B. and Simpson, T. W. 1988. Metal availability in sludge-amended soils with elevated metal levels. J. Environ. Qual. 17: 42-47.

Réveillé, V., Mansuy, L., Jardé, É. and Garnier-Sillam, É. 2003. Characterisation of sewage sludge-derived organic matter: lipids and humic acids. Organic Geochemistry 34: 615-627.

Rumpel, C., Kögel-Knabner, I. and Bruhn, F. 2002. Vertical distribution, age, and chemical composition of organic carbon in two forest soils of different pedogenesis. Organic Geochemistry 33: 1131-1142.

Rumpel, C., Rabia, N., Derenne, S., Quenea, K., Eusterhues, K., Kögel-Knabner, I. and Mariotti, A. 2006. Alteration of soil organic matter following treatment with hydrofluoric acid (HF). Organic Geochemistry 37: 1437-1451.

Salati, S., Adani, F., Cosentino, C. and Torri, G. 2008. Studying soil organic matter using 13C CP-MAS NMR: The effect of soil chemical pre-treatments on spectra quality and representativity. Chemosphere 70: 2092-2098.

Schmidt, M. W. I., Knicker, H., Hatcher, P. G. and Kogel-Knabner, I. 1997. Improvement of ^{13}C and ^{15}N CPMAS NMR spectra of bulk soils, particle size fractions and organic material by treatment with 10% hydrofluoric acid. European Journal of Soil Science 48: 319-328.

Schnaak, W., Küchler, T., Kujawa, M., Henschel, K. P., Süßenbach, D. and Donau, R. 1997. Organic contaminants in sewage sludge and their ecotoxicological significance in the agricultural utilization of sewage sludge. Chemosphere 35: 5-11.

Schnitzer, M. and Khan, S. U. 1978. Soil organic matter. Elsevier Scientific Pub. Co. New York, NY.

Schöning, I., Morgenroth, G. and Kögel-Knabner, I. 2005. O/N-alkyl and alkyl C are stabilised in fine particle size fractions of forest soils. Biogeochemistry 73: 475-497.

Schulten, H. R. and Leinweber, P. 1991. Influence of long-term fertilization with farmyard manure on soil organic matter: Characteristics of particle-size fractions. Biology and Fertility of Soils 12: 81-88.

Simpson, M. J. and Hatcher, P. G. 2004. Determination of black carbon in natural organic matter by chemical oxidation and solid-state 13C nuclear magnetic resonance spectroscopy. Organic Geochemistry 35: 923-935.

Simpson, T. W., Donohue, S. J., Hawkins, G. W., Monnett, M. M. and Baker, J. C. 1993. The development and implementation of the Virginia agronomic land use evaluation system (VALUES). Blacksburg, VA., Department of Crop and Soil Environmental Sciences, Virginia Tech.

Six, J., Conant, R. T., Paul, E. A. and Paustian, K. 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant and Soil 241: 155-176.

Skjemstad, J. O., Clarke, P., Taylor, J. A., Oades, J. M. and Newman, R. H. 1994. The removal of magnetic materials from surface soils - a solid state ^{13}C CP/MAS NMR study. Australian Journal of Soil Research 32: 1215-1229.

Smernik, R. J., Oliver, I. W. and Merrington, G. 2003. Characterization of sewage sludge organic matter using solid-state carbon-13 nuclear magnetic resonance spectroscopy. J. Environ. Qual. 32: 1516-1522.

Smith, M. T. E., Smernik, R. J., Merrington, G. and Tibbett, M. 2008. Changes in sewage sludge carbon forms along a treatment stream. Chemosphere 72: 981-985.

Sojka, R. E., Upchurch, D. R. and Borlaug, N. E. 2003. Quality soil management or soil quality management : performance versus semantics. Advances in Agronomy, Academic Press. Volume 79: *pp.* 1-68.

Sollins, P., Glassman, C., Paul, E. A., Swanston, C., Lajtha, K., Heil, J. W. and Elliott, E. T. 1999. Soil carbon and nitrogen: pools and fractions. Standard Soil Methods for Long-term Ecological Research: 89-105.

Spargo, J. T., Evanylo, G. K. and Alley, M. M. 2006. Repeated compost application effects on phosphorus runoff in the Virginia Piedmont. J. Environ. Qual. 35: 2342-2351.

Stewart, C. E., Follett, R. F., Wallace, J. and Pruessner, E. G. 2012. Impact of biosolids and tillage on soil organic matter fractions: Implications of carbon saturation for conservation management in the Virginia Coastal Plain. Soil Sci. Soc. Am. J.: In progress.

Stone, A. G., Traina, S. J. and Hoitink, H. A. J. 2001. Particulate organic matter composition and pythium damping-off of cucumber. Soil Sci. Soc. Am. J. 65: 761-770.

Stover, H. D. H. and Frechet, J. M. J. 1989. Direct polarization carbon-13 and proton magic angle spinning NMR in the characterization of solvent-swollen gels. Macromolecules 22: 1574-1576.

Sukkariyah, B. F., Evanylo, G. K., Zelazny, L. and Chaney, R. L. 2005. Cadmium, copper, nickel, and zinc availability in a biosolids-amended Piedmont soil years after application. J. Environ. Qual. 34: 2255-2262.

Tiessen, H. and Stewart, J. W. B. 1983. Particle-size fractions and their use in studies of soil organic matter: II. Cultivation effects on organic matter composition in size fractions. Soil Sci. Soc. Am. J. 47: 509-514.

Tuomela, M., Vikman, M., Hatakka, A. and Itävaara, M. 2000. Biodegradation of lignin in a compost environment: a review. Bioresource Technology 72: 169-183.

Ussiri, D. A. N. and Johnson, C. E. 2003. Characterization of organic matter in a northern hardwood forest soil by ^{13}C NMR spectroscopy and chemical methods. Geoderma 111: 123-149.

Vinceslas-Akpa, M. and Loquet, M. 1997. Organic matter transformations in lignocellulosic waste products composted or vermicomposted (eisenia fetida andrei): Chemical analysis and ^{13}C CPMAS NMR spectroscopy. Soil Biology and Biochemistry 29: 751-758.

Virginia Cooperative Extension 1992. Pest management guide for field crops. Virginia Tech: Blacksburg, VA, VA Coop. Ext. Publ. No. 456-016: 1-276.

Wander, M. M., Traina, S. J., Stinner, B. R. and Peters, S. E. 1994. Organic and conventional management effects on biologically active soil organic matter pools. Soil Sci. Soc. Am. J. 58: 1130-1139.

Wilson, M. A. 1987. NMR techniques and applications in geochemistry and soil chemistry. Oxford, Pergamon Press.

Wilson, M. A., Pugmire, R. J., Zilm, K. W., Goh, K. M., Heng, S. and Grant, D. M. 1981. Cross-polarization ^{13}C-NMR spectroscopy with /`magic angle/' spinning characterizes organic matter in whole soils. Nature 294: 648-650.

Figure Captions

Figure 4.1. Spectral editing for identification of functional groups in 1993 BS from Site II and BS+SD from Site III. (a) Unselective CP/TOSS spectra for reference; (b) Corresponding dipolar-dephased CP/TOSS spectra with nonprotonated C and mobile CH_2 and CH_3 and (c) Selection of sp^3-hybridized carbon signals by a ^{13}C CSA filter, which in particular identifies O-C-O carbons, near 105 ppm.

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Figure 4.2. Semi-quantitative ^{13}C CP/TOSS NMR spectra at a spinning speed of 13 kHz of (a) CON, PYC, BSC and PL from Site I; (b) 1993 BS and 2011 BS from Site II and (c) CON, BS, and BS+SD from Site III. CON = control; PL = poultry litter; BSC = biosolids-woodchip compost; PYC = poultry litter-yard waste compost; BS = biosolids; SD = sawdust.

Figure 4.3. The ratio of alkyl C to O-alkyl C as measured by DP/MAS NMR versus the C to N ratio for particulate organic matter extracted from organic residual amended soils from the three field sites. CON = control; PL = poultry litter; BSC = biosolids-woodchip compost; PYC = poultry litter-yard waste compost; BS = biosolids; SD = sawdust.

Table 4.1. Soil series, treatments management and sampling depths in the three study sites.

Site	Soil Series	Complete Treatment	Frequency	Selected Treatment	Sampling Depth
Site I	Fauquier silty clay loam (*Fine, mixed, mesic Ultic Hapludalfs*)	Control, poultry litter, poultry litter yard waste compost, and biosolids woodchip compost; all were based on the agronomic N rate.	Continuous from 2000-2004	Control, poultry litter, poultry litter yard waste compost, and biosolids woodchip compost	0-7.5 cm
Site II	Davidson clay loam (*Clayey, kaolinitic, thermic, Rhodic Paleudult*)	Six rates of aerobically digested biosolids (0, 42, 84, 126, 168, and 210 dry Mg ha^{-1}); the agronomic rate of biosolids was 42 Mg ha^{-1}.	Single in 1984	210 dry Mg ha^{-1} biosolids in 1993 and 2011	0-15 cm
Site III	Pamunkey sandy loam (*Fine-loamy, mixed, thermic Ultic Hapludalfs*)	Five rates of anaerobically digested biosolids (0, 14, 42, 70, and 98 dry Mg ha^{-1}), with and without sawdust to adjust the C:N ratio; the agronomic rate of biosolids was 14 Mg ha^{-1}.	Single in 1996	Control, 98 dry Mg ha^{-1} biosolids and 98 dry Mg ha^{-1} biosolids with sawdust	0-7.5 cm

Table 4.2. Selected chemical properties of the organic residuals applied to the three study sites.

Study Site	Treatment	C:N	C	TKN	NH_4-N	NO_3-N	Organic-N	Total P	K
			--	g kg^{-1}	------------------------				---
Site I	PL†	8	474	56.1	8.2	0.19	48	17.6	26.3
	BSC‡	10	239	26.0	3.2	0.88	22	15.6	1.1
	PYC§	20	379	19.3	0.2	0.73	18	4.2	6.8
Site II	BS¶	20	320	16	<0.008	--	16	31.7	1.1
Site III	BS	8	358	44.7	6.4	0.30	38	17	1.2
	SD#	198	475	2.4	--	--	2.4	--	--

†PL = poultry litter; ‡BSC = biosolids-woodchip compost; §PYC = poultry litter-yard waste compost; ¶BS = biosolids; #SD = sawdust.

Table 4.3. Carbon expressed as a fraction of whole soil mass and carbon recovery rate in density fractions and in hydrogen fluoride (HF) treatment from different organic residual-amended soils at the three sites.

Site	Treatment	C, g kg^{-1} whole soil	C expressed as a fraction of whole soil, g kg^{-1}						% C recovery in density fractions	% POM-C recovery in HF
			POM[††]	clay	silt	sand	sum	HF-POM		
Site I	CON[‡‡]	23.2	2.4	5.5	7.6	6.9	22.3	1.5	96.3	61.0
	PYC[§]	47.4	2.8	9.7	16.4	2.8	31.8	1.7	67.0	60.1
	BSC[‡]	37.5	3.2	8.2	14.2	9.2	34.8	2.3	92.8	71.2
	PL[†]	23.7	2.0	5.0	13.5	6.2	26.7	1.2	112.6	60.3
Site II	1993 BS[¶]	23.4	2.2	5.2	6.1	5.0	18.5	2.2	78.9	98.8
	2011 BS	18.8	2.6	5.9	4.3	5.3	18.1	2.6	96.3	98.7
Site III	CON	15.0	1.0	3.8	4.0	2.0	10.8	1.0	71.8	100.5
	BS	19.1	1.2	4.8	5.5	3.8	15.3	1.2	80.0	100.8
	BS +SD[#]	18.8	1.6	4.0	5.7	2.8	14.1	1.6	75.0	101.1

†PL = poultry litter; ‡BSC = biosolids-woodchip compost; §PYC = poultry litter-yard waste compost; ¶BS = biosolids; #SD = sawdust; ††POM=particulate organic matter. ‡‡CON = control.

Table 4.4. Percentages of total spectral area assigned to different functional groups obtained by semi-quantitative ^{13}C cross polarization / magic angle spinning (CP/TOSS) NMR and spectral-editing techniques.

	ppm	220-190	190-163	163-142	142-109	109-94	94-60	60-45	45-0
		Ketone/aldehyde	COO/N-C=O	Arom. C-O	Arom. C-C	Di-O-Alkyl	O-Alkyl	OCH_3/NCH	Alkyl
Site I	CON††	0.6	4.7	4.2	12.1	11.2	48.2	8.3	10.7
	PYC§	1	6.5	7	15.5	8.6	34.7	10.7	16
	BSC‡	0.4	4.8	4.7	13.2	9.8	40.9	10.5	15.7
	PL†	1	5.4	4.7	12.1	11.3	47.7	7.6	10.3
Site II	1993 BS¶	0.6	8.8	4.8	12.6	6.4	24.1	10	32.7
	2011 BS	0.9	8	4.7	15.6	6.5	28.1	9.1	27
Site III	CON	0.9	6.8	5.6	15.7	8.8	36.2	10	16
	BS	1.1	6.7	5.6	14.6	8.8	36.2	10	17
	BS +SD#	0.7	6.9	5.7	16.3	7.8	32	10.3	20.3

The percentages of anomerics were obtained based on ^{13}C CP/TOSS NMR combined with ^{13}C chemical-shift-anisotropy (CSA) filter. †PL = poultry litter; ‡BSC = biosolids-woodchip compost; §PYC = poultry litter-yard waste compost; ¶BS = biosolids; #SD = sawdust; ††CON = control.

Table 4.5. Percentages of total spectral area assigned to different functional groups obtained by quantitative ^{13}C direct polarization / magic angle spinning (DP / MAS) NMR and spectral-editing techniques.

	ppm	220-190	190-163	163-142	142-109	109-94	94-60	60-45	45-0
		Ketone/aldehyde	COO/N-C=O	Arom. C-O	Arom. C-C	Di-O-Alkyl	O-Alkyl	OCH_3/NCH	Alkyl
Site I	CON††	3.7	8.6	6.7	21.3	6.5	34.3	6	13
	PYC§	5.5	10.6	9.9	23.6	4.1	22	6..9	17.3
	BSC‡	2.4	8.6	7.2	22.3	5.3	28.2	7.4	18.5
	PL†	6	9.6	7.2	20.8	6.2	32.8	5.4	12.1
Site II	1993 BS¶	8.6	13.7	6.7	18.6	2.5	13.8	5.7	30.4
	2011 BS	4.8	12.6	6.4	22.2	3	17.2	5.7	28.1
Site III	CON	5	11.3	8	24.1	4.4	23.2	6.6	17.5
	BS	6.1	11.1	8	22.3	4.4	23.1	6.5	18.5
	BS +SD#	3.9	11.3	8	24.1	3.8	20.2	6.7	21.9

The percentages of anomerics were obtained based on ^{13}C DP / MAS combined with ^{13}C chemical-shift-anisotropy (CSA) filter. †PL = poultry litter; ‡BSC = biosolids-woodchip compost; §PYC = poultry litter-yard waste compost; ¶BS = biosolids; #SD = sawdust; ††CON = control.

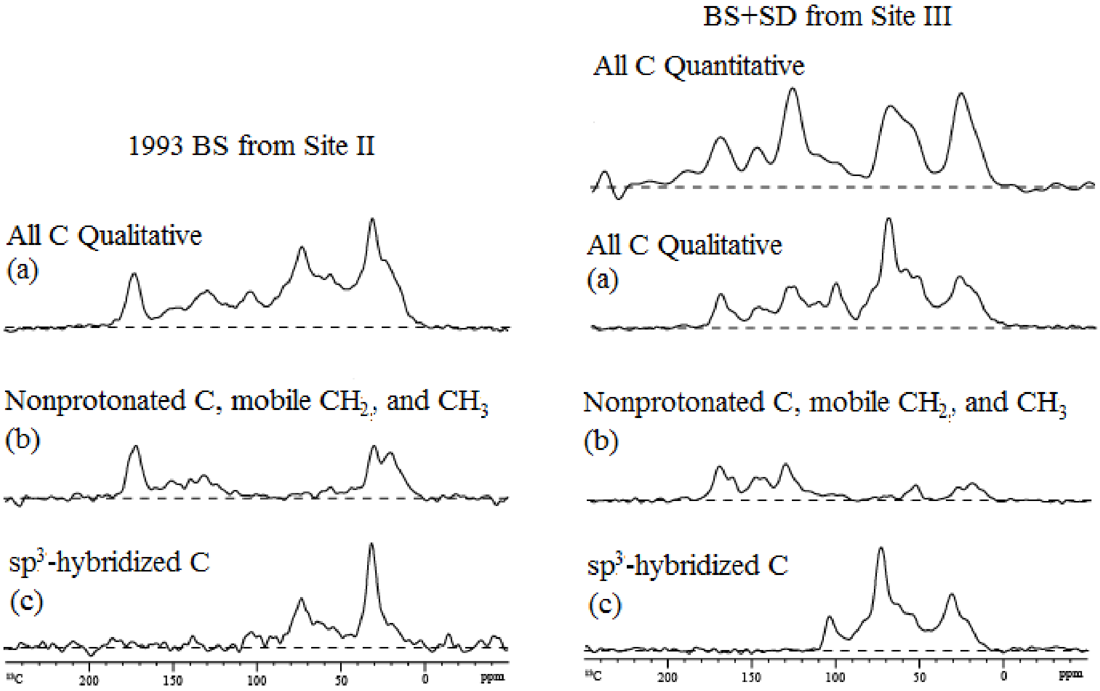
BS+SD from Site III
All C Quantitative
1993 BS from Site II
All C Qualitative
(a)
All C Qualitative
(a)
Nonprotonated C, mobile CH$_2$, and CH$_3$
(b)
Nonprotonated C, mobile CH$_2$, and CH$_3$
(b)
sp^3-hybridized C
(c)
sp^3-hybridized C
(c)
^{13}C
200
150
100
50
0
ppm
^{13}C
200
150
100
50
0
ppm

Figure 4.1

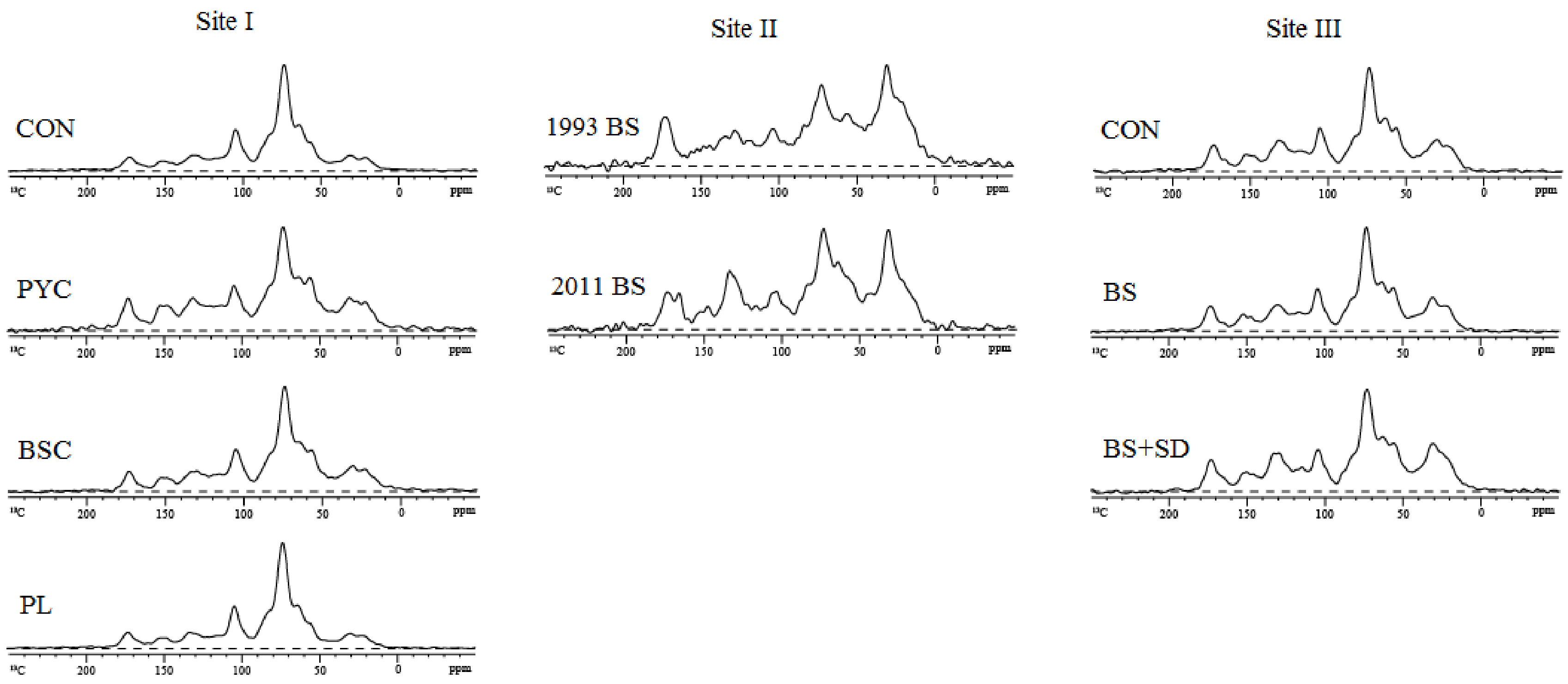
Site I
CON
PYC
BSC
PL
Site II
1993 BS
2011 BS
Site III
CON
BS
BS+SD
13C
200
150
100
50
0
ppm

Figure 4.2.

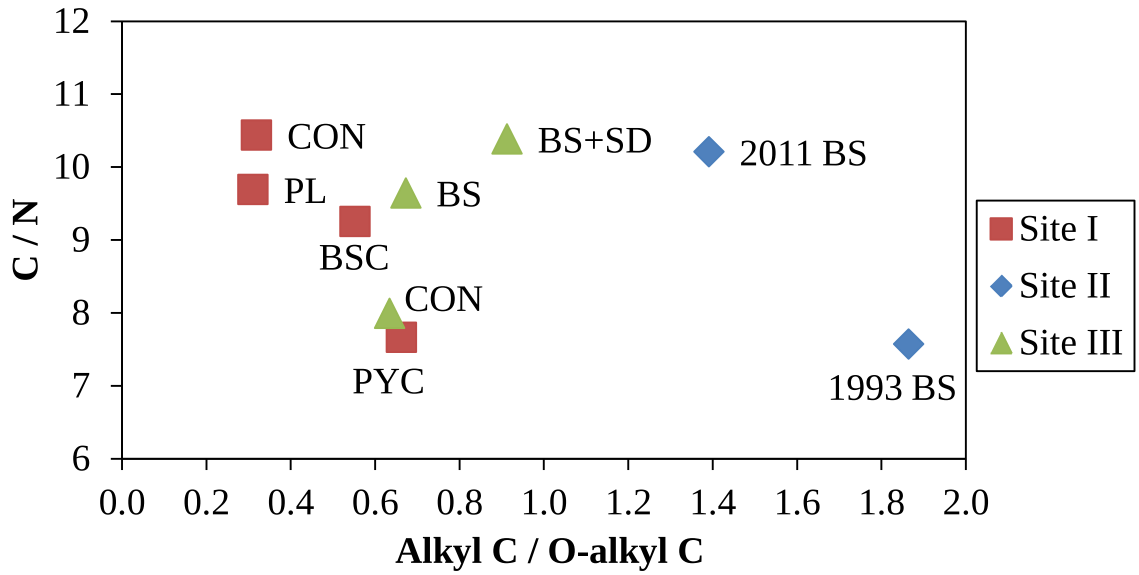
12
11
10
9
8
7
6
C / N
CON
PL
BSC
PYC
BS
BS+SD
CON
2011 BS
1993 BS
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
Alkyl C / O-alkyl C
Site I
Site II
Site III

Figure 4.3.

5. Carbon (1s) K-edge Near Edge X-ray Absorption Fine Structure (NEXAFS) Spectroscopy for Carbon Dynamics from Long-term Application of Organic Residual

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5.1 Abstract

Application of organic residuals (e.g. biosolids and composts) to soil may provide an effective method for sequestering carbon (C), but the long term stability of such C is not well known. Three field sites across Virginia were investigated in 2011 to characterize C status remaining in soils following a history of amendment with biosolids and composts. Pre-treated C fractions were obtained by physical separation methods in order to extract particulate organic matter (POM). Carbon (1s) near edge X-ray absorption fine structure spectroscopy (NEXAFS) was used to investigate the development of major organic C speciation in the POM fraction of the organic residual-amended soils. The NEXAFS spectra revealed the average presence of O-alkyl C (41.2%), aromatic C (17.1%), alkyl C (15.7%), carboxylic C (14.9%), and phenolic C (11.2%) in the POM of all soils. The alkyl C / O-alkyl C ratio was within a range of 0.19-0.69 in the POM of all soils, suggesting a broad degree of soil organic C decomposition. These results reveal that the chemistry of organic amendments imposes long term effects in C dynamics.

5.2. Introduction

The Intergovernmental Panel on Climate Change (IPCC) has confirmed that increasing atmospheric carbon dioxide (CO_2) concentration is the main factor leading to global warming (IPCC, 1990). Carbon (C) sequestration may play an important role in capturing carbon dioxide emission and avoiding climate change (Batjes, 1998; Lal, 2004). Land application of organic amendments has been demonstrated to increase soil organic matter and, potentially, sequester C in the Mid-Atlantic region of the United States (Sukkariyah *et al.*, 2005; Spargo *et al.*, 2006; Franzluebbers, 2010; Stewart *et al.*, 2012). The application of organic residuals affects the amount and the composition of soil organic carbon (SOC) via organic matter decomposition and humification processes.

The soil organic matter (SOM), of which carbon is a major component, is a heterogeneous mixture composed of living biomass of microorganism, fresh and partially decomposed plant, animal and microbial residues, and well-decomposed humic substances (Schnitzer and Khan, 1978). The amount, structural composition and stability of SOM, however, can be dramatically influenced by climate, time, soil, and anthropogenic activities, e.g., addition of organic amendments (Parton *et al.*, 1987; Six *et al.*, 2002; Solomon *et al.*, 2007). These influences create significant analytical problems and, thus, have made studies on SOM composition and their implications for the effects of organic residuals on C sequestration challenging.

Soil organic matter fractions with turnover times of years to decades, such as particulate organic matter (POM), often respond more rapidly to management-induced changes in the SOC pool than more stabilized, mineral-associated fractions with longer turnover times (Sollins *et al.*, 1999). POM is thought to represent partly decomposed or undecayed plant materials at an early stage of decomposition, thus representing a transitional stage in the humification process (Christensen, 2001; Leifeld and Kögel-Knabner, 2005). Application of organic residuals can increase the SOC pools and change soil quality before net organic matter contents change (Wander *et al.*, 1994; Kumar and Goh, 1999; Sojka *et al.*, 2003; Lal, 2006).

To isolate SOM fractions, numerous fractionation schemes have been developed and applied in SOM studies, among which physical and chemical fractionations are the most widely used procedures (Post and Kwon, 2000; Christensen, 2001). Chemical extractions were dominant until recent decades, when physical extractions became increasingly popular and more elaborate (Olk and Gregorich, 2006). Physical fractionations have been used widely to investigate soil C turnover and decomposition, allowing for the separation of physically uncomplexed organic matter based on density or size of primary (clay-, silt-, and sand sized particles) or secondary (aggregates) organo-mineral complexes (Golchin *et al.*, 1994a). The distribution of SOM can be assessed by the separation of physical fractions based on particle size or density (Buyanovsky *et al.*, 1994), via shaking and sonication (Elliott and Cambardella, 1991). The POM, including the free light fraction and the occluded particulate OM, can be separated from mineral particles by flotation on a liquid with density of 1.8 g mL^{-1}.

Recent investigations of soil organic matter using synchrotron-based C (1s) near-edge X-ray absorption fine structure (NEXAFS) spectroscopy have demonstrated that it can be used to identify and fingerprint the complex structural characteristics of SOC, as well as to investigate the impact of management on the composition and biogeochemical cycling of organic C at the molecular level (Sollins *et al.*, 2007). NEXAFS spectroscopy is an advanced tool for SOC characterization because: 1) Unlike solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy which is sensitive to interferences by paramagnetic minerals, NEXAFS is elemental specific and, therefore, there is less interference from other elements; 2) it is an nondestructive technique allowing for thorough analysis with minimal damage to the sample; 3) it can detect and identify individual C components of a given soil with low C content.

NEXAFS uses the intense, tunable, polarized X-ray beams generated by a synchrotron light source to probe the electronic states of a sample (Watts *et al.*, 2006). It probes the X-ray absorption cross section of a sample through inner-shell excitation processes. At photon energy close to an atomic absorption edge, inner shell electrons are excited to an unoccupied energy level, creating resonance peaks in the absorption spectra (Stöhr, 2003). Carbon NEXAFS features arise when incident photon energy from synchrotron radiation is increased throughout the absorption K-edge, which is at a specific energy level for each element (284 -290 eV for C). At this energy level, core electrons (in the K shell) are promoted to higher orbitals (above the K-edge) or completely removed (above the ionization threshold) by the photons (Lehmann and Solomon, 2010). Using a tunable monochromator, C NEXAFS spectra close to the K-edge can be collected either by measuring the absorption of the photons, in fluorescent mode, where the emitted photons are monitored, or by determining total electron yield, in which the neutralization current from the sample is recorded (Schulze and Bertsch, 1995). Generally, C (1s) NEXAFS spectra are split into five main regions: aromatic C, phenolic C, alkyl C, carboxylic C and O-alkyl C. This technique renders an almost-quantitative or semi-quantitative way to compare functional groups of organic C in a sample

(Schumacher, 2005). During organic matter decomposition, the C functional groups in the soil change in characteristic ways: O-alkyl C is lost initially and alkyl C is increasing accordingly (Golchin *et al.*, 1994b). Therefore, investigating dynamic C structures via NEXAFS could provide essential information of the degree of decomposition of SOM.

Carbon (1s) NEXAFS has been effectively employed in the past to study soil colloids (Schumacher *et al.*, 2005), soil aggregates (Wan *et al.*, 2007; Solomon *et al.*, 2012), humic substances (Solomon *et al.*, 2005; Sedlmair *et al.*, 2009), and black carbon (Heymann *et al.*, 2011). Solomon *et al.* (2009) used NEXAFS spectroscopy to determine spectral signatures and peak positions of major organic molecules, i.e., carbohydrates, amino sugars and amino acids, etc. in natural organic matter (NOM). Lehmann *et al.* (2008) investigated forest soils and demonstrated that SOC speciation detected below 50 nm resolution had no similarity to the organic carbon speciation of total soil, which were remarkably similar between soils from several temperate and tropical forests with very distinct vegetation composition and soil mineralogy. However, few studies have reported on the C speciation of various size fractions characterized by NEXAFS. Solomon *et al.* (2005) used C (1s) NEXAFS to speciate C in the humic substances extracted from clay and silt fractions from natural forest, tea and Cupressus plantations, and cultivated fields in Ethiopia. The spectra showed that carboxylic-C and O-alkyl-C functional groups were the dominant forms of organic C, followed by moderate amounts of aromatic-C and phenolic-C groups, and the aliphatic-C forms contributed the least portion of the total SOM in the humic substances extracted from the two size fractions.

So far, no NEXAFS study has attempted to characterize the chemistry of SOC from different types of organic amendments within Mid-Atlantic region of the United

States. The main objectives of this research were (i) to characterize the organic C chemistry of soils from field sites with various history of organic amendments, (ii) to determine the value of NEXAFS in distinguishing functional groups of SOC from field sites with various histories of organic amendments, and (iii) to compare the NEXAFS results with the those obtained using ^{13}C NMR.

5.3. Materials and Methods

5.3.1. Experimental sites

Study site I

This experimental site was located at the Northern Piedmont Agricultural Research and Extension Center (NPAREC) on a Fauquier silty clay loam (*Fine, mixed, mesic Ultic Hapludalfs*) in Orange, VA. Eight treatments were established in the spring of 2000 to investigate the agronomic and environmental effects of compost use in the Virginia Piedmont (Table 5.1) (Bowden *et al.*, 2010). The soil properties prior to organic amendment application are shown in Table 5.2. The treatments were arranged in a randomized complete block design with four replicates. Each plot measured 3.6 m wide by 7.5 m long. The following four treatments were selected for reporting in this study as: unamended control (CTL); poultry litter (PL); poultry litter-yard waste compost (PYC); and biosolids-woodchip compost (BSC).

The PL was annually applied at rates estimated to meet crop N requirements. The compost treatments established in 2000 were biennial (2000, 2002) agronomic N rates of poultry litter-yard waste compost with (2001) and without supplemental inorganic fertilizer N. Then beginning in the spring of 2003, two of the four replicates of each of the two biennial compost treatments continued to receive annual (2003, 2004) applications of agronomic N rates of poultry litter-yard waste compost, and the other two replicates from the same treatments began to receive annual (2003, 2004) applications of agronomic N rates of a biosolids-woodchip compost. No organic amendment or inorganic fertilizer was applied beyond spring 2005. However, in August 2010, N fertilizer was applied to the entire site for tall fescue maintenance. Lime was applied in April 2004 to achieve a target pH of 7 to all plots except the BSC treatment, as lime was added during treatment of biosolids .

The poultry litter-yard waste compost (PYC) was a commercially-produced material (PYC; Panorama Farms, Earlysville, Virginia), whose poultry litter and yard waste feedstocks were combined at a ratio of 1:2 (v/v) and composted using turned windrow technology for 120 days. The biosolids-woodchip compost (BSC) was produced at the Rivanna Water and Sewer Authority (Charlottesville, Virginia) from anaerobically-digested biosolids dewatered with $Ca(OH)_2$ and composted with wood chips (1:2 ratio of biosolids and woodchips) for 21 days, including five consecutive days at 66°C, via static pile technology. The material was cured for an additional 10 days after screening through a 0.95 cm sieve to remove oversized woodchips. A commercially-processed, screened poultry litter (PL; Glen Hill Farm, Harrisonburg, Virginia) was used for the poultry litter treatment. Chemical properties of the organic residuals applied are summarized in Table 5.3. Soil amendments were hand-applied and incorporated within 24 h associated with seedbed preparation by roto-tilling.

The initial crops included pumpkin (*Cucurbita pepo* V. Magic Lantern) in 2000, sweet corn (*Zea mays* L. V. Silver Queen) in 2001, and bell pepper (*Capsicum annuum*

V. Aristotle) in 2002. Following modification of the treatments in spring 2003, corn (*Zea mays* L. V. Pioneer 31G20) was grown in 2003 and 2004 and soybean (*Glycine max* V. Delta Pine 4933RR) was grown in 2005. Cereal rye (*Secale cereal* L.) was planted in all plots in the autumn of 2000-2005 as a winter cover crop. Tall fescue (*Festuca arundinacea* Schreb.) was planted in spring 2006 and has been maintained in all plots with twice annual bush hogging as the only management practice.

Study site II

This experimental site was also located at the NPAREC on a Davidson clay loam (*Clayey, kaolinitic, thermic, Rhodic Paleudults*). Six rates of aerobically digested biosolids (0, 42, 84, 126, 168, and 210 dry Mg ha^{-1}; the agronomic rate of biosolids was 42 Mg ha^{-1} for corn) were applied in single applications in the spring of 1984 (Table 5.1) (Rappaport *et al.*, 1988). Soil properties prior to organic amendment application are shown as Table 5.2. The treatments were arranged in a randomized complete block design with four replicates. Each plot consisted of a volume of soil 2.3 m long x 1.5 m wide x 0.9 m high isolated by plastic wrapping below ground and wooden boards above ground to prevent lateral movement of biosolids constituents. Only one treatment as 210 dry Mg ha^{-1} biosolids (BS) was selected for reporting in this study.

The aerobically digested biosolids were obtained from a Bristol, VA wastewater treatment plant having high industrial inputs. The chemical properties of the organic residuals applied are shown in Table 5.3. The biosolids were dewatered on sand beds, which allowed for maximum NH_3 volatilization and NO_3^- leaching losses; therefore, the biosolids contained negligible amounts of inorganic N (<8 mg kg^{-1}). The biosolids were incorporated into the soils to a depth of approximately 20 cm in spring 1984 prior to planting. The plots were rototilled every year to a depth of 15 cm from 1984-2005.

Corn (*Zea mays* L.) was grown each year from 1984-2000, except in 1994 when sorghum (*Sorghum bicolor* L. V. Dekalb 41Y) was planted instead of corn because of drought. Corn varieties were Pioneer 3192 in 1984–1990 and 1996–2000 and Pioneer 3136 in 1991–1995. Radish (*Raphanus sativus* L.) and Romaine lettuce (*Lactuca sativa* V. longifolia) cultivar Parris Island Cos replaced corn in the cropping system from 2001 to 2004. Winter barley (*Hordeum vulgare* L. V. Nomini) was grown in 2002, 2004 and 2005. The plots had been left fallow since 2005, with an occasional herbicide application to reduce weed pressure. Lime applications in 1989 and 1998 were made to raise the pH to 6.0.

Study site III

This experimental site was located on a Pamunkey sandy loam (*Fine-loamy, mixed, thermic Ultic Hapludalfs*) in Charles City County, VA. Five rates of anaerobically digested biosolids (BS) (0, 14, 42, 70, and 98 dry Mg ha^{-1}; the agronomic rate of biosolids was 14 Mg ha^{-1} for corn), with and without sawdust (SD) to adjust the C:N ratio to 20:1, were applied in single applications in March 1996 (Table 5.1) (Daniels *et al.*, 2003). Soil properties prior to organic amendment application are summarized in Table 5.2. The treatments were arranged in a randomized complete block design with four replicates. Each plot was approximately 36 x 15 m in size. The entire area of each experimental block was approximately 3 ha. The following three treatments were selected for reporting in this study: Control (CON), 98 dry Mg ha^{-1} biosolids (BS) and 98 dry Mg ha^{-1} biosolids+sawdust (BS +SD).

The anaerobically digested secondary biosolids was obtained from Chesterfield, VA. The chemical properties of the organic residuals applied are shown in Table 5.3. Biosolids N composition required a dry biosolids : sawdust ratio of 0.75:1 to attain the desired C:N ratio (20:1). The sawdust had a bulk C: N ratio of 198:1. The biosolids with and without sawdust were surface-applied to the soils in the spring of 1996 prior to planting.

A crop rotation of corn (*Zea mays* L.; planted April 1996), wheat (*Triticum aestivum;* planted November 1996), soybean (*Glycine max*; planted July 1997) and cotton (*Gossypium spp*.; planted in 1998) have been grown at the site since the establishment of the treatments. After the application of biosolids in 1996, this crop rotation has been managed as no-till system from 1997-2011 with only essential N, P, and K fertilizers applied to all the plots.

At each study site, nitrogen needs for crops were determined with the Virginia Agronomic Land Use Evaluation System (VALUES) (Simpson *et al.*, 1993). Phosphorus and potassium requirements were determined by Virginia Cooperative Extension soil testing results (Donohue and Heckendorn, 1994). Agronomic practices, seedbed preparation, pest control, and weeding control were performed according to Virginia Cooperative Extension recommendations (Virginia Cooperative Extension, 1992). The lime requirement was determined by Virginia Cooperative Extension soil testing results (Donohue and Heckendorn, 1994).

5.3.2. Soil sampling and processing

Soil sampling

Three soil cores measuring 5 cm in diameter were collected at a depth of 0-7.5 cm from selected treatments plots identified above in 2011. Surface soil samples from 0-15 cm depth of the highest rate biosolids treatment were collected from site II in 1993 and in 2011. Nine treatment plots in the three field sites were selected for further analysis in this study: CON, PYC, BSC, and PL from site I; 1993 BS and 2011 BS from site II; CON, BS, and BS+SD from site III. Each soil core of 0-7.5 cm (0-15 cm for site II) including the three subsamples and the four replicates from these nine treatments was composited and processed for analysis.

Soil processing

For each treatment, 50 g of air-dried soil (≤2.0 mm) was dispersed in 250 mL of distilled water at 22 J mL^{-1} (Leifeld and Kögel-Knabner, 2005) with a 1.2 cm-diameter probe sonifier (Sonicator 3000, Misonix Inc., Farmingdale, NY). The suspension was passed through a 53-µm sieve and the filtrate was collected in a tall 1-L beaker for further separation. The residue on the sieve (sand plus POM) was transferred quantitatively to a 250-mL centrifuge tube and was stirred, soaked and centrifuged at 1949g for 20 min in 50 mL of sodium iodide (NaI) adjusted to a density of 1.8 g mL^{-1} with water. The light-weight POM was siphoned into a tared beaker and washed three times with DI water. The remaining sand fraction was washed, dried and weighed.

The filtrate (clay plus silt) was adjusted to a final volume of approximately 500 mL before it was dispersed at 450 J mL^{-1}. The clay fraction was separated by exhaustive sedimentation and decantation, coagulated with 0.5M $MgCl_2$. The remaining sediment was the silt fraction. Both clay and silt fractions were freeze-dried and weighed to determine yield for each fraction.

To make the comparison between the NEXAFS and the NMR spectroscopic methods, our samples were pretreated and analyzed using both techniques. The collected POM was washed twice with 1 M HF (2% v/v) and then twice with DI water and finally freeze-dried. The samples were subjected to this procedure to remove iron which can interfere with the ^{13}C NMR measurement (Keeler and Maciel, 2003).

A subsample of the freeze-dried POM and the remaining size-fractions, i.e., clay, silt, and sand, as well as the whole soils, were ground to pass a 0.5 mm sieve for total SOC concentration by dry combustion in a Vario Max CNS macro elemental analyzer (Elementar, GER).

5.3.3. C (1s) K-edge NEXAFS experiment

Sample preparation

The freeze-dried POM was mixed with C-free nanopure water (0.5 mg mL^{-1} water) in 5 ml Eppendorf vials lowered into an ultrasound bath to achieve homogeneous wetting of the samples. Then 1 ml of sample solution was deposited onto Au coated Si wafers. Deposition was at 10^{-5} Torr and, once prepared, the substrates were left under vacuum until shortly before NEXAFS measurements.

Sample measurement

C (1*s*) K-edge NEXAFS spectra were obtained on beamline 11ID-1 at the Canadian Light Source (CLS) of the University of Saskatchewan, Saskatoon, Canada. The beamline was equipped with a spherical grating monochromator (SGM) designed for high resolution soft X-ray spectroscopy (Regier *et al.*, 2007). The detector was at the same elevation as the sample, making an angle of 43^0 with respect to the beam axis. The prepared sample plate was loaded into a vacuum chamber (1e^{-7} Torr). The beamline was configured for a resolving power of 7500 at the K edge (exit slit gap 50 µm) and the photon energy was scanned from 270 to 310 eV. Dwell time was 0.5 s. Background measurements were taken by measuring a- blank Au-coated wafer on each sample plate loaded into the chamber. Normalization current was also measured during each scan by collecting the photodiode from an Au mesh. The mesh was monitored for C contamination and was periodically refreshed using an in situ Au evaporator incorporated into the beamline vacuum system. A Ti filter was used in the beamline to reduce the effects of 2nd order oxygen in the pre-edge region (Heymann *et al.,* 2011).

Ten measurements were taken for each sample at different spots on the sample to make sure that the resulting spectra were identical. Spectra were background subtracted as follows:

$$I = [I_s - I_p]/I_0$$

where I corresponds to the subtracted signal, I_s to sample current, I_p to the current from a Au plate, and I_0 to the current from a Au mesh monitor located upstream from the sample. In order to correct for C contamination in the beamline optics, a linear transformation was applied to normalize the pre-edge region of the sample with the pre-edge region of clean Au measured at the beginning of the experimental run. Carbon NEXAFS spectra close to the K-edge can be collected either by measuring the absorption of the photons, in fluorescent mode, where the emitted photons are monitored, or by determining total electron yield, in which the neutralization current from the sample is recorded.

Spectra processing

All data were normalized prior to curve fitting using ATHENA 0.8.052 software (Ravel and Newville, 2005). The photon energy was calibrated to the C 1s-π*resonance in CO_2 gas at 287.38 eV. A fitting scheme based on six Gaussians (labeled G1-G6) was applied to the normalized spectra in the range 280–310 eV. The specific positions for the Gaussian peaks are described in Table 3. An arctangent function was used to model the ionization step and was fixed at 290 eV. The full width at half maximum of the bands was set at 0.4 ± 0.2 eV, while the amplitude was floated during the fit. Spectral regions represented by Gaussian curves were described as being generally attributed to the following functional groups: overall aromatic type C was represented by the sum of the (G1 + G2) peaks; phenolic C by the G3 peak; alkyl C by the G4 peak; carboxylic C by the G5 peak, and O-alkyl C by the G6 peak.

5.3.4. ^{13}C Cross Polarization/total sideband suppression (CP/TOSS) NMR

^{13}C NMR analyses were performed using a Bruker Avance III 300 spectrometer at 75 MHz. All experiments were run in a double-resonance probe head using 4-mm sample rotors. Semi-quantitative compositional information was obtained with good sensitivity using ^{13}C cross polarization/magic angle spinning NMR technique, with a spinning speed of 5 kHz, contact time 1 ms, and ^{1}H 90º pulse-length 4 μs. Four-pulse total suppression of sidebands (TOSS) (Shrestha *et al.*, 2008) was employed before detection, with a two-pulse phase modulated (TPPM) decoupling applied for optimum resolution.

5.3.5. Statistical analysis

The linear relationship and correlation coefficients between the organic C functional groups of POM identified by C (1s) NEXAFS and ^{13}C NMR chemical shifts were analyzed using the PROC REG procedure of SAS 9.2 (SAS Institute, 2008)

5.4. Results and Discussion

5.4.1. Soil organic carbon concentration from size fractions

Carbon expressed on a basis of whole soil mass and carbon recovery rates in density fractions and in HF treatment at the three sites are shown in Table 5.4. The size fractionation procedure yielded mass recoveries between 92.8 and 97.2% (data not shown). More POM-C was extracted from the compost- and biosolids-treated whole soils than from the control, suggesting that organic amendments were capable of maintaining active soil C pool. These results are descriptive of the soil C status and thus not statistically analyzed. Details of the C concentration and C stock of the whole soils at different depths from these three sites were analyzed and reported in a previous study Chapter 3).

The mass recovery rate from HF-treatment of POM is within the range of 17.1-54.4% (data not shown). No significant difference in C loss was observed with 2% HF acids washes (Skjemstad *et al.*, 1994; Schmidt *et al.*, 1997). However, it is worthwhile to note that there was a C loss around 30-40% from the HF treatment in site I. The detailed information can be found in our previous studies (Chapter 4). We assumed that this HF-treatment did not change the SOC speciation measured by C NEXAFS spectroscopy in our study.

5.4.2. Soil organic carbon speciation from NEXAFS

The typical C (1s) NEXAFS spectra with the main 1*s*-*π** transitions and the arctangent step function for the selected sample are shown as Figure 5.1. The spectra revealed multiple peaks in the fine structure of the C NEXAFS region (284–290 eV) indicating the presence of various C functional groups in the POM extracted from the soils under investigation. However, regions of slight overlap between the bands associated especially with 1*s*–3*p*/σ* transitions of alkyl-C near 287.6 eV and 1*s*–*π** transitions carboxylic-C near 288.3 eV may not be excluded. The existence of regions of overlap in C K-edge NEXAFS near 287 and 288 eV have also been reported by Cody *et al.* (1998). The multiple peaks (G1-G6) in the fine structure of the C NEXAFS region (284-290 eV) indicate the presence of various C functional groups in the sample. The C 1*s*-*π** transition near 284.5 eV (G1) correspond to quinone type C (e.g., benzoquinore) and to protonated and alkylated aromatic C. The resonance near 285.4 eV (G2) represents aromatic C (protonated and alkylated to carbonyl-substituted aromatic C) and possible olefinic-C. The sum of these two organic C functional groups (G1+G2) was used throughout this paper to represent aromatic C. The absorption band near 286.2 V (G3) is associated with phenolic C (e.g., O-substituted aryl-C), which is indicative of lignin. The 1s-3ρ/σ*/1*s*-*π** transition near 287.6 eV (G4) was due to aliphatic-C, whereas the strong absorption band near 288.3 eV (G5) was assigned to a 1*s*-*π** transition of carboxylic C. The sharp absorption band near 289.3 eV (G6) corresponds to the 1*s*-*π** of O-alkyl C group (e.g., polysaccharides, alcohol and ether C) (Lehmann *et al.*, 2009).

Main 1*s*-*π** transitions in the fine structure regions of C (1s) K-edge spectra span an energy range of 284–290 eV. The apparent broad resonance bands in the post-edge region (290-310 eV) are mainly due to overlapping resonances of σ^* transitions observed for alkyl, O-alkyl, carboxyl and carbonyl, H and C-substituted aromatic, as well as phenolic carbon. Therefore, only the main $1s$-π^* transitions were used for interpretation of the NEXAFS results. The characteristic double peaks near 300 eV indicated potassium presence in the C-edge spectra. Potassium salts are expected to be associated with agricultural soils due to addition of inorganic fertilizers or organic residuals. The potassium double peaks do not interfere with the organic C functional group of the NEXAFS spectra.

The C (1s) NEXAFS spectra and the relative proportions of different C functional groups in the POM of the three field sites in total electron yield (TEY) are shown in Figure 5.2 and Table 5.5. The main characteristic of C (1s) NEXAFS spectra was a broad, well resolved absorption band near 285 eV corresponding to the $1s$-$3p/\sigma^*$ C-H transition, indicating that the POM was dominated by strong O-alkyl C, which represented an average of about 41.2% of the total organic C. The aromatic C (17.1%), alkyl C (15.7%) and carboxylic C (14.9%) accounted for almost similar proportions of total organic C, while the phenolic C (11.2%) took up the least proportions of total organic C. The alkyl C / O-alkyl C ratio showed a range of 0.19-0.69.

The relative proportions of different C functional groups in the POM of the three field sites in total fluorescence yield are shown as Table 5.6. The C functional group proportions showed contrasting results between the two methods in alkyl C region (G4) (15.7% for TEY vs. 32.2% for TFY), as well as in O-alkyl C region (G6) (41.2% for TEY vs. 28.2% for TFY). Clearly, TFY detected twice the amount of alkyl C than TEY did. Values for the other regions were similar (within 7% difference) for the two methods.

Due to the abnormally different values of alkyl C for TFY spectra, the alkyl C / O-alkyl C ratio was mostly increased to greater than 1. Apparently, TFY detected values were not in agreement with TEY to have similar proportions of absorption intensity within the alkyl C region. Thus, only TEY measurements were used for the interpretation of spectrum in this study. The sample measurements with TFY suffered from self-absorption, which caused distortion in the spectra and impacted negatively on data quality (Tröger *et al.*, 1992; Heymann *et al.*, 2011). This was a result of the material characteristics and not avoidable for most C NEXAFS studies. Jokic et al. (2003) used C NEXAFS to characterize organic carbon structures in a series of wetland soils in Saskatchewan, Canada, and reported large proportions of O-alkyl-C and carboxylic-C followed by aromatic-C and alkyl-C using TEY mode that probes the surface layers (approximately 10 nm) into the sample. However, TFY that probes deeper (approximately 100 nm) in to the sample revealed an increase in the proportion of alkyl-C compared with both carboxylic-C and aromatic-C from the same soils.

Additionally, since absorption intensity and transition intensity reflect only the relative concentration of functional groups, absolute concentration based on peak intensity cannot be determined with NEXAFS. Proportions used in the context of this study are meant to be a comparative tool and not as an absolute quantification of functional groups, particularly where comparisons are made with values obtained from other instrumental methods (such as ^{13}C NMR).

Study site I

The aromatic C region (G1+G2) follows the decreasing order as: BSC (19.0%) > PYC (18.2%) > CON (14.0%) > PL (11.5%). Aromatic C accounted for the second largest proportion of total SOC in the POM samples. The aromatic C was higher for both compost-amended soil samples than CON and PL. The results may suggest that decomposition of the added composts in soil is as an ongoing humification process of the composts themselves, as aromatic C is related to humification factor (Melillo *et al.*, 1982). Aromatic C in the organic amendments increase during the composting process (Chen and Su, 1996). Tuomela *et al.* (2000) investigated mature compost and found it contains more aromatic structures and carboxyl groups and less carbohydrate components than immature compost.

Both compost-amended soil samples maintained higher phenolic C than CON and PL. The phenolic C region (G3) covers carbonyl C in aromatic ring, aromatic C attached to amide group, phenol C, carbonyl C, and pyrimidine C. The acetonitrile, acetic acid and phenol fragment tended to increase with composting (Hernández *et al.*, 2006). This phenolic C in the compost-amended soils increased because the applied composts contained more lignin, which altered little even after prolonged decomposition of the composts in soil (Leifeld *et al.*, 2002). At processing composting stages, the composition of organic matter underwent a progressive mineralization of lipidic and peptidic structures and an enrichment in stable ligno-cellulosic material (Spaccini and Piccolo, 2008). It was hypothesized that lignin in compost would persist in the POM fraction beyond a growing season, potentially altering the decomposition rate of plant residues and the turnover time of POM (Fortuna *et al.*, 2003). Furthermore, the nature of plant debris (C:N ratio, lignin content, and phenolic compound content) affects the rate of SOC sequestration (Blanco-Canqui and Lal, 2004). The more phenolic C and aromatic C in the two compost treated-soils might indicate a higher potential for C sequestration, which agrees with our previous studies (Chapter 3).

Both compost treatments and the control showed lower proportions of carboxylic C (G5) than PL treatments (13.9%). The carboxylic C region is commonly assigned to carboxylic C, carboxyamide C, and carbonyl C (insert reference). This result suggests that PL containing greater carboxylic C might persist in the soil for a long time with little degradation (Prasad and Sinha, 1980).

The alkyl C region (G4) decreased in the order: BSC (18.2%) > PYC (17.2%) > CON (15.0%)> PL (10.0%). The O-alkyl C region (G6) decreased in the order: PL (54.2%) > CON (51.1%) > BSC (42.5%) > BSC (40.9%). The two compost-amended POM had higher alkyl C and lower O-alkyl C than CON and PL treatments. The alkyl C region is assigned to terminal methyl groups, methylene groups in aliphatic rings and chains. This is typical of long-chain CH_2 arising from a mixture of surface waxes and cutin. The O-alkyl C region includes polysaccharides, alcohols, and other hydroxylated and ether-linked C. Polysaccharide C is the most common O-alkyl C in the SOM and comprise the greatest proportion of C in the composts (Chefetz *et al.*, 1996). Generally, O-alkyl C (sugar) can be rapidly degraded, because it contains hydrolytic bonds that are decomposed by a ubiquitous group of enzymes known as hydrolases which are able to hydrolyze ester, glycoside, and ether (Lützow *et al.*, 2006).

The alkyl C / O-alkyl C ratio followed the order: BSC (0.43) > PYC (0.42) > CON (0.29) > PL (0.19). There was more alkyl C and less O-alkyl C in compost-treated soil samples compared with PL and CON treatments, which is consistent with CPMAS ^{13}C NMR testing results. The concomitant increase and decrease in alkyl and O-alkyl C contents, respectively, suggest that the ratio of alkyl C to O-alkyl C may provide a sensitive index of the extent of decomposition (Baldock *et al.*, 1997). The higher ratio revealed that the POM from the two compost-treated soils was more decomposed than those from CON and PL treatments. Helfrich *et al.* (2006) analyzed the SOM in different density fractions in silty soils under land use (spruce, grassland, and maize) and found that decomposition of spruce litter resulted in a decreasing O-alkyl-C content and an increasing alkyl-C content.

The composition of SOM 7 years after compost applications were made changed mainly by increases in the aromatic C and alkyl C, and a decrease in the O-alkyl C due to decomposition. These results suggest that decomposition of the added composts in soil is as an ongoing humification process of the composts themselves. However, very slight differences of C functional groups were found between the two composts treatment (PYC and BSC). The effects of various composting treatment processes and the raw solid wastes could not be verified by NEXAFS spectroscopy.

Study site II

The aromatic C region (G1+G2) shows strongly contrasting values between the two samples from site II (11.8% for 1993 BS and 22.1% for 2011 BS). These results indicated that the biosolids residual C might have gone through a higher degree of humification and generated higher proportions of aromatic C during the 18 years of agricultural management. Furthermore, the higher proportions of aromatic C in the 2011 BS sample might have resulted from the partial decomposition and decay of crop residue which contains esters and ethers associated with plant cell wall structures (Montgomery, 2004).

The phenolic C (G3) is lower in 1993 BS (10.2%) than 2011 BS (11.4%). And the carboxylic C (G5) is higher for 1993 BS (34.3%) than 2011 BS (24.3%). Phenolic compounds are found in biosolids products, and they are also toxic and recalcitrant (Polymenakou and Stephanou, 2005). The phenolic C region is typical of a grass lignin, with a mixture of syringyl, guaiacyl, and hydroxyphenylpropane units (Almendros *et al.*, 1992). Phenolic C can be related to SOM mineralization. Rowell *et al.* (2001a) reported that net N mineralization rates were best predicted by a model incorporating the initial organic N concentration and the proportion of phenolic C in the biosolids.

The alkyl C region (G4) accounts for 11.0 % for 1993 BS and 8.0% for 2011 BS. The O-alkyl C region (G6) follows the increasing order as: 1993 BS (32.7%) < 2011 BS (34.2%). The alkyl C and O-alkyl C proportions for both two samples are similar. The alkyl C / O-alkyl C ratio is 0.34 for 1993 BS and 0.23 for 2011 BS. Since the alkyl C / O-alkyl C ratio of a soil indicates the degree of SOM decomposition, this result indicated that the C from 1993 BS was more decomposed and thus more stable than 2011 BS. This is an unexpected result since it indicated that, after 18 years of agricultural management, higher proportions of sugar of polysaccharides were produced rather than degraded. It seems that considerable amounts of crop residue had accumulated and partly decomposed into active soil C pool in the plots, although the site has been kept fallow since 2005.

The C (1s) NEXAFS spectra reflect an increase of O-alkyl structures and the continual aromatization of the SOC, but a decrease of alkyl components during 18 years of agricultural management on the study sites. The crop residues and biosolids residual C might help generate the active C pool which contains more O-alkyl C and less alkyl C. It is questionable whether the sample collected is sufficient to verify the assumption that time affects the decomposition degree of SOC. However, observations obtained by ^{13}C NMR spectroscopic techniques showed the same results and confirmed the C dynamics of the biosolids-amended soils in this site (Chapter 4). Such findings in this study might be caused by other environmental and/or systematic errors but not the limitations of spectroscopic techniques.

Study site III

The aromatic C region (G1+G2) decreases as: BS+SD (22.2%) > BS (19.9%) > CON (15.0%). The both organic residual-treated samples maintained higher proportions of aromatic C than the control, indicating either that aromatic C from organic amendments could persist in soils for a long time, or that SOC in organic residual-amended soils went through a higher degree of humification. Particularly, the BS+SD sample showed a higher aromatic C intensity than the BS sample. This might be caused by the higher proportional lignin from the sawdust, which produced aromatic C during chemical humification processes (Kögel-Knabner, 2002) and, thus, plays an important role in humus formation.

The phenolic C region (G3) accounts for highest proportions in BS+SD (12.1%), compared with CON (11.6%) and BS (11.1%). Phenolic compounds can directly affect the composition and activity of decomposer communities, thus influencing the rate of SOM decomposition (Hättenschwiler and Vitousek, 2000). Additionally, lignin breakdown, as well as microbial synthesis from nonphenolic compounds, can also contribute to the pool of polyphenols in the soil (Jones and Hartley, 1999; Hättenschwiler and Vitousek, 2000). This higher aromatic polymer (22.2%) along with polyphenols (12.1%) might represent a possible extent of humification processes in BS+SD sample.

The carboxylic C (G5) proportions are almost similar between the BS (11.7%) and BS+SD (12.4%) treatments. By contrast, the CON treatment only maintain 7% carboxylic C of the total SOC. The higher carboxylic C from the two organic residual-amended soil samples might resulted from the biosolids processing treatment, i.e., anaerobic digestion (Aiello-Mazzarri *et al.*, 2005). The assimilable end-products of biosolids are reported to consist mainly of carboxylic acids (Weemaes *et al.*, 2000). Furthermore, the organic residuals might go through a higher degree of humification, in which loss of methoxyl (O-CH_3) groups occurs with generation of hydroxyphenol and oxidation of aliphatic side chains to form COOH groups (Popa *et al.*, 2010).

The alkyl C region (G4) follows the decreasing order as: BS+SD (21.7%) > BS (20.5%) > CON (19.5%), while the O-alkyl C follows the decreasing order as: CON (46.8%) >BS > (36.9%) > BS + SD (31.7%). The alkyl C / O-alkyl C ratio is decreasing as: BS+SD (0.69) > BS (0.56) > CON (0.42). The BS+SD maintained higher alkyl C / O-alkyl C ratio and, thus, indicated higher degree of decomposition than the BS. Apparently, the addition of sawdust increased the degree of SOC decomposition. Evidence has demonstrated that fresh added C could increase SOC mineralization / decomposition and results less C sequestration (Fontaine *et al.*, 2004; Abiven *et al.*, 2009).

The C (1s) NEXAFS spectral features reflect the degradation and loss of O-alkyl structures and the continual aromatization and aliphatization of the SOC during the decomposition process following long-term application of biosolids and sawdust. The addition of sawdust to biosolids treatment enhanced the decomposition of SOC. The C input from sawdust did not persist in the soils and decomposes or humifies into humus or even CO_2. Our previous studies (Chapter 3) confirmed the findings that C saturation might have occur in this site. These observations complement the results obtained by ^{13}C NMR spectroscopic techniques.

5.4.3. Comparison between NMR and NEXAFS

The linear relationships of the C speciation between C (1s) NEXAFS and CPMAS ^{13}C NMR spectroscopy is shown as Figure 5.3. The C functional groups, including aromatic C, O-alkyl C, carboxylic C and phenolic C revealed a good fitness ($P < 0.05$; $n = 9$; $R^2 = 0.57\text{-}0.90$) between the two spectroscopic methods.

The correlation coefficients between the C functional groups from POM identified by C (1s) NEXAFS and CPMAS ^{13}C NMR spectroscopy from site I and site III showed positive correlation ($P < 0.05$; $n = 7$) between the aromatic-C ($r = 0.68$), phenolic-C ($r = 0.89$), alkyl C ($r=0.92$), carboxylic-C ($r = 0.53$), and O-alkyl-C ($r = 0.91$), and alkyl C / O-alkyl C ($r=0.95$). However, when the two site II biosolids NMR data points were included, the correlations ($P < 0.05$; $n = 9$) with C functional groups were as follows: aromatic-C ($r = 0.75$), phenolic-C ($r = 0.84$), alkyl C ($r=-0.33$), carboxylic-C ($r = 0.84$), and O-alkyl-C ($r = 0.91$), and alkyl C / O-alkyl C ($r=-0.06$). Clearly, the weak and negative relationship between the two spectroscopic methods at alkyl C was caused by the inconsistencies between NEXAFS and NMR techniques.

Despite wide range of origin and chemical heterogeneity of the SOC, the results of C (1s) NEXAFS spectra compared very well with the results of the ^{13}C NMR spectroscopy in our study. Scheinost *et al.* (2001) investigated humic acid and fulvic acid from a Humic Gleysol in Switzerland and reported significant correlations between aromatic-C ($r=0.91$), phenolic-C ($r=0.87$), alkyl-C ($r=0.71$), carboxylic-C ($r=0.71$), and O-alkyl-C ($r=0.57$). Schäfer *et al.* (2003) reported correlations for aromatic-C ($r=0.91$), phenolic-C (r=0.98), aliphatic-C (r=0.99) and carbonyl-C (r=0.95) for fulvic acid samples extracted from ground water. Solomon *et al.* (2005) reported positive correlation between the aromatic-C (r = 0.64), phenolic-C (r = 0.74), carboxylic-C (r = 0.62), alkyl-C (0.12), and O-alkyl-C (r = 0.79) in the humic substances extracted from the clay and silt fractions of the soils. Solomon *et al.* (2007) reported positive correlation for aromatic-C (r=0.72), phenolic-C (r=0.88), aliphatic-C (r=0.70), carboxylic-C (r=0.65) and O-alkyl-C (r=0.63) from humic substances extracted from forest and plantation soils in Kenya. These authors concluded that C (1s) NEXAFS is a well suited method for characterization of organic C functional groups.

The linear regression suffers from abnormal distortion as the ^{13}C NMR highlights alkyl C in the two samples from site II, which made comparison between the two methods complicated. The limitations of CP ^{13}C NMR are not likely to be responsible for the problems observed with the fits to the NEXAFS data, as the quantitative direct polarization (DP) ^{13}C NMR has confirmed the C intensities (Chapter 4). The overestimation of alkyl may be due to relative reduction of aromatics in CP/TOSS and/or the presence of alkyl C with high molecular mobility that approximately 30% of the CPMAS NMR signal went undetected (Smernik *et al.*, 2003). Although fitting problems may have occurred because of poorly resolved overlapping bands, C K-edge XANES spectroscopy is still a useful technique for detecting organic C functional groups and providing C dynamics both qualitatively and semi-quantitatively in SOC.

5.5. Conclusions

The NEXAFS spectra showed that O-alkyl C functional groups were the dominant forms of organic C, followed by moderate amounts of aromatic C, alkyl C, and carboxylic C groups. The phenolic C forms contributed only to a small portion of the total SOC in POM extracted from organic residual amended soils. Carbon K-edge NEXAFS spectroscopy exhibited good selectivity and revealed various degree of decomposition caused by organic residual species by time, or by organic residual combination (i.e., biosolids combined with sawdust) among those organic residual amended soils. The results of our investigation provide clear evidence that the C (1s) K-edge NEXAFS technique used in the present investigation has good sensitivity for the different organic C functionalities compared with other spectroscopic methods, i.e., ^{13}C NMR. Hence, it is imperative to use a suite of complementary spectroscopic techniques, i.e., NEXAFS, NMR, FTIR, and Pyrolysis GC/MS, etc., in an integrated manner to accurately fingerprint the structural composition of SOC and to critically assess the long-term impacts of organic amendments on the speciation and structural chemistry of SOC to provide process-oriented data for global C agroecosystems.

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5.7. References

Abiven, S., Menasseri, S. and Chenu, C. 2009. The effects of organic inputs over time on soil aggregate stability – A literature analysis. Soil Biology and Biochemistry 41: 1-12.

Aiello-Mazzarri, C., Coward-Kelly, G., Agbogbo, F. and Holtzapple, M. 2005. Conversion of municipal solid waste into carboxylic acids by anaerobic countercurrent fermentation. Applied Biochemistry and Biotechnology 127: 79-93.

Almendros, G., Martinez, A. T., Gonzalez, A. E., Gonzalez-Vila, F. J., Fruend, R. and Luedemann, H. D. 1992. CPMAS carbon-13 NMR study of lignin preparations from wheat straw transformed by five lignocellulose-degrading fungi. Journal of Agricultural and Food Chemistry 40: 1297-1302.

Baldock, J. A., Oades, J. M., Nelson, P., Skene, T., Golchin, A. and Clarke, P. 1997. Assessing the extent of decomposition of natural organic materials using solid-state ^{13}C NMR spectroscopy. Australian Journal of Soil Research 35: 1061-1083.

Batjes, N. H. 1998. Mitigation of atmospheric CO_2 concentrations by increased carbon sequestration in the soil. Biology and Fertility of Soils 27: 230-235.

Blanco-Canqui, H. and Lal, R. 2004. Mechanisms of carbon sequestration in soil aggregates. Critical Reviews in Plant Sciences 23: 481-504.

Bowden, C. L., Evanylo, G. K., Zhang, X., Ervin, E. H. and Seiler, J. R. 2010. Soil carbon and physiological responses of corn and soybean to organic amendments. Compost Science & Utilization 18: 162-173.

Buyanovsky, G. A., Aslam, M. and Wagner, G. H. 1994. Carbon turnover in soil physical fractions. Soil Sci. Soc. Am. J. 58: 1167-1173.

Chefetz, B., Hatcher, P. G., Hadar, Y. and Chen, Y. 1996. Chemical and biological characterization of organic matter during composting of municipal solid waste. J. Environ. Qual. 25: 776-785.

Chen, Y.-H. and Su, S. Y. W. 1996. Implementation and evaluation of parallel query processing algorithms and data partitioning heuristics in object-oriented databases. Distributed and Parallel Databases 4: 107-142.

Christensen, B. T. 2001. Physical fractionation of soil and structural and functional complexity in organic matter turnover. European Journal of Soil Science 52: 345-353.

Cody, G. D., Ade, H., Wirick, S., Mitchell, G. D. and Davis, A. 1998. Determination of chemical-structural changes in vitrinite accompanying luminescence alteration using C-NEXAFS analysis. Organic Geochemistry 28: 441-455.

Daniels, W. L., Evanylo, G. K., Nagle, S. M. and Schmidt, J. M. 2003. Effects of biosolids loading rate on nitrate leaching potentials in sand and gravel mine reclamation in Virginia. Proceedings of the Water Environment Federation 2003: 271-278.

Donohue, S. J. and Heckendorn, S. E. 1994. Soil test recommendations for Virginia. Blacksburg, VA., Virginia Cooperative Extension Service Publication. 834.

Elliott, E. T. and Cambardella, C. A. 1991. Physical separation of soil organic matter. Agriculture, Ecosystems & Environment 34: 407-419.

Evanylo, G., Sherony, C., Spargo, J., Starner, D., Brosius, M. and Haering, K. 2008. Soil and water environmental effects of fertilizer-, manure-, and compost-based fertility practices in an organic vegetable cropping system. Agriculture, Ecosystems & Environment 127: 50-58.

Fontaine, S., Bardoux, G., Abbadie, L. and Mariotti, A. 2004. Carbon input to soil may decrease soil carbon content. Ecology Letters 7: 314-320.

Fortuna, A., Harwood, R. R. and Paul, E. A. 2003. The Effects of compost and crop rotations on carbon turnover and the particulate organic matter fraction. Soil Science 168: 434-444.

Franzluebbers, A. J. 2010. Achieving soil organic carbon sequestration with conservation agricultural systems in the southeastern United States. Soil Sci. Soc. Am. J. 74: 347-357.

Golchin, A., Oades, J., Skjemstad, J. and Clarke, P. 1994. Soil structure and carbon cycling. Soil Research 32: 1043-1068.

Hättenschwiler, S. and Vitousek, P. M. 2000. The role of polyphenols in terrestrial ecosystem nutrient cycling. Trends in Ecology & Evolution 15: 238-243.

Helfrich, M., Ludwig, B., Buurman, P. and Flessa, H. 2006. Effect of land use on the composition of soil organic matter in density and aggregate fractions as revealed by solid-state ^{13}C NMR spectroscopy. Geoderma 136: 331-341.

Hernández, T., Masciandaro, G., Moreno, J. I. and García, C. 2006. Changes in organic matter composition during composting of two digested sewage sludges. Waste Management 26: 1370-1376.

Heymann, K., Lehmann, J., Solomon, D., Schmidt, M. W. I. and Regier, T. 2011. C 1s K-edge near edge X-ray absorption fine structure (NEXAFS) spectroscopy for characterizing functional group chemistry of black carbon. Organic Geochemistry 42: 1055-1064.

IPCC 1990. Climate change: the IPCC impacts assessment. Canberra, Australia, Australian Govt. Pub. Service.

Jokic, A., Cutler, J. N., Ponomarenko, E., van der Kamp, G. and Anderson, D. W. 2003. Organic carbon and sulphur compounds in wetland soils: insights on structure and transformation processes using K-edge XANES and NMR spectroscopy. Geochimica et Cosmochimica Acta 67: 2585-2597.

Jones, C. G. and Hartley, S. E. 1999. A Protein competition model of phenolic allocation. Oikos 86: 27-44.

Keeler, C. and Maciel, G. E. 2003. Quantitation in the solid-state ^{13}C NMR analysis of soil and organic soil fractions. Analytical Chemistry 75: 2421-2432.

Kögel-Knabner, I. 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biology and Biochemistry 34: 139-162.

Kumar, K. and Goh, K. M. 1999. Crop residues and management practices: Effects on soil quality, soil nitrogen dynamics, crop yield, and nitrogen recovery. Advances in Agronomy. Donald, L. S., Academic Press. Volume 68: *pp.* 197-319.

Lal, R. 2004. Soil carbon sequestration impacts on global climate change and food security. Science 304: 1623-1627.

Lal, R. 2006. Enhancing crop yields in the developing countries through restoration of the soil organic carbon pool in agricultural lands. Land Degradation & Development 17: 197-209.

Lehmann, J. and Solomon, D. 2010. Chapter 10 - Organic carbon chemistry in soils observed by synchrotron-based spectroscopy. Developments in Soil Science. Balwant, S. and Markus, G., Elsevier. Volume 34: *pp.* 289-312.

Lehmann, J., Solomon, D., Brandes, J., Fleckenstein, H., Jacobsen, C. and Thieme, J. 2009. Synchrotron-based near-edge X-ray Spectroscopy of natural organic matter in soils and sediments. Biophysico-Chemical Processes Involving Natural Nonliving Organic Matter in Environmental Systems. Hoboken, NJ: Wiley. *pp*: 723-775.

Lehmann, J., Solomon, D., Kinyangi, J., Dathe, L., Wirick, S. and Jacobsen, C. 2008. Spatial complexity of soil organic matter forms at nanometre scales. Nature Geosci 1: 238-242.

Leifeld, J. and Kögel-Knabner, I. 2005. Soil organic matter fractions as early indicators for carbon stock changes under different land-use. Geoderma 124: 143-155.

Leifeld, J., Siebert, S. and Kögel-Knabner, I. 2002. Changes in the chemical composition of soil organic matter after application of compost. European Journal of Soil Science 53: 299-309.

Lützow, M. v., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B. and Flessa, H. 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions – a review. European Journal of Soil Science 57: 426-445.

Melillo, J. M., Aber, J. D. and Muratore, J. F. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63: 621-626.

Montgomery, R. 2004. Development of biobased products. Bioresource Technology 91: 1-29.

Olk, D. C. and Gregorich, E. G. 2006. Overview of the symposium proceedings, “Meaningful pools in determining soil carbon and nitrogen dynamics”. Soil Sci. Soc. Am. J. 70: 967-974.

Parton, W. J., Schimel, D. S., Cole, C. V. and Ojima, D. S. 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. Soil Sci. Soc. Am. J. 51: 1173-1179.

Polymenakou, P. N. and Stephanou, E. G. 2005. Effect of temperature and additional carbon sources on phenol degradation by an indigenous soil Pseudomonad. Biodegradation 16: 403-413.

Popa, V. I., Stingu, A. and Volf, I. 2010. Lignins an polyphenols in bioremediation. Bioremediation Technology: Recent Advances: 100.

Post, W. M. and Kwon, K. C. 2000. Soil carbon sequestration and land-use change: processes and potential. Global Change Biology 6: 317-327.

Prasad, B. and Sinha, M. 1980. Physical and chemical characterization of soil and poultry litter humic and fulvic metal complexes. Plant and Soil 54: 223-232.

Rappaport, B. D., Martens, D. C., Reneau, R. B. and Simpson, T. W. 1988. Metal availability in sludge-amended soils with elevated metal levels. J. Environ. Qual. 17: 42-47.

Ravel, B. and Newville, M. 2005. ATHENA, ARTEMIS, HEPHAESTUS: data analysis for X-ray absorption spectroscopy using IFEFFIT. Journal of Synchrotron Radiation 12: 537-541.

Regier, T., Krochak, J., Sham, T. K., Hu, Y. F., Thompson, J. and Blyth, R. I. R. 2007. Performance and capabilities of the Canadian Dragon: The SGM beamline at the Canadian Light Source. Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment 582: 93-95.

Rowell, D. M., Prescott, C. E. and Preston, C. M. 2001. Decomposition and nitrogen mineralization from biosolids and other organic materials Douglas. Australia. J. Environ. Qual. 30: 1401-1410.

SAS Institute 2008. SAS/STAT 9.2 User's Guide. Cary, NC, SAS Institute, Inc.

Schäfer, T., Hertkorn, N., Artinger, R., Claret, F. and Bauer, A. 2003. Functional group analysis of natural organic colloids and clay association kinetics using C(1s) spectromicroscopy. J. Phys. IV France 104: 409-412.

Scheinost, A. C., Kretzschmar, R., Christl, I. and Jacobsen, C. 2001. Carbon group chemistry of humic and fulvic acid: a comparison of C-1s NEXAFS and ^{13}C-NMR spectroscopies. Humic substances: Structures, models and functions. . Ghabbour, E. A. and Davies, G. Gateshead, UK., Royal Soc. Chem. 273: *pp*. 39-50.

Schmidt, M. W. I., Knicker, H., Hatcher, P. G. and Kogel-Knabner, I. 1997. Improvement of ^{13}C and ^{15}N CPMAS NMR spectra of bulk soils, particle size fractions and organic material by treatment with 10% hydrofluoric acid. European Journal of Soil Science 48: 319-328.

Schnitzer, M. and Khan, S. U. 1978. Soil organic matter. Elsevier Scientific Pub. Co., New York, NY.

Schulze, D. G. and Bertsch, P. M. 1995. Synchrotron X-ray techniques in soil, plant, and environmental research. Advances in Agronomy. Donald, L. S., Academic Press. Volume 55: *pp.* 1-66.

Schumacher, M. 2005. Microheterogeneity of soil organic matter investigated by C-1s NEXAFS spectroscopy and X-ray microscopy, GöttingenSierke Verlag.

Schumacher, M., Christl, I., Scheinost, A. C., Jacobsen, C. and Kretzschmar, R. 2005. Chemical heterogeneity of organic soil colloids investigated by scanning transmission X-ray microscopy and C-1s NEXAFS microspectroscopy. Environmental Science & Technology 39: 9094-9100.

Sedlmair, J., Geber, S.-C., Peth, C., Mann, K. and Thieme, J. 2009. NEXAFS spectroscopy with a laser plasma x-ray source on soil samples. Journal of Physics: Conference Series 186: 012034.

Shrestha, M., Abraham, W.-R., Shrestha, P. M., Noll, M. and Conrad, R. 2008. Activity and composition of methanotrophic bacterial communities in planted rice soil studied by flux measurements, analyses of pmoA gene and stable isotope probing of phospholipid fatty acids. Environmental Microbiology 10: 400-412.

Simpson, T. W., Donohue, S. J., Hawkins, G. W., Monnett, M. M. and Baker, J. C. 1993. The development and implementation of the Virginia agronomic land use evaluation system (VALUES). Blacksburg, VA., Department of Crop and Soil Environmental Sciences, Virginia Tech.

Six, J., Conant, R. T., Paul, E. A. and Paustian, K. 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant and Soil 241: 155-176.

Skjemstad, J., Clarke, P., Taylor, J., Oades, J. and Newman, R. 1994. The removal of magnetic materials from surface soils - a solid state ^{13}C CP/MAS NMR study. Soil Research 32: 1215-1229.

Smernik, R. J., Oliver, I. W. and Merrington, G. 2003. Characterization of sewage sludge organic matter using solid-state carbon-13 nuclear magnetic resonance spectroscopy. J. Environ. Qual. 32: 1516-1522.

Sojka, R. E., Upchurch, D. R. and Borlaug, N. E. 2003. Quality soil management or soil quality management : performance versus semantics. Advances in Agronomy, Academic Press. Volume 79: *pp.* 1-68.

Sollins, P., Glassman, C., Paul, E. A., Swanston, C., Lajtha, K., Heil, J. W. and Elliott, E. T. 1999. Soil carbon and nitrogen: pools and fractions. Standard Soil Methods for Long-term Ecological Research: 89-105.

Sollins, P., Swanston, C. and Kramer, M. 2007. Stabilization and destabilization of soil organic matter—a new focus. Biogeochemistry 85: 1-7.

Solomon, D., Lehmann, J., Harden, J., Wang, J., Kinyangi, J., Heymann, K., Karunakaran, C., Lu, Y., Wirick, S. and Jacobsen, C. 2012. Micro- and nano-environments of carbon sequestration: Multi-element STXM–NEXAFS spectromicroscopy assessment of microbial carbon and mineral associations. Chemical Geology 329: 53-73.

Solomon, D., Lehmann, J., Kinyangi, J., Amelung, W., Lobe, I., Pell, A., Riha, S., Ngoze, S., Verchot, L. O. U., Mbugua, D., Skjemstad, J. A. N. and SchÄFer, T. 2007. Long-term impacts of anthropogenic perturbations on dynamics and speciation of organic carbon in tropical forest and subtropical grassland ecosystems. Global Change Biology 13: 511-530.

Solomon, D., Lehmann, J., Kinyangi, J., Liang, B., Heymann, K., Dathe, L., Hanley, K., Wirick, S. and Jacobsen, C. 2009. Carbon (1s) NEXAFS spectroscopy of biogeochemically relevant reference organic compounds. Soil Sci. Soc. Am. J. 73: 1817-1830.

Solomon, D., Lehmann, J., Kinyangi, J., Liang, B. and Schäfer, T. 2005. Carbon K-edge NEXAFS and FTIR-ATR spectroscopic investigation of organic carbon speciation in soils. Soil Sci. Soc. Am. J. 69: 107-119.

Spaccini, R. and Piccolo, A. 2008. Spectroscopic characterization of compost at different maturity stages. CLEAN – Soil, Air, Water 36: 152-157.

Spargo, J. T., Evanylo, G. K. and Alley, M. M. 2006. Repeated compost application effects on phosphorus runoff in the Virginia Piedmont. J. Environ. Qual. 35: 2342-2351.

Stewart, C. E., Follett, R. F., Wallace, J. and Pruessner, E. G. 2012. Impact of biosolids and tillage on soil organic matter fractions: Implications of carbon saturation for conservation management in the Virginia Coastal Plain. Soil Sci. Soc. Am. J.: In progress.

Stöhr, J. 2003. NEXAFS spectroscopy. Berlin; New York, Springer.

Sukkariyah, B. F., Evanylo, G. K., Zelazny, L. and Chaney, R. L. 2005. Cadmium, copper, nickel, and zinc availability in a biosolids-amended Piedmont soil years after application. J. Environ. Qual. 34: 2255-2262.

Tröger, L., Arvanitis, D., Baberschke, K., Michaelis, H., Grimm, U. and Zschech, E. 1992. Full correction of the self-absorption in soft-fluorescence extended x-ray-absorption fine structure. Physical Review B 46: 3283-3289.

Tuomela, M., Vikman, M., Hatakka, A. and Itävaara, M. 2000. Biodegradation of lignin in a compost environment: a review. Bioresource Technology 72: 169-183.

Virginia Cooperative Extension 1992. Pest management guide for field crops. Virginia Tech: Blacksburg, VA, VA Coop. Ext. Publ. No. 456-016: 1-276.

Wan, J., Tyliszczak, T. and Tokunaga, T. K. 2007. Organic carbon distribution, speciation, and elemental correlations within soil microaggregates: Applications of STXM and NEXAFS spectroscopy. Geochimica et Cosmochimica Acta 71: 5439-5449.

Wander, M. M., Traina, S. J., Stinner, B. R. and Peters, S. E. 1994. Organic and conventional management effects on biologically active soil organic matter pools. Soil Sci. Soc. Am. J. 58: 1130-1139.

Watts, B., Thomsen, L. and Dastoor, P. C. 2006. Methods in carbon K-edge NEXAFS: Experiment and analysis. Journal of Electron Spectroscopy and Related Phenomena 151: 105-120.

Weemaes, M., Grootaerd, H., Simoens, F. and Verstraete, W. 2000. Anaerobic digestion of ozonized biosolids. Water Research 34: 2330-2336.

Figure Captions

Figure 5.1. Carbon (1s) total electron yield NEXAFS spectrum of particulate organic matter from poultry litter-yard waste compost (PYC) treated soils of site I. The spectrum is deconvoluted using a series of Gaussian curves (G) at energy positions of known transitions, along with a step function of arctangent at the edge.

Figure 5.2. Carbon (1s) total electron yield NEXAFS spectra for particulate organic matter extracted from organic residuals amended soils of the three field sites. CON = control; PYC = poultry litter-yard waste compost; BSC = biosolids-woodchip compost; PL = poultry litter; BS= biosolids; BS+SD=biosolids+sawdust.

Figure 5.3. Correlation plots of organic C functional groups as a fraction of total carbon (%) identified by C (1s) NEXAFS and ^{13}C NMR spectroscopy of particulate organic matter from organic residuals amended soils of the three field sites. The two samples from site II reveal abnormal values from NMR. BS=biosolids.

Table 5.1. Soil series, treatments management and sampling depths in the three study sites.

Site	Soil Series	Complete Treatment	Frequency	Selected Treatment	Sampling Depth
Site I	Fauquier silty clay loam (*Fine, mixed, mesic Ultic Hapludalfs*)	Control, poultry litter, poultry litter yard waste compost, and biosolids woodchip compost; all were based on the agronomic N rate.	Continuous from 2000-2004	Control, poultry litter, poultry litter yard waste compost, and biosolids woodchip compost	0-7.5 cm
Site II	Davidson clay loam (*Clayey, kaolinitic, thermic, Rhodic Paleudult*)	Six rates of aerobically digested biosolids (0, 42, 84, 126, 168, and 210 dry Mg ha^{-1}); the agronomic rate of biosolids was 42 Mg ha^{-1}.	Single in 1984	210 dry Mg ha^{-1} biosolids in 1993 and 2011	0-15 cm
Site III	Pamunkey sandy loam (*Fine-loamy, mixed, thermic Ultic Hapludalfs*)	Five rates of anaerobically digested biosolids (0, 14, 42, 70, and 98 dry Mg ha^{-1}), with and without sawdust to adjust the C:N ratio; the agronomic rate of biosolids was 14 Mg ha^{-1}.	Single in 1996	Control, 98 dry Mg ha^{-1} biosolids and 98 dry Mg ha^{-1} biosolids with sawdust	0-7.5 cm

Table 5.2. Selected chemical properties of the organic residuals applied to the three study sites.

Study Site	Treatment	C:N	C	TKN	NH_4-N	NO_3-N	Organic-N	Total P	K
			----------	----------	----------	----------g kg^{-1}	----------	----------	----------
Site I	PL[†]	8	474	56.1	8.2	0.19	48	17.6	26.3
	BSC[‡]	10	239	26.0	3.2	0.88	22	15.6	1.1
	PYC[§]	20	379	19.3	0.2	0.73	18	4.2	6.8
Site II	BS[¶]	20	320	16	<0.008	--	16	31.7	1.1
Site III	BS	8	358	44.7	6.4	0.30	38	17	1.2
	SD[#]	198	475	2.4	--	--	2.4	--	--

†PL = poultry litter; ‡BSC = biosolids-woodchip compost; §PYC = poultry litter-yard waste compost; ¶BS = biosolids; #SD = sawdust.

Table 5.3. C(1s) NEXAFS approximate transition energy ranges and assignments of primary absorption peaks.

C form	Bond	Transition	Peak energy (eV)	Peak	Fit position
Aromatic C and quinone C	C=O	1*s*-*π**	283–284.5	G1	284.5
Aromatic C and double-bonded alkyl C	C=C	1*s*-*π**	284.9–285.5	G2	285.4
Aromatic C with side chain and N-substituted aromatic C (Phenolic C)	C–OH C=O R–(C=O)–R′ C=N, C–N	1*s*-*π**	286.0–287.4	G3	286.1
Alkyl C	C-H	1*s*-*π**/ 1*s*-3*p*/σ*	287–287.6	G4	287.6
Carboxylic C	R–COOH COO C=O (NH2)–C–O R–(NH2)–R′	1*s*-*π**	288.0–288.7	G5	288.4
O-alkyl C	C–OH	1*s*-*π**/ 1*s*-3*p*/σ*	289.2–289.5	G6	289.2

Table 5.4. Carbon expressed as a fraction of whole soil mass and carbon recovery rate in density fractions and in hydrogen fluoride (HF) treatment from different organic residual-amended soils at the three sites.

Site	Treatment	C g kg^{-1} whole soil	C expressed as a fraction of whole soil g kg^{-1}						% C recovery in density fractions	% POM-C recovery in HF
			POM[††]	clay	silt	sand	sum of C	HF-POM		
Site I	CON[‡‡]	23.2	2.4	5.5	7.6	6.9	22.3	1.5	96.3	61.0
	PYC[§]	47.4	2.8	9.7	16.4	2.8	31.8	1.7	67.0	60.1
	BSC[‡]	37.5	3.2	8.2	14.2	9.2	34.8	2.3	92.8	71.2
	PL[†]	23.7	2.0	5.0	13.5	6.2	26.7	1.2	112.6	60.3
Site II	1993 BS[¶]	23.4	2.2	5.2	6.1	5.0	18.5	2.2	78.9	98.8
	2011 BS	18.8	2.6	5.9	4.3	5.3	18.1	2.6	96.3	98.7
Site III	CON	15.0	1.0	3.8	4.0	2.0	10.8	1.0	71.8	100.5
	BS	19.1	1.2	4.8	5.5	3.8	15.3	1.2	80.0	100.8
	BS+SD[#]	18.8	1.6	4.0	5.7	2.8	14.1	1.6	75.0	101.1

†PL = poultry litter; ‡BSC = biosolids-woodchip compost; §PYC = poultry litter-yard waste compost; ¶BS = biosolids; #SD = sawdust; ††POM=particulate organic matter. ‡‡CON = control.

Table 5.5. Carbon functional groups proportions of particulate organic matter using C (1s) NEXAFS total electron yield.

Site	Treatment	Proportion of absorption regions (%)							
		G1 284.5eV	G2 285.4eV	G3 286.1eV	G4 287.6eV	G5 288.4eV	G6 289.2eV	G1+G2	G4/G6
				Phenolic	Alkyl	Carboxylic	O-alkyl	Aromatic	Alkyl/O-alkyl
Site I	CON††	1.9	12.1	8.5	15.0	11.5	51.1	14.0	0.29
	PYC§	1.8	16.4	13.2	17.2	10.5	40.9	18.2	0.42
	BSC‡	1.5	17.5	11.8	18.2	8.5	42.5	19.0	0.43
	PL†	4.7	6.7	10.4	10.0	13.9	54.2	11.5	0.19
Site II	1993 BS¶	1.5	10.3	10.2	11.0	34.3	32.7	11.8	0.34
	2011 BS	5.7	16.3	11.4	8.0	24.3	34.2	22.1	0.23
Site III	CON	1.7	13.4	11.6	19.5	7.0	46.8	15.0	0.42
	BS	1.3	18.6	11.1	20.5	11.7	36.9	19.9	0.56
	BS+SD#	4.5	17.7	12.1	21.7	12.4	31.7	22.2	0.69

†PL = poultry litter; ‡BSC = biosolids-woodchip compost; §PYC = poultry litter-yard waste compost; ¶BS = biosolids; #SD = sawdust; ††CON = control.

Table 5.6. Carbon functional groups proportions of particulate organic matter using C (1s) NEXAFS total fluoresce yield.

Site	Treatment	Proportion of absorption regions of TFY (%)							
		G1 284.5eV	G2 285.4eV	G3 286.1eV	G4 287.6eV	G5 288.4eV	G6 289.2eV	G1+G2	G4/G6
				Phenolic	Alkyl	Carboxylic	O-alkyl	Aromatic	Alkyl/O-alkyl
Site I	CON††	4.0	9.3	17.2	31.5	10.2	27.8	13.3	1.13
	PYC§	3.5	8.5	14.8	36.7	8.9	27.6	12.0	1.33
	BSC‡	4.4	7.9	15.0	34.7	10.0	27.9	12.3	1.25
	PL†	3.5	9.6	16.6	31.9	10.1	28.3	13.1	1.13
Site II	1993 BS¶	3.0	8.0	14.5	25.6	16.9	32.0	10.9	0.80
	2011 BS	4.6	9.0	15.5	29.2	15.9	25.7	13.7	1.14
Site III	CON	4.4	9.0	15.5	34.8	9.2	27.1	13.4	1.28
	BS	0.0	15.1	12.7	33.5	10.4	28.3	15.1	1.18
	BS+SD#	0.0	17.2	11.3	31.8	10.5	29.2	17.2	1.09

†PL = poultry litter; ‡BSC = biosolids-woodchip compost; §PYC = poultry litter-yard waste compost; ¶BS = biosolids; #SD = sawdust; ††CON = control.

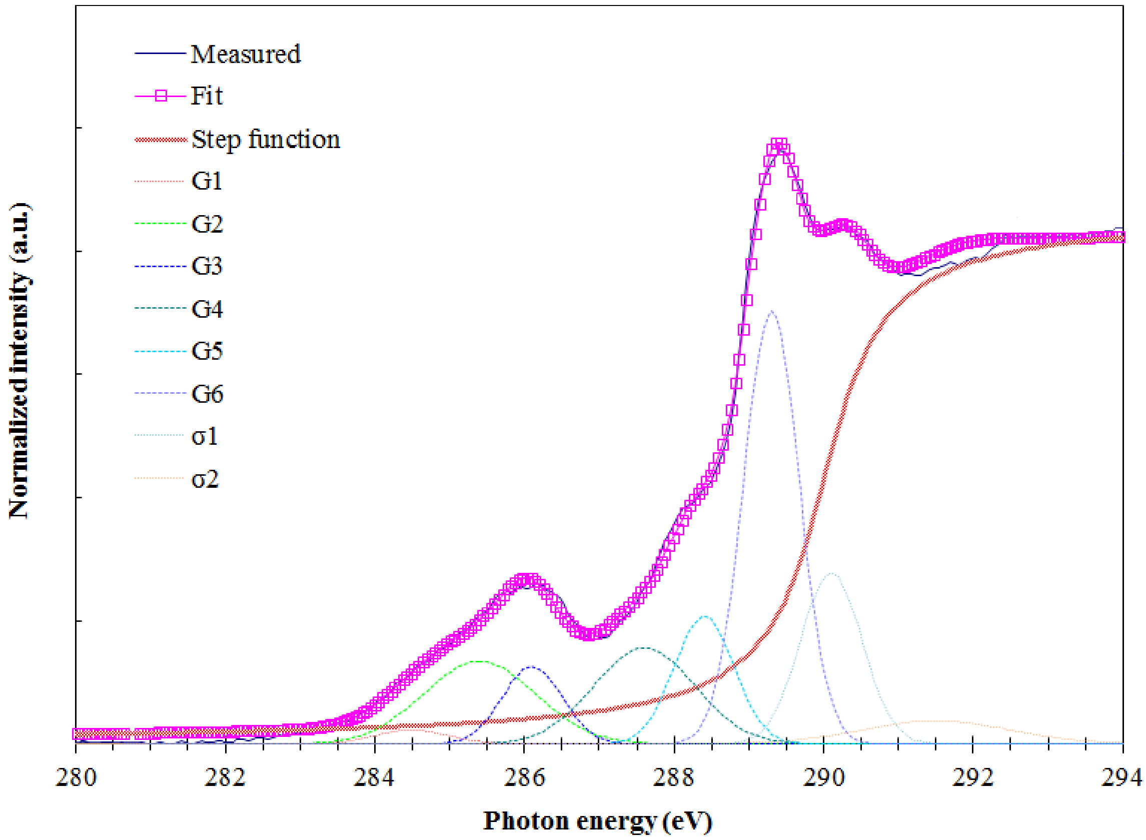
Measured
Fit
Step function
G1
G2
G3
G4
G5
G6
σ1
σ2
Normalized intensity (a.u.)
280
282
284
286
288
290
292
294
Photon energy (eV)

Figure 5.1.

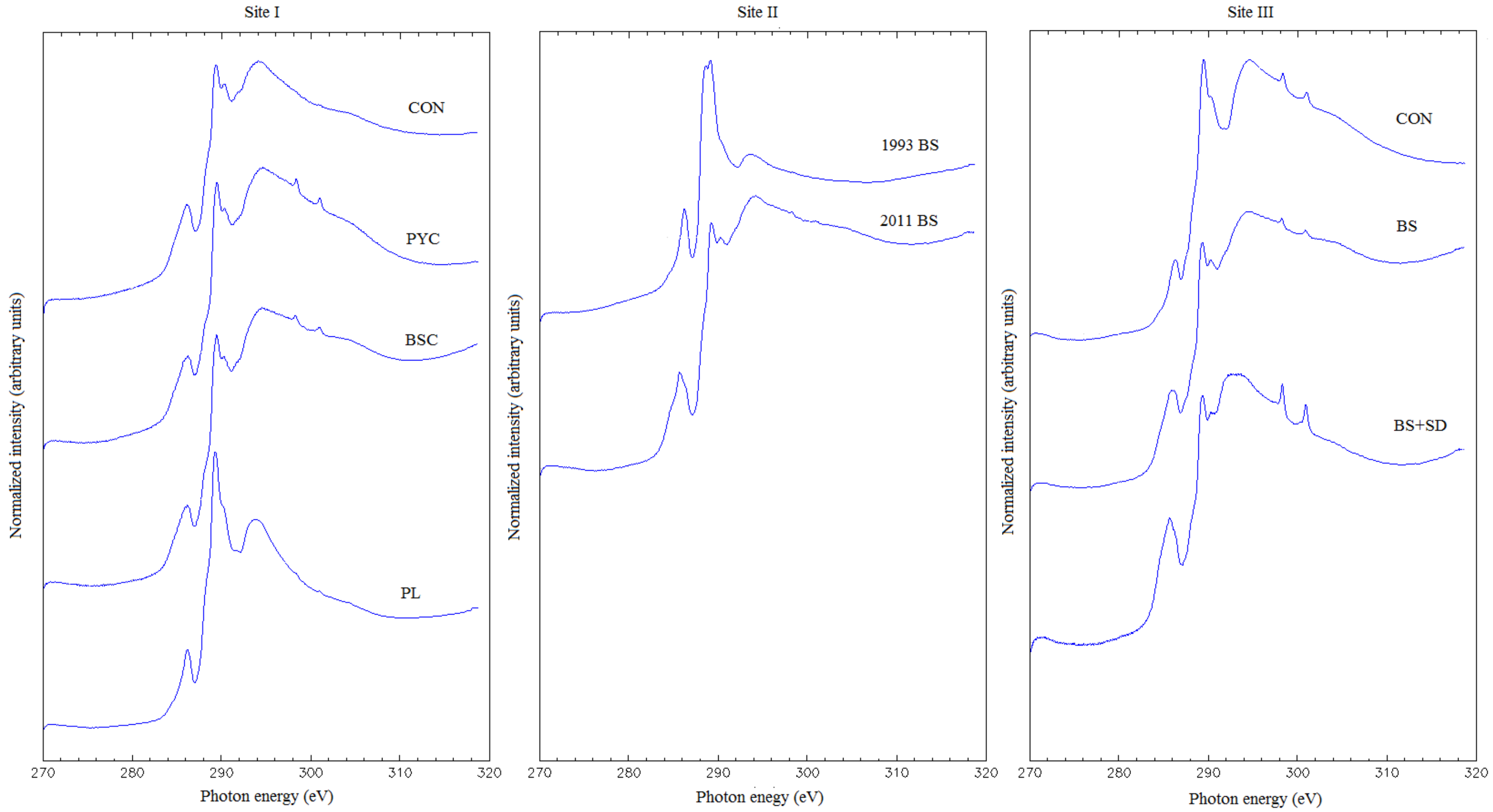
Site I
Site II
Site III
CON
PYC
BSC
PL
1993 BS
2011 BS
CON
BS
BS+SD
Normalized intensity (arbitrary units)
Photon energy (eV)
Photon enegy (eV)
270
280
290
300
310
320

Figure 5.2.

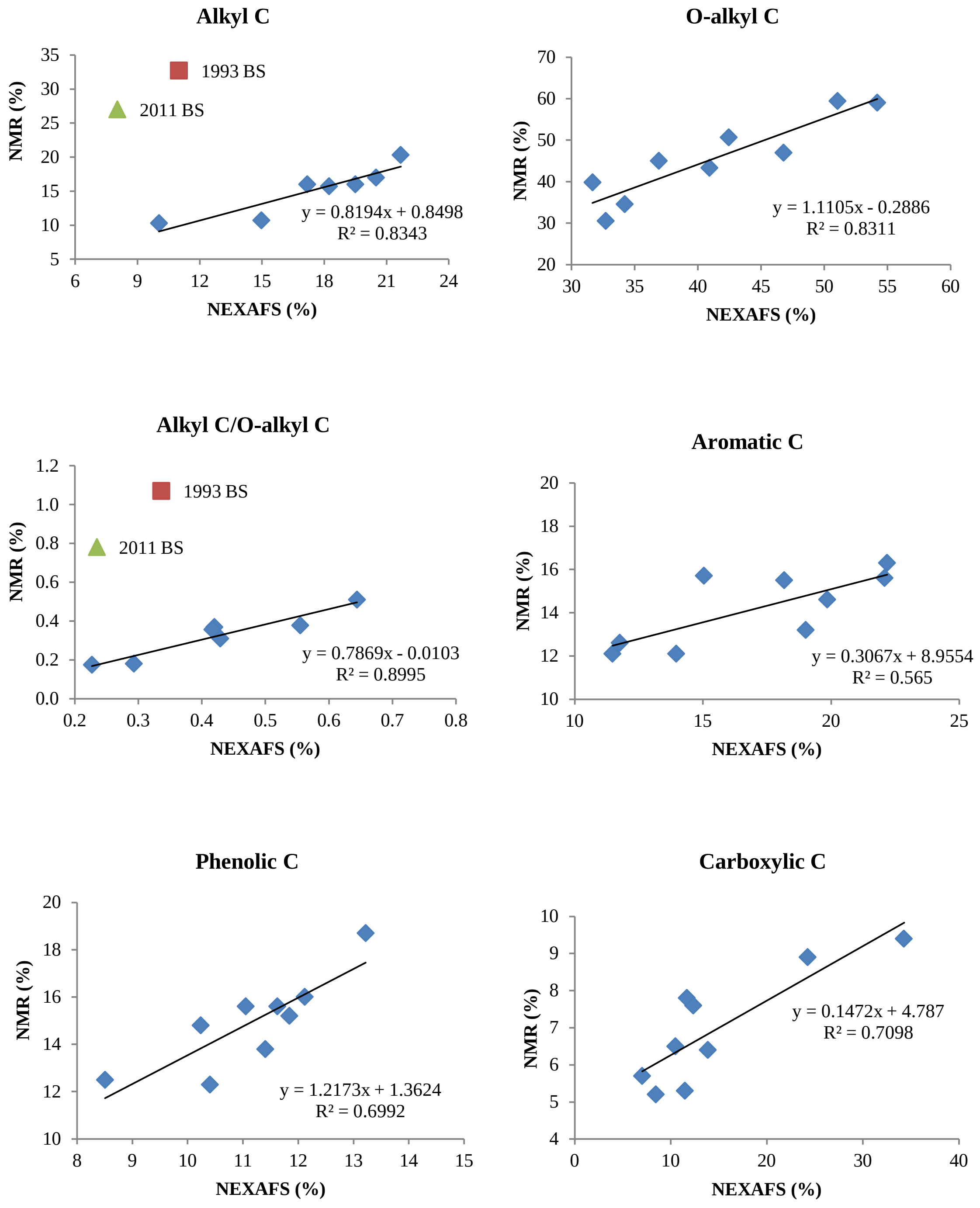
Alkyl C
NMR (%)
NEXAFS (%)
1993 BS
2011 BS
y = 0.8194x + 0.8498
R² = 0.8343
35
30
25
20
15
10
5
6
9
12
15
18
21
24
O-alkyl C
NMR (%)
NEXAFS (%)
y = 1.1105x - 0.2886
R² = 0.8311
70
60
50
40
30
20
30
35
40
45
50
55
60
Alkyl C/O-alkyl C
NMR (%)
NEXAFS (%)
1993 BS
2011 BS
y = 0.7869x - 0.0103
R² = 0.8995
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Aromatic C
NMR (%)
NEXAFS (%)
y = 0.3067x + 8.9554
R² = 0.565
20
18
16
14
12
10
10
15
20
25
Phenolic C
NMR (%)
NEXAFS (%)
y = 1.2173x + 1.3624
R² = 0.6992
20
18
16
14
12
10
8
9
10
11
12
13
14
15
Carboxylic C
NMR (%)
NEXAFS (%)
y = 0.1472x + 4.787
R² = 0.7098
10
9
8
7
6
5
4
0
10
20
30
40

Figure 5.3.

6. Effects of Biosolids Treatment Processes on N Cycling and C Accumulation under Various Tillage Practices

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6.1. Abstract

Biosolids are typically injected or incorporated into soils by tillage; however, little research has been conducted on biosolids effects on nitrogen (N) availability under no or minimum tillage for crop production conditions. We conducted a three-year field study to investigate the effects of lime-stabilized (LS) and anaerobically digested (AD) biosolids on crop response and soil N availability in a corn (*Zea mays* L.)-soybean (*Glycine max* L.) rotation under both conventional tillage (CT) and no-tillage (NT) practices. Research was established on an Orangeburg loamy sand (*Fine-loamy, kaolinitic, thermic Typic Kandiudults*) in 2009-2011 in Sussex County, VA. The results showed that both LS and AD biosolids increased spring soil nitrate N, plant tissue N at silking, post-season corn stalk nitrate N, grain yield, and total soil N by the end of the growing season. Both biosolids types increased soil C accumulation and plant available water in the coarse-textured, conventionally-tilled soils in 2011. The same prediction factors used to calculate plant available N for soil-incorporated biosolids can be used on biosolids applied to no-till systems in coarse-textured soils of the Mid-Atlantic Coastal Plain.

6.2. Introduction

Land application of biosolids has been demonstrated to be a safe and effective means for recovery of plant nutrients (mainly N and P) (Magdoff and Amadon, 1980; O'Riordan *et al.*, 1987; Epstein, 2003). Increased crop yields have been obtained with land application of biosolids (Giordano *et al.*, 1975; Cripps *et al.*, 1992; Warman and Termeer, 2005). Land application of biosolids also replenishes valuable organic matter, while simultaneously improving soil structure, water retention, nutrient capacity, and microbiological properties (Epstein, 1975; Khalilian *et al.*, 1998; Novak and Watts, 2004; Garland *et al.*, 2010).

Biosolids are traditionally incorporated into soil or injected directly below the surface to reduce odors, runoff risk, and soil compaction resulting from the application (Gupta *et al.*, 1976; Bruggeman and Mostaghimi, 1993). No-till practices have been increasingly implemented for row crop production (Evanylo, 1991; Roygard *et al.*, 2002; Licht and Al-Kaisi, 2005). Incorporation of biosolids into soil increases N availability for loss through rapid microbial decomposition, while no-tillage generally results in slower biosolids decomposition and a net increase in soil organic C (Menelik *et al.*, 1990; Tian *et al.*, 2009). Field studies throughout the eastern United States have demonstrated that long-term N conservation and C storage can be increased through the land application of biosolids under continuous no-tillage practices (Spargo *et al.*, 2008a; Spargo *et al.*, 2008b; Stewart *et al.*, 2012). However, few studies report the short-term effects of tillage management on biosolids N availability and C accumulation under no-tillage practices for row crops, especially in the Mid-Atlantic region.

The method of biosolids application, the biosolids processing methods used, and the environmental properties (esp., temperature, moisture, and aeration) control biosolids plant available N (PAN). NH_4-N can be lost via NH_3 volatilization when biosolids are applied on soil surface (Adamsen and Sabey, 1987; Quemada *et al.*, 1998), resulting in a reduction in available N and lower yields (Cripps *et al.*, 1992). Incorporation has been shown to produce more biosolids N mineraliation compared to surface-applied biosolids (King, 1973; Castillo *et al.*, 2011). NO_3-N generated from mineralization and nitrification is more easily lost through leaching when biosolids are incorporated to soil (Cripps *et al.*, 1992; Cartron and Weil, 1998; Sierra *et al.*, 2001).

The type of biosolids treatment processes can also influence PAN (Sommers *et al.*, 1981; Smith *et al.*, 1998; Gilmour *et al.*, 2003). Organic N in lime-stabilized biosolids may mineralize to a greater extent than anaerobically digested biosolids during the first season after application (U.S. Environmental Protection Agency, 1995; Cartron and Weil, 1998). Anaerobically digested biosolids contain significant proportions of NH_4-N, which can be more vulnerable to loss through ammonia volatilization during and immediately after application and incorporation (Smith *et al.*, 1998; Gilmour *et al.*, 2003; Evanylo, 2006).

Edaphic factors may also influence biosolids PAN, e.g., soil temperature, moisture, and aeration (Kelling *et al.*, 1978; Gilmour and Clark, 1988). Leaching potential is greater in coarse-textured soils possessing high hydraulic conductivity (Gupta *et al.*, 1976; Evanylo, 2003; Correa *et al.*, 2006). Mineralization rates are generally greater in coarse textured soils due to the greater aeration (Sukkariyah *et al.*, 2007).

Both soil and plant tissue testing diagnostic tools can be used to evaluate the supplying capacity of N sources, such as biosolids, for corn. The pre-sidedress soil nitrate test (PSNT) has proven useful for assessing available soil N sufficiency when corn is 25 to 30 cm tall (Evanylo and Alley, 1997). The ear leaf N test at silking provides reliable assessment of corn plant N sufficiency/deficiency (McWilliams *et al.*, 1999) that can be used to interpret tillage and biosolids effects on PAN. The end-of-season corn stalk nitrate test (CSNT) provides post-season evaluation of N status because corn plants store excessive NO_3-N in the bottom of stalks that can be re-mobilized upon crop needs (Binford, 1990; Blackmer *et al.*, 1992). Corn grain yield is an excellent indicator of N availability if all other potentially limiting growth factors are optimal (Schmidt *et al.*, 2011). End-of-season soil properties, such as soil C and N contents, can be used to measure the accumulation of soil organic matter among various soil building processes (e.g., no-till) (Bremner *et al.*, 1996), and the plant available water (PAW) capacity can provide direct information about soil aggregate and soil water infiltration (Cassel *et al.*, 1986). We proposed measuring these indicators to evaluate biosolids PAN for corn growth under various tillage practices. Our objectives were to compare biosolids types (i.e., lime-stabilized and anaerobically digested biosolids) and tillage practices (i.e., conventional tillage and no-tillage) on short-term N availability, soil C accumulation, and plant available water in a corn-soybean rotation in the Mid-Atlantic Coastal Plain.

6.3. Materials and Methods

6.3.1. Experimental site

This study was conducted on a commercial farm in Sussex County, VA on an Orangeburg loamy sand (*Fine-loamy, kaolinitic, thermic Typic Kandiudults*), where grain yield potential is estimated to be 8.80 Mg ha^{-1} according to Virginia Department of Conservation and Recreation (2005). Identical treatments, varying only in crop rotation, were laid out on each of two adjacent parcels of land. The dimensions of both study sites were 22.86 m x 87.78 m (2007 m^2).

6.3.2. Experimental design

A split-plot design with tillage types randomly assigned to the main plot and fertility treatments randomly assigned to subplots was implemented. Each treatment was replicated four times, resulting in a total of 48 experimental plots per each crop rotation. Each experimental plot had an area of 9.14 m x 3.66 m (33.45 m^2). Two types of tillage practices were employed: conventional tillage (CT) and no-tillage (NT). Conventional tillage consisted of disking to a depth of 10cm, and no-tillage consisted of seed drilling directly into the undisturbed soil through the stubble remaining from the previously harvested crop. The six fertility treatments were four fertilizer N rates (0x, 0.5x, 1x, 1.5x agronomic N rate), and lime-stabilized (LS; Blue Plains Wastewater Treatment Plant, DC: http://www.epa.gov/owm/septic/pubs/alkaline_stabilization.pdf) and anaerobically digested (AD; Alexandria Sanitation Authority, VA: http://epa.gov/OWM/mtb/multi-stage.pdf) biosolids, each applied at the agronomic N rate. The agronomic N rate for corn grain of 156 kg ha^{-1} was reduced by either 51 kg N ha^{-1} (following peanut) or 23 kg N ha^{-1} (following soybean) each year based on residual N availability of previous legume crop (Virginia Department of Conservation and Recreation, 2005).

A corn-soybean rotation was implemented at each site during 2009-2011. Study site I was previously planted with peanuts in 2008, followed by corn (*Zea mays* L.) in 2009, soybean (*Glycine max* L.) in 2010, and corn in 2011. The adjacent study site II was planted with corn in 2009-2010 and soybean in 2011. Thus, the three-year study consisted of two growing seasons of corn in study site I (2009 and 2011) and one growing season of corn in study site II (2010).

6.3.3. Biosolids and fertilizer application

Biosolids application rates were calculated from previous biosolids analyses. The actual nutrient rates applied were calculated each year from analyses of the biosolids applied. Ten subsamples were randomly collected from the biosolids stockpiled at the site, placed on ice and sent to a commercial laboratory (A&L Eastern Laboratories) for property analysis. Analyses performed included total solids (SM-2540G), total Kjeldahl N (SM-4500- TKN), ammonium-N (SM-4500-NH_3) (Standard Methods for the Examination of Water and Wastewater, 1992), phosphors (SW-846-6010C), potassium (SW-846-6010C) (U.S. Environmental Protection Agency, 1986), and other macro and micro nutrients.

Fresh biosolids were weighed in the field, surface-applied to each plot, and uniformly raked. The commercial urea N fertilizer [$CO(NH_2)_2$] were applied to plots by hand before planting the corn. All experimental plots also received supplemental basal inorganic P ($NH_4H_2PO_4$) and K (KCl) fertilizers based on soil testing results (Donohue and Heckendorn, 1994).

The conventional tillage treatments were disked each spring prior to planting corn to incorporate biosolids and fertilizers into soil. A field cultivator was used following disking to further mix the material and prepare a seedbed. Corn (*Zea mays* L. Pioneer 31G71) was planted in mid-April each year with row spacing of 91 cm and a seeding rate of 69,300 kernel ha^{-1} (resulting in a plant population of 63,000 plant ha^{-1}). Soybean (*Glycine max* L. Pioneer 95M82) was rotated on the plots in 2010 (study site I) and 2011 (study site II), respectively at a seeding rate of 334,000 seed ha^{-1} (resulting a plant population of 304,000 plant ha^{-1}). Pest and weed control was implemented according to standard Virginia Cooperative Extension recommendations (Virginia Cooperative Extension, 1992), including a pre-plant roundup herbicide-glyphosate before planting, a 7.8 kg ha^{-1} Counter TM insecticide for corn in seed furrow at planting, and a 0.14 kg ha^{-1} Karate TM insecticide for soybean at moth egg threshold in August.

Estimated biosolids PAN were calculated from published Virginia regulatory ammonia-N volatilization and organic N mineralization factors under CT and NT (Virginia Department of Conservation and Recreation, 2005) http://www.dcr.virginia.gov/documents/StandardsandCriteria.pdf). For LS biosolids, ammonia-N availability factor for the first year after application is 0.75 for conventional tillage and 0.25 for no-tillage. For AD biosolids, ammonia-N availability factor for the first year after application is 0.85 for conventional tillage and 0.5 for no-tillage. For both types of biosolids, the organic N mineralization factor for the first year after application is 0.3 under both tillage practices. Biosolids PAN was calculated as the sum of the expected mineralized organic N and the non-volatilized ammonium/ammonia.

6.3.4. Sampling and analysis

Soil routine test

Each fall from 2008 to 2011, soil cores with a diameter of 1.9 cm were randomly collected from the 0-15 cm and 15-30 cm depth in the two study sites (2008) and in every treatment plot (2009-2011), air-dried, ground to pass a 2 mm sieve and sent to Virginia Tech Soil Testing Laboratory for routine soil test analysis of Mehlich 1 extractable P, K, Ca, Mg, and pH (Donohue and Friedericks, 1984).

Pre-sidedress soil nitrate test

In spring, when corn plants were 25-30 cm tall, ten soil cores with a diameter of 1.9 cm were collected from the 0-15 cm and 15-30cm depth in each plot for pre-sidedress soil nitrate test (PSNT) analysis as an in-season indicator of available soil inorganic N (Magdoff *et al.*, 1984; Evanylo and Alley, 1997; Pennsylvania Cooperative Extension, 1999). All samples were stored on ice and transported to laboratory, where they were extracted with 2 M KCl for nitrate (NO_3-N) and ammonium (NH_4-N) analysis (Keeney and Nelson, 1982) by flow injection analysis on Lachat 8000 (Lachat Instruments, US).

End-of-season soil N, C and plant available water

After harvest each autumn, ten soil cores with a diameter of 1.9 cm were collected from the 0-15 cm depth in each plot, air-dried, and ground to pass a 0.5 mm sieve for total soil N analysis by dry combustion on a Vario Max CNS macro elemental analyzer (Elementar, GER).

After corn was harvested in 2011, two soil cores from each replication of the conventionally tilled 1x N fertilizer plots, LS biosolids plots, and AD biosolids plots at study site I were sampled. Bulk density samples were collected with a drop hammer using the core method (Rogers and Carter, 1987). The cores were air-dried and weighed to calculate bulk density based on moisture correction, and ground to pass a 0.5 mm sieve for soil organic C analysis by dry combustion in a Vario Max CNS macro elemental analyzer (Elementar, GER). The C stock was calculated from bulk density and soil organic C concentration. Plant available water capacity was determined on the bulk density soil cores by a pressure plate method (Cassel *et al.*, 1986). The amount of water held by soil under 33 kPa of pressure (field capacity) minus the amount of water held at 1500 kPa of pressure (wilting point) was considered the plant available water capacity and was reported as % water in soil (v/v) on an oven-dried basis.

Corn ear leaf total N

Each July, ten corn ear leaf samples from each plot were randomly collected at the early silking (R1) stage (McWilliams *et al.*, 1999), dried at 65 °C and ground in a Wiley mill to pass a 0.5 mm sieve for determination of nitrogen. Total Kjeldahl N (TKN) of these plant samples were determined colorimetrically by flow injection analysis on Lachat 8000 (Lachat Instruments, US).

End-of-season corn stalk nitrate test

Before harvest, end-of-season corn stalk NO_3-N was determined to evaluate the adequacy (or excess) of the N fertility program for the current growing season. Corn segments (20 cm) were taken 15 cm above-ground from ten plants in each plot. Stalk segments were cut, dried at 65 °C and ground in a Wiley mill to pass a 0.5 mm sieve for analysis of NO_3-N content via electrode analysis (Binford, 1990; Blackmer *et al.*, 1992).

Corn grain yield

Each September, corn ears were hand-picked in 6.1-m of the two center rows in each plot, dried at 65 °C, and shelled to obtain grain yield estimates (0.155 g moisture g^{-1} dry weight).

6.3.5. Statistical analysis

The split plot design (Bingham *et al.*, 2004) was implemented using a mixed model procedure of Statistical Analysis System (SAS) 9.2 (SAS Institute, 2008). Analysis Of Variance (ANOVA) and Least Significant Difference (LSD) measurement were applied at level of 0.05 to compare differences between treatment means (e.g., corn yield). The relationship between soil or plant tissue N level and fertilizer N application rate was examined with linear and quadratic regression analyses using the PROC REG procedure of SAS 9.2 (SAS Institute, 2008). A randomized complete block design was used to statistically analyze the soil C stocks and plant available water capacity obtained in November, 2011.

6.4. Results and Discussion

6.4.1. Experimental site

The soil properties from 0-15 cm and 15-30 cm depths at the two sites prior to treatments applied are shown in Table 6.1. The pH values were above 6.2, and no additional liming was required. Soil testing (i.e., Mehlich I extractable) indicated a need for P and K that was provided by biosolids and/or supplemental fertilization (Donohue and Heckendorn, 1994). The total organic C concentration was typical for coarse-textured Virginia Coastal Plain soils. The low nutrient and C contents of the coarse-textured soils made the application of organic residuals (i.e., biosolids) a desirable practice.

Monthly temperature and precipitation data for the study period are shown in Table 6.2. The mean temperature during the growing season (April-August) was 21.9 °C, 23.2 °C, and 22.5 °C for the three years, respectively. The total precipitation during the growing season (April-August) was 498 mm, 363 mm, and 683 mm for the three years, respectively. To supplement rainfall and prevent crop failure due to drought, plots were irrigated between June and July with two applications of 2 cm of water; in 2010 plots were irrigated between June and August with four applications of 2 cm of water.

6.4.2. Biosolids properties

The attributes of the two biosolids are shown in Table 6.3. The AD biosolids contained almost twice as much TKN and a higher proportion of NH_4-N than the LS biosolids. The AD biosolids also contain higher concentrations of P and S but no calcium carbonate equivalent (CCE), lower Ca and pH than the LS biosolids. The target and estimated actual biosolids PAN applied during 2009-2011 are shown as Table 6.4. The target agronomic N rate was not exactly achieved because the actual N composition of the material varied from the analyses used to calculate the application rates. The biosolids application rates to NT were higher than to CT to account for N volatilization loss; therefore, application rates of CCE and nutrients other than N were higher to NT than to CT treatments.

6.4.3. Soil properties and crop response

Pre-sidedresss soil nitrate N

The results from the pre-sidedress soil nitrate test (PSNT) are presented in Table 6.6 and Figure 6.1a. Tillage did not affect soil NO_3-N levels in any fertility treatment plots, likely because organic and ammonium N were not differentially mineralized and/or nitrified under the different tillage systems in the coarse-textured Coastal Plain soils. This result is consistent with Meisinger *et al.* (1992).

The response of soil NO_3-N concentration to increasing fertilizer N rate followed a similar pattern according to the near equal slopes of the regression lines in 2010 and 2011, but the slope in 2009 was reduced by higher volume of rainfall, which likely increased leaching loss beyond the zone of sampling. The 1x fertilizer N rate resulted in NO_3-N concentration at or above the critical level of 20 mg kg^{-1} (Evanylo and Alley, 1997) for corn production in 2010-2011, but not in 2009. These responses demonstrate the susceptibility to leaching loss of N in the coarse-textured Coastal Plain soils receiving sufficient precipitation in early May (Sims *et al.*, 1995). There were no significant differences among fertility treatments in soil NH_4-N (data not shown) across all three years because ammonium is rapidly converted to nitrate through nitrification in the coarse-textured soils (Meisinger *et al.*, 1992).

The biosolids maintained soil NO_3-N concentrations with a range of 21-45 mg kg^{-1}, during 2009-2011. According to PSNT diagnostic criteria, both biosolids types should provide sufficient NO_3-N for optimal corn production, necessitating no additional N (Evanylo and Alley, 1997). Soil NO_3-N concentrations in the biosolids treatments were lower than with the 1x fertilizer N rate in 2010, with soil NO_3-N lower in the AD (24 mg kg^{-1}) than the LS biosolids (43 mg kg^{-1}). The U.S. Environmental Protection Agency (1995) recommends higher annual first-year organic N mineralization rate for LS (30%) than AD (20%) biosolids. However, Gilmour *et al.* (2003) determined that climate plays a greater role than processing treatment in mineralization rates, and there should be little difference in mineralization rate between lime stabilized and digested biosolids. In our studies, rainfall in April and May of 2010 were considerably below average, which may have reduced microbially facilitated N transformations, such as mineralization and nitrification, in the biosolids. This could have decreased the NO_3-N concentration of biosolids during this period, particularly in AD biosolids.

Our studies found that leaching losses of NO_3-N occur in coarse-textured soil under conditions of high soil moisture, thus reducing biosolids PAN in spring 2009. Al-Kaisi and Kwaw-Mensah (2007) reported that the PSNT on a Kenyon loam soil was decreased by deep leaching of NO_3–N due to above normal rainfall in early April. These findings indicate a need to reassess N availability while using the PSNT for interpreting biosolids PAN where NO_3 leaching loss may be greater in coarse-textured Coastal Plain soils.

End-of-season soil N, C and plant available water

Tillage only influenced end-of-season soil total N concentration in 2009 ($p=0.039$), with a higher level under NT (388 mg kg^{-1}) than CT (355 mg kg^{-1}) (Table 6.5). Increased tillage intensity decrease the soils capacity to immobilize and conserve mineral N (Spargo *et al.*, 2008a). Gallaher and Ferrer (1987) reported that no-tillage resulted in 20% more total Kjeldahl N than conventional tillage at the 0 to 5 cm soil depth in a 3-year study. However, short-term tillage management effects on soil C and N dynamics are often complex and variable. Al-Kaisi *et al.* (2005) reported that tillage effects on soil organic C and total N were negligible at the end of only 3 years of tillage practices.

Results showed that end-of-season soil total N concentration did not increase with increasing fertilizer N rates during any of the three years (Table 6.6 and Figure 6.1b). The soluble fertilizer N was likely rapidly assimilated by plants or leached through the soil, resulting in lower residual N levels than biosolids. The sufficient volume of early-spring rainfall in 2009 may have increased leaching NO_3–N losses, which reduced soil N availability.

Soil N concentrations were higher where biosolids were applied than where the commercial fertilizers were applied. Biosolids contain recalcitrant N which remains in organic form for a long time after application. The slowly available organic N pool is made up of more resistant N compounds that take months or years for complete decomposition (Henry *et al.*, 1999). Biosolids N inputs increase plant residues and improve microbial activity which depletes labile SOC pool (Nave *et al.*, 2009). The 2011 field site also received biosolids in 2009, resulting in higher end-of-season soil total N concentration caused by biosolids residual N effects.

The end-of-season soil C stocks in 2011 ($p=0.062$) were decreased in the conventionally-tilled soils in the order: LS biosolids (4.2 Mg ha^{-1}) > AD biosolids (3.6 Mg ha^{-1}) = 1x N rate (3.5 Mg ha^{-1}). Biosolids application increased soil C stocks in the short term, because biosolids added C inputs into soils. Other research has shown that short-term added biosolids increased soil C accumulation (Fernandes *et al.*, 2005; Dai *et al.*, 2009). However, such increase in soil C accumulation cannot be regarded as sequestration, as C sequestration is unlikely to occur in such soils following a few biosolids applications (Powlson *et al.*, 2012). The supply of fresh C may accelerate the decomposition of soil C and induce a negative C balance (Fontaine *et al.*, 2004). Additionally, N additions from biosolids likely perturb the balance between substrate and microbial biomass C:N ratio, leading to shifts from net immobilization to mineralization (Epstein *et al.*, 1978; Petersen *et al.*, 2003).

Biosolids application increased volumetric soil plant available water (PAW) (p=0.074) in the conventionally-tilled soils in the order: 1xN rate (44.4 mg/g) < LS biosolids (45.8 mg/g) < AD biosolids (52.4 mg/g). The increase in soil organic matter (as measured by soil C) likely increased soil moisture holding capacity (Zebarth *et al.*, 1999).

Corn ear leaf N

In 2011, tillage practices were significant for ear leaf N (p=0.002), with ear leaf N concentration higher under CT (23.2 g kg^{-1}) than NT (21.2 g kg^{-1}) (Table 5). Although PSNT showed no differences between tillage systems, tillage may have promoted the mineralization of the previously grown soybean stubble as well as biosolids residual N, which appeared to be increased with tillage.

Corn ear leaf N concentration increased with fertilizer N rate and followed a similar quadratic pattern across all three years (Table 6.6 and Figure 6.2a). The variability in weather among the 2009-2011 seasons apparently did not influence fertilizer induced corn leaf N concentration.

During the rainy 2009 season, both biosolids maintained higher ear leaf N concentrations than 1.5x fertilizer N rate. There were no differences in ear leaf N between the two biosolids types. The LS biosolids gave higher ear leaf N concentration than AD biosolids in two of the three years of the study (2010, 2011), possibly due to higher mid-season soil N maintenance. The LS biosolids have a high pH (>12) and a higher proportion of slow release organic N than AD biosolids. The AD biosolids gave lower ear leaf N concentration than 1x fertilizer N rate in 2010-2011, but higher in 2009, when soluble fertilizer N would have been more likely to leach under the high rainfall.

Although PSNT showed that spring soil NO_3-N may have been lost through leaching under high rainfall in 2009, the corn ear leaf N concentration response to the various rates and sources of N did not vary considerably over the three years.

Corn stalk nitrate N

There were no significant differences of corn stalk NO_3-N concentrations between conventional tillage and no-tillage during the growing seasons of 2009-2011.The tillage practices did not affect the NO_3-N concentrations accumulation at the bottom of the corn stalks, as plants can absorb and utilized the same amounts of N under these two types of tillage managements.

There was a positive quadratic relationship between corn stalk NO_3-N concentration and fertilizer N rate (Table 6.6 and Figure 6.2b). Corn stalk NO_3-N concentration remain steady and does not increase with N applied rate until the maximum yield is reached (Balkcom *et al.*, 2000). The optimum levels of corn stalk NO_3-N are 450-2000 mg kg^{-1} (Iversen *et al.*, 1985; Fox *et al.*, 1989; Hooker and Morris, 1999; Pennsylvania Cooperative Extension, 1999). The upper boundary was never attained by any of our fertilizer treatments and only by the LS biosolids in 2010. In fact, insufficient N (CSNT<450 mg kg^{-1}) diagnosis was more often indicated than excessive N throughout the three years of the study. This was likely due to the application of optimal or less PAN rates and/or nitrate-leaching rainfalls.

Both biosolids maintained higher corn stalk NO_3-N concentrations than 1x fertilizer N rate during 2009-2011. The LS biosolids maintained higher corn stalk NO_3-N concentration than AD biosolids in 2010, but slightly lower than AD in 2009 and equal to AD biosolids in 2011.The excessive corn stalk NO_3-N concentration with LS biosolids in

2010 (2212 mg kg^{-1}) was consistent with early season high N assimilation followed by biomass-reducing drought (Balkcom *et al.*, 2000).

Because CSNT provides a direct measure of N sufficiency and deficiency for plant growth during the growing season, these measurements provide evidence that sufficiency of N for plant growth has been obtained in 2010-2011, but not 2009.

Corn grain yield

Tillage significantly (p=0.011) affected corn grain yield only in 2011, after the study had been in NT the longest time. A higher yield for no-till than for conventional tillage (Table 5) has been reported by others for Coastal Plain soils (Evanylo, 1991; Spargo *et al.*, 2008a). The typical explanation for such advantages of NT over CT are that improved water availability and water use efficiency increase corn grain yields (Wagger and Cassel, 1993). No-till decreases soil bulk density and increases water infiltration and water holding capacity, consequently increasing plant growth and crop yields (Reicosky *et al.*, 1977; Stewart *et al.*, 2012).

Grain yields increased with the increasing fertilizer N rate and surpassed the expected yield for this soil (8.80 Mg ha^{-1}; Virginia Department of Conservation and Recreation, 2005) in two of the three years of the study (2009, 2011) (Figure 6.2c). The lowest yields occurred during the droughty 2010 season, in which the 1.5x fertilizer N rate attained a yield of 5.14 Mg ha^{-1}.

LS biosolids resulted in higher yields than AD biosolids due to greater PAN, as supported by PSNT, corn ear leaf N test, and CSNT. The higher yields with LS biosolids may have been due to the maintenance of higher PAN concentrations later in the season from the potentially mineralizable resource or from some other benefits provided by the organic materials (Zhang *et al.*, 2012). Both biosolids types produced higher yields than 1.5x fertilizer N rate in 2009-2011, justifying biosolids as a valuable inorganic fertilizer replacement.

Biosolids appeared to increase corn grain yield compared to inorganic fertilizer following repeated applications (2011), possibly due to a multiplicative effect of biosolids biostimulants and nutrients on crop drought stress tolerance (Zhang *et al.*, 2012) and/or the increase in soil PAW and soil C accumulation. Such yield-increasing effects under drought have been previously reported (Zebarth *et al.*, 1999; Zhang *et al.*, 2012).

6.5. Conclusions

We determined that surface-applied and unincorporated biosolids should be treated the same as biosolids incorporated into the soil by tillage for the purposes of estimating plant available N using the mineralization and volatilization factors employed in the mid-Atlantic U.S. Biosolids PAN was not affected by tillage practices management on this very coarse-textured soil containing little organic matter. Both lime-stabilized biosolids and anaerobically digested biosolids were equally capable of providing the PAN required for optimal corn grain yield. Furthermore, we found additional evidence that biosolids use provides a yield advantage over inorganic fertilizers under repeated application. The causes of this effect may be a result of improved soil physical properties (e.g., plant available water) or some plant biostimulant effect.

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6.7. References

Adamsen, F. J. and Sabey, B. R. 1987. Ammonia volatilization from liquid digested sewage sludge as affected by placement in soil. Soil Sci. Soc. Am. J. 51: 1080-1082.

Al-Kaisi, M. and Kwaw-Mensah, D. 2007. Effect of tillage and nitrogen rate on corn yield and nitrogen and phosphorus uptake in a corn-soybean rotation. Agron. J. 99: 1548-1558.

Al-Kaisi, M. M., Yin, X. and Licht, M. A. 2005. Soil carbon and nitrogen changes as influenced by tillage and cropping systems in some Iowa soils. Agric. Ecosyst. Environ. 105: 635-647.

Balkcom, K. S., Blackmer, A. M., Hansen, D. J., Morris, T. F. and Mallarino, A. P. 2000. Testing soils and cornstalks to evaluate nitrogen management on the watershed scale. J. Environ. Qual. 32: 1015-1024.

Binford, G. D., A.M. Blackmer, and N.M. El-Hout. 1990. 1990. Tissue test for excess nitrogen during corn production. Agron. J. 82: 124-129.

Bingham, D., Schoen, E. and Sitter, R. 2004. Designing fractional factorial split-plot experiments with few whole-plot factors. J. Roy. Stat. Soc. C-App. 53: 325-339.

Blackmer, A., Morris, T. and Binford, G. 1992. Predicting N fertilizer needs for corn in humid regions: Advances in Iowa. Predicting N fertilizer needs for corn in humid regions. Bull. Y-226. Tennessee Valley Authority, Muscle Shoals, AL: 57-72.

Bremner, J., Sparks, D., Page, A., Helmke, P., Loeppert, R., Soltanpour, P., Tabatabai, M., Johnston, C. and Sumner, M. 1996. Nitrogen-total. Methods of Soil Analysis. Part 3-Chemical Methods: 1085-1121.

Bruggeman, A. C. and Mostaghimi, S. 1993. Sludge application effects on runoff, infiltration, and water quality. J. Am. Water Resour. As. 29: 15-26.

Cartron, J. M. and Weil, R. R. 1998. Seasonal trends in soil nitrogen from injected or surface-incorporated sewage sludge applied to corn. Comm. Soil Sci. Plant Anal. 29: 121-139.

Cassel, D., Nielsen, D. and Klute, A. 1986. Field capacity and available water capacity. Methods of soil analysis. Part I. Physical and Mineralogical Methods: 901-926.

Castillo, M. S., Sollenberger, L. E., Vendramini, J. M. B., Woodard, K. R., O'Connor, G. A., Silveira, M. L. and Sartain, J. B. 2011. Incorporation of municipal biosolids affects organic nitrogen mineralization and elephantgrass biomass production. Agron. J. 103: 899-905.

Correa, R., White, R. and Weatherley, A. 2006. Risk of nitrate leaching from two soils amended with biosolids. Water Resources 33: 453-462.

Cripps, R. W., Winfree, S. K. and Reagan, J. L. 1992. Effects of sewage sludge application method on corn production. Comm. Soil Sci. Plant Anal. 23: 1705-1715.

Dai, X., Vietor, D. M., Hons, F. M., Provin, T. L., White, R. H., Boutton, T. W. and Munster, C. L. 2009. Effect of composted biosolids on soil organic carbon storage during establishment of transplanted sod. HortScience 44: 503-507.

Donohue, S. J. and Friedericks, J. B. 1984. Laboratory Procedures. VPI&SU, Blacksburg, VA., Virginia Coop. Ext. Serv. Publ. No. 452-881.

Donohue, S. J. and Heckendorn, S. E. 1994. Soil test recommendations for Virginia. Blacksburg, VA., Virginia Cooperative Extension Service Publication. 834.

Epstein, E. 1975. Effect of sewage sludge on some soil physical properties. J. Environ. Qual. 4: 139-142.

Epstein, E. 2003. Land application of sewage sludge and biosolids, CRC.

Epstein, E., Keane, D. B., Meisinger, J. J. and Legg, J. O. 1978. Mineralization of nitrogen from sewage sludge and sludge compost. J. Environ. Qual. 7: 217-221.

Evanylo, G. K. 1991. No-till corn response to nitrogen rate and timing in the middle Atlantic coastal plain. Journal of Production Agriculture 4: 180-185.

Evanylo, G. K. 2003. Effects of biosolids application timing and soil texture on nitrogen availability for corn. Comm. Soil Sci. Plant Anal. 34: 125-143.

Evanylo, G. K. 2006. Land application of biosolids. The Mid-Atlantic Nutrient Management Handbook: 226-162.

Evanylo, G. K. and Alley, M. M. 1997. Presidedress soil nitrogen test for corn in Virginia. Comm. Soil Sci. Plant Anal. 28: 1285-1301.

Faulkner, D. 2001. Applying biosolids: Issues for Virginia agriculture, Virginia Cooperative Extension.

Fernandes, S. A. P., Bettiol, W., Cerri, C. C. and Camargo, P. 2005. Sewage sludge effects on gas fluxes at the soil–atmosphere interface, on soil δ13C and on total soil carbon and nitrogen. Geoderma 125: 49-57.

Fontaine, S., Bardoux, G., Abbadie, L. and Mariotti, A. 2004. Carbon input to soil may decrease soil carbon content. Ecology Letters 7: 314-320.

Fox, R. H., Roth, G. W., Iversen, K. V. and Piekielek, W. P. 1989. Soil and Tissue Nitrate Tests Compared for Predicting Soil Nitrogen Availability to Corn. Agron. J. 81: 971-974.

Gallaher, R. N. and Ferrer, M. B. 1987. Effect of no-tillage vs. conventional tillage on soil organic matter and nitrogen contents. Comm. Soil Sci. Plant Anal. 18: 1061-1076.

Garland, J. L., Mackowiak, C. L. and Zabaloy, M. C. 2010. Organic waste amendment effects on soil microbial activity in a corn–rye rotation: Application of a new approach to community-level physiological profiling. Appl. Soil Ecol. 44: 262-269.

Gilmour, J. T. and Clark, M. D. 1988. Nitrogen release from wastewater sludge: A site specific approach. Water Poll. Control Fed. J. 60: 494-498.

Gilmour, J. T., Cogger, C. G., Jacobs, L. W., Evanylo, G. K. and Sullivan, D. M. 2003. Decomposition and plant-available nitrogen in biosolids. J. Environ. Qual. 32: 1498-1507.

Giordano, P. M., Mortvedt, J. J. and Mays, D. A. 1975. Effect of municipal wastes on crop yields and uptake of heavy metals. J. Environ. Qual. 4: 394-399.

Gupta, S. C., Dowdy, R. H. and Larson, W. E. 1976. Hydraulic and thermal properties of a sandy soil as influenced by incorporation of sewage sludge. Soil Sci. Soc. Am. J. 41: 601-605.

Henry, C., Dan, S., Rynk, R., Dorsey, K. and Cogger, C. 1999. Managing nitrogen from biosolids, Washington State Department of Ecology.

Hooker, B. A. and Morris, T. F. 1999. End-of-season corn stalk test for excess nitrogen in silage corn. Journal of production agriculture. 12: 282-288.

Hudson, B. D. 1994. Soil organic matter and available water capacity. J. Soil Water Conserv. 49: 189-194.

Iversen, K. V., Fox, R. H. and Piekielek, W. P. 1985. The Relationships of Nitrate Concentrations in Young Corn Stalks to Soil Nitrogen Availability and Grain Yields1. Agron. J. 77: 927-932.

Keeney, D. R. and Nelson, D. W. 1982. Nitrogen-inorganic forms. Methods of Soil Analysis, Part 2. Chemical and microbial properties. A. L. Page, R. H. Miller and D. R. Keeney, Agronom: 643-694.

Kelling, K. A., Walsh, L. M., Keeney, D. R., Ryan, J. A. and Peterson, A. E. 1978. A field study of the agricultural use of sewage sludge: II. Effect on soil N and P. J. Environ. Qual. 6: 345-352.

Khalilian, A., Sullivan, M. J., Mueller, J. D., Wolack, F. J., Williamson, R. E. and Lippert, R. M. 1998. Composted municipal solid waste applications impacts on cotton yield and soil properties. Composting in The Southeast. 193-202.

King, L. D. 1973. Mineralization and gaseous loss of nitrogen in soil-applied liquid sewage sludge. J. Environ. Qual. 2: 356-358.

Licht, M. A. and Al-Kaisi, M. 2005. Corn response, nitrogen uptake, and water use in strip-tillage compared with no-tillage and chisel plow. Agron. J. 97: 705-710.

Magdoff, F. R. and Amadon, J. F. 1980. Nitrogen availability from sewage sludge. J. Environ. Qual. 9: 451-455.

Magdoff, F. R., Ross, D. and Amadon, J. 1984. A soil test for nitrogen availability to corn. Soil Sci. Soc. Am. J. 48: 1301-1304.

McWilliams, D. A., Berglund, D. R. and Endres, G. J. 1999. Corn growth and management quick guide. Retrieved June, 1999, from http://www.ag.ndsu.edu/pubs/plantsci/rowcrops/a1173/a1173w.htm.

Meisinger, J. J., Bandel, V. A., Angle, J. S., O'Keefe, B. E. and Reynolds, C. M. 1992. Presidedress soil Nitrate test evaluation in Maryland. Soil Sci. Soc. Am. J. 56: 1527-1532.

Menelik, G., Reneau, R. B., Martens, D. C., Simpson, T. W. and Hawkins, G. W. 1990. Effects of tillage and nitrogen fertilization on nitrogen losses from soils used for corn production. Blacksburg, Virginia, Virginia Water Resources Research Center.

Nave, L. E., Vance, E. D., Swanston, C. W. and Curtis, P. S. 2009. Impacts of elevated N inputs on north temperate forest soil C storage, C/N, and net N-mineralization. Geoderma 153: 231-240.

Novak, J. M. and Watts, D. W. 2004. Increasing the phosphorus sorption capacity of southeastern coastal plain soils using water treatment residuals. Soil Sci. 169: 206-214.

O'Riordan, E., Dodd, V., Tunney, H. and Fleming, G. 1987. Fertiliser value of sewage sludge: 2. Phosphorus. Irish J. Agr. Res. 26: 53-61.

Pennsylvania Cooperative Extension 1999. Pre-sidedress soil nitrate test for corn. Agronomy Facts 17, Penn State University Cooperative Extension.

Petersen, S. O., Petersen, J. and Rubæk, G. H. 2003. Dynamics and plant uptake of nitrogen and phosphorus in soil amended with sewage sludge. Applied Soil Ecology 24: 187-195.

Powlson, D. S., Bhogal, A., Chambers, B. J., Coleman, K., Macdonald, A. J., Goulding, K. W. T. and Whitmore, A. P. 2012. The potential to increase soil carbon stocks through reduced tillage or organic material additions in England and Wales: A case study. Agric. Ecosyst. Environ. 146: 23-33.

Quemada, M., Lasa, B., Lamsfus, C. and Aparicio-Tejo, P. M. 1998. Ammonia volatilization from surface or incorporated biosolids by the addition of dicyandiamide. J. Environ. Qual. 27: 980.

Reicosky, D. C., Cassel, D. K., Blevins, R. L., Gill, W. R. and Naderman, G. C. 1977. Conservation tillage in the Southeast. J. Soil Water Conserv. 32: 13-39.

Rogers, J. S. and Carter, C. E. 1987. Soil core sampling for hydraulic conductivity and bulk density. Soil Sci. Soc. Am. J. 51: 1393-1394.

Roygard, J. K. F., Alley, M. M. and Khosla, R. 2002. No-Till corn yields and water balance in the Mid-Atlantic coastal plain. Agron. J. 94: 612-623.

SAS Institute 2008. SAS/STAT 9.2 User's Guide. Cary, NC, SAS Institute, Inc.

Schmidt, J. P., Sripada, R. P., Beegle, D. B., Rotz, C. A. and Hong, N. 2011. Within-field variability in optimum nitrogen rate for corn linked to soil moisture availability. Soil Sci. Soc. Am. J. 75: 306-316.

Sierra, J., Fontaine, S. and Desfontaines, L. 2001. Factors controlling N mineralization, nitrification, and nitrogen losses in an Oxisol amended with sewage sludge. Soil Research 39: 519-534.

Simpson, T. W., Donohue, S. J., Hawkins, G. W., Monnett, M. M. and Baker, J. C. 1993. The development and implementation of the Virginia agronomic land use evaluation system (VALUES). Blacksburg, VA., Department of Crop and Soil Environmental Sciences, Virginia Tech.

Sims, J. T., Vasilas, B. L., Gartley, K. L., Milliken, B. and Green, V. 1995. Evaluation of soil and plant nitrogen tests for maize on manured soils of the Atlantic Coastal Plain. Agron. J. 87: 213-222.

Smith, S. R., Woods, V. and Evans, T. D. 1998. Nitrate dynamics in biosolids-treated soils. I. Influence of biosolids type and soil type. Bioresource Technol. 66: 139-149.

Sommers, L., Parker, C. and Meyers, G. 1981. Volatilization, plant uptake and mineralization of nitrogen in soils treated with sewage sludge, Indiana Water Resources Research Center. 133.

Spargo, J., Alley, M., Follett, R. and Wallace, J. 2008a. Soil nitrogen conservation with continuous no-till management. Nutr. Cycl. Agroecosys. 82: 283-297.

Spargo, J. T., Alley, M. M., Follett, R. F. and Wallace, J. V. 2008b. Soil carbon sequestration with continuous no-till management of grain cropping systems in the Virginia coastal plain. Soil Till. Res. 100: 133-140.

Standard Methods for the Examination of Water and Wastewater 1992. SM-4500. Washington , DC, American Public Health Association.

Stewart, C. E., Follett, R. F., Wallace, J. and Pruessner, E. G. 2012. Impact of biosolids and tillage on soil organic matter fractions: Implications of carbon saturation for conservation management in the Virginia Coastal Plain. Soil Sci. Soc. Am. J.: In progress.

Sukkariyah, B., Evanylo, G. and Zelazny, L. 2007. Distribution of copper, zinc, and phosphorus in Coastal Plain soils receiving repeated liquid biosolids applications. J. Environ. Qual. 36: 1618-1626.

Tian, G., Granato, T. C., Cox, A. E., Pietz, R. I., Carlson, C. R. and Abedin, Z. 2009. Soil Carbon Sequestration Resulting from Long-Term Application of Biosolids for Land Reclamation. J. Environ. Qual. 38: 61-74.

U.S. Environmental Protection Agency 1986. EPA/SW-846. Test Methods for Evaluating Solid Waste. Washington, DC, U.S. Environmental Protection Agency.

U.S. Environmental Protection Agency 1995. Process design for agricultural utilization. In Process design manual—Land application of sewage sludge and domestic septage. EPA/625/R-95/001. Washington, DC., U.S. Environmental Protection Agency: 6:1-6:48.

Virginia Cooperative Extension 1992. Pest management guide for field crops. Virginia Tech: Blacksburg, VA, VA Coop. Ext. Publ. No. 456-016: 1-276.

Virginia Department of Conservation and Recreation 2005. Virginia Nutrient Management Standards and Criteria. Virginia Department of Conservation and Recreation. Richmond, VA.

Wagger, M. G. and Cassel, D. K. 1993. Corn yield and water-use efficiency as affected by tillage and irrigation. Soil Sci. Soc. Am. J. 57: 229-234.

Warman, P. R. and Termeer, W. C. 2005. Evaluation of sewage sludge, septic waste and sludge compost applications to corn and forage: yields and N, P and K content of crops and soils. Bioresource Technol. 96: 955-961.

Zebarth, B. J., Neilsen, G. H., Hogue, E. and Neilsen, D. 1999. Influence of organic waste amendments on selected soil physical and chemical properties. Canadian Journal of Soil Science 79: 501-504.

Zhang, X., Ervin, E. H., Evanylo, G. K. and Li, J. 2012. Corn and soybean physiological responses to biosolids under conventional tillage and no-till cropping systems. I. grain yield, photochemical efficiency and hormone metabolism. In progress.

Figure Captions

Figure 6.1a. Effect of fertilizer N rate and estimated biosolids plant available N on spring soil pre-sidedress nitrate-N test under conventional tillage and no-tillage systems during 2009-2011. LS=lime stabilized biosolids, AD=anaerobically digested biosolids.

Figure 6.1b. Effect of fertilizer N rate and estimated biosolids plant available N on fall soil total N concentration under conventional tillage and no-tillage systems during 2009-2011. LS=lime stabilized biosolids, AD=anaerobically digested biosolids.

Figure 6.2a. Effect of fertilizer N rate and estimated biosolids plant available N on corn ear leaf N concentration under conventional tillage and no-tillage systems during 2009-2011. LS=lime stabilized biosolids, AD=anaerobically digested biosolids.

Figure 6.2b. Effect of fertilizer N rate and estimated biosolids plant available N on end-of season corn stalk nitrate-N test under conventional tillage and no-tillage systems during 2009-2011. LS=lime stabilized biosolids, AD=anaerobically digested biosolids.

Figure 6.2c. Effect of fertilizer N rate and estimated biosolids plant available N on corn grain yield (on a basis of 15.5% of moisture) under conventional tillage and no-tillage systems during 2009-2011. LS=Lime stabilized biosolids, AD=Anaerobically digested biosolids.

Table 6.1. Chemical properties of soils at study site prior to treatment application in October, 2008.

Depth (cm)	pH	Mehlich 1 P	K	Ca	Mg	Zn	Mn	C
		---------------------------------mg kg^{-1}---------------------------						g kg^{-1}
0-15	6.67	14	73	442	45	1	5.6	34
15-30	6.55	9	57	399	43	0.9	3.8	28

Table 6.2. Monthly temperature and precipitation means site during 2009-2011 and the 30-yr average for the study †.

	---------Temperature (°C)---------				------------------Precipitation (mm)-------------			
Month	30-yr mean	2009	2010	2011	30-yr mean	2009	2010	2011
Jan	2.8	2.8	1.9	1.4	108	48.3	113	39.1
Feb	4.5	4.8	1.6	5.7	84.8	15.5	72.6	33.5
Mar	9.1	7.9	10.1	8.7	119	139	110	112
Apr	14.2	14.6	15.9	15.7	87.9	35.8	37.6	47.0
May	19.2	20.1	20.5	19.7	110	140	98.6	83.6
Jun	23.8	24.1	26.3	24.8	103	128	101	175
Jul	26.0	24.4	27.3	26.8	115	59.7	36.1	143
Aug	24.6	26.2	26.2	25.3	113	134	89.2	234
Sep	21.1	20.4	23.3	22.2	112	143	192	226
Oct	14.3	14.0	15.6	14.6	88.9	46.7	91.9	70.6
Nov	9.4	11.1	8.9	11.2	79.2	222	25.9	154
Dec	4.6	3.8	0.2	7.3	74.2	223	84.6	47.0
Annual	14.5	14.4	14.8	15.3	1195	1334	1053	1365

†The data were obtained from the U.S. Department of Commerce National Climatic Data Center Stony Creek 2 N weather station, Stony Creek, VA (36°58'N / 77°24'W and 32.0m (105') above s/l). http://www.ncdc.noaa.gov/oa/ncdc.html

Table 6.3. Attributes of Blue Plains lime-stabilized and Alexandria anaerobically digested biosolids applied at study sites in spring 2009-2011.

Attribute	Lime-stabilized biosolids			Anaerobically digested biosolids		
	2009	2010	2011	2009	2010	2011
Solids (g kg^{-1})	421	322	335	282	263	271
Volatile solids (g kg^{-1})	546	629	609	627	635	642
Total Kjeldahl N (g kg^{-1})	30.4	42.2	40.1	60.2	76.5	56.8
NH_4-N (g kg^{-1})	1.1	2.6	2.3	17.2	21.4	14.7
Organic N (g kg^{-1})	29.3	39.6	37.7	42.8	55.1	42.1
Phosphorus (g kg^{-1})	10.2	10.9	13.6	37.5	33.5	35.9
Potassium (g kg^{-1})	1.8	2.0	2.3	1.5	1.7	1.7
Sulfur (g kg^{-1})	5.9	4.5	4.6	10.4	9.4	9.7
Calcium (g kg^{-1})	127	101	124	25.3	20.4	22.1
Magnesium (g kg^{-1})	3.0	2.5	2.4	3.5	3.3	3.1
pH	12.4	12.2	12.3	8.2	8.3	8.8
Calcium Carbonate Equivalent (CCE) (g kg^{-1})	238	166	108	--	--	--

Table 6.4. Target and actual nitrogen (estimated) from lime-stabilized and anaerobically digested biosolids applied at study sites in spring 2009-2011.

Rate	Tillage	Lime-stabilized biosolids			Anaerobically digested biosolids		
		2009	2010	2011	2009	2010	2011
Target PAN† (kg ha^{-1})	CT‡	106	157	135	106	157	135
	NT§	106	157	135	106	157	135
Actual biosolids (dry Mg ha^{-1})	CT	10.2	11.6	8.0	4.8	5.3	4.4
	NT	10.9	12.8	8.7	6.0	6.9	5.7
Actual organic N (kg ha^{-1})	CT	90	137	90	62	89	56
	NT	95	152	98	71	114	73
Actual NH_4^+-N (kg ha^{-1})	CT	8	23	14	77	97	56
	NT	3	8	5	51	73	42
Actual PAN (kg ha^{-1})	CT	98	160	104	133	186	112
	NT	98	160	103	128	187	115

†PAN=plant available nitrogen; ‡CT=conventional tillage; §NT=no-tillage.

Table 6.5. Mean concentrations and p-values of fall soil total N in 2009, corn ear leaf N in 2011, and grain yield in 2011 under various tillage practices.

	Tillage		p-value
	Conventional tillage	No-tillage	
Fall Soil N, 2009 (mg kg^{-1})	355	388	0.039
ELN†, 2011 (g kg^{-1})	23.2	21.2	0.002
Grain yield, 2011 (Mg ha^{-1})	9.3	10.9	0.011

†ELN = Corn Ear Leaf N.

Table 6.6. Regression equation†, R^2 and p-value for soil or crop N response as function of N applied rate.

Dependent variable	Year	Regression Equation	R^2	p-value
PSNT‡ (mg kg^{-1})	2009	$y = 0.077x + 8.2893$	0.9864	<.0001
	2010	$y = 0.3423x + 6.6251$	0.9411	<.0001
	2011	$y = 0.2577x + 11.29$	0.9636	<.0001
Fall soil N (mg kg^{-1})	2009	$y = 0.0002x + 0.3339$	0.9141	0.0001
	2010	$y = -7E-05x + 0.4238$	0.0389	0.0372
	2011	$y = 0.0046x^2 - 0.5952x + 422.07$	0.9092	0.004
ELN§ (g kg^{-1})	2009	$y = -0.0001x^2 + 0.0675x + 16.208$	0.9406	<.0001
	2010	$y = -0.0003x^2 + 0.129x + 13.483$	0.9935	<.0001
	2011	$y = -0.0002x^2 + 0.1052x + 13.757$	0.9986	<.0001
CSNT# (mg kg^{-1})	2009	$y = 0.0137x^2 - 1.1719x + 334.01$	0.9608	0.0157
	2010	$y = 0.0433x^2 - 3.8906x + 440.08$	0.9963	<.0001
	2011	$y = 0.0091x^2 - 0.4822x + 414.45$	0.9922	0.043
Grain yield (Mg ha^{-1})	2009	$y = -0.0002x^2 + 0.053x + 10.29$	0.9805	<.0001
	2010	$y = -9E-05x^2 + 0.0306x + 2.7929$	0.9458	<.0001
	2011	$y = -0.0002x^2 + 0.071x + 5.1754$	0.9189	<.0001

†Dependent variable (y) = crop or soil response; Independent variable (x)=N applied rate.
‡PSNT = Pre-sidedress Nitrate Test; §ELN = Corn Ear Leaf N; #CSNT = Corn Stalk Nitrate Test.

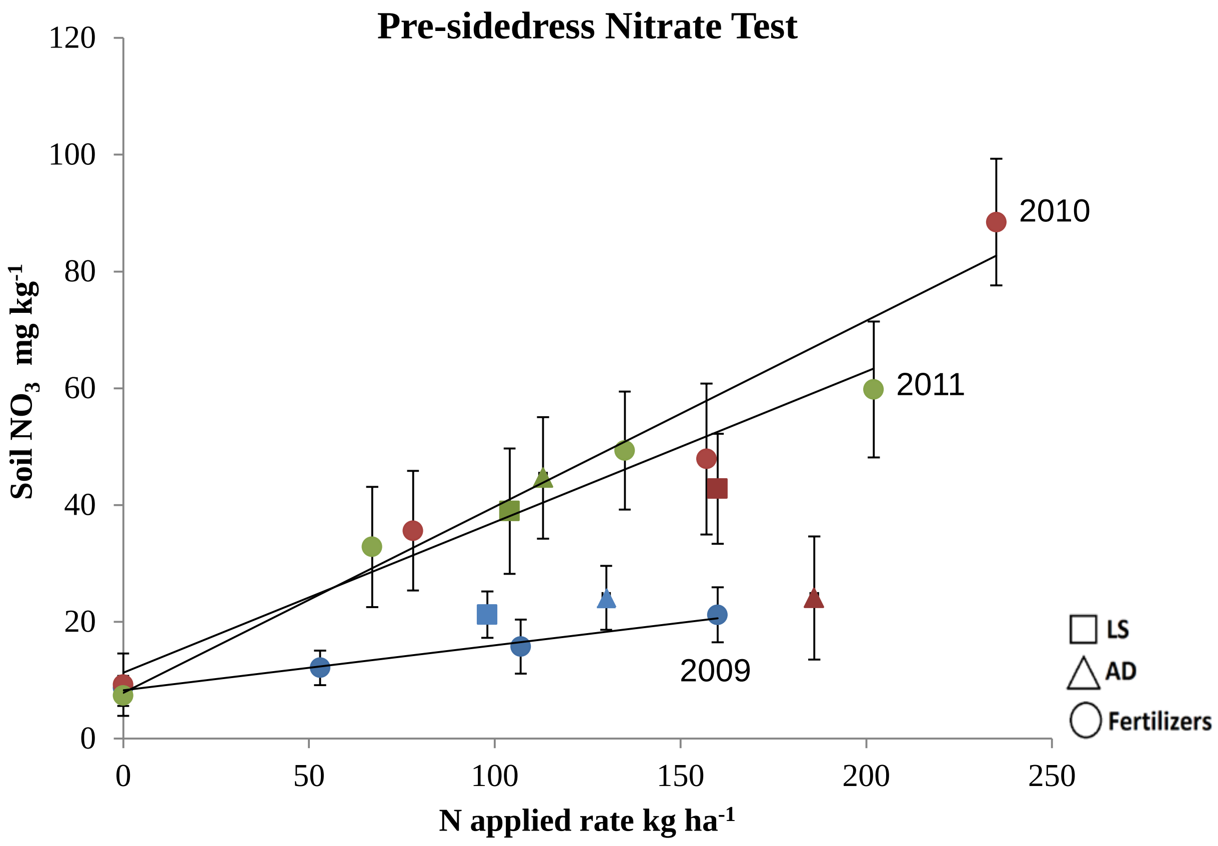
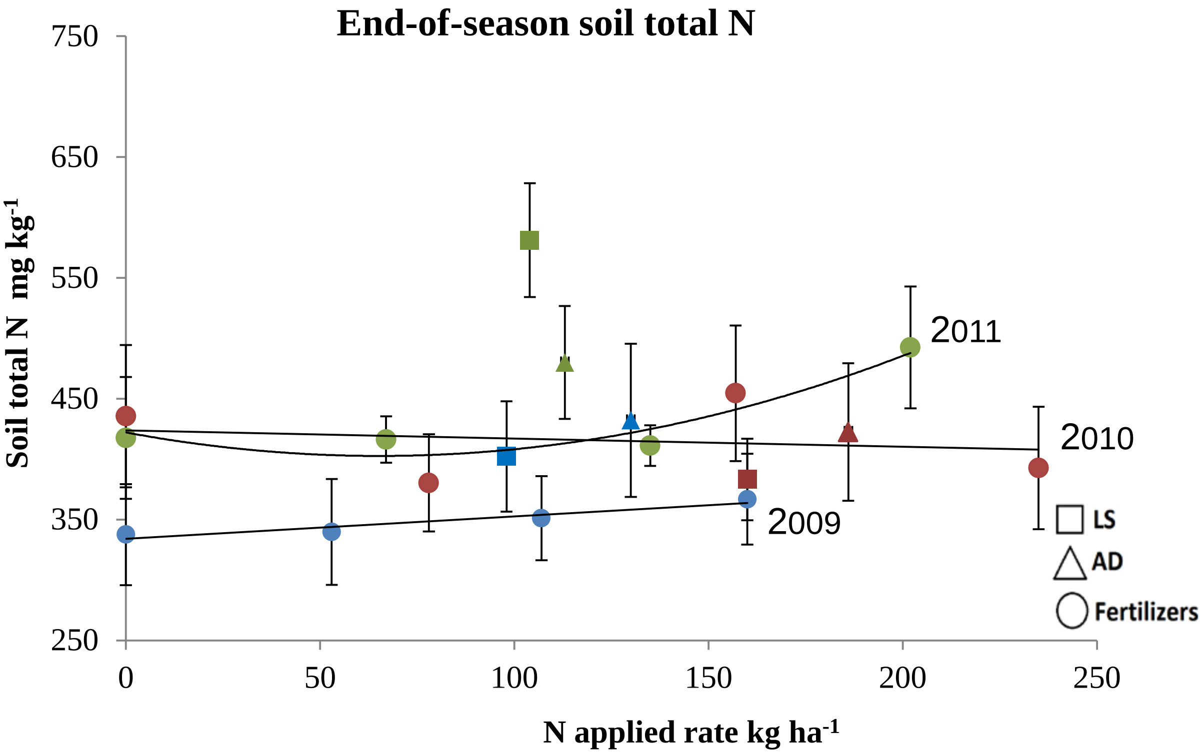

Pre-sidedress Nitrate Test
Soil NO3 mg kg-1
N applied rate kg ha-1
0
20
40
60
80
100
120
0
50
100
150
200
250
2010
2011
2009
LS
AD
Fertilizers

Figure 6.1a



End-of-season soil total N
Soil total N mg kg-1
N applied rate kg ha-1
750
650
550
450
350
250
0
50
100
150
200
250
2011
2010
2009
LS
AD
Fertilizers

Figure 6.1b

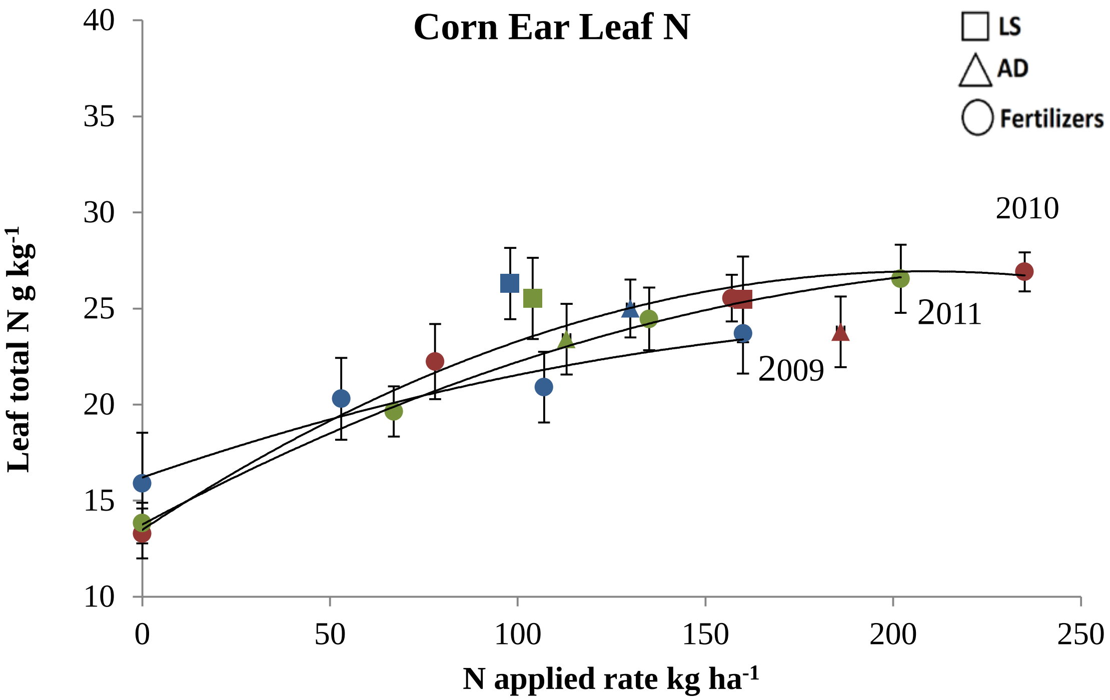

Corn Ear Leaf N
LS
AD
Fertilizers
2010
2011
2009
Leaf total N g kg^{-1}
N applied rate kg ha^{-1}
40
35
30
25
20
15
10
0
50
100
150
200
250

Figure 6.2a

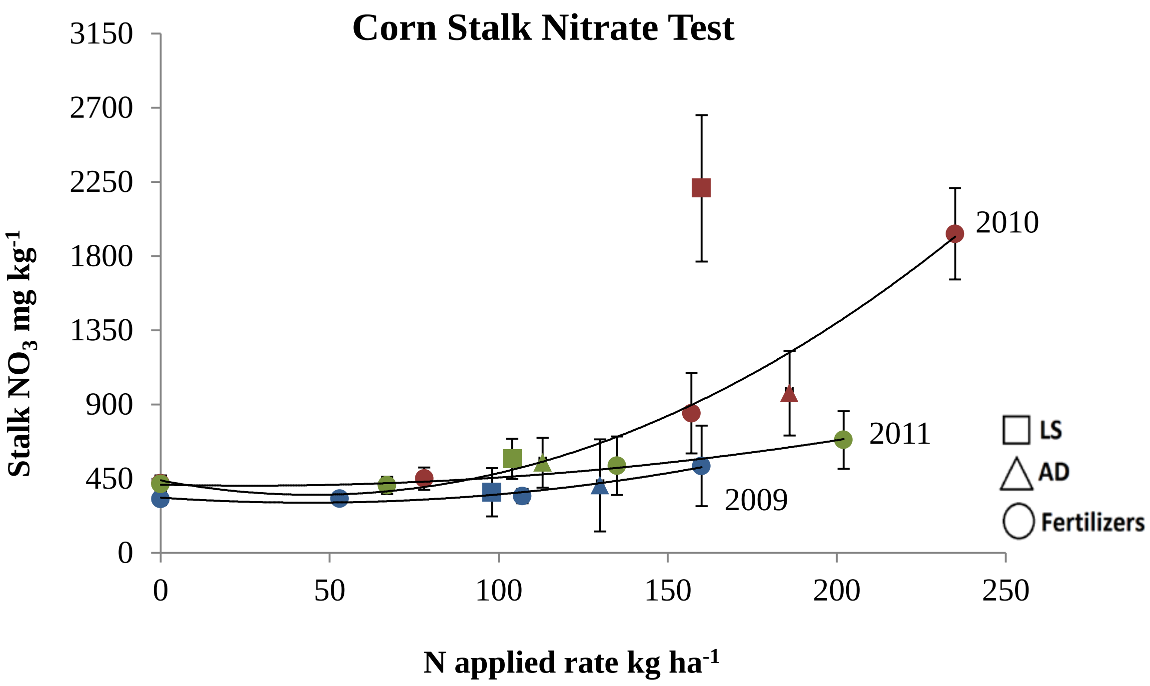

Corn Stalk Nitrate Test
3150
2700
2250
1800
1350
900
450
0
Stalk NO$_3$ mg kg^{-1}
0
50
100
150
200
250
N applied rate kg ha^{-1}
2010
2011
2009
LS
AD
Fertilizers

Figure 6.2b

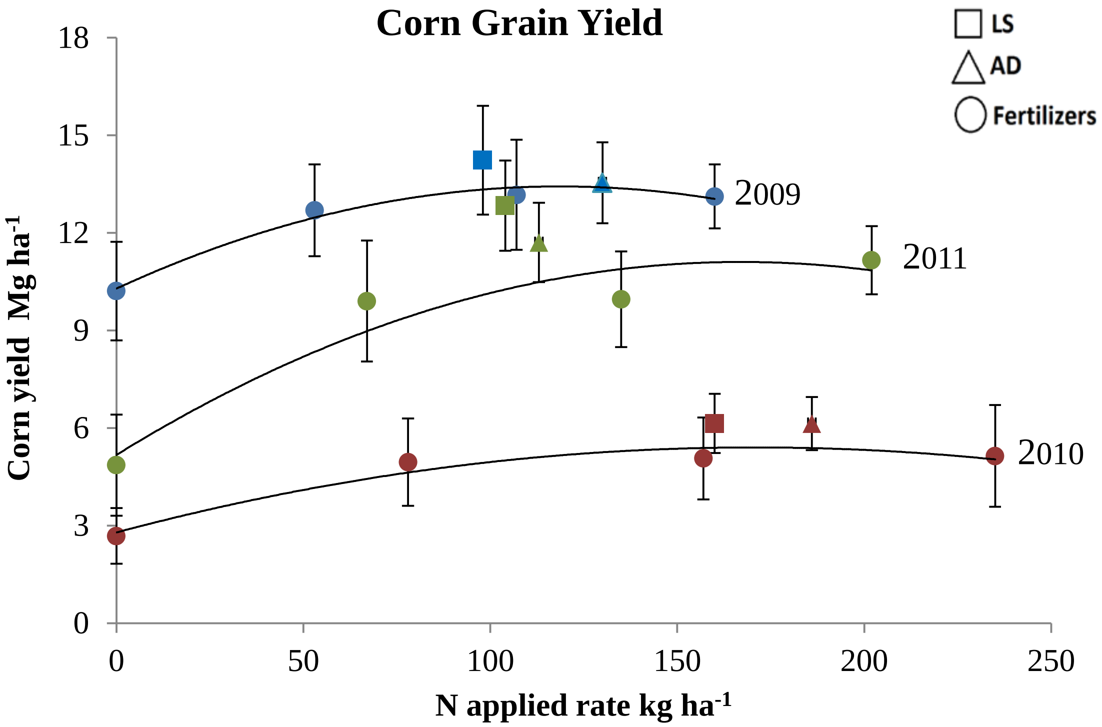

Corn Grain Yield
LS
AD
Fertilizers
18
15
12
9
6
3
0
0
50
100
150
200
250
Corn yield Mg ha-1
N applied rate kg ha-1
2009
2011
2010

Figure 6.2c

7. Conclusions

Biosolids are created through the treatment of sewage sludge in a municipal wastewater treatment facility. The benefits of recycling biosolids onto agricultural land include providing essential nutrients for crop needs and organic matter for improving soil tilth, water-holding capacity, soil aeration, and an energy source for soil biota and beneficial microorganisms. Land application of biosolids has been demonstrate to improve nutrient availability (mainly N) and improve organic matter in the soils, but the effects of biosolids on C sequestration and N cycling in the Mid-Atlantic region are still not well known. This dissertation focused on effects of biosolids on long-term C sequestration and short-term N availability in a Mid-Atlantic agroecosystem. To accomplish this, a series of experiments were designed to quantify the C concentration in soils with a history of biosolids and other organic residuals, i.e., compost and manure, to qualify the C chemistry in the soils using spectroscopic methods, and finally to evaluate the two types of biosolids plant available N under various tillage practices in a crop rotation commonly found in the coastal plain in Virginia.

To evaluate the quantity and quality of soil C from application of biosolids, in 2011 we investigated three experimental sites that had been amended with biosolids and composts. The three study sites were located on a Fauquier silty clay loam (*Ultic Hapludalfs*), a Davidson clay loam (*Rhodic Paleudults*) and a Pamunkey sandy loam (*Ultic Hapludalfs*). Soil cores were obtained at a depth of 0-60 cm. The biosolids and composts accumulated more C (Mg ha^{-1}) in the surface soil depth (< 15 cm), ranging from 2 to 12 % of applied C across all three sites over long periods of time. Soil C sequestration could be expected to occur not only in the Piedmont where slow deposition of biosolids was caused by cold or dry conditions, but also in the Coastal Plain where fast soil organic matter mineralization was caused by hot or wet conditions. However, the evidence for C saturation was found in the Coastal Plain soils with single high application of biosolids in 1996, additional soil C sequestration may not be achievable by increasing C inputs from biosolids to the soil. The changes in soil C appeared immediately after application and can continue decades after application of organic residuals, and their intensity for the most part remained after 27 years. Furthermore, although soil organic C from profiles in the field sites was not different at depths below the plow layer (>15 cm), future studies are still needed to investigate the C mobility or decomposition in the deeper soil profiles from application of biosolids, as subtle but persistent changes in soil C accumulation in the deep depth may continue long after a steady state had been approached.

We also conducted a series of spectroscopic experiments to characterize soil C in the surface soils from application of biosolids. We used particle size and density fractionations to concentrate soil organic C into particulate organic matter (POM) fractions successfully. Solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy obtained structural information about the POM-C functional groups from the biosolids-amended soils. We compared the two ^{13}C NMR techniques cross polarization/total sideband suppression (CP/TOSS) and direct polarization-magic angle spinning (DP/MAS) spectroscopies to reveal the proportions of POM-C functional groups. The quantitative NMR analyses allowed us to conclude that O-alkyl C functional groups were the dominant forms of organic C, followed by moderate amounts of aromatic C, alkyl C, COO/N-C=O, aromatic C-O, OCH_3 / NCH and ketones and aldehydes, particularly the aliphatic carbon and the aromatic components were enriched but the O-alkyl C was decreased in the biosolids-amended soils. The changes in chemical composition indicated that the biosolids-derived POM-C was more decomposed and thus more stable than the unamended control treatment. The addition of sawdust with biosolids into soils enhanced soil C decomposition. The obtained POM fraction provided information about the partly decomposed organic matter which is affected most by application of biosolids and other organic residuals. However, since long-term application of biosolids might influence the soil humification processes, future studies should focus on the humic acids and fulvic acids extracted from soils where biosolids have been applied. Also future work should evaluate clay and silt size fractions to investigate the organic matter-mineral association with the soil C.

The ^{13}C NMR technique suffers from some aforementioned limitations: paramagnetic minerals in the soils may mess up the spectrum and result in broadening bands and over laps; also the technique poorly detected and identified individual C components when soil C content was low. To solve these problems, the alternative C (1s) K-edege near edge X-ray absorption fine structure (NEXAFS) spectra was used to measure the C functional groups in the biosolids-amended soils. The NEXAFS spectra showed that O-alkyl C functional groups were the dominant forms of organic C, followed by moderate amounts of aromatic C, alkyl C, and carboxylic C groups. The phenolic C forms contributed only to a small portion of the total SOC in POM extracted from biosolids-amended soils. The results were similar to those obtained from NMR analysis, which emphasized that biosolids enriched soils with recalicitrant C. The changes in the inherent molecular structures of the soil C may exert considerable influence on biogeochemical cycling of C as part of complex organic biomolecules, and may significantly affect the sustainability of agriculture, as well as the potential for soils to sequester C in these agroecosystems within the Mid-Atlantic region. The regression and correlation analyses on the proportions of C functional groups between NEXAFS and NMR provided clear evidence that the NEXAFS technique used in the present investigation had good sensitivity for the different organic C functionalities and is an advanced tool for characterization of SOC which is not limited by interferences on NMR (i.e., iron and C content). Future studies can be conducted on a suite of complementary spectroscopic techniques, i.e., NEXAFS, NMR, FTIR, and Pyrolysis GC/MS, etc., in an integrated manner to accurately fingerprint the structural composition of soil organic C with application of biosolids.

To evaluate the short-term various types of biosolids C and N availability, we conducted a three-year field study with two kinds of tillage systems under a corn-soybean rotation in the Coastal Pain soils during 2009-2011. Our studies showed that both surface-applied and incorporated biosolids were equally capable of providing plant available N required for corn grain yield. However, no interactive effects of biosolids and tillage practices were detected. We also found additional evidence that biosolids use provides a yield advantage over inorganic fertilizers under repeated application. The causes of this effect may be a result of improved soil physical properties (e.g., plant available water) or some plant biostimulant effect. These results could provide necessary and additional background information for biosolids N effects studies in coarse-textured sandy soils in Mid-Atlantic region. Our studies only focused on short-term N cycling; future work should evaluate the long-term effects of continuous biosolids applications on soil N availability and crop responses. Future research will add to the knowledge base to refine the conditions and rates under which the application of biosolids provides economic and/or environmental benefits.

Appendix

Appendix A. Sampling depth, bulk density (BD), and concentration of total soil C and N, and soil C stocks of Site I in Chapter 3.

Rep	Treat†	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock
		g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}
		0-7.5 cm				7.5-15 cm				15-30 cm				30-45 cm				45-60 cm			
1	1	0.81	1.87	2.29	13.97	0.95	1.08	1.26	8.98	1.03	1.14	1.24	19.18	0.96	0.62	0.59	8.57	0.87	0.60	0.55	7.17
1	1	0.94	1.59	1.79	12.67	0.99	1.29	1.28	9.52	1.01	0.98	1.17	17.69	0.91	0.67	0.56	7.63	0.83	0.75	0.43	5.37
1	1	0.77	1.98	2.23	12.79	1.08	1.17	1.21	9.74	1.07	1.49	1.14	18.31	0.84	0.68	0.59	7.43	0.80	0.58	0.52	6.19
1	2	0.77	3.65	4.21	24.25	0.95	1.99	2.27	16.13	1.05	1.00	0.98	15.45	0.92	0.46	0.42	5.72	0.84	0.58	0.38	4.79
1	2	0.72	3.47	3.90	21.12	0.89	1.71	1.78	11.96	1.00	1.01	1.06	16.02	0.95	0.53	0.48	6.87	0.92	0.54	0.40	5.44
1	2	0.75	3.86	4.66	26.20	1.02	1.48	1.57	11.97	1.03	1.00	1.07	16.51	0.95	0.57	0.51	7.31	0.84	0.57	0.31	3.95
1	3	0.71	3.07	3.63	19.41	0.94	1.56	1.64	11.59	1.12	0.96	1.17	19.58	0.89	0.71	0.60	8.03	0.91	5.84	0.46	6.22
1	3	0.67	3.26	4.11	20.73	0.89	1.42	1.67	11.12	1.05	2.27	2.44	38.55	0.94	0.61	0.51	7.28	0.92	0.64	0.45	6.19
1	3	0.68	3.51	3.84	19.55	1.02	1.69	1.80	13.78	0.98	1.32	1.18	17.36	1.01	0.86	0.48	7.28	0.90	0.49	0.46	6.16
1	4	0.89	1.09	1.27	8.46	0.88	1.84	2.16	14.16	1.02	0.59	0.54	8.31	1.08	0.94	0.88	14.27	0.87	0.47	0.55	7.09
1	4	0.85	0.62	0.53	3.36	1.06	1.16	1.23	9.79	1.05	1.09	1.14	18.00	0.93	0.94	0.75	10.49	0.88	0.48	0.34	4.53
1	4	0.88	1.98	1.96	12.88	1.01	1.23	1.31	9.91	1.01	1.40	1.53	23.07	0.93	0.65	0.60	8.40	0.87	0.46	0.50	6.58
2	1	0.79	1.94	2.38	14.12	0.91	1.44	1.73	11.88	1.06	1.01	1.20	19.14	0.94	0.53	0.49	6.91	1.04	0.33	0.22	3.39
2	1	0.78	1.67	1.88	10.98	0.93	1.26	1.26	8.82	1.07	1.10	1.19	19.05	1.02	0.51	0.49	7.50	0.86	0.28	0.27	3.50
2	1	0.66	2.15	2.50	12.31	1.03	1.58	1.34	10.38	1.08	2.00	1.15	18.53	0.97	0.47	0.46	6.65	1.00	0.32	0.25	3.82
2	2	0.63	3.09	3.96	18.79	0.93	1.81	2.00	14.00	1.05	1.05	1.26	19.90	1.03	1.01	0.92	14.31	1.03	0.59	0.40	6.21
2	2	0.65	3.37	4.03	19.60	0.98	1.64	1.87	13.77	1.10	1.15	1.21	19.87	0.99	0.71	0.69	10.24	1.05	0.56	0.45	7.12
2	2	0.63	4.05	4.66	21.93	0.93	2.20	2.31	16.03	1.02	1.12	1.30	19.98	1.03	0.84	0.48	7.40	1.00	0.49	0.38	5.71
2	3	0.67	2.70	3.10	15.50	0.94	1.78	1.94	13.63	1.13	1.02	1.16	19.73	1.04	0.80	0.72	11.18	0.91	0.39	0.31	4.28
2	3	0.68	3.16	3.68	18.77	0.91	1.49	1.81	12.36	1.06	1.07	1.29	20.51	1.08	0.58	0.59	9.46	0.95	0.47	0.36	5.12
2	3	0.68	3.16	3.49	17.69	0.82	4.05	1.72	10.49	1.05	1.50	1.67	26.30	1.06	0.61	0.60	9.51	1.01	0.37	0.34	5.07
2	4	0.69	1.98	2.29	11.87	0.91	1.71	1.65	11.20	1.06	0.94	1.11	17.73	0.98	0.67	0.62	9.11	0.93	0.37	0.49	6.85

Appendix A continued

Rep	Treat†	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock
		g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}
		0-7.5 cm				7.5-15 cm				15-30 cm				30-45 cm				45-60 cm			
2	4	0.61	2.27	2.81	12.78	0.98	1.40	1.53	11.20	1.03	1.02	1.22	18.80	1.01	0.64	0.45	6.86	0.89	0.48	0.40	5.38
2	4	0.84	2.01	2.28	14.40	0.94	0.95	1.07	7.57	0.99	2.01	2.11	31.19	0.99	0.68	0.57	8.53	0.97	0.47	0.51	7.40
3	1	0.92	1.70	2.15	14.81	1.06	1.18	1.36	10.84	1.13	1.10	1.10	18.59	1.06	1.19	1.01	16.05	0.93	0.49	0.39	5.38
3	1	0.66	1.95	2.36	11.75	0.98	1.75	1.76	12.94	1.08	1.06	1.13	18.38	1.12	0.93	1.06	17.81	0.93	0.43	0.39	5.49
3	1	0.77	1.81	2.18	12.57	1.04	1.12	1.32	10.23	1.03	1.07	1.24	19.10	1.09	1.65	1.50	24.56	0.91	0.56	0.48	6.62
3	2	0.49	3.86	4.38	16.17	0.81	2.64	2.83	17.16	0.97	1.34	1.68	24.38	1.15	1.05	1.07	18.39	0.99	0.58	0.43	6.36
3	2	0.55	4.30	5.13	21.15	0.79	3.07	3.51	20.78	0.88	1.60	1.74	23.06	0.94	1.01	1.05	14.75	0.98	0.47	0.47	6.95
3	2	0.59	4.63	5.25	23.25	0.80	3.15	3.27	19.69	0.92	1.49	1.71	23.65	1.09	1.04	1.06	17.23	1.01	0.42	0.53	8.03
3	3	0.79	3.95	3.45	20.41	0.88	1.42	1.63	10.77	1.01	1.09	1.22	18.46	1.02	0.87	0.92	14.06	0.84	0.67	0.45	5.74
3	3	0.83	3.40	3.51	21.85	0.93	1.77	1.89	13.14	1.09	1.31	1.26	20.60	1.12	1.67	1.28	21.61	0.91	0.59	0.43	5.83
3	3	0.52	4.27	4.93	19.33	0.94	2.66	2.88	20.34	1.05	0.98	1.16	18.29	1.08	0.91	0.95	15.46	1.00	0.58	0.43	6.43
3	4	0.84	2.26	2.54	16.01	1.01	1.37	1.52	11.57	1.07	1.12	1.20	19.20	1.02	1.06	1.12	17.08	0.89	0.63	0.58	7.78
3	4	0.83	2.37	2.67	16.63	0.98	1.29	1.42	10.39	0.98	1.18	1.21	17.74	1.00	0.93	1.01	15.11	0.89	0.81	0.63	8.42
3	4	0.67	2.33	2.59	13.08	0.91	1.40	1.29	8.82	0.98	1.23	1.19	17.51	1.02	1.34	1.09	16.79	0.97	0.69	0.68	9.88
4	1	0.78	1.75	2.01	11.79	1.01	1.24	1.48	11.16	1.12	1.41	1.71	28.79	1.12	0.84	0.94	15.75	0.99	0.33	0.37	5.49
4	1	0.85	2.39	2.22	14.07	1.07	1.21	1.36	10.97	1.16	1.14	1.01	17.52	1.10	1.05	1.03	16.95	1.00	0.67	0.51	7.73
4	1	0.70	2.10	2.38	12.50	0.97	1.53	1.39	10.07	1.07	1.00	1.09	17.56	1.13	0.50	0.53	9.03	0.99	0.60	0.56	8.30
4	2	0.69	3.82	4.51	23.39	0.76	2.44	3.27	18.63	0.98	1.15	1.24	18.23	1.12	0.93	1.08	18.24	1.08	0.59	0.55	8.85
4	2	0.50	4.39	5.20	19.67	0.96	1.85	2.24	16.06	1.03	1.41	1.30	20.12	1.17	0.99	1.04	18.29	1.09	0.45	0.56	9.24
4	2	0.38	4.04	4.73	13.40	0.46	3.68	4.20	14.58	0.90	1.48	1.43	19.31	1.04	0.98	1.07	16.75	1.13	0.65	0.77	13.02
4	3	0.70	2.99	3.23	17.05	0.96	1.43	1.56	11.21	1.11	1.13	1.27	21.03	1.03	1.03	1.11	17.17	0.95	0.44	0.67	9.53
4	3	0.64	3.35	3.71	17.92	0.93	1.65	1.80	12.62	1.03	1.25	1.30	19.96	1.07	1.09	1.17	18.79	1.04	0.85	0.92	14.30
4	3	0.49	0.80	0.45	1.63	0.92	2.42	2.37	16.38	0.99	1.32	1.34	19.86	1.14	0.99	1.12	19.25	0.98	0.72	0.67	9.76
4	4	0.57	2.27	2.80	12.01	0.87	1.95	2.08	13.58	0.99	1.31	1.35	20.16	1.08	0.86	1.00	16.23	0.95	0.59	0.56	8.03

Appendix A continued

Rep	Treat†	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock
		g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}
		0-7.5 cm				7.5-15 cm				15-30 cm				30-45 cm				45-60 cm			
4	4	0.84	1.91	2.23	14.10	0.92	1.62	1.52	10.49	1.04	1.26	1.36	21.18	1.15	1.23	1.04	17.92	0.95	0.36	0.31	4.46
4	4	0.66	2.17	2.71	13.40	0.97	1.79	1.71	12.49	1.02	1.11	0.95	14.65	1.13	0.92	1.00	16.88	0.96	0.87	0.39	5.56

Treat†: 1-control; 2-poultry litter-yard waste compost; 3-biosolids-wood chip compost; 4-poultry litter.

Appendix B. Sampling depth, bulk density (BD), and concentration of total soil C and N, and soil C stocks of Site II in Chapter 3.

Rep	Treat†	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock
		g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}
		0-7.5 cm				7.5-15 cm				15-30 cm				30-45 cm				45-60 cm			
1	0	0.45	1.58	19.13	6.44	0.64	1.21	12.05	5.78	0.93	0.99	9.82	13.73	0.94	0.43	3.96	5.60	0.84	0.31	2.13	2.70
1	0	0.64	1.42	14.44	6.94	0.78	1.03	10.34	6.03	0.87	0.93	9.62	12.62	0.83	0.48	4.08	5.10	0.73	0.40	1.27	1.40
1	0	0.26	2.30	24.30	4.82	0.70	1.46	12.26	6.44	1.02	0.92	8.50	12.98	0.95	0.44	3.93	5.60	0.88	0.38	2.99	3.95
1	1	0.55	1.71	16.14	6.70	0.67	1.57	15.16	7.66	0.93	1.17	7.89	11.02	0.97	0.49	4.08	5.97	0.84	0.43	3.12	3.91
1	1	0.49	2.13	22.76	8.39	0.77	1.40	12.77	7.36	1.00	1.04	9.38	14.08	0.96	0.45	3.79	5.46	0.94	0.38	3.40	4.80
1	1	0.91	1.58	16.00	10.88	0.77	1.33	12.24	7.08	0.88	1.09	8.84	11.68	0.93	0.65	3.51	4.89	0.81	0.51	3.24	3.95
1	2	0.71	1.54	17.44	9.33	0.79	1.25	11.00	6.52	1.02	0.75	7.37	11.26	0.92	0.46	3.40	4.67	1.02	0.31	2.10	3.21
1	2	0.81	1.59	16.84	10.26	0.90	1.10	11.50	7.77	0.97	0.93	8.10	11.82	0.98	0.39	3.09	4.56	0.96	0.53	2.96	4.28
1	2	0.73	1.87	17.64	9.69	0.88	1.16	11.95	7.85	0.97	0.78	8.06	11.72	0.94	0.42	3.24	4.56	1.01	0.58	3.76	5.70
1	3	0.75	1.76	19.60	11.06	0.70	1.44	11.53	6.08	0.99	0.93	7.65	11.40	0.95	0.63	4.05	5.80	0.91	0.30	2.66	3.63
1	3	0.86	1.72	16.64	10.72	0.86	1.29	11.83	7.62	1.04	0.68	6.57	10.25	0.96	0.55	3.14	4.52	0.95	0.50	2.50	3.54
1	3	0.81	1.76	18.41	11.24	0.88	1.23	11.71	7.77	1.07	0.79	7.47	12.03	0.92	0.59	3.54	4.87	1.01	0.43	2.70	4.10
1	4	0.76	2.09	20.33	11.61	0.95	1.06	11.03	7.83	0.92	8.28	7.18	9.95	0.92	5.84	3.18	4.41	0.85	0.37	2.24	2.86
1	4	0.78	1.89	20.16	11.79	0.85	1.01	10.46	6.65	0.94	0.79	6.99	9.84	0.93	0.52	3.77	5.26	0.88	0.31	2.23	2.96
1	4	0.74	2.26	22.73	12.54	1.03	1.23	11.11	8.61	0.89	1.06	7.81	10.46	0.91	0.52	3.74	5.13	0.84	0.27	2.32	2.91
1	5	0.77	2.39	25.62	14.85	0.75	1.33	15.47	8.64	0.94	1.19	9.17	12.91	0.93	0.89	4.45	6.19	0.90	0.87	3.94	5.31
1	5	0.77	2.18	22.33	12.85	0.78	1.41	15.15	8.91	0.89	1.16	8.65	11.56	0.94	0.65	4.06	5.75	0.90	0.75	3.87	5.25
1	5	0.73	2.11	21.84	11.95	0.68	1.61	20.73	10.53	0.93	1.15	9.58	13.42	0.89	0.83	5.60	7.51	0.89	0.61	3.56	4.75
2	0	0.64	1.45	16.15	7.81	0.86	1.09	12.02	7.78	0.91	0.79	8.01	10.92	0.88	0.43	3.29	4.35	0.79	0.38	2.97	3.51
2	0	0.65	1.51	17.59	8.57	0.80	0.68	7.09	4.26	0.89	0.87	8.03	10.78	0.89	0.44	3.53	4.72	0.89	0.68	4.45	5.97
2	0	0.63	1.64	15.62	7.38	0.74	1.23	12.24	6.83	0.92	0.73	8.12	11.27	0.91	0.43	3.33	4.53	0.86	0.39	2.74	3.53
2	1	0.69	1.45	15.92	8.21	0.71	1.10	11.09	5.95	0.99	0.61	5.92	8.82	0.92	0.70	3.12	4.33	0.89	0.25	2.52	3.37
2	1	0.49	1.57	18.39	6.74	0.81	1.05	10.96	6.67	0.98	1.05	8.02	11.74	0.95	0.54	3.37	4.78	0.90	0.29	2.34	3.15
2	1	0.76	1.77	18.68	10.69	0.85	1.28	11.50	7.31	1.07	1.15	7.96	12.78	0.98	0.50	2.54	3.73	0.96	0.58	3.85	5.55

Appendix B continued

Rep	Treat†	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock
		g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}
		0-7.5 cm				7.5-15 cm				15-30 cm				30-45 cm				45-60 cm			
2	2	0.73	1.65	14.75	8.04	0.94	1.33	10.76	7.57	0.99	0.85	5.79	8.56	0.99	0.42	3.16	4.67	0.91	0.49	2.84	3.90
2	2	0.76	1.34	13.39	7.63	0.83	1.01	9.59	5.99	0.96	0.72	7.11	10.28	0.97	0.52	3.83	5.59	0.94	0.39	2.55	3.59
2	2	0.72	1.86	17.17	9.26	0.87	1.01	9.12	5.95	1.00	0.88	5.85	8.73	0.99	0.53	3.58	5.30	0.98	0.36	2.37	3.48
2	3	0.76	1.62	16.81	9.52	0.74	1.54	15.32	8.51	0.95	0.91	6.35	9.09	0.94	0.66	3.83	5.37	0.83	0.59	2.92	3.63
2	3	0.77	1.95	19.97	11.53	0.97	1.00	11.77	8.55	0.96	0.69	6.47	9.32	0.92	0.51	3.64	5.03	0.86	0.57	2.87	3.71
2	3	0.72	2.19	22.68	12.30	0.69	1.44	16.79	8.75	1.04	1.12	7.53	11.71	0.95	0.44	3.50	4.98	0.81	0.60	3.15	3.82
2	4	0.62	2.37	24.84	11.56	0.75	1.11	12.61	7.11	0.96	1.22	8.78	12.69	0.92	0.49	3.80	5.27	0.93	0.63	2.75	3.82
2	4	0.67	1.84	20.49	10.22	0.82	1.41	15.87	9.80	0.89	0.85	8.17	10.88	0.96	0.43	3.07	4.42	0.94	0.32	2.22	3.13
2	4	0.62	2.19	22.21	10.32	0.80	1.37	14.31	8.64	0.93	0.81	8.21	11.49	0.95	0.34	2.90	4.11	0.93	0.32	2.99	4.18
2	5	0.70	2.34	24.09	12.67	0.77	1.70	18.10	10.40	0.91	0.98	9.93	13.57	0.88	0.42	3.71	4.93	0.88	0.52	4.71	6.20
2	5	0.71	2.01	23.22	12.36	0.61	1.87	23.04	10.57	1.05	0.96	8.96	14.05	0.91	0.49	3.80	5.18	0.91	0.31	2.52	3.42
2	5	0.74	1.88	23.42	13.00	0.72	1.33	15.49	8.34	0.94	0.95	6.33	8.95	0.96	0.41	2.85	4.08	0.92	0.41	2.63	3.63
3	0	0.73	1.42	16.11	8.87	1.07	0.92	9.25	7.42	0.95	0.73	7.52	10.67	0.95	0.38	2.86	4.08	0.98	0.59	2.37	3.49
3	0	0.71	2.12	26.56	14.17	0.94	0.98	8.88	6.29	1.02	0.72	7.40	11.29	1.01	0.48	3.16	4.80	0.96	0.64	2.26	3.24
3	0	0.83	2.51	23.92	14.89	0.96	1.38	9.92	7.17	1.01	0.63	6.17	9.34	1.04	0.48	3.61	5.63	1.00	0.51	2.86	4.28
3	1	0.73	1.45	15.30	8.42	1.00	0.95	8.89	6.68	1.05	0.61	5.62	8.85	0.94	0.44	3.44	4.83	0.98	0.46	2.76	4.07
3	1	0.70	1.59	15.43	8.12	0.92	0.97	8.96	6.15	1.05	0.67	6.18	9.70	1.04	0.82	3.80	5.95	0.94	0.38	2.75	3.88
3	1	0.58	1.70	19.40	8.37	0.98	1.00	8.54	6.27	1.03	0.61	5.97	9.18	1.00	0.41	3.14	4.71	1.02	0.37	3.18	4.87
3	2	0.68	1.70	17.00	8.66	0.94	0.96	9.51	6.71	1.00	0.69	7.38	11.04	0.94	0.51	3.31	4.66	1.00	0.38	2.69	4.03
3	2	0.73	1.76	19.81	10.91	0.98	1.37	11.80	8.66	1.04	0.82	7.34	11.40	1.00	0.49	3.78	5.69	1.03	0.42	3.32	5.13
3	2	0.74	1.62	16.07	8.86	0.90	1.05	9.78	6.61	1.02	0.83	7.60	11.66	0.97	0.56	3.25	4.73	0.96	0.54	2.74	3.96
3	3	0.68	1.92	21.48	10.94	0.83	1.15	12.80	7.98	0.97	0.84	7.84	11.39	0.99	0.36	2.67	3.95	0.90	0.36	2.61	3.54
3	3	0.74	2.50	23.43	13.02	0.82	1.34	11.97	7.38	0.93	0.95	8.51	11.89	0.91	0.42	3.06	4.20	0.91	0.49	3.16	4.31
3	3	0.73	2.05	22.10	12.15	0.77	1.19	12.39	7.20	0.99	1.19	9.58	14.26	0.87	0.48	4.33	5.66	0.88	0.34	2.90	3.84

Appendix B continued

Rep	Treat†	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock
		g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}
		0-7.5 cm				7.5-15 cm				15-30 cm				30-45 cm				45-60 cm			
3	4	0.79	1.83	19.73	11.70	0.92	8.26	13.38	9.23	1.02	0.84	7.52	11.56	0.97	0.47	3.93	5.70	1.06	0.45	3.27	5.19
3	4	0.92	2.28	24.01	16.50	0.94	4.35	13.78	9.77	0.93	0.74	6.57	9.17	0.96	0.39	2.77	3.99	0.90	0.43	2.87	3.89
3	4	0.76	1.74	19.13	10.91	0.96	1.22	11.35	8.14	0.90	0.81	7.13	9.59	0.96	0.40	3.10	4.47	0.97	0.47	2.73	3.99
3	5	0.67	2.12	22.59	11.33	0.84	1.51	11.90	7.45	0.90	0.88	8.40	11.29	0.93	0.97	4.72	6.56	0.87	0.43	3.23	4.23
3	5	0.70	2.19	22.23	11.61	0.81	1.32	13.32	8.13	0.87	0.88	8.26	10.79	0.90	0.82	3.44	4.64	0.90	0.42	2.65	3.58
3	5	0.82	1.95	20.22	12.49	0.80	1.14	11.55	6.97	0.84	0.90	9.06	11.46	0.88	0.54	4.32	5.70	1.00	0.42	3.03	4.53
4	0	0.72	1.47	15.78	8.52	0.85	1.08	9.32	5.92	0.96	0.65	5.46	7.90	1.04	0.44	2.77	4.30	1.09	0.45	2.36	3.85
4	0	0.75	1.13	12.96	7.32	0.88	1.06	9.43	6.23	1.00	0.45	4.15	6.24	1.02	0.37	2.98	4.58	1.03	0.65	3.52	5.43
4	0	0.52	1.26	13.15	5.16	0.86	1.06	9.85	6.39	0.99	0.66	5.32	7.90	0.99	0.39	3.05	4.55	1.00	0.34	2.41	3.61
4	1	0.90	1.50	15.53	10.48	0.79	1.12	11.17	6.62	0.96	0.73	7.36	10.60	0.89	0.45	3.39	4.52	0.93	0.47	2.61	3.63
4	1	0.86	1.81	16.12	10.38	0.83	1.07	10.93	6.77	0.89	0.62	7.29	9.73	0.98	0.39	3.37	4.96	0.87	0.36	2.51	3.26
4	1	0.79	2.46	20.95	12.38	0.72	0.98	9.81	5.33	0.68	0.86	8.09	8.21	0.93	0.73	3.18	4.44	0.75	0.77	6.02	6.82
4	2	0.72	1.69	18.93	10.28	0.97	1.16	11.26	8.16	0.91	0.59	5.66	7.72	0.93	0.41	3.03	4.21	0.90	0.39	3.21	4.33
4	2	0.96	1.58	18.21	13.08	0.75	0.99	9.88	5.59	0.92	0.66	5.96	8.25	0.89	0.59	3.89	5.19	0.89	0.34	2.35	3.14
4	2	0.75	1.63	19.26	10.90	0.79	1.06	12.49	7.45	0.93	0.75	6.72	9.36	0.93	0.50	2.57	3.57	0.90	0.38	2.57	3.47
4	3	0.96	1.67	18.38	13.23	0.80	1.24	11.65	6.95	0.97	0.81	7.06	10.32	0.95	0.45	3.80	5.43	1.01	0.44	2.62	3.98
4	3	0.74	1.81	18.17	10.13	0.88	1.11	11.40	7.49	0.97	0.77	6.47	9.39	0.93	0.70	3.65	5.06	0.93	0.45	2.80	3.92
4	3	0.80	1.91	19.02	11.44	0.90	1.06	10.82	7.32	0.96	0.63	6.37	9.14	0.99	0.49	3.85	5.70	0.96	0.40	2.37	3.41
4	4	0.83	1.57	18.66	11.55	0.82	0.96	10.41	6.40	0.92	1.10	9.93	13.66	0.90	0.39	3.83	5.14	0.86	0.54	4.03	5.22
4	4	0.76	1.85	20.66	11.70	0.85	1.32	12.02	7.68	0.93	0.67	6.54	9.15	0.87	0.46	3.94	5.16	0.85	0.39	3.30	4.23
4	4	0.79	1.54	18.93	11.25	0.73	1.30	12.11	6.60	0.91	0.88	7.94	10.79	0.91	0.44	4.22	5.78	0.86	0.47	4.16	5.35
4	5	0.73	2.00	19.84	10.90	0.89	1.36	13.44	8.95	1.06	0.79	7.52	11.94	1.05	0.49	4.27	6.72	1.04	0.51	3.90	6.09
4	5	0.75	1.79	18.08	10.12	0.79	1.13	12.60	7.43	1.02	0.68	6.10	9.36	1.05	0.77	3.23	5.10	0.94	0.54	3.75	5.28
4	5	0.73	1.90	20.85	11.42	0.85	1.24	12.75	8.12	1.01	0.98	7.45	11.29	1.05	0.68	4.76	7.49	0.85	0.44	3.42	4.38

Treat†: 0-control (CTL); 1-42 dry Mg ha^{-1} biosolids (BS 1x); 2-84 dry Mg ha^{-1} biosolids (BS 2x); 3-126 dry Mg ha^{-1} biosolids (BS 3x); 4-168 dry Mg ha^{-1} biosolids (BS 4x); and 5-210 dry Mg ha^{-1} biosolids (BS 5x).

Appendix C. Sampling depth, bulk density (BD), and concentration of total soil C and N, and soil C stocks of Site III in Chapter 3.

Reps	Treat†	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock
		g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}
		0-7.5 cm				7.5-15 cm				15-30 cm				30-45 cm				45-60 cm			
1	1	0.85	1.37	12.82	8.19	1.08	1.65	9.47	7.70	1.22	0.61	5.49	10.08	1.09	0.67	3.18	5.19	1.16	0.60	2.85	4.97
1	1	0.81	1.36	12.95	7.87	1.27	1.33	8.82	8.39	1.11	0.70	5.73	9.56	0.98	0.85	3.27	4.81	1.28	0.51	2.28	4.36
1	1	0.96	1.45	11.96	8.58	1.16	0.66	7.53	6.55	1.18	0.65	5.46	9.69	1.13	0.60	2.66	4.51	1.21	0.48	1.96	3.56
1	2	0.88	1.45	13.52	12.37	1.06	0.89	8.41	6.66	1.14	0.62	4.65	7.95	1.17	0.64	2.65	4.64	1.24	0.73	2.45	4.55
1	2	0.58	1.70	16.21	7.05	1.14	1.02	10.09	8.63	1.16	0.76	6.93	12.05	1.15	0.65	2.89	4.97	1.23	0.69	2.53	4.68
1	2	0.68	1.87	17.38	8.90	1.18	1.06	9.93	8.81	1.11	0.74	6.27	10.41	1.18	0.57	2.71	4.81	1.27	0.75	2.49	4.73
1	3	0.84	1.75	15.45	9.71	1.08	1.86	10.38	8.43	1.16	0.75	5.66	9.80	1.20	5.16	3.15	5.66	1.15	6.17	2.54	4.40
1	3	0.75	1.38	13.60	7.69	1.26	1.20	8.68	8.17	1.18	0.91	5.38	9.50	1.18	0.50	4.29	7.63	1.13	0.59	2.41	4.09
1	3	0.94	1.47	15.15	10.69	1.04	1.75	9.47	7.37	1.11	0.62	5.39	8.99	1.21	0.59	2.24	4.08	1.19	0.70	3.17	5.66
1	4	1.01	1.60	16.05	12.10	0.96	1.32	11.93	8.57	1.14	1.13	5.82	9.93	1.16	0.62	3.07	5.33	1.14	0.79	2.71	4.62
1	4	0.76	2.08	20.33	11.62	0.95	1.68	12.85	9.17	1.08	1.14	6.45	10.44	1.11	0.72	3.13	5.22	1.09	0.53	7.85	12.83
1	4	0.92	1.92	21.07	14.56	1.05	1.19	11.56	9.11	1.16	0.75	7.13	12.45	1.15	0.74	4.22	7.31	1.18	0.75	3.04	5.39
1	5	0.85	1.89	16.95	10.78	1.17	1.34	12.09	10.60	1.19	0.94	6.89	12.27	1.07	0.82	3.37	5.40	1.08	0.77	2.74	4.43
1	5	0.94	1.83	19.46	13.72	1.06	1.43	11.23	8.91	1.19	1.06	6.40	11.47	1.06	0.82	3.54	5.62	1.18	0.63	2.57	4.53
1	5	0.83	2.27	23.27	14.49	1.08	1.29	12.32	9.99	1.18	0.87	7.68	13.56	1.13	0.72	3.40	5.79	1.12	0.66	2.80	4.70
1	6	0.87	1.47	16.64	10.83	1.33	0.76	8.16	8.13	1.14	0.79	6.45	11.06	1.18	6.98	2.65	4.70	1.16	0.58	3.16	5.49
1	6	0.84	1.50	16.51	10.39	1.12	0.90	8.50	7.16	1.15	0.84	5.88	10.12	1.19	0.53	2.73	4.86	1.18	0.59	3.62	6.39
1	6	0.81	1.34	17.75	10.77	1.10	0.84	8.78	7.27	1.07	0.73	6.15	9.85	1.20	0.56	3.16	5.71	1.11	0.61	3.73	6.21
1	7	0.71	1.70	14.69	7.78	1.13	1.07	10.33	8.78	1.13	0.84	6.14	10.38	1.19	0.80	2.66	4.75	1.15	0.64	3.38	5.85
1	7	0.93	2.42	21.86	15.27	0.96	1.52	14.21	10.23	1.15	0.58	6.35	10.95	1.02	0.61	4.12	6.28	1.14	0.63	3.75	6.41
1	7	0.84	2.16	20.17	12.75	1.02	1.52	12.34	9.43	1.17	0.62	5.55	9.76	1.12	0.59	3.26	5.47	1.23	0.64	2.55	4.70
2	1	0.95	1.74	16.18	11.47	1.13	1.20	9.47	7.99	1.15	0.84	4.99	8.58	1.17	0.61	3.19	5.61	1.12	0.60	3.19	5.35
2	1	0.88	1.42	15.97	10.53	1.13	1.01	9.29	7.87	1.05	0.71	6.76	10.70	1.08	0.94	3.98	6.42	1.42	0.51	2.13	4.55
2	1	1.14	1.21	13.41	11.52	1.14	0.96	9.21	7.91	1.15	0.92	5.95	10.29	1.16	0.60	2.93	5.09	1.23	0.55	2.55	4.70

Appendix C continued

Reps	Treat†	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock
		g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}
		0-7.5 cm				7.5-15 cm				15-30 cm				30-45 cm				45-60 cm			
2	2	0.92	1.76	16.67	11.51	1.07	1.12	9.23	7.40	1.10	0.85	5.86	9.71	1.20	0.51	2.40	4.31	1.16	0.56	3.09	5.40
2	2	0.87	1.55	16.67	10.89	1.05	1.04	8.25	6.50	1.12	0.85	5.93	10.00	1.13	0.65	3.45	5.85	1.09	0.73	2.14	3.50
2	2	0.63	1.42	15.04	7.13	0.71	1.14	12.03	6.36	1.16	0.82	6.93	12.03	1.10	0.67	3.56	5.87	1.17	0.78	2.42	4.23
2	3	0.85	1.43	14.31	9.18	1.06	1.06	10.30	8.21	1.10	0.62	5.90	9.69	1.18	0.50	2.66	4.71	1.19	0.62	2.13	3.78
2	3	0.86	1.89	20.49	13.24	1.01	0.95	10.85	8.19	1.08	0.91	6.38	10.32	1.15	0.50	2.67	4.59	1.24	0.68	2.17	4.04
2	3	0.90	2.55	26.95	18.16	0.96	0.98	10.11	7.30	1.18	0.95	10.45	18.57	1.08	0.55	4.21	6.80	1.13	0.77	3.32	5.63
2	4	0.79	1.98	19.09	11.35	0.94	1.23	11.66	8.19	1.13	0.91	4.85	8.21	1.15	0.72	2.83	4.86	1.23	0.61	2.54	4.67
2	4	0.79	0.75	6.11	3.60	1.06	1.51	13.01	10.39	1.09	0.96	7.07	11.54	1.18	0.61	2.97	5.25	1.25	0.81	2.96	5.52
2	4	0.87	2.02	19.77	12.87	0.98	1.44	14.01	10.25	1.11	1.52	16.53	27.55	1.17	0.67	3.23	5.68	1.20	0.54	2.06	3.71
2	5	0.93	1.62	16.45	11.47	0.96	1.51	10.69	7.71	1.15	0.77	6.25	10.81	1.19	0.44	2.59	4.63	1.17	0.56	2.16	3.79
2	5	0.84	1.75	17.36	10.91	1.33	1.24	10.53	10.47	1.18	0.63	5.90	10.45	1.11	0.90	3.04	5.08	1.21	0.72	5.86	10.65
2	5	0.91	1.57	17.06	11.69	1.03	1.50	14.42	11.11	1.23	0.58	4.91	9.03	1.17	0.63	3.72	6.50	1.21	0.60	1.81	3.28
2	6	0.85	0.85	17.38	11.12	0.96	0.95	11.05	7.92	1.10	0.75	8.46	13.99	1.07	0.56	3.66	5.88	1.21	0.47	2.66	4.83
2	6	0.88	0.79	15.24	10.08	1.04	1.10	11.42	8.89	1.16	0.86	7.41	12.88	1.18	5.65	3.96	6.99	1.23	0.52	2.49	4.61
2	6	0.84	0.85	17.65	11.18	1.01	0.95	9.87	7.44	1.09	0.67	6.47	10.59	1.10	0.51	4.04	6.68	1.26	0.58	2.75	5.17
2	7	1.02	2.44	25.64	19.59	1.19	0.95	10.76	9.61	1.13	0.65	6.76	11.49	1.15	0.50	3.00	5.20	1.12	0.72	3.17	5.33
2	7	0.78	1.70	18.01	10.56	1.04	1.17	11.05	8.62	1.08	0.60	5.22	8.46	1.12	0.59	3.12	5.23	1.19	0.44	1.89	3.36
2	7	0.99	1.41	15.52	11.49	1.07	1.00	10.12	8.09	1.03	0.93	6.56	10.14	1.12	0.65	3.22	5.41	1.18	0.56	2.42	4.28
3	1	0.92	1.26	12.97	8.91	1.06	1.03	10.27	8.15	1.16	0.68	4.98	8.69	1.16	0.63	3.61	6.30	1.12	0.60	2.79	4.67
3	1	0.80	1.60	15.53	9.32	1.11	0.95	9.15	7.64	1.13	0.77	6.12	10.38	1.14	0.57	3.27	5.62	1.18	0.60	2.72	4.80
3	1	0.86	1.65	10.39	6.67	1.06	0.95	9.84	7.83	1.15	0.75	5.87	10.16	1.16	0.59	3.66	6.34	1.15	0.55	2.47	4.25
3	2	0.87	1.70	16.56	10.83	1.15	0.69	8.03	6.94	1.17	0.65	4.77	8.37	1.13	0.66	2.84	4.80	1.13	0.70	3.00	5.09
3	2	0.89	1.63	15.20	10.18	1.09	0.87	9.56	7.84	1.18	0.64	4.87	8.60	1.15	0.46	2.34	4.05	1.22	0.45	1.57	2.87
3	2	0.87	1.43	14.82	9.62	1.13	1.01	9.83	8.33	1.16	0.74	4.54	7.89	1.24	0.48	2.50	4.64	1.17	0.44	2.22	3.91

Appendix C continued

Reps	Treat†	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock
		g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}
		0-7.5 cm				7.5-15 cm				15-30 cm				30-45 cm				45-60 cm			
3	3	0.75	1.95	18.68	10.57	1.15	1.15	10.88	9.37	1.07	0.81	7.41	11.94	1.11	0.56	3.41	5.68	1.15	0.72	3.82	6.56
3	3	0.73	1.82	17.70	9.66	0.95	1.34	11.26	8.04	1.08	0.93	7.67	12.45	1.10	0.62	4.90	8.07	1.20	0.89	3.06	5.49
3	3	0.68	2.07	19.39	9.95	0.92	1.30	12.36	8.53	1.19	0.79	6.52	11.64	1.18	0.60	3.17	5.60	1.19	0.63	3.01	5.38
3	4	0.93	2.15	17.76	12.37	1.00	1.38	10.17	7.60	1.15	0.65	4.75	8.17	1.16	0.69	3.40	5.91	1.20	0.69	3.25	5.84
3	4	0.99	1.99	19.72	14.58	1.01	1.43	11.74	8.91	1.05	1.19	12.16	19.07	1.15	0.72	4.83	8.36	1.21	0.79	3.30	5.98
3	4	0.92	1.67	16.57	11.48	1.16	1.32	11.40	9.88	1.11	1.04	6.13	10.19	1.18	0.62	2.42	4.29	1.19	0.66	3.05	5.43
3	5	0.90	1.89	21.32	14.33	0.95	1.77	15.52	11.08	1.22	0.83	5.40	9.92	1.24	0.58	3.19	5.93	1.15	0.46	2.19	3.78
3	5	1.10	1.63	17.20	14.19	1.19	1.09	10.95	9.80	1.10	0.66	4.54	7.52	1.16	0.68	3.15	5.48	1.22	0.61	2.74	5.01
3	5	0.87	1.69	17.69	11.48	1.06	1.13	10.89	8.70	1.21	1.25	6.73	12.24	1.15	0.60	2.91	5.02	1.27	0.53	1.62	3.08
3	6	0.79	1.15	18.98	11.26	0.91	1.21	11.38	7.77	1.11	1.26	13.01	21.58	1.03	0.51	3.66	5.66	1.22	0.53	4.44	8.10
3	6	0.76	1.65	17.40	9.98	1.07	1.28	12.11	9.72	1.08	1.09	7.93	12.79	1.17	0.56	4.15	7.30	1.24	0.71	6.04	11.22
3	6	0.86	2.00	21.10	13.54	0.99	1.40	14.50	10.74	1.07	0.84	8.59	13.76	1.10	0.53	5.91	9.74	1.22	0.50	2.25	4.13
3	7	0.58	2.20	18.84	8.21	1.17	1.33	11.40	9.97	1.18	1.47	10.07	17.86	1.14	0.49	2.05	3.53	1.24	0.62	1.70	3.18
3	7	0.75	1.91	19.52	10.91	1.13	1.45	12.98	10.97	1.21	1.03	5.91	10.76	1.14	0.70	2.95	5.05	1.21	0.83	2.47	4.47
3	7	0.86	1.89	19.04	12.25	1.15	1.12	10.53	9.07	1.17	0.88	6.74	11.87	1.17	0.61	2.81	4.94	1.23	0.59	2.70	4.98
4	1	1.03	1.57	15.34	11.90	0.96	0.95	8.59	6.17	1.15	1.06	12.15	20.96	1.10	0.65	2.68	4.42	1.28	0.54	2.22	4.26
4	1	1.26	1.08	10.62	10.00	1.13	0.90	7.71	6.54	1.10	0.85	5.56	9.16	1.19	0.56	3.24	5.78	1.24	0.89	3.07	5.69
4	1	1.04	1.22	12.61	9.82	1.08	0.88	8.40	6.83	1.10	0.63	4.83	7.95	1.15	0.52	2.60	4.48	1.21	0.57	2.08	3.79
4	2	0.95	1.62	16.82	12.03	1.13	1.05	11.54	9.75	1.12	-28.76	19.62	33.06	1.24	0.88	3.09	5.74	1.24	0.68	2.73	5.06
4	2	0.82	1.82	17.66	10.87	1.16	1.20	11.18	9.70	1.08	0.70	6.37	10.32	1.21	0.56	2.83	5.15	1.13	0.73	2.85	4.81
4	2	0.87	1.75	15.89	10.32	1.15	1.17	9.01	7.80	1.06	0.83	6.17	9.84	1.16	0.51	2.92	5.06	1.20	0.67	2.93	5.29
4	3	0.85	1.55	16.78	10.65	1.15	1.35	10.53	9.08	1.09	0.93	6.81	11.18	1.19	0.80	3.31	5.93	1.20	0.61	2.50	4.49
4	3	0.93	1.48	16.99	11.85	1.18	1.05	10.39	9.19	1.19	0.63	4.74	8.45	1.21	0.72	2.65	4.81	1.16	0.89	3.79	6.61
4	3	1.04	1.43	14.57	11.32	1.20	0.94	10.38	9.36	1.14	0.63	4.51	7.70	1.25	0.48	2.04	3.84	1.17	0.45	2.08	3.64

Appendix C continued

Reps	Treat†	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock	BD	Soil N	Soil C	C stock
		g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}	g cm^{-3}	g kg^{-1}	g kg^{-1}	Mg ha^{-1}
		0-7.5 cm				7.5-15 cm				15-30 cm				30-45 cm				45-60 cm			
4	4	0.46	2.04	18.84	6.54	1.08	1.65	12.37	10.05	1.12	0.88	6.36	10.73	1.26	0.54	3.70	7.00	1.18	0.44	1.66	2.96
4	4	0.81	1.83	16.83	10.28	1.11	0.92	8.70	7.21	1.22	0.56	4.97	9.07	1.18	0.61	2.59	4.58	1.16	0.46	1.90	3.29
4	4	1.05	1.67	15.65	12.27	0.85	0.83	8.77	5.56	1.15	0.59	5.75	9.96	1.18	0.46	2.33	4.13	1.19	0.44	1.64	2.92
4	5	0.85	2.36	23.73	15.20	0.95	1.45	12.64	9.01	1.16	1.00	5.82	10.09	1.13	0.66	3.75	6.37	1.09	0.97	4.41	7.18
4	5	1.10	1.69	16.96	13.95	1.16	1.00	10.18	8.87	1.12	0.64	4.59	7.69	1.23	0.69	2.85	5.25	1.17	0.76	3.54	6.24
4	5	0.84	1.75	19.08	12.07	1.18	1.01	9.63	8.51	1.09	1.24	7.14	11.69	1.19	0.80	3.21	5.72	1.17	0.70	2.93	5.15
4	6	0.74	1.75	19.95	11.01	1.08	1.30	11.62	9.38	1.05	1.02	7.20	11.32	1.17	0.77	3.80	6.68	1.19	0.57	1.89	3.39
4	6	0.71	1.80	18.42	9.75	1.08	0.90	10.49	8.46	1.19	0.70	5.46	9.76	1.10	0.47	2.71	4.46	1.16	0.45	2.53	4.39
4	6	0.88	1.79	19.54	12.94	1.11	1.00	10.52	8.79	1.12	0.66	6.84	11.51	1.14	0.65	3.36	5.76	1.29	0.36	1.37	2.65
4	7	0.88	1.97	19.83	13.05	0.88	1.74	13.67	9.02	1.06	0.90	8.12	12.97	1.15	0.77	3.73	6.43	1.16	0.63	2.31	4.03
4	7	0.94	2.28	22.36	15.76	0.98	1.26	11.21	8.21	1.16	1.02	7.05	12.29	1.12	0.60	3.19	5.35	1.20	0.61	2.72	4.90
4	7	0.91	2.05	23.38	15.98	1.19	1.28	10.42	9.31	1.07	0.69	6.32	10.14	1.21	0.83	4.17	7.59	1.16	0.60	1.91	3.32

Treat†: 1-Control (CTL); 2-14 dry Mg ha^{-1} biosolids (BS 1x); 3-42 dry Mg ha^{-1} biosolids (BS 3x); 4-70 dry Mg ha^{-1} biosolids (BS 5x); 5-98 dry Mg ha^{-1} biosolids (BS 7x); 6-42 dry Mg ha^{-1} biosolids+sawdust (BS 3x+SD); and 7-98 dry Mg ha^{-1} biosolids+sawdust (BS 7x+SD).

Appendix D. Soil properties and corn responses from treatment and tillage in 48 plots during 2009-2011 in Chapter 6.

	2009		Spring PSNT†			Corn leaf at silking		Fall soil test			Fall CSNT#	
rep	CT/NT‡	treat§	NO_3 mg kg^{-1}	NH_4 mg kg^{-1}	NO_3+NH_4 mg kg^{-1}	TKN g kg^{-1}	TKP g kg^{-1}	Soil C g kg^{-1}	Soil N g kg^{-1}	C:N	stalk NO_3 mg kg^{-1}	corn yield Mg ha^{-1}
1	1	1	7.60	2.74	10.34	14.48	1.61	4.43	0.36	12.28	349.53	7.31
1	1	2	12.75	3.62	16.37	19.25	1.90	3.73	0.33	11.19	378.86	10.48
1	1	3	19.46	5.74	25.20	23.35	2.14	4.20	0.38	11.11	368.44	11.43
1	1	4	19.97	3.78	23.75	19.23	1.82	3.65	0.33	11.08	969.21	11.10
1	1	5	21.09	4.45	25.54	26.50	2.55	4.67	0.44	10.71	721.97	12.14
1	1	6	21.30	5.32	26.62	20.88	2.10	4.28	0.39	11.09	1097.16	11.48
1	2	1	8.81	3.87	12.68	15.98	1.83	4.28	0.37	11.50	333.65	10.06
1	2	2	17.46	4.02	21.48	18.90	2.09	3.96	0.35	11.35	300.00	10.75
1	2	3	15.58	3.19	18.77	16.98	1.79	4.60	0.39	11.68	373.04	11.34
1	2	4	21.27	3.46	24.73	24.60	2.36	4.58	0.40	11.41	321.46	11.95
1	2	5	20.72	3.15	23.87	22.58	2.43	5.14	0.46	11.11	316.52	13.13
1	2	6	27.15	7.82	34.97	28.00	2.83	5.09	0.40	12.62	310.68	12.03
2	1	1	13.27	3.82	17.09	10.85	1.39	3.02	0.30	10.03	300.27	6.77
2	1	2	8.43	2.97	11.40	16.65	1.72	3.43	0.33	10.45	363.90	9.48
2	1	3	9.38	2.81	12.19	20.98	1.97	3.75	0.35	10.66	343.09	8.51
2	1	4	28.37	3.51	31.88	18.80	1.78	3.97	0.38	10.33	519.77	10.67
2	1	5	26.25	4.08	30.33	26.50	2.63	4.76	0.42	11.33	369.58	9.75
2	1	6	25.93	3.46	29.39	22.38	2.26	4.20	0.40	10.57	302.14	9.17
2	2	1	7.50	2.26	9.76	17.23	2.06	4.25	0.39	10.86	357.19	8.61
2	2	2	13.50	2.11	15.61	13.95	1.55	4.77	0.43	11.13	322.46	9.43
2	2	3	13.38	2.75	16.13	18.15	1.95	4.14	0.39	10.69	329.53	10.00
2	2	4	14.99	1.21	16.20	31.50	3.13	4.46	0.41	10.88	493.08	10.38
2	2	5	23.71	3.95	27.66	31.50	3.15	4.41	0.42	10.42	326.48	10.96
2	2	6	22.11	2.50	24.61	23.20	2.42	5.23	0.50	10.49	380.04	11.82

Appendix D continued

	2009		Spring PSNT†			Corn leaf at silking		Fall soil test			Fall CSNT#	
rep	CT/NT‡	treat§	NO_3	NH_4	NO_3+NH_4	TKN	TKP	Soil C	Soil N	C:N	stalk NO_3	corn yield
			mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}		mg kg^{-1}	Mg ha^{-1}
3	1	1	8.05	2.94	10.99	16.55	1.92	3.99	0.32	12.66	325.47	8.57
3	1	2	11.00	2.64	13.64	23.65	2.33	4.08	0.35	11.52	346.29	12.91
3	1	3	13.70	1.51	15.21	20.05	2.05	3.73	0.34	11.12	277.88	10.59
3	1	4	26.75	2.59	29.34	26.75	2.63	3.75	0.32	11.55	516.55	11.51
3	1	5	24.54	2.71	27.25	22.68	2.33	4.52	0.41	10.98	303.07	12.78
3	1	6	24.84	3.49	28.33	22.85	2.30	4.13	0.37	11.26	306.86	11.68
3	2	1	9.27	2.64	11.91	19.90	2.38	3.58	0.27	13.45	271.91	10.30
3	2	2	12.07	2.42	14.49	23.65	2.55	3.40	0.30	11.45	278.74	11.88
3	2	3	24.91	2.12	27.03	17.50	1.90	3.29	0.29	11.35	325.47	12.27
3	2	4	15.51	2.95	18.46	20.15	2.03	4.73	0.40	11.79	277.88	11.61
3	2	5	15.15	3.57	18.72	26.00	2.60	4.48	0.39	11.42	275.30	12.58
3	2	6	22.59	3.03	25.62	27.75	2.83	6.04	0.55	10.89	274.45	10.94
4	1	1	6.65	1.52	8.17	14.90	1.84	3.55	0.33	10.73	389.58	9.55
4	1	2	13.42	2.22	15.64	29.75	2.85	3.14	0.28	11.06	332.61	10.89
4	1	3	15.28	2.20	17.48	28.75	2.80	3.56	0.33	10.72	424.91	11.71
4	1	4	20.96	3.08	24.04	29.25	2.93	3.38	0.32	10.59	1196.65	11.88
4	1	5	22.53	6.34	28.87	26.50	2.80	3.46	0.33	10.52	342.02	14.05
4	1	6	14.86	1.29	16.15	31.00	3.20	4.76	0.42	11.48	331.58	11.60
4	2	1	8.53	1.40	9.93	17.38	1.95	4.09	0.36	11.28	292.01	7.88
4	2	2	8.31	1.46	9.77	16.33	1.79	3.78	0.35	10.95	314.56	10.00
4	2	3	14.50	2.10	16.60	21.48	2.20	3.79	0.34	11.16	321.46	13.15
4	2	4	21.81	1.82	23.63	18.93	1.96	4.61	0.37	12.60	322.46	9.58
4	2	5	15.85	6.12	21.97	28.00	2.85	3.77	0.34	10.99	284.85	10.82
4	2	6	33.95	1.98	35.93	23.68	2.42	4.71	0.44	10.80	280.47	12.76

Appendix D continued.

	2010		Spring PSNT†			Corn leaf at silking		Fall soil test			Fall CSNT#	
rep	CT/NT‡	treat§	NO_3 mg kg^{-1}	NH_4 mg kg^{-1}	NO_3+NH_4 mg kg^{-1}	TKN g kg^{-1}	TKP g kg^{-1}	Soil C g kg^{-1}	Soil N g kg^{-1}	C:N	stalk NO_3 mg kg^{-1}	corn yield Mg ha^{-1}
1	1	1	12.36	7.98	20.34	11.53	2.17	6.94	0.46	15.00	409.49	3.11
1	1	2	24.03	3.57	27.60	25.50	3.08	3.61	0.33	11.10	415.05	4.63
1	1	3	40.74	8.94	49.68	25.75	2.88	4.93	0.55	9.00	504.12	2.92
1	1	4	101.43	14.42	115.85	27.00	2.73	3.76	0.38	10.00	817.39	3.84
1	1	5	52.81	26.10	78.91	26.50	2.53	4.42	0.38	11.60	1173.69	6.31
1	1	6	31.66	44.22	75.88	28.00	3.20	4.54	0.46	9.90	502.76	6.20
1	2	1	20.18	5.33	25.51	12.58	1.98	4.83	0.46	10.60	422.97	1.83
1	2	2	42.43	5.51	47.94	24.53	2.43	4.61	0.40	11.70	392.18	3.55
1	2	3	52.61	7.16	59.77	25.25	2.36	5.35	0.44	12.20	527.80	6.62
1	2	4	159.95	14.80	174.75	27.25	2.53	4.07	0.39	10.60	1286.54	3.72
1	2	5	24.25	6.80	31.05	23.00	1.96	3.89	0.36	11.00	1248.89	6.65
1	2	6	15.64	26.49	42.13	22.08	2.42	4.89	0.49	10.00	576.98	4.00
2	1	1	7.06	4.84	11.90	12.28	2.10	4.60	0.48	9.60	494.68	1.18
2	1	2	36.59	6.98	43.57	22.03	2.70	4.41	0.44	10.10	546.65	4.09
2	1	3	40.94	14.34	55.28	23.73	2.19	3.90	0.39	9.90	612.29	3.30
2	1	4	129.76	6.57	136.33	27.25	3.75	5.41	0.47	11.40	1807.88	3.56
2	1	5	35.58	18.24	53.82	24.60	2.85	4.15	0.40	10.20	943.14	4.40
2	1	6	26.30	12.60	38.90	24.63	2.95	4.20	0.41	10.30	545.18	5.53
2	2	1	5.91	6.04	11.95	12.43	2.00	4.12	0.39	10.50	441.65	2.16
2	2	2	17.87	6.72	24.59	26.25	3.13	4.10	0.39	10.50	396.43	1.80
2	2	3	60.85	6.09	66.94	27.25	2.53	4.30	0.42	10.20	567.71	3.76
2	2	4	145.56	4.72	150.28	26.00	2.40	4.07	0.41	9.90	592.77	3.67
2	2	5	85.86	5.73	91.59	25.50	2.45	4.34	0.40	10.80	1562.61	3.05
2	2	6	12.70	11.50	24.20	21.93	2.55	3.88	0.38	10.20	501.40	5.55

Appendix D continued.

	2010		Spring PSNT†			Corn leaf at silking		Fall soil test			Fall CSNT#	
rep	CT/NT‡	treat§	NO_3 mg kg^{-1}	NH_4 mg kg^{-1}	NO_3+NH_4 mg kg^{-1}	TKN g kg^{-1}	TKP g kg^{-1}	Soil C g kg^{-1}	Soil N g kg^{-1}	C:N	stalk NO_3 mg kg^{-1}	corn yield Mg ha^{-1}
3	1	1	5.47	4.83	10.30	14.20	2.25	3.70	0.35	10.70	435.72	3.42
3	1	2	33.91	6.89	40.80	22.75	2.78	4.07	0.36	11.40	468.68	4.82
3	1	3	26.56	4.43	30.99	26.75	2.95	4.94	0.49	10.10	475.05	3.80
3	1	4	45.92	7.21	53.13	26.25	2.95	3.89	0.34	11.50	2361.87	4.21
3	1	5	37.13	6.06	43.19	28.25	3.13	3.60	0.32	11.20	2479.50	5.42
3	1	6	44.33	23.38	67.71	20.08	2.33	5.11	0.51	10.00	1164.22	4.53
3	2	1	11.29	3.01	14.30	14.00	1.83	4.62	0.44	10.40	448.86	2.35
3	2	2	31.62	3.98	35.60	23.18	2.58	4.27	0.38	11.30	557.08	5.49
3	2	3	44.33	7.01	51.34	26.25	2.73	4.60	0.44	10.40	1722.12	3.80
3	2	4	66.40	7.98	74.38	28.00	2.73	3.81	0.38	10.10	2703.25	7.26
3	2	5	32.40	4.75	37.15	28.00	2.50	4.01	0.38	10.60	1500.59	5.46
3	2	6	13.86	19.65	33.51	24.28	2.48	3.74	0.35	10.60	1798.14	4.86
4	1	1	8.35	2.78	11.13	15.48	2.22	5.43	0.53	10.30	402.91	2.27
4	1	2	29.85	4.17	34.02	13.83	1.84	4.12	0.33	12.30	429.88	4.13
4	1	3	21.87	2.85	24.72	24.13	2.58	5.62	0.51	11.00	1722.12	3.60
4	1	4	27.20	4.75	31.95	28.25	3.23	4.11	0.33	12.50	2554.24	3.27
4	1	5	43.56	5.18	48.74	26.00	3.15	4.18	0.39	10.80	4882.98	5.29
4	1	6	24.30	4.00	28.30	27.50	3.30	3.73	0.37	10.00	879.20	5.55
4	2	1	3.40	2.80	6.20	13.88	1.99	3.98	0.38	10.50	329.05	1.83
4	2	2	68.49	3.11	71.60	19.85	2.16	4.32	0.43	10.10	398.58	4.99
4	2	3	95.22	5.14	100.36	25.25	2.63	4.17	0.39	10.60	646.27	6.46
4	2	4	81.06	4.81	85.87	25.25	2.70	4.56	0.45	10.10	3364.08	5.25
4	2	5	30.71	4.80	35.51	21.93	2.19	4.61	0.43	10.60	3902.65	4.99
4	2	6	23.87	11.84	35.71	21.83	2.41	4.29	0.41	10.50	1788.46	5.27

Appendix D continued.

	2011		Spring PSNT†			Corn leaf at silking	Fall soil test			Fall CSNT#	
rep	CT/NT‡	treat§	NO_3 mg kg^{-1}	NH_4 mg kg^{-1}	NO_3+NH_4 mg kg^{-1}	leaf N g kg^{-1}	Soil C g kg^{-1}	Soil N g kg^{-1}	C:N	stalk NO_3 mg kg^{-1}	corn yield Mg ha^{-1}
1	1	1	6.00	9.29	15.29	12.98	0.38	3.85	10.16	366.92	2.51
1	1	2	42.15	9.95	52.10	22.32	0.45	5.01	11.06	478.74	7.46
1	1	3	67.80	11.63	79.43	24.36	0.39	4.46	11.38	727.17	8.16
1	1	4	82.36	4.79	87.15	29.84	0.39	4.22	10.88	846.54	9.96
1	1	5	32.53	9.79	42.33	29.15	0.44	4.65	10.67	443.70	10.08
1	1	6	65.40	10.58	75.98	25.16	0.41	4.16	10.10	727.17	9.90
1	2	1	7.31	9.86	17.17	12.56	0.37	4.16	11.13	395.90	3.85
1	2	2	42.20	10.91	53.11	20.23	0.42	4.30	10.27	373.96	8.36
1	2	3	35.71	10.25	45.96	24.71	0.41	4.35	10.62	373.96	9.20
1	2	4	47.10	9.36	56.46	24.28	0.48	4.91	10.31	478.74	10.41
1	2	5	26.54	13.64	40.18	24.64	0.59	6.30	10.62	452.21	11.18
1	2	6	29.06	19.96	49.02	23.44	0.47	4.98	10.48	435.35	8.98
2	1	1	6.33	8.24	14.57	14.76	0.47	3.97	8.39	478.74	3.86
2	1	2	26.12	9.95	36.07	20.39	0.39	3.59	9.11	353.24	7.38
2	1	3	32.04	8.86	40.89	26.69	0.41	3.78	9.29	546.84	5.86
2	1	4	47.73	3.00	50.72	25.94	0.97	8.17	8.44	403.49	5.66
2	1	5	40.60	8.96	49.55	25.00	0.54	5.04	9.38	487.92	10.60
2	1	6	50.38	12.34	62.72	25.19	0.48	4.49	9.31	546.84	9.53
2	2	1	7.39	8.31	15.71	15.86	0.51	5.88	11.53	478.74	5.02
2	2	2	13.46	8.32	21.78	14.52	0.42	4.35	10.36	411.23	7.76
2	2	3	59.81	10.35	70.16	25.40	0.41	4.50	10.98	419.12	10.08
2	2	4	58.17	9.11	67.28	25.64	0.41	4.27	10.49	478.74	9.53
2	2	5	26.30	10.12	36.42	25.27	0.73	5.39	7.36	506.82	11.29
2	2	6	27.45	9.28	36.73	22.40	0.48	4.98	10.38	578.92	8.93

Appendix D continued.

			Spring PSNT†			Corn leaf at silking	Fall soil test			Fall CSNT#	
rep	CT/NT‡	treat§	NO_3 mg kg^{-1}	NH_4 mg kg^{-1}	NO_3+NH_4 mg kg^{-1}	leaf N g kg^{-1}	Soil C g kg^{-1}	Soil N g kg^{-1}	C:N	stalk NO_3 mg kg^{-1}	corn yield Mg ha^{-1}
3	1	1	6.78	7.81	14.59	13.51	0.41	4.04	9.82	478.74	4.46
3	1	2	29.76	9.35	39.11	21.04	0.39	3.98	10.19	497.28	9.23
3	1	3	42.55	8.08	50.63	25.79	0.44	4.46	10.23	497.28	7.81
3	1	4	69.60	17.83	87.43	28.35	0.43	4.51	10.51	1104.51	8.31
3	1	5	57.51	12.06	69.57	23.74	0.52	6.02	11.61	497.28	11.28
3	1	6	46.55	3.49	50.04	24.05	0.47	4.52	9.55	814.97	9.69
3	2	1	10.99	9.53	20.52	13.36	0.41	4.26	10.32	366.92	4.57
3	2	2	49.75	11.45	61.21	20.23	0.42	4.41	10.60	366.92	11.16
3	2	3	48.73	8.74	57.48	23.03	0.43	4.46	10.26	403.49	8.76
3	2	4	54.05	4.04	58.10	25.72	0.45	4.69	10.40	366.92	11.03
3	2	5	23.04	9.52	32.56	22.58	0.63	6.59	10.50	411.23	12.52
3	2	6	46.38	13.02	59.40	19.59	0.50	5.20	10.37	427.16	11.43
4	1	1	5.49	8.54	14.04	13.54	0.37	3.76	10.26	395.90	2.29
4	1	2	38.16	8.56	46.71	23.63	0.42	3.98	9.59	411.23	9.54
4	1	3	52.80	12.42	65.22	24.17	0.39	3.60	9.25	862.78	8.99
4	1	4	58.91	13.20	72.11	25.67	0.41	4.06	9.88	1361.25	9.88
4	1	5	66.82	14.01	80.83	27.68	0.61	5.30	8.62	1361.25	6.54
4	1	6	60.86	13.87	74.73	24.46	0.58	4.98	8.65	427.16	9.15
4	2	1	8.57	8.52	17.10	14.14	0.42	4.43	10.47	388.45	6.32
4	2	2	21.07	7.99	29.06	14.75	0.42	4.37	10.33	388.45	6.08
4	2	3	55.20	11.48	66.68	21.51	0.41	4.03	9.74	403.49	8.49
4	2	4	60.21	9.92	70.12	26.90	0.40	4.18	10.33	443.70	10.63
4	2	5	38.37	11.06	49.43	26.15	0.59	5.51	9.36	403.49	13.29
4	2	6	31.12	9.39	40.51	22.95	0.45	4.53	10.05	427.16	11.50

CT/NT‡: 1-conventianl tillage; 2-no-tillage.
treat§: 1-0xN; 2-0.5xN; 3-1xN; 4-1.5xN; 5-lime stabilized biosolids; 6-anaerobically digested biosolids.
Spring PSNT†:‡PSNT = Pre-sidedress Nitrate Test.
Fall CSNT#: Corn Stalk Nitrate Test.

Appendix E. Bulk density (BD), water holding capacity (WHC) and soil C for selected samples from corn plots under conventional tillage in November, 2011 for Chapter 6.

rep	treat§	BD	Pressure			WHC	Soil C	C stocks
			1/3 bar	3 bar	15 bar			
		g cm^{-3}	% (v/v)				g kg^{-1}	Mg ha^{-1}
1	3	1.67	15.13	11.77	10.09	5.04	4.46	11.43
1	3	1.67	13.23	9.82	8.73	4.51	4.46	10.22
1	5	1.60	13.38	9.95	8.35	5.03	4.65	11.90
1	5	1.72	14.00	11.47	10.47	3.54	4.65	8.36
1	6	1.64	16.52	11.81	10.45	6.07	4.16	12.85
1	6	1.43	12.88	9.16	7.94	4.95	4.16	10.46
2	3	1.71	12.94	9.87	8.77	4.17	3.78	8.00
2	3	1.59	12.74	8.81	7.74	4.99	3.78	9.59
2	5	1.45	13.26	9.18	7.86	5.40	5.04	13.83
2	5	1.66	12.90	9.69	8.21	4.69	5.04	12.02
2	6	1.56	12.20	9.52	7.95	4.25	4.49	9.69
2	6	1.39	12.03	8.82	7.04	4.98	4.49	11.38
3	3	1.60	12.36	9.55	8.23	4.13	4.46	9.36
3	3	1.72	13.86	10.93	9.41	4.45	4.46	10.08
3	5	1.54	15.83	12.21	10.48	5.35	6.02	16.36
3	5	1.59	12.99	10.22	9.34	3.65	6.02	11.16
3	6	1.70	14.04	10.10	8.83	5.21	4.52	11.96
3	6	1.60	13.20	9.01	7.66	5.54	4.52	12.72
4	3	1.69	13.22	10.63	9.66	3.56	3.60	6.51
4	3	1.69	13.04	9.39	8.36	4.68	3.60	8.57
4	5	1.54	14.06	10.34	8.82	5.24	5.30	14.09
4	5	1.49	13.78	11.29	10.07	3.71	5.30	9.99
4	6	1.44	14.21	10.03	8.83	5.37	4.98	13.61
4	6	1.60	15.75	10.55	10.21	5.54	4.98	14.03

treat§: 1-0xN; 2-0.5xN; 3-1xN; 4-1.5xN; 5-lime stabilized biosolids; 6-anaerobically digested biosolids.

Appendix F. Soil characteristics of total 48 plots in spring and fall 2009 for Chapter 6.

rep	CT/NT	treat	pH	P	K	Ca	Mg	Zn	Mn	Cu	Fe	CEC
	Spring, 2009			Mehlich 1 extractable mg kg^{-1}								
1	1	1	6.69	21	73	385	45	0.9	5.2	0.4	6.9	2.5
1	1	2	6.41	23	65	396	46	0.9	6	0.4	7.6	2.6
1	1	3	5.91	15	81	344	50	0.9	4.9	0.3	8.4	2.4
1	1	4	6.14	16	70	376	50	0.9	6.5	0.4	8.3	2.6
1	1	5	6.21	26	87	615	50	1.6	8.9	0.6	18.9	3.7
1	1	6	6.03	28	83	364	50	1.3	8.1	0.4	20.7	2.5
1	2	1	6.63	20	67	384	43	0.9	4.8	0.4	7.4	2.5
1	2	2	6.35	35	93	447	48	1.2	5.5	0.4	7.6	2.9
1	2	3	6.39	33	67	447	46	1.1	5.3	0.4	7.9	2.8
1	2	4	6.14	30	69	426	45	1.1	5.3	0.5	7.7	2.7
1	2	5	6.45	21	68	465	42	1	5.6	0.5	8.2	2.9
1	2	6	5.94	26	69	437	47	1.3	9.9	0.5	14.7	2.8
2	1	1	6.14	12	62	306	32	0.8	4.5	0.3	10	2
2	1	2	6.54	12	75	344	48	0.8	4.8	0.3	7.5	2.4
2	1	3	6.22	11	65	324	42	0.8	4.9	0.3	8	2.2
2	1	4	5.5	16	98	339	42	1.1	7.3	0.4	8.7	2.5
2	1	5	6.31	34	72	1018	39	2.5	9.6	0.8	30.8	5.6
2	1	6	5.51	22	66	330	40	1.5	8.6	0.5	21.2	2.4
2	2	1	6.19	28	78	371	38	1.2	5.2	0.3	10.2	2.4
2	2	2	5.9	20	66	318	33	1	3.6	0.4	8.3	2.1
2	2	3	5.95	52	65	404	35	1.6	4.8	0.4	9.8	2.6
2	2	4	5.73	22	65	324	32	1.1	5.1	0.4	10	2.6
2	2	5	6.31	23	75	555	36	1.5	7.1	0.5	17.3	3.3
2	2	6	5.54	19	70	375	35	1.2	6	0.4	14.2	2.7
3	1	1	6.55	31	65	428	49	1.2	5.5	0.4	8	2.8
3	1	2	6.38	21	64	354	43	1	4.5	0.3	8.4	2.3
3	1	3	6.2	21	61	367	43	1	5.1	0.3	7.6	2.4
3	1	4	5.86	32	66	398	44	1.2	5.6	0.4	8.6	2.6
3	1	5	6.47	28	58	537	43	1.5	6.7	0.5	11.4	3.2
3	1	6	5.98	36	61	386	46	1.7	8.7	0.5	2.3	2.5
3	2	1	6.63	30	83	416	46	1.2	5.3	0.4	7.6	2.7
3	2	2	6.29	28	77	405	44	1.1	5.2	0.4	8	2.6
3	2	3	6.13	29	69	473	47	1.1	6.5	0.4	8.1	3
3	2	4	6.2	47	77	460	46	1.4	5.7	0.4	8.3	2.9
3	2	5	6.57	29	72	591	47	1.5	7.3	0.5	11.4	3.6
3	2	6	6.21	32	72	480	52	1.7	9	0.5	13.8	3.1
4	1	1	6.27	16	64	365	37	0.9	4	0.3	9.6	2.3
4	1	2	5.77	22	67	332	29	1	5	0.4	9.1	2.4
4	1	3	6.15	18	54	322	39	0.9	3.8	0.3	8.7	2.1
4	1	4	5.5	21	64	329	30	1	6.9	0.3	10.3	2.6

Appendix F continued

rep	CT/NT	treat	pH	P	K	Ca	Mg	Zn	Mn	Cu	Fe	CEC
	Spring, 2009			Mehlich 1 extractable mg kg^{-1}								
4	1	5	6.81	38	54	972	42	2.6	9.8	0.7	33.1	5.3
4	1	6	5.61	21	64	316	38	1.3	5.8	0.4	17	2.4
4	2	1	6.23	24	76	368	39	1.2	4.8	0.4	8.6	2.5
4	2	2	6.28	26	59	382	46	1.1	5.1	0.4	8.1	2.5
4	2	3	6	24	67	346	39	1	4	0.4	7.7	2.3
4	2	4	5.74	21	67	346	42	1	4.9	0.4	8.6	2.5
4	2	5	6.96	38	64	1130	45	2.7	10.7	0.8	31.4	6.2
4	2	6	5.69	30	61	391	44	1.6	8.5	0.5	19	2.9
	Fall, 2009											
1	1	1	6.91	11	49	404	54	0.7	5.5	0.5	6.3	2.6
1	1	2	6.78	13	42	348	41	0.7	4.9	0.4	6.4	2.2
1	1	3	7.14	13	62	346	54	0.7	6	0.4	6.4	2.3
1	1	4	7.05	12	52	347	52	0.7	5.8	0.4	6.2	2.3
1	1	5	7.72	33	73	1030	47	2.5	10	0.9	28.3	5.7
1	1	6	7.04	34	52	389	54	1.7	7.3	0.7	28.1	2.5
1	2	1	7.01	36	50	406	44	1	5.2	0.5	8.5	2.5
1	2	2	6.86	22	57	369	42	0.8	4.8	0.4	7.3	2.4
1	2	3	6.7	40	55	437	42	1.1	5.4	0.5	7.4	2.7
1	2	4	6.57	20	46	384	43	0.9	5.4	0.5	7.4	2.4
1	2	5	7.53	42	42	1153	40	2.8	9.7	0.9	26.3	6.2
1	2	6	6.45	31	34	374	43	1.7	7.1	0.7	23	2.3
2	1	1	6.78	10	65	298	34	0.7	5.3	0.4	8.1	2
2	1	2	6.23	9	73	272	31	0.7	5.8	0.4	7.3	1.9
2	1	3	6.49	11	54	289	39	0.7	5.4	0.4	6.7	1.9
2	1	4	6.31	8	84	268	32	0.6	5.8	0.4	6.5	1.9
2	1	5	6.82	20	51	595	33	1.7	7.1	0.7	17	3.4
2	1	6	6.24	30	62	301	33	1.7	6.7	0.7	29.2	2
2	2	1	6.52	20	69	314	40	1	5.5	0.5	11	2.1
2	2	2	6.52	36	82	338	43	1.2	6	0.4	10.7	2.3
2	2	3	6.44	18	59	298	33	1	5.2	0.4	8	1.9
2	2	4	6.29	21	73	281	32	1	5.7	0.5	9.8	1.9
2	2	5	7.17	25	64	607	32	1.6	7.6	0.7	18.6	3.4
2	2	6	6.13	53	69	331	34	2.7	8.9	0.9	48.8	2.2
3	1	1	6.89	19	43	345	39	1	5.2	0.5	10	2.2
3	1	2	6.77	18	43	384	43	0.9	5.5	0.5	7.9	2.4
3	1	3	6.82	32	43	409	43	1.1	5.9	0.5	7.9	2.5
3	1	4	6.52	20	39	332	37	1	4.8	0.5	7.4	2.1
3	1	5	7.36	26	47	579	33	1.6	8.1	0.6	14.8	3.3
3	1	6	6.48	40	35	381	40	1.8	7.8	0.7	32.8	2.3
3	2	1	6.85	39	50	421	44	1.4	6.9	0.5	9.4	2.6

Appendix F continued

rep	CT/NT†	treat#	pH	P	K	Ca	Mg	Zn	Mn	Cu	Fe	CEC
	Fall, 2009			Mehlich 1 extractable mg kg^{-1}								
3	2	2	6.63	29	49	398	41	1.1	6.6	0.5	7.5	2.5
3	2	3	6.74	22	41	371	38	1	6.2	0.5	7.3	2.3
3	2	4	6.4	23	49	347	38	1	6.3	0.4	7.4	2.2
3	2	5	7.41	29	42	667	38	1.6	8.5	0.7	15.7	3.7
3	2	6	6.21	38	39	396	37	1.8	8.4	0.7	24	2.4
4	1	1	6.66	17	44	315	36	0.8	5.5	0.4	90.9	2
4	1	2	6.3	16	46	290	27	0.8	5.3	0.5	8.1	1.8
4	1	3	6.24	11	52	246	26	0.9	5.9	0.4	8.8	1.6
4	1	4	5.92	13	50	227	23	0.8	5.4	0.4	8.5	1.5
4	1	5	7.29	23	45	630	25	1.6	8.9	0.6	18.9	3.5
4	1	6	5.97	27	44	279	27	1.5	6.7	0.7	27.7	1.8
4	2	1	6.85	22	61	381	46	1.1	6.8	0.5	9.3	2.5
4	2	2	6.63	39	76	401	45	1.2	7	0.5	8.1	2.6
4	2	3	6.54	23	54	407	69	1	7	0.5	8.5	2.8
4	2	4	6.47	21	53	314	42	1	5.9	0.5	8.8	2.1
4	2	5	6.57	21	39	441	21	1.3	6.7	0.7	17.1	2.5
4	2	6	6.26	40	51	329	34	2.2	8.2	0.7	32.3	2.1

†CT/NT: 1-conventianl tillage; 2-no-tillage.

#treat: 1-0xN; 2-0.5xN; 3-1xN; 4-1.5xN; 5-lime stabilized biosolids; 6-anaerobically digested biosolids.

Appendix G. Soil characteristics of total 48 plots in spring and fall 2010 for Chapter 6.

rep	CT/NT†	treat#	pH	P	K	Ca	Mg	Zn	Mn	Cu	Fe	CEC
	Spring, 2010			Mehlich 1 extractable mg kg^{-1}								
1	1	1	6.46	11	57	349	43	0.6	5.7	0.4	6.8	2.3
1	1	2	6.16	11	66	368	42	0.8	6.9	0.5	6.1	2.4
1	1	3	5.74	12	61	354	45	0.8	6.9	0.5	6.6	2.3
1	1	4	5.43	12	65	339	41	0.7	7.4	0.5	7.2	2.2
1	1	5	6.83	13	59	497	45	1.1	7.1	0.6	11	3
1	1	6	6.24	28	76	382	49	1.6	12.4	0.7	23.1	2.5
1	2	1	6.15	10	62	305	34	0.6	5.7	0.5	6.6	2
1	2	2	5.77	8	91	334	42	0.8	5.6	0.5	7.1	2.3
1	2	3	5.58	7	61	317	39	0.6	5.5	0.5	6.9	2.1
1	2	4	5.14	7	70	308	43	0.6	7.2	0.5	7.6	2.2
1	2	5	6.19	5	89	348	44	0.6	4.7	0.5	6.4	2.4
1	2	6	6.12	9	77	333	44	1	7.4	0.5	11.4	2.3
2	1	1	6.62	21	78	381	53	0.7	5.8	0.4	7.4	2.6
2	1	2	6.09	10	70	337	42	0.7	5	0.5	6.8	2.2
2	1	3	5.74	9	67	391	58	0.7	6.7	0.5	6.8	2.6
2	1	4	5.62	20	69	378	54	0.8	6.1	0.5	7.1	2.6
2	1	5	6.73	16	71	583	53	1.1	6.4	0.6	13	3.5
2	1	6	6.05	14	69	406	55	1.1	7.5	0.6	10.8	2.7
2	2	1	6.87	9	83	401	57	0.6	5.1	0.4	6.4	2.7
2	2	2	6.44	9	84	428	57	0.7	4.7	0.6	8.6	2.8
2	2	3	6.18	8	77	405	60	0.6	5.4	0.4	6.1	2.7
2	2	4	5.89	10	76	427	70	0.6	6	0.4	6.3	3
2	2	5	6.44	6	81	420	59	0.6	5.5	0.5	6.8	2.8
2	2	6	6.47	12	88	421	59	1	8.7	0.5	10.8	2.8
3	1	1	6.48	11	72	365	45	0.8	5.9	0.5	7.1	2.4
3	1	2	5.89	13	63	325	40	0.6	5.4	0.5	7	2.2
3	1	3	5.87	15	61	348	43	0.7	5.7	0.5	6.6	2.3
3	1	4	5.51	12	68	327	40	0.7	6.6	0.5	7	2.2
3	1	5	6.35	13	66	427	48	0.8	6.2	0.5	9.6	2.7
3	1	6	5.84	14	76	341	47	1	7.7	0.6	11.6	2.3
3	2	1	6.39	12	65	351	46	0.7	5.7	0.5	6.5	2.3
3	2	2	6.05	18	64	374	49	0.8	5.4	0.4	6.9	2.5
3	2	3	5.79	13	57	348	48	0.7	5.2	0.4	5.9	2.3
3	2	4	5.85	10	52	352	50	0.7	5.5	0.4	6.5	2.3
3	2	5	6.22	9	66	351	41	1.2	4.8	0.6	6	2.3
3	2	6	6.15	22	77	356	45	5.1	11.3	2.4	20.8	2.4
4	1	1	6.56	14	69	385	54	0.7	6	0.5	6.8	2.6
4	1	2	6.21	14	76	422	57	0.8	6.5	0.5	6.5	2.8
4	1	3	5.78	13	78	365	51	0.7	6.5	0.5	6.1	2.5
4	1	4	5.78	10	72	373	49	0.7	7	0.5	7.6	2.5

Appendix G continued

rep	CT/NT†	treat#	pH	P	K	Ca	Mg	Zn	Mn	Cu	Fe	CEC
	Spring, 2010			Mehlich 1 extractable mg kg^{-1}								
4	1	5	6.29	9	76	393	47	0.9	6.3	0.6	6.4	2.6
4	1	6	6.34	10	76	374	54	0.8	6.3	0.5	6.9	2.5
4	2	1	6.83	11	60	379	52	0.8	5.8	0.5	5.9	2.5
4	2	2	6.04	11	70	407	57	0.8	7.1	0.5	7.2	2.7
4	2	3	5.53	15	75	426	53	1	7.3	0.6	7.9	2.8
4	2	4	5.72	13	64	392	54	0.8	6.7	0.5	6.7	2.6
4	2	5	6.4	10	70	445	57	0.9	5.6	0.6	7.4	2.8
4	2	6	6.08	15	68	385	49	1.1	8	0.6	11.9	2.5
	Fall, 2010											
1	1	1	6.73	9	65	300	37	0.6	4	0.4	6.1	2
1	1	2	6.66	13	89	297	33	0.6	3.7	0.4	5.7	2
1	1	3	6.46	17	58	300	35	0.6	3.6	0.4	8.3	2
1	1	4	4.42	8	72	268	31	0.6	4.3	0.5	73.7	2.4
1	1	5	7.49	32	65	849	37	1.7	7.8	0.7	22.6	4.7
1	1	6	6.83	20	76	345	45	1.1	7	0.6	15.3	2.3
1	2	1	6.82	7	71	297	38	0.6	5	0.4	5.4	2
1	2	2	6.54	7	104	313	38	0.7	5.1	0.4	8.4	2.2
1	2	3	6.53	6	66	254	33	0.6	5	0.4	5.2	1.7
1	2	4	6.35	7	96	240	32	0.6	5.8	0.4	6.7	1.7
1	2	5	6.86	9	71	447	41	0.8	5.5	0.5	9.2	2.8
1	2	6	6.55	15	92	333	46	1.1	6.5	0.6	15.7	2.3
2	1	1	7.02	9	87	324	45	0.6	3.8	0.4	5	2.2
2	1	2	6.77	10	83	315	43	0.7	4.2	0.4	5.9	2.2
2	1	3	6.79	8	86	297	42	0.6	3.6	0.4	5.9	2.1
2	1	4	6.74	8	76	300	39	0.6	3.3	0.4	7.2	2
2	1	5	6.89	9	89	352	37	0.7	3.7	0.5	6.9	2.3
2	1	6	6.74	11	84	304	41	0.8	3.8	0.5	8.6	2.1
2	2	1	7.11	8	87	374	51	0.5	3.7	0.4	6.2	2.5
2	2	2	7.1	16	92	443	57	0.7	4.3	0.4	8.2	2.9
2	2	3	6.92	5	91	315	44	0.5	3.7	0.4	5.2	2.2
2	2	4	6.72	7	83	288	40	0.5	3.4	0.4	5.7	2
2	2	5	7.22	9	98	437	40	0.7	4.7	0.4	7.4	2.8
2	2	6	6.9	11	109	379	49	0.8	4.7	0.5	10.3	2.6
3	1	1	6.61	11	62	269	30	0.5	3.5	0.4	5.3	1.8
3	1	2	6.67	14	88	295	35	0.7	4.3	0.4	5.5	2
3	1	3	6.53	8	60	271	30	0.6	3.7	0.4	5.9	1.8
3	1	4	6.37	10	63	270	33	0.7	4	0.4	5.8	1.8
3	1	5	7.07	17	55	482	29	1	4.6	0.6	12	2.8
3	1	6	6.52	25	77	301	35	1.7	5.2	0.7	20.2	2
3	2	1	6.74	9	54	291	34	0.7	3.7	0.5	6.1	1.9

Appendix G continued

rep	CT/NT†	treat#	pH	P	K	Ca	Mg	Zn	Mn	Cu	Fe	CEC
	Fall, 2010			Mehlich 1 extractable mg kg^{-1}								
3	2	2	6.88	9	84	297	36	0.6	3.9	0.4	6.8	2
3	2	3	6.6	10	61	267	31	0.6	4	0.4	6.4	1.8
3	2	4	6.55	8	59	273	33	0.6	4.1	0.4	5.5	1.8
3	2	5	7.29	35	58	805	40	1.8	7.2	1	26.2	4.5
3	2	6	6.35	20	59	274	34	1.7	6.4	0.7	18.5	1.8
4	1	1	6.88	11	67	321	43	0.6	4	0.4	6.1	2.2
4	1	2	6.89	15	83	332	43	0.7	3.9	0.4	7.1	2.2
4	1	3	7.06	7	98	314	44	0.7	3.8	0.4	5.9	2.2
4	1	4	6.45	11	80	308	43	0.7	4.1	0.4	6.1	2.2
4	1	5	7.14	16	105	496	37	1	5.1	0.5	10.4	3
4	1	6	6.68	14	91	317	45	1.2	4.7	0.6	11.3	2.2
4	2	1	7.08	12	76	351	47	0.7	4.3	0.4	5.9	2.3
4	2	2	7.01	9	65	334	43	0.6	3.7	0.4	5.2	2.2
4	2	3	6.67	9	81	313	35	0.8	3.7	0.5	7.3	2.1
4	2	4	6.7	9	74	305	36	0.7	3.8	0.4	6.5	2
4	2	5	7.21	23	67	606	39	1.3	5.2	0.7	14.5	3.5
4	2	6	6.53	26	85	380	52	2	7	0.7	19.4	2.6

†CT/NT: 1-conventianl tillage; 2-no-tillage.

#treat: 1-0xN; 2-0.5xN; 3-1xN; 4-1.5xN; 5-lime stabilized biosolids; 6-anaerobically digested biosolids.

Appendix H. Soil characteristics of total 48 plots in spring and fall 2011 for Chapter 6.

rep	CT/NT†	treat#	pH	P	K	Ca	Mg	Zn	Mn	Cu	Fe	CEC
	Spring, 2011			Mehlich 1 extractable mg kg^{-1}								
1	1	1	6.7	16	75	369	50	0.8	4.9	0.5	6.2	2.5
1	1	2	6.06	31	94	404	56	0.9	6	0.5	7.5	2.8
1	1	3	5.69	16	96	372	60	0.8	8	0.5	9	2.9
1	1	4	5.72	20	100	385	60	1	7.4	0.5	9.5	2.7
1	1	5	6.7	11	103	491	51	1	8.2	0.6	13.5	3.1
1	1	6	6.02	69	122	442	72	2.8	13.5	1.1	63	3.2
1	2	1	6.62	22	79	351	42	0.9	3.9	0.5	8.1	2.3
1	2	2	6.2	23	98	414	51	0.9	4.4	0.5	7.7	2.8
1	2	3	6.27	20	82	414	47	0.9	5.1	0.5	6.7	2.7
1	2	4	5.93	33	84	417	50	0.9	4.9	0.5	6.9	2.8
1	2	5	7.11	28	76	608	33	1.3	6.4	0.7	14.2	3.5
1	2	6	6.15	43	103	397	50	1.9	6.5	0.8	29.4	2.7
2	1	1	6.84	15	68	282	30	0.8	4.2	0.7	9.2	2.1
2	1	2	5.76	14	73	329	47	0.8	4.9	0.4	8	2.3
2	1	3	5.61	12	71	307	40	0.8	5.2	0.5	8.1	2.3
2	1	4	5.38	11	81	315	39	0.7	7.1	0.4	7.1	2.6
2	1	5	7.02	25	94	618	31	1.5	7.6	0.8	21.7	3.6
2	1	6	5.45	48	88	322	42	1.9	9.2	0.9	45.8	2.4
2	2	1	6.24	21	82	310	35	1	4.9	0.6	8.5	2.2
2	2	2	6.05	14	63	331	31	0.9	4.4	0.6	9.1	2.4
2	2	3	5.57	13	93	314	34	0.9	6.7	0.6	8.7	2.4
2	2	4	5.41	21	84	323	43	1	6.8	0.6	9.2	2.7
2	2	5	7.19	26	59	619	24	1.5	7	0.8	24.5	3.4
2	2	6	5.94	27	122	351	43	1.6	5.8	0.8	21.5	2.5
3	1	1	6.45	25	84	422	49	1	5.5	0.5	9.2	2.8
3	1	2	6.22	23	86	400	60	1	5.6	0.5	10.3	2.8
3	1	3	5.58	25	93	372	56	0.9	8.2	0.4	7.3	2.7
3	1	4	5.69	29	130	388	53	1.1	8.5	0.5	8.2	2.9
3	1	5	7.22	45	77	922	41	2.2	9.8	0.8	27.4	5.1
3	1	6	5.89	49	71	398	50	2	7.1	0.8	37.3	2.6
3	2	1	6.43	32	97	410	53	1.1	5.3	0.5	7.4	2.8
3	2	2	6.22	27	131	459	58	1.1	5.8	0.5	8.2	3.2
3	2	3	6.09	22	109	404	55	1	4.8	0.5	6.4	2.8
3	2	4	5.86	24	83	397	52	1	5.1	0.5	8.3	2.7
3	2	5	7.37	32	72	712	36	1.7	7.3	0.7	18.4	4
3	2	6	6.09	47	107	478	63	2	7.4	0.8	25.6	3.2
4	1	1	6.17	15	90	300	36	0.8	4.6	0.5	7.8	2.1
4	1	2	5.84	26	84	365	45	1	5.9	0.5	8.8	2.6
4	1	3	5.44	21	100	310	41	0.9	9	0.5	8.4	2.5
4	1	4	5.66	24	80	364	44	1.1	8.2	0.5	10.7	2.6

Appendix H continued

rep	CT/NT†	treat#	pH	P	K	Ca	Mg	Zn	Mn	Cu	Fe	CEC
	Spring, 2011			Mehlich 1 extractable mg kg^{-1}								
4	1	5	7.18	43	98	904	44	2.2	10.7	0.9	32.7	5.1
4	1	6	5.53	60	102	387	50	2.3	10.5	1	55.8	3.1
4	2	1	6.28	21	81	319	35	0.8	4.2	0.5	7.7	2.1
4	2	2	6.01	19	74	330	37	0.9	4.4	0.5	7.4	2.3
4	2	3	5.62	18	96	336	44	1.1	6	0.5	7.8	2.4
4	2	4	5.56	21	99	348	45	1	6.3	0.5	7.5	2.7
4	2	5	7.06	31	76	638	31	1.6	7.3	0.9	23.7	3.6
4	2	6	5.86	45	85	378	45	1.8	7.5	0.9	33.4	2.8
	Fall, 2011											
1	1	1	6.97	17	58	360	48	0.8	4.7	0.5	6.7	2.3
1	1	2	6.91	22	55	365	44	0.9	5.4	0.5	7.9	2.4
1	1	3	6.94	16	75	361	50	0.9	6.2	0.5	10.4	2.4
1	1	4	6.8	18	60	316	45	0.9	4.8	0.5	8.9	2.1
1	1	5	7.43	33	66	688	44	2.1	8.4	0.9	23	4
1	1	6	6.86	47	69	388	50	2	6.9	1	40.5	2.6
1	2	1	7.01	21	53	366	42	1	4.9	0.5	8.7	2.3
1	2	2	6.99	25	72	446	52	1.2	6.4	0.6	8	2.8
1	2	3	7.12	23	63	434	52	1.1	12	0.5	10.5	2.8
1	2	4	6.75	34	52	436	54	1.1	6.6	0.5	8.2	2.8
1	2	5	7.97	53	57	1107	43	2.6	16.4	1.1	36.6	6
1	2	6	6.8	51	58	439	53	2.3	6.7	1	38.4	2.8
2	1	1	6.7	23	63	320	36	1	6	0.6	11.5	2.1
2	1	2	6.7	14	64	306	44	0.7	4.9	0.5	6.5	2.1
2	1	3	6.65	10	62	298	38	0.7	4.3	0.4	6.9	2
2	1	4	6.47	25	62	317	38	0.7	4.1	0.4	6.9	2.1
2	1	5	7.72	32	49	759	29	2.4	9.6	1	31.9	4.2
2	1	6	6.39	34	53	318	45	2.2	5.5	1.1	36.2	2.1
2	2	1	6.57	23	76	337	38	1.1	5.7	0.6	9.4	0.2
2	2	2	6.73	16	62	296	35	0.9	5.1	0.5	8.3	2
2	2	3	6.75	20	60	367	39	1.1	5.9	0.5	10.6	2.3
2	2	4	6.46	19	69	311	41	1	6.3	0.5	9.7	2.1
2	2	5	7.97	57	58	1225	37	3.2	12.1	1.2	43.7	6.6
2	2	6	6.24	41	55	291	34	2.2	7.7	0.9	37.4	1.9
3	1	1	6.91	26	49	376	44	1	4.8	0.5	8.7	2.4
3	1	2	6.88	20	53	343	43	0.9	4.5	0.5	8.5	2.2
3	1	3	6.88	24	53	373	49	0.9	5.5	0.4	6.5	2.4
3	1	4	6.62	21	49	354	43	1	4.5	0.5	8	2.3
3	1	5	7.67	43	51	882	38	2.5	8.2	0.9	26.3	4.8
3	1	6	6.66	53	48	385	45	2.3	6.3	1	48	2.4
3	2	1	6.75	23	63	365	44	1	4.8	0.5	6.8	2.4

Appendix H continued

rep	CT/NT†	treat#	pH	P	K	Ca	Mg	Zn	Mn	Cu	Fe	CEC
	Fall, 2011			Mehlich 1 extractable mg kg^{-1}								
3	2	2	7.02	23	61	424	45	1.4	4.9	0.6	9	2.6
3	2	3	6.69	23	50	356	44	1	4.7	0.5	6.9	2.3
3	2	4	6.69	28	54	359	44	1.1	4.5	0.5	7.8	2.3
3	2	5	7.87	65	59	1439	47	3.2	11.1	1.1	31.2	7.7
3	2	6	6.69	38	52	415	48	1.7	5.5	0.8	21.5	2.6
4	1	1	6.71	24	59	318	38	0.9	6.3	0.5	10.5	2.1
4	1	2	6.78	22	46	374	48	1.1	5.6	0.5	10.4	2.4
4	1	3	6.83	15	52	314	39	1	12	0.5	13.2	2
4	1	4	6.35	23	51	291	31	0.9	5.5	0.4	8.9	1.9
4	1	5	7.84	31	64	724	38	2	9.3	0.8	26.6	4.1
4	1	6	6.58	53	82	349	41	2.3	8.4	1	52.4	2.3
4	2	1	6.84	16	58	340	52	0.9	5.6	0.5	7.9	2.3
4	2	2	6.9	17	63	321	43	0.9	5.6	0.5	7.5	2.1
4	2	3	6.43	21	53	299	37	1.1	5.4	0.5	10.5	2
4	2	4	6.39	15	59	275	34	0.9	4.7	0.5	7.7	1.8
4	2	5	7.88	49	49	1056	32	3.6	10.1	1.2	45.3	5.6
4	2	6	6.41	52	61	330	38	2.2	6.2	1	42.9	2.2

†CT/NT: 1-conventianl tillage; 2-no-tillage.

#treat: 1-0xN; 2-0.5xN; 3-1xN; 4-1.5xN; 5-lime stabilized biosolids; 6-anaerobically digested biosolids.

Appendix I: Fall soil C and N concentration and soybean yield in 48 plots in Chapter 6.

rep	CT/NT†	treat#	moisture	yield	soil N	soil C	C:N	moisture	yield	soil N	soil C	C:N
			%	Mg ha^{-1}	g kg^{-1}	g kg^{-1}		%	Mg ha^{-1}	g kg^{-1}	g kg^{-1}	
1	1	1	17.2	0.93	0.33	3.8	11.4	13.8	3.54	0.4	3.74	9.32
1	1	2	17.2	0.92	0.35	4.16	11.9	14.2	3.39	0.48	5.07	10.51
1	1	3	15.8	0.38	0.35	4.05	11.5	13.6	3.86	0.43	4.03	9.27
1	1	4	16.4	0.79	0.33	3.88	11.6	14	2.96	0.45	4.39	9.7
1	1	5	16.3	0.41	0.49	4.66	9.6	14	3.45	0.46	4.02	8.8
1	1	6	16.1	0.73	0.37	4.03	10.9	13.9	3.17	0.52	4.91	9.45
1	2	1	16.2	0.85	0.31	4.66	15.2	14.1	3.1	0.36	3.46	9.71
1	2	2	16.9	0.81	0.36	4.32	12	14.1	3.13	0.5	5	10.06
1	2	3	16.8	0.84	0.39	4.65	11.9	14.2	2.7	0.39	3.68	9.32
1	2	4	16.1	0.84	0.4	4.8	12	14.7	2.6	0.38	3.45	9
1	2	5	15.8	0.84	0.38	4.51	12	15.2	3.12	0.5	5.03	10.13
1	2	6	15.5	1.04	0.39	4.59	11.8	15.8	3.06	0.47	4.54	9.71
2	1	1	16.5	0.27	0.34	3.96	11.5	14.5	2.35	0.42	4.14	9.81
2	1	2	16.3	0.54	0.37	4.37	11.7	14.1	3.01	0.53	4.28	8.03
2	1	3	15.5	0.83	0.32	3.31	10.3	14.5	3.06	0.46	4.23	9.16
2	1	4	16.2	1.09	0.33	3.51	10.6	14.2	2.97	0.49	4.68	9.53
2	1	5	15.8	1.37	0.43	4.88	11.4	14.1	3.11	0.62	5.62	9
2	1	6	17.1	1.62	0.37	3.97	10.7	14.5	2.84	0.6	5.56	9.3
2	2	1	16.2	0.27	0.4	4.43	11	14.5	2.82	0.49	4.43	9.02
2	2	2	16.7	0.54	0.38	4.25	11.1	14.2	2.36	0.49	4.51	9.27
2	2	3	16.8	0.81	0.39	4.63	12	15	3.4	0.44	3.91	8.82
2	2	4	16.4	1.09	0.31	4.13	13.3	14.5	3	0.4	3.83	9.51
2	2	5	16.9	1.35	0.49	5.04	10.4	14.9	2.61	0.45	4.62	10.24
2	2	6	16.3	1.63	0.38	4.21	11.2	14.2	2.9	0.55	4.56	8.31
3	1	1	15.8	0.97	0.37	4.5	12.3	14.1	3.1	0.43	4.14	9.72
3	1	2	15.9	0.55	0.34	4.71	13.8	14.3	3.66	0.38	3.87	10.09
3	1	3	15.1	0.6	0.37	4.24	11.6	13.7	3.17	0.42	3.94	9.32
3	1	4	16.1	0.98	0.35	4.11	11.8	14	3.11	0.48	4.61	9.61
3	1	5	16.3	0.95	0.44	5.11	11.5	13.8	3.87	0.46	4.9	10.58
3	1	6	15.5	0.66	0.39	5.01	12.7	14.5	2.74	0.47	4.5	9.66
3	2	1	16.1	0.96	0.34	4.06	12	13.9	3.3	0.45	4.14	9.16
3	2	2	15.9	0.93	0.41	4.98	12	13.5	3.23	0.41	3.98	9.81
3	2	3	15.7	1.01	0.4	4.8	12	13.6	3.29	0.41	3.89	9.57
3	2	4	15.7	0.96	0.62	5.06	8.1	13.7	3.31	0.4	3.56	8.94
3	2	5	16.4	1.03	0.48	5.85	12.1	14.5	2.79	0.49	4.46	9.2
3	2	6	15	1.01	0.47	5.5	11.8	14.2	3.17	0.56	5.33	9.6
4	1	1	16.3	0.27	0.33	3.7	11.3	14.2	2.92	0.4	4.27	10.64
4	1	2	15.5	0.55	0.37	4.4	11.8	14.4	3.11	0.56	4.54	8.18
4	1	3	15.8	0.82	0.35	3.76	10.7	13.9	3	0.48	4.18	8.69

Appendix I continued.

rep	CT/NT†	treat#	moisture	yield	soil N	soil C	C:N	moisture	yield	soil N	soil C	C:N
			%	Mg ha^{-1}	g kg^{-1}	g kg^{-1}		%	Mg ha^{-1}	g kg^{-1}	g kg^{-1}	
4	1	4	15.4	1.1	0.34	3.81	11.2	13.8	3.14	0.63	4.77	7.61
4	1	5	16.2	1.36	0.41	4.65	11.2	14.1	3.01	0.59	5.57	9.52
4	1	6	15.6	1.65	0.37	4.08	10.9	14.3	3.3	0.67	4.67	6.95
4	2	1	15.8	0.27	0.38	5.11	13.6	13.9	3.11	0.41	3.67	8.98
4	2	2	16.3	0.54	0.38	4.45	11.6	14.3	2.98	0.43	4.11	9.63
4	2	3	16.4	0.82	0.43	4.55	10.5	14.6	2.31	0.48	4.62	9.64
4	2	4	16.9	1.08	0.38	4.11	11	14.1	3.34	0.48	4.19	8.74
4	2	5	16.2	1.36	0.4	4.51	11.2	14.7	2.83	0.66	4.54	6.87
4	2	6	15.7	1.65	0.42	4.71	11.2	13.8	3.1	0.57	5.38	9.52

†CT/NT: 1-conventianl tillage; 2-no-tillage.

#treat: 1-0xN; 2-0.5xN; 3-1xN; 4-1.5xN; 5-lime stabilized biosolids; 6-anaerobically digested biosolids.