To the Graduate Council:

I am submitting herewith a dissertation written by Jason Patrick Wight entitled "Soil Carbon and Microfauna Changes as Influenced by a Combination of Bio-covers and Cropping Sequences of Glyphosate Tolerant Corn, Soybean, and Cotton." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plants, Soils and Insects.

Fred L. Allen, Major Professor

I have read this dissertation
and recommend its acceptance:

Donald D. Tyler

Patricia A. Donald

Timothy G. Rials

Arnold M. Saxton

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean
of the Graduate School

(Original signatures are on file with official student records.)

Soil Carbon and Microfauna Changes as Influenced by Combinations of Bio-covers and Cropping Sequences of Glyphosate Tolerant Corn, Soybean, and Cotton

A Dissertation

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Abstract

The objective of this research was to compare the effects of combinations of a wide range of cropping sequences and soil bio-covers on soil organic carbon (SOC) and *Heterodera glycines* under no-tillage. The experiment used a split-block design with four replications at the Milan (RECM) and Middle TN (MTREC) Research & Education Centers. The whole-block treatment was cropping sequences of corn (*Zea mays*), soybean (*Glycine max*), and cotton (*Gossypium hirsutum*). The split-block was bio-covers of winter wheat (*Triticum aestivum*), hairy vetch (*Vicia villosa*), poultry litter, and fallow. Soil samples were taken over four years of experimentation. A novel technique was developed to measure SOC called near infrared reflectance spectroscopy (NIR). Partial least squares regression was used to predict SOC as measured by NIR. Results were compared to assess the reliability of NIR determination of organic carbon. NIR analysis correlated well (r greater than 0.9) with SOC as determined by combustion. NIR was therefore considered sufficiently accurate for quantifying soil organic carbon.

Overall, both sites showed a small but consistent loss in carbon over all treatments. The average losses of SOC at the 0-5 cm depth were 1.06 and 1.40 Mg ha^{-1} at RECM and MTREC, respectively. In the subsurface, mean SOC loss at RECM was 1.43 Mg ha^{-1}. Conversely, the subsurface SOC at MTREC was 3.14 Mg ha^{-1} higher than pre-experiment levels. Crop sequence had a significant effect on change in SOC ($P \leq 0.05$) in both surface and subsurface regions at RECM. Sequences with two or more years of cotton tended to lose significantly more ($P \leq 0.05$) SOC than those with two or more

years of soybean. The poultry litter bio-cover lost less surface SOC (0.58 Mg ha^{-1}) than those under vetch (1.33 Mg ha^{-1}) or fallow (1.8 Mg ha^{-1}).

Soybean cyst nematode (SCN) eggs were found in sequences containing no soybean throughout the experiment, indicating survival of over seven years without soybean. Sequences with two or more years of soybean had significantly higher ($P \leq 0.01$) SCN egg density than others, with continuous soybean having the greatest egg population density. One year of rotation with a non-host crop typically reduced SCN egg population density by more than 50%.

Preface

A major point of debate at the November, 2000 meeting of the United Nations Framework for Climate Change in The Hague was whether countries would be allowed to claim credits for carbon stored in soils and forests. Disagreement over carbon credits eventually led to a suspension of the talks (Fuller, 2000). Although talks have resumed, several countries are in disagreement over potential carbon storage credits.

To date, the United States is not participating in the Kyoto Treaty to reduce greenhouse emissions, but is independently developing a system of rules and guidelines for carbon sequestration. Part of these guidelines is a voluntary carbon dioxide emission and sequestration reporting system (US Department of Energy form EIA 1605 2003). If successful, this system may ultimately lead to the creation of a Federal “carbon credit system” which would give incentives to industry and agriculture for reducing CO_2 emission and sequestration. Currently, carbon credits in the 1605(b) plan tailored to detailed soil management strategies have not been developed due to a general lack of knowledge about management effects in specific systems (Jim Hrubovcak, USDA 2003). Even so, the Chicago Climate Exchange™ has taken the initiative to develop an independent carbon trading market and has enlisted the help of organizations such as the Nation Farmers Credit Union to administer a system that pays agricultural producers for sequestering greenhouse gasses to later be sold to industry. Currently, farmers can earn up to two and four dollars per acre for grass and no-tillage production, respectively (National Farmers Credit Union, 2007).

Agriculture is responsible for approximately 5% of total U.S. carbon emissions of which carbon mineralization from conventional tillage practices make up a significant part. Tillage destroys crop residue and exposes soil organic matter to oxygen, thereby promoting decomposition and mineralization of crop residue and existing soil C stocks. Agriculture can reduce CO_2 emission by shifting production methods away from conventional tillage. One alternative to conventional tillage is known as "no-till."

No-till is a crop production strategy where the soil is essentially undisturbed and crop residue is left on the surface rather than plowed underground. Crop production without tillage has been shown to increase soil carbon as compared to conventional tillage (Campbell et al., 1999; Angers and Giroux, 1996; Karlen et al., 1994). Under no-till management, atmospheric C fixed by crops can be transferred to the undisturbed subsoil. There, it is protected from rapid oxidation and may become part of the recalcitrant soil carbon fraction referred to as humus (Ding et al., 2002). Using no-till, agricultural systems can play a positive role in the global carbon cycle by converting arable soils from a carbon source to a carbon sink. In a review of 39 paired studies ranging in length from 5 to 20 years, Pautisian et al. (1997) concluded that no-till increased soil carbon an average of 285 g per m^2 with respect to conventional tillage. Lal et al. (1998) estimated that the total amount of C that can be sequestered by managers of U.S. croplands following "recommended" practices to be between 2,500 and 3,500 million megatons. Land under optimized no-till production could therefore be an important resource in a carbon credit system. However, it remains unclear how specific cropping sequences and bio-covers affect soil carbon. A controlled study of carbon dynamics under several no-till production practices will help optimize both carbon storage and productivity.

In cropland converted from conventional tillage to no-till, location and availability of soil nutrients is expected to change. Tillage and management induced enhancement of soil organic matter can affect nutrient cycling throughout the soil profile by reducing erosion, increasing cation exchange capacity (CEC), and releasing plant nutrients upon breakdown (Carter and Stewart, 1996). Tillage can also affect nutrient cycling by causing stratification, in which applied fertilizer (Blevins et al., 1983), lime (Dick, 1983), and organic matter (Franzluebbers and Hons, 1996; Ismail et al., 1994) accumulate near the soil surface. Nutrient stratification may result from reduced mechanical mixing while increases in CEC may allow an increase in overall soil nutrients (Mahboubi et al. 1993).

Soil organic carbon may be affected by crop sequence complexity, mulch rate, and mulch quality. Studies have found increasing crop residue input has a direct correlation with soil carbon (Clapp et al., 2000; Duiker and Lal, 1999). Furthermore, in a survey of soil carbon studies, West and Post (2002) found that when farmland is converted from conventional till to no-till, increasing rotation complexity enhances carbon sequestration. However, the group also presented contrary results for systems already under no-till. In existing no-till systems, enhancing complexity did not directly result in increased soil carbon.

Duiker and Lal (2002) concluded that mulch rate of bio-covers was directly related to gains in soil organic carbon. As overall system productivity increases, mulch returned to the soil surface should also increase. However, this relationship may be confounded by mulch characteristics. In addition to mulching rate, quality can also affect soil carbon. Drinkwater et al. (1998) suggest low carbon-to-nitrogen organic residues increased carbon retention in soil. This study helps clarify how soil carbon is affected by bio-cover, cropping sequence, and their interactions under no-till production.

Production methods including cropping sequence and bio-cover can also affect nematode populations. Nematodes are a diverse, complex group of microscopic roundworms that can be found in all soils. Plant-parasitic species can severely impact agricultural productivity. An estimated $100 billion in annual yield losses are due to nematode damage (Sasser and Freckman, 1987; Koenning et al., 1999). Nematodes can play such a significant role, that when other limiting factors are taken into account, their populations can be used as predictors of yield losses in annual crops (Barker et al., 1985). Compounding the nematode-plant predation problem, the United States has implemented a phase-out of the most effective chemical nematicides due to environmental quality concerns (UNEP, 1992). These restrictions have already caused growers to incur increased nematode crop damage (Roberts, 1993). Therefore, improvements in alternative methods of nematode control are critical to maintain agricultural productivity and profitability.

Crop rotation represents one such method of nematode control, and has been used successfully for centuries. This control strategy involves alternating susceptible crops with non-host species. Years in which non-host crop production takes place allow nematode populations to starve and die back over time, essentially waiting out the harmful species.

The amount of time that critical plant-parasitic species can survive in soil with no host is dependent on a variety of factors, with annual temperature pattern being a major one. Cool temperatures promote survival of soybean cyst nematode (*Heterodera glycines*) (Kinloch, 1998). Therefore, the length of rotations with non-host crops required to reduce these priority nematode species to sub-threshold levels is dependant on regional weather conditions.

Winter cover crops are an important part of no-till cultivation; reducing erosion and promoting soil and water quality. They can also effect nematode populations. Winter covers that act as alternative host plants can increase nematode populations by allowing reproductive or over-wintering sites. The cover crops can break nematodes' dormancy, causing them to be exposed to harsh conditions and killed. Other covers act as trap crops; nematodes enter roots, but do not leave or reproduce.

The effect on nematode communities is unknown when cropping sequence and winter cover are used in conjunction with no-till cultivation methods. Conservation tillage systems may not have the same beneficial effects on nematode populations as disking or plowing. Mechanical tillage causes root material to be brought to the surface, causing it to be exposed to harsh environmental conditions, which can reduce overall nematode survival (Duncan and Noling, 1988). However, no-tillage systems have been shown to reduce SCN survival (Tyler et al., 1987). Intact crop residue in no-till systems has been shown to increase water infiltration (Dao, 1993) and reduce surface soil temperatures (Ross, 1964), both of which can effect SCN development (Ross, 1964; Alston and Schmitt, 1987) and infectivity (Young and Heatherly, 1988). Stubble can also provide habitat for nematode antagonists, negatively impacting nematode species

(Bernard et al., 1996). SCN is the pathogen that is responsible for the most yield loss of soybean in North America. Its' survival and reproduction can be effected by environmental and management conditions. This study documents the effect of cropping sequence and bio-cover on SCN egg population density in a silt loam soil in southwestern Tennessee.

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TABLE OF CONTENTS

Part III: Impact of cropping sequence and bio-covers on the soybean cyst nematode, *Heterodera glycines*

LIST OF TABLES

LIST OF FIGURES

Part I

Development of Near-infrared Reflectance Spectroscopy for Measurement of Soil Carbon

Abstract

As interest in soil carbon dynamics and sequestration grows, so does the need for rapid, accurate, and inexpensive methods for quantifying soil organic carbon. The objective of this research was to evaluate the potential of near infrared reflectance (NIR) spectroscopy to determine soil organic carbon (SOC) content in a variety of soils collected from across Tennessee. Soils were collected from 14 sites at three depths. All samples were analyzed via dry combustion (SOC), a Walkley-Black chemical soil organic matter (SOM), and NIR methods. In addition, samples were air-dried and processed to give five surface roughness levels to determine the effect of particle size on NIR measurement quality. Spectra were obtained using a LabSpec Pro® near infrared spectrometer. Partial least squares regression was used to develop a model able to predict SOC as measured by NIR. Results from the three methods; SOC, SOM, and NIR, were compared to assess the reliability of NIR determination of organic carbon. Both NIR and SOM analysis correlated well (r greater than 0.9) with SOC as determined by combustion. NIR is therefore considered to be a sufficiently accurate method of quantifying soil organic carbon.

CHAPTER I

Introduction

Due to the recent trend of increasing atmospheric CO_2 concentrations, interest in carbon dynamics and sequestration has increased. Soil carbon sequestration has the potential to be an inexpensive and widely utilized form of carbon storage. However, the size and nature of SOC pools can be affected by a variety of factors including local management practice, climate, and soil type. Therefore, studies across a wide array of systems must be undertaken to understand soil carbon sequestration. This has created a need for a rapid, accurate, and inexpensive method for quantifying SOC.

One method that has recently demonstrated the potential to fulfill this demand is near-infrared reflectance spectroscopy (NIR). NIR based technology has been successfully used for grain characterization for almost 35 years (Ben-Gera and Norris, 1968), and has recently been expanded to other areas. By 1986, Dalal and Henry had used NIR to predict organic matter in soil using NIR. In 1995, Ben-Dor and Banin had achieved high correlations between NIR signal intensity at specific wavelengths correlated to soil organic matter functional groups. More recently McCarty and Reeves (2000) presented a refined analytical and statistical technique resulting in a NIR model with r^2 value of 0.90 encompassing a wide range of Midwestern soils. However, one drawback of this method is that it requires a soil library and calibration model to be developed so that NIR signals can be correlated to traditionally analyzed soil properties.

NIR relies on the principle that photons striking a surface interact with molecules of that material. During this interaction, photons may be reflected, refracted, or absorbed.

A full explanation of photon interface phenomena has been produced by Chalmers and Griffiths (2001) or Williams and Norris (2001). The aspects relevant to near infrared reflectance spectroscopy of soil organic carbon will be briefly discussed.

Photons can be absorbed if their wavelengths match either the bond length between atoms of a molecule or the vibrational angles between bonded atoms. Each molecule has 3*n*-6 different modes of vibration, where *n* is the number of constituent atoms of the molecule (Clark, 1999). For example, H_2O has three atoms, so it has three fundamental vibrations. Each of these vibration modes has a fundamental frequency. If this frequency matches the wavelength of an incoming photon, the photon can be absorbed. Spectral patterns formed by adsorption at fundamental frequencies are known as fundamental tones.

The frequency of bond vibration can also occur in approximate multiples of the original fundamental frequency. Therefore, photon adsorption at multiples of the fundamental frequency can also occur. This type of adsorption is referred to as an overtone, and typically is 30 to 100 times weaker than the fundamental frequency. Adsorption can also occur at wavelengths involving two or more different fundamental overtones. The wavelengths that this third type of adsorption occurs at are known as combination tones.

The various photon-surface interactions at fundamental tones, overtones, and combination tones can lead to several absorption bands within an NIR spectrum for even simple molecules. Many molecules and functional groups have specific signatures in the near-infrared. This trait allows NIR spectroscopy to be routinely used to obtain qualitative information about the makeup of various minerals (Hunt and Salisbury, 1970;

Hunt et. al, 1971; Hunt, 1989) including carbonates (Gaffey, 1986), aluminosilicate clays (Ben-Dor and Banin, 1990; Hauff et. al, 1990; Madejova, and Komadel, 2001), and the interactions of water with minerals (Idso et. al, 1975; Cariati et. al, 1981; and Cariati et. al, 1983).

In addition to qualitative analysis, Clark and Roush (1984) noted the potential to quantitatively describe mineral abundances in a multimineral surface using physical models based on reflectance spectra. Early on, there were successes using direct physical modeling that relied on the intensity of absorption bands (reflectance [R], absorption [$A = \log 1/R$]) at specific wavelengths to determine the concentration of the constituents of interest. Dalal and Henry (1986) used this technique with multiple regression of absorption at specific infrared wavelengths. They achieved high correlations ($r>0.9$) between predicted and measured concentrations of gravimetric moisture content, Kjeldahl total nitrogen, and Walkley-Black organic matter in soils ground to less than 0.25 mm. However, their results were significantly worse when samples were not finely ground (< 2 mm particle size) or when the method was applied to soils with a wide range of colors.

Ben-Dor and Banin (1995) were also able to develop a model to predict the properties of 12 groups of Israeli soils based on multiple regression of specific absorption bands of NIR spectra. Although they did not achieve correlations that were as strong as Dalal and Henry, they did obtain high correlations ($r>0.74$) for several soil properties; including clay content, specific surface area, cation exchange capacity, hygroscopic moisture, organic matter, and calcium carbonate. The group suggested that careful selection of calibration samples based on the soil constituents and the population of interest in order to improve results.

One reason for the difficulty in predicting soil organic matter is that it is complex, consisting of many different types of chemical functional groups (Bowers and Hanks, 1965). Because the wide range of functional groups have absorption bands at fundamental tones, overtones, and combination tones, organic matter is spectroscopically active across the entire NIR region (Ben-Dor and Banin, 1995).

An additional complication results from the fact that spectral regions of absorption shift due to interactions with molecules that are part of a heterogeneous structured solid like soil, or because of subtle changes in a materials' crystalline structure (Clark, 1999). An example can be seen within the lattice of Ca-montmorillonite, a 2:1 layer silicate with a calcium saturated interlayer. For this material, OH groups absorb at 1410 nm and 2206 nm (combination of stretching and vibration) (Ben-Dor and Banin, 1990). The stretching bond at 1400 has been shifted to 1410nm. Absorption features can even shift from one type of smectitic clay to another. The hydroxyl sorption feature of aluminum enriched smectite is between 2160 and 2170 nm while the OH functional group of iron enriched smectite hydroxyl groups absorb between 2210 and 2240 nm. For magnesium enriched smectite, adsorption is also shifted, with the hydroxyl band appearing at 2300 nm (Ben-Dor and Banin, 1990).

The complexity of absorbance features arising within solids of mixed and variable components discouraged the use of theoretical models based on the specific absorption signatures caused by photons interacting with a solid surface. The advent of more powerful computing hardware combined with software that uses advanced statistical techniques has allowed the development and implementation of chemometrics.

This approach often produces models that have high predictive accuracy. Several groups have achieved good results for soil organic carbon, with r-squared values greater than 0.90 and correspondingly low prediction errors (McCarty et al. 2002; Sorensen and Dalsgaard, 2005). Others have expanded the technique to simultaneously measure several soil properties such as total nitrogen (Confalonieri et al., 2001) and potential nitrogen mineralization, (Fystro, 2002), texture (Sorensen and Dalsgaard, 2005), and trace metals (Chang et al., 2001; Udelhoven et al., 2003; Moron and Cozzolino, 2003).

Chemometrics adds to the applicability of NIR by allowing several properties to be determined simultaneously using empirical models. Using this approach requires the development of spectral libraries with which to calibrate NIR spectral signals. These libraries are made up of reference soils whose constituent of interest are known. Typically, libraries are developed to represent the physical region the user is studying. Having a broad calibration range may allow a user to characterize heterogeneous sample sets, but often, restriction of calibration sets improves predictive ability for specific traits of interest (McCarty and Reeves, 2000; Chang et al., 2001; Chodak et al., 2004).

Inherent with the empirical approach is an uncertainty in the robustness of calibration (Groenigen, et al., 2003; Udelhoven et al., 2003). This method often does not link the precise spectral features contributing to the detection of the material of interest. Therefore, the reliability of calibrations is unknown and must be determined through experimentation (Confalonieri et al., 2001). For users to deal with calibration uncertainty, they must validate their predictions using samples with known composition over time and as methods are expanded to different soils.

Spectra are highly complex, which makes it difficult to relate soil characteristics to specific features. Geologic heterogeneity (Udelhoven et al., 2003), climate, topography, parent material, age, the organic matter, texture (Baumgardner et al., 1970; Sorensen and Dalsgaard, 2003; Cozzolino and Moron, 2006), and water content (Muller and Décamps, 2001; Lobell and Asner, 2002), and characteristics of a sample may all effect NIR models (Cohen et al., 2005). In spite of these complexities, several groups have had success determining SOC using NIR.

As early as 1986, Dalal and Henry found that absorption at 1744, 1870, and 2052 nm highly correlated with Walkley-black soil organic matter. Others have found that organic matter has multiple absorption bands between 2100 and 2400 nm (Chang et al, 2001; Malley and Williams, 1997; Morra et al., 1991). Humic substances and pigments derived from phenolic compounds and chlorophyll have been associated with absorbtion in the spectral region between 700 and 800 nm (Daughtry, 2001; Fidencio et al., 2002). Some groups have correlated absorption bands of specific organic functional to soil organic carbon. Ben-Dor and Banin (1995) found that amines, carboxyl and phenols absorb NIR energy at 2388, 1585 and 2016 nm, respectively. Cozzolino and Moron (2006) correlated absorption due to methyl overtones (1700 nm), aliphatic groups (2200 nm), and phenolic compounds (2300 nm) with soil carbon. The objective of this research was to develop a near-infrared soil carbon detection method and to compare its performance to benchmark combustion and chemical methods.

CHAPTER II

Materials and Methods

Two soil sample subsets were used. Subset 1 consisted of a group of 55 random samples taken from those collected by landowners from across Tennessee and submitted to the University of Tennessee Soil Test Lab in Nashville. These soil samples were selected to represent a wide range of soil types and carbon levels. They were air dried and lightly ground to pass through a 2 mm sieve.

Subset 2 consisted of soil samples taken from specific locations with a 2.5 cm diameter soil corer. These soils were sampled from 14 selected sites across Tennessee. To yield a range of carbon contents, the soils were taken from the following depths: 0-5, 5-15, and 0-15 cm. The samples were then air dried, lightly ground, and sieved into five particle size classes to determine the effect of surface roughness on NIR SOC carbon detection (Table 1.1; Note: all tables and figures are placed in the Appendix to Part I). All soil samples tested negative for calcium carbonate, which can interfere with organic carbon determination.

Organic Carbon Determination

Soil organic matter (SOM) was measured using the Walkley-Black chromic acid oxidation method (Walkley and Black, 1934). This gave an organic matter value for each sample expressed in percent by mass. The organic matter values were regressed against those taken by combustion to yield the relationship

$$[1]\ Y = 0.692 - 0.018x,$$

where Y equals carbon determined by combustion and x is the SOM value (R = 0.87).

Soil organic carbon (SOC) was determined by combustion with a Flash EA 1112 Series NC Soil Analyzer (Thermo Electron Corporation, Waltham, MA). Samples were combusted after being ground to pass through a 250 μm sieve, yielding a measure of organic carbon in percent by mass. The results from the combustion analyses were used as the benchmark standards for purposes of comparing the methods for determining SOC in this study.

NIR Spectra Capture

NIR spectra were made with an LabSpec Pro® Field Spectrometer (Analytical Spectral Devices, boulder, CO) at wavelengths between 400-2100 nm, using a rotating sample cup. Light was provided by a DC lamp set at 30^{o} above the sample, directed towards the sample center. A fiber optic probe placed 7.0 cm above the surface gave an optical scanning field with 3.55 cm diameter. Captured spectra were then transferred from the ASD to an Unscrambler® file (CAMO Technologies, 2003). Five spectra per sample were collected and averaged together to reduce signal noise. The data set was further reduced by averaging spectral data collected at 1 nm intervals to 4nm in order to reduce file size and computation. Then, reflectance (R) was transformed to absorbance (A), A=log (1/R) and a mean normalization transformation was performed. A multiplicative scatter correction data transform was performed (Beebe et al., 1998) to reduce the negative effects of light scattering from the irregular sample surface. To improve peak discrimination, the Norris derivative (CAMO Software, 2003) was taken of each 4 nm segment. Partial least squares (PLS) models were then constructed to correlate spectral features with SOC and SOM. The PLS models were fully cross validated to ensure robustness with the number of principal components determined by the software.

Effect of Surface Irregularity on NIR Prediction of Soil Organic Carbon

Irregularities at the surface of a soil affect the way light scatters. It may therefore interfere with NIR determination of SOC. To determine how roughness at the surface of a soil sample affects NIR prediction of SOC, a completely randomized factorial design was used with two treatment factors. Each sample from each location in subset 2 was subjected to a different preparation treatment to give samples of different particle size. They were: (A) ground to less than 250um, (B) ground to less than 2 mm, (C) 1:1 mix by mass of A and B, (D) less than 2mm but larger than 250 um, and (E) sifted to pass through a 2 mm mesh.

Carbon content of each sample was measured using both NIR and combustion methods. Differences in SOC as measured by the two procedures were analyzed using Mixed Model procedures (SAS, 1997). These procedures produce Type III F-values without printing mean square values or error terms for each model term. Mean separation was performed by the SAS macro “pdmix800” (Saxton, 1998) using a Fishers Least Significant Difference test with a type I error rate less than 0.05%. Analysis of variance tests were performed to identify differences caused by particle size class, measurement method, and their interactions.

CHAPTER III

Results and Discussion

Spectroscopy of Soil Samples

Although the soils were collected from different agricultural systems across the seven physiographic regions of Tennessee, the major features of their spectra were similar (Fig. 1.1). Some signature patterns of soil mineralogical characteristics can be discerned from the raw absorbance spectra. All spectra had strong absorbance in the visible region (< 700 nm) and two distinctive absorbance peaks were noted in the near-infrared around 1450 nm, which is the first overtone and combination vibration modes of OH groups (Madejova and Komadel, 2001), and also around 1950 nm, which is due to a combination of stretching and bending vibrations of structural water of clay minerals.

The spectra taken from two soils at long term pasture sites are shown in Fig. 1.1. The samples from the Plateau Research and Education Center (PREC) had a lower reflectance than those from Ames Plantation, and lower reflectance was seen from the surface 0 – 5 cm samples than those taken from 5 – 15 cm. Also, the absorbance bands around 1440 and 1940 are more strongly accented in the surface soils in comparison to the subsurface. This is likely due to the carbon content of each soil.

Soil humic material has a wide range of functional groups that have absorption bands at fundament tones, overtones, and combination tones, organic matter is spectroscopically active across the entire NIR region (Ben-Dor and Banin, 1995). Increasing carbon content of the soils is likely responsible for the observed spectral shifts.

Surface Texture Effects

Although reflectance from soil surfaces increases exponentially with decreasing particle size (Bowers and Hanks, 1965), this did not seem to effect NIR prediction of SOC in this case (Fig. 1.2). NIR predictions of SOC were slightly higher than those measured by combustion, but differences were less than 1 g kg^{-1} for all particle size classes. Also, variation amongst the NIR measurements was slightly higher than those derived from combustion, being 5.78 g kg^{-1} in samples measured with combustion and 6.198 g kg^{-1} in those measured with NIR (Fig. 1.3). Notably, there was no significant difference between the two carbon measurements derived from the two techniques ($P > 0.8$).

For these soils, NIR was relatively robust and insensitive to sample particle size. Others have theorized that variability in particle size can hinder the prediction of soil carbon using NIR (Cozzolino and Moron, 2006). The multiplicative scatter correction and Norris derivative data transformations used, which are designed to account for the variability of rough surfaces, may have compensated for any particle size effect.

NIR Prediction of Soil Organic Carbon and Organic Matter

Because the molecular makeup of soil is very complex, spectra resulting from NIR are actually a series of overlapping peaks and troughs. In order to characterize specific features and correlate them with the presence of the constituent of interest, the statistical method known as chemometrics was used. Chemometrics uses partial least squares regression (PLS) to link spectral features to constituent concentrations. This method is based on decomposing the spectral data to isolate sources of variance (PLS/plus IQ User's guide, 1997). The decomposition of the spectra and concentration data are performed simultaneously, so that the resulting eigenvectors are directly related the constituent of interest and represent the principal components of variation amongst in the dataset. For the data set in this analysis, 13 principal components were chosen because collectively they explained 99% of the variation in the data, gave the smallest root means standard error of calibration (RMSEC) and (RMSEV), and the highest correlation between measured and predicted values of soil carbon.

The NIR model was fully cross-validated. Cross-validation is a technique that estimates a model's prediction error by separating the population into groups. One group is excluded and used for validation, while the rest are used to develop the calibration. The process is repeated until all groups (five in this case) have been used for both validation and calibration (Shenk and Westerhaus, 1993; Moron and Cozzolino, 2003). All computations were performed using Unscrambler® 9.0 (CAMO Technologies, Software Inc., Woodbridge NJ).

Due to the diversity of functional groups in organic matter, it produces many overtones and combination absorption bands in the near-infrared. Additionally, strong signals from other soil components, including hydroscopic and crystal lattice water, make it difficult to assign a particular organic matter component to a single absorption band (Ben-Dor and Banin, 1995). The wavelengths with the greatest influence on PLS predictions of carbon content can be seen in Fig. 1.3. Wavelengths that correlated with SOC also correlated with SOM. The first principal component (PC) explains 38% of the spectral variance related to SOC Fig 1.3 (A). The visible range (<700 nm), the portion of the spectrum that is responsible for soil color, correlated with SOC and SOM. Organic material has a melanization effect in soil, darkening it. The region between 700 and 800 nm is also correlated with carbon prediction. These wavelengths are associated with pigments derived from the decomposition of humic substances, chlorophyll, and phenolic compounds (Daughtry, 2001; Fidencio et al., 2002). The organic functional groups that are strongly correlated with NIR prediction of carbon and organic matter can be seen in the fourth PC (Fig. 1.3 (B)). The third overtone of methyl bonds is between 800 and 950 nm (Burns and Ciurzak, 2001). Also, the absorbance of the second overtone of methyl, amide, and alcohol groups around 1440 nm is highly correlated with SOC and SOM. The first overtone of carboxyl (1880 nm) (Dalal and Henry, 1986) and amide (1920 nm) (Burns and Ciurzak, 2001) are also used to determine SOC and SOM.

Early in the model development process it was observed that models for higher carbon (SOC > 70 g kg^{-1}) samples did not apply well to those for lower carbon samples (SOC < 70 g kg^{-1}). Because most of the samples were low in carbon, further analysis was directed towards these low C samples. This caused the omission of 9 of the 235 samples, and improved model performance by reducing prediction error from ± 9.1 g kg^{-1} to ± 4.9 g kg^{-1} (data not shown).

Analysis resulted in a strong (r=0.92) overall relationship between SOC predicted using NIR (Fig. 1.4). Table 1.1 shows in detail the correlations, standard errors, and fitting parameters between the different sample groups, NIR signal, SOC and SOM. Generally, NIR correlated better with SOC than SOM (Walkley-Black).

Standard errors displayed trends similar to the correlations with those of SOM being smaller (1.1 to 2.5 g kg^{-1} C) than those of SOM (3.6 to 9.0 g kg^{-1}). The fully cross-validated models followed the same trends, with accordingly lower correlations and higher standard errors. Cross-validated NIR correlations with SOC were r = 0.80 or above with standard errors of prediction ranging from 1.6 g kg^{-1} to 5.3 g kg^{-1}. NIR showed lower correlation with SOM (r = 0.65) than SOC (combustion), with standard prediction errors being higher (4 to 10 g kg^{-1} C).

It should be noted that while the subset 1 samples showed the same NIR prediction trends for SOC relative to SOM, overall model quality was much lower than those based on subset 2. This may be due to a number of factors including more variability within each individual sample or the fact that the samples represent wider spatial and taxonomic variety. However, when the subset 1 samples were combined with subset 2, overall model quality was improved to a 0.92 correlation and 4.2 g carbon per kg soil standard error of prediction for the validated model, with the performance on the calibration set being r = 0.92 with an error of ± 4.0 g carbon per kg soil. Data for 0 - 5 cm and 5 - 15 cm depth increments were used for model development, but comparisons between C detection methods at depths are not shown. Accuracy between methods followed similar trends for each depth. The overall linear regression of Walkley-Black determined SOM versus NIR predicted SOM correlation was 0.87 with a standard error of 5.5 g kg^{-1}. All NIR predictions of SOC compared favorably to the Walkley-Black chemical method to determine soil carbon (Table 1.1).

Table 1.2 shows in detail the performance of NIR on subset 2. NIR prediction of SOC was good, and the prediction of SOC using NIR was more accurate than using the Walkley-Black method to predict SOC using equation [1]. Although the acceptable degree of accuracy is dependant upon the intent of the individual user, for this experiment, NIR measurements deviated from those derived from combustion by well below 10 percent in most cases. Compared to the combustion results, the errors of NIR predictions of SOC were generally less than 2 g kg^{-1}. The best performance was seen for soils whose SOC values were towards the middle of the data range. Poorer performance was seen at the extreme ends of the dataset, with very low carbon values such as those in tilled fields being underpredicted and high carbon values like those seen at forested sites being overpredicted. Although performance was adequate, improvement in accuracy should be achieved by the inclusion of additional low and high carbon samples in the calibration. McCarty and Reeves (2000) showed that the addition of relatively few samples with properties similar to those being predicted can correct for bias in predicting outlying samples.

CHAPTER IV

Conclusions

To understand the effects of agronomic practices on soil carbon dynamics, rapid and inexpensive testing techniques must be developed. NIR is an alternative soil carbon detection method that may fill this role. Once calibrated, it is capable of rapid analysis with less soil preparation than combustion techniques. NIR also produces no toxic chemical byproducts and requires no reagents.

In this study, a NIR technique was developed and applied to soils from across Tennessee. It was found that the method was relatively unaffected by soil particle size. SOC predictive accuracy for samples passed through a 2 mm sieve was similar to that of the same samples ground to 250 um or less. High correlations were found between NIR predictions and SOC and SOM measured using standard techniques. NIR produced better measures of total soil organic carbon than those extrapolated from Walkley-Black organic matter measurements. It is concluded from this research that, when calibrated with a representative set of soil samples, the NIR procedure is an accurate alternative to the combustion and Walkley-Black methods for determining soil organic carbon.

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Trade names or commercial products were mentioned solely for the purpose of providing specific information and does not constitute an endorsement or recommendation by the University of Tennessee.

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APPENDIX

Part I

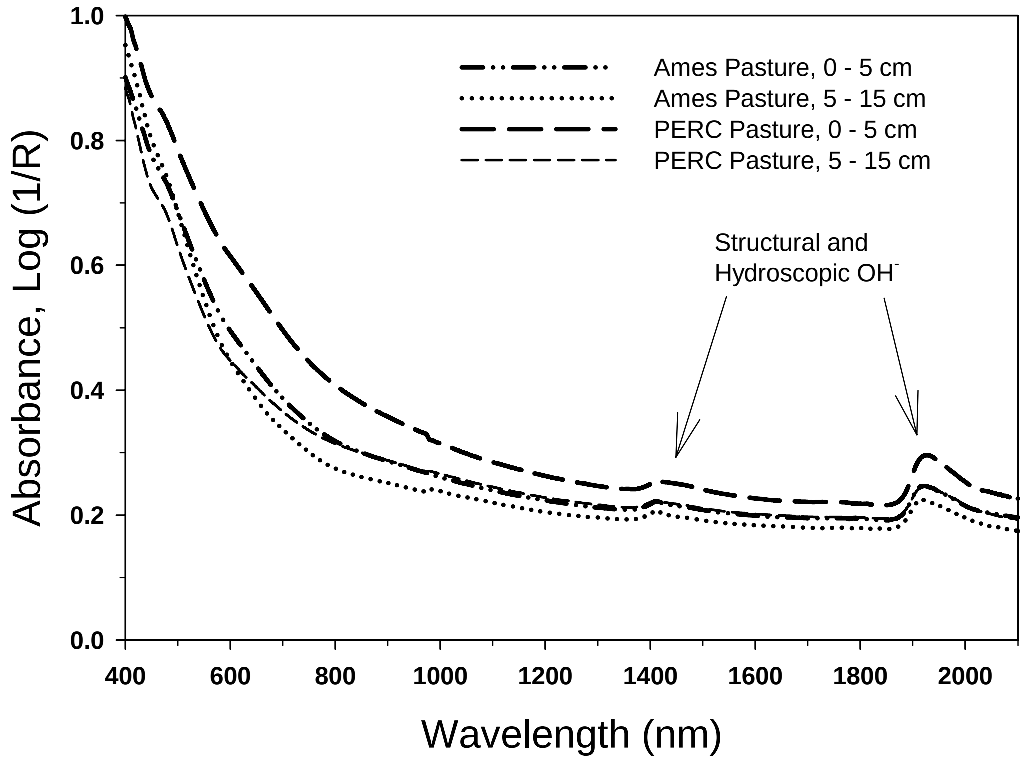


Fig.1.1. The average near-infrared and visible spectra for soils collected at Ames Plantation in western Tennessee and at the Plateau Research and Education Center (PREC) in eastern Tennessee.

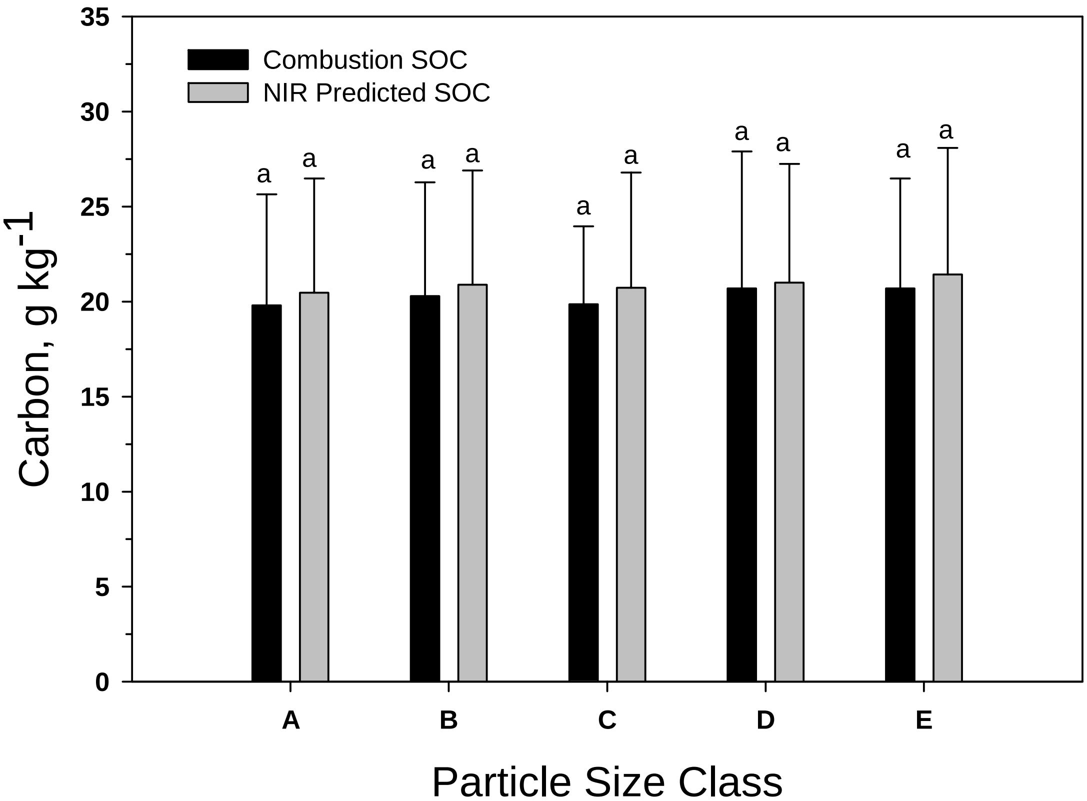


Fig. 1.2. Effect of particle size class on measured soil carbon as determined by near-infrared spectroscopy or combustion. Classes are: (A) ground to less than 250um, (B) ground to less than 2 mm, (C) 1:1 mix by mass of A and B, (D) less than 2mm but larger than 250 um, and (E) sifted to pass through a 2 mm mesh.

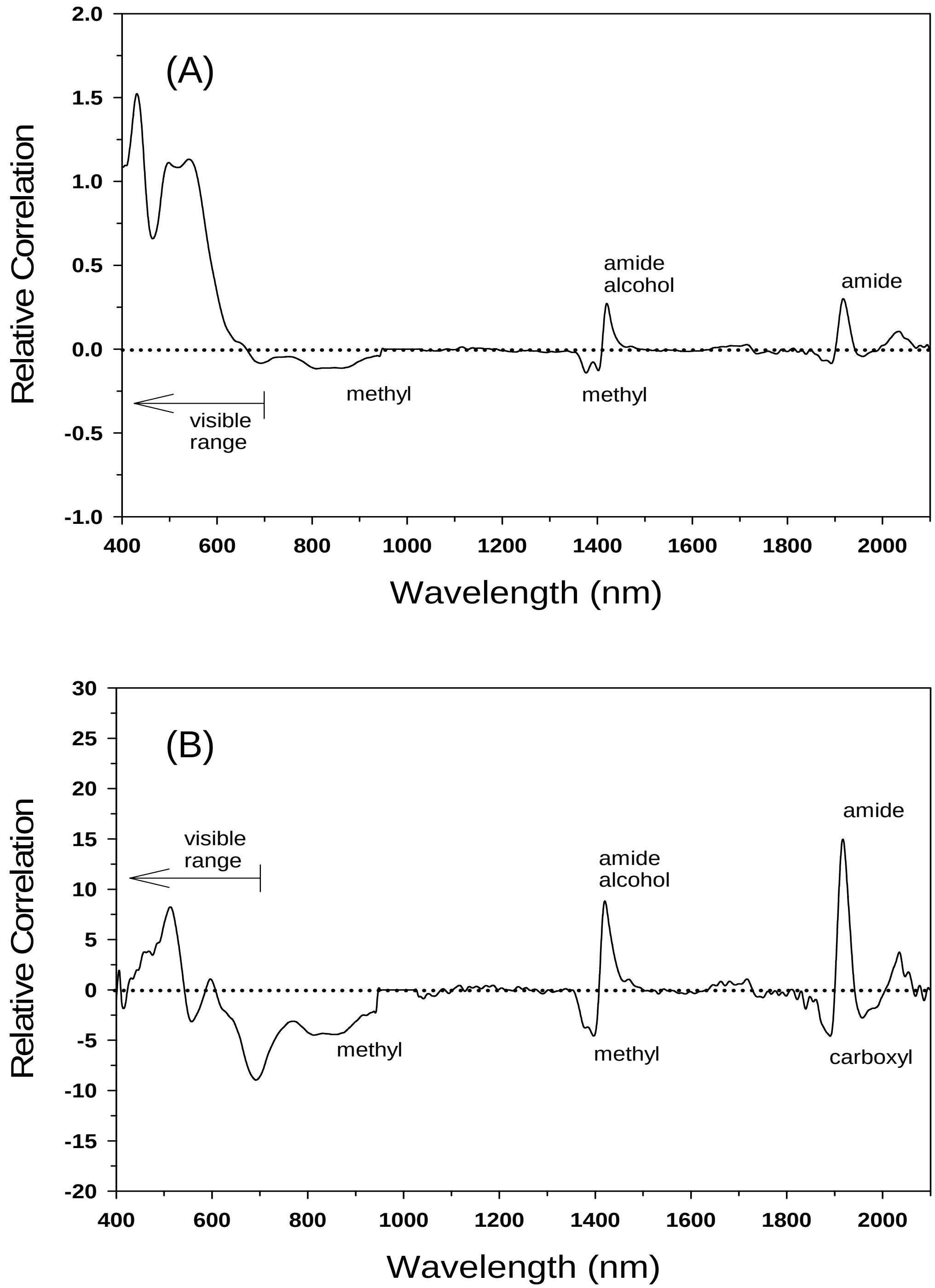
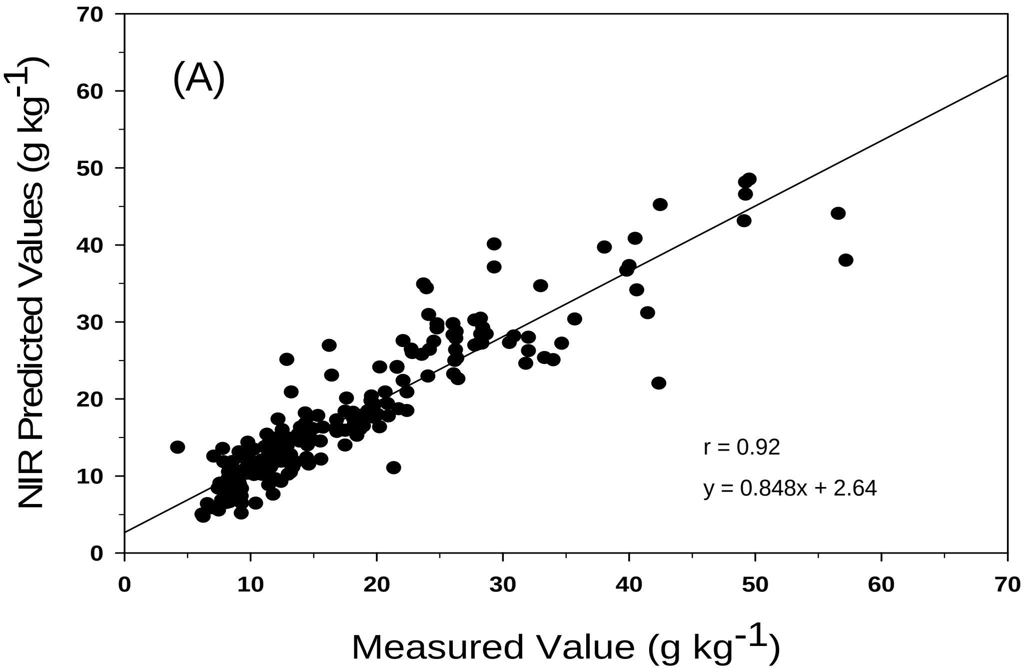


Fig. 1.3. Two of the 13 principal components (PC's) correlated to soil organic carbon in the partial least squares regression on near-infrared spectra of soils taken from across Tennessee. PC 1 (A), and PC 4 (B), Combined, the two PC's account for 50% of the variation in the spectral data.



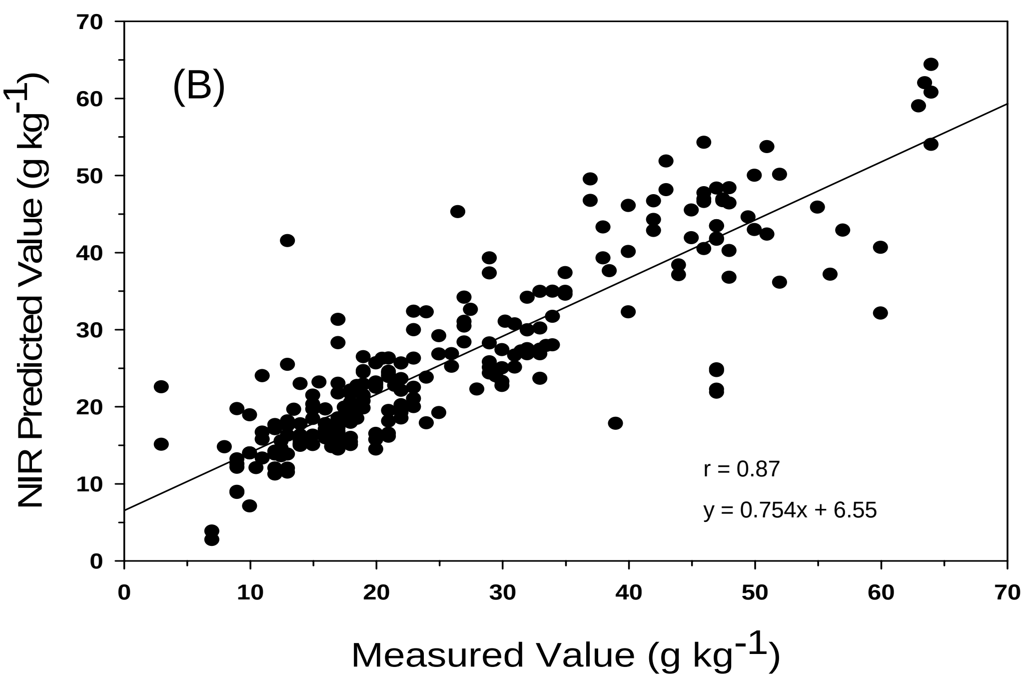


Fig. 1.4. Plot of measured near-infrared reflectance spectroscopy (NIR) predicted soil organic carbon by combustion (A) and of soil organic matter measured using the Walkley-Black method (B).

Table 1.1. Performance of models used to predict the benchmark; soil organic carbon (C) as determined by combustion, for two sets of soils taken from across Tennessee. Models using organic matter to predict C were based on linear regression while near-infrared reflectance spectroscopy (NIR) used partial least squares regression to predict C. The correlation, slope, offset, and error parameters are shown for both the calibration and fully cross-validated sample sets.

Model Calibration												
	--------NIR Prediction of Combustion C------				---------------NIR Prediction of OM---------------				-------OM Prediction of Combustion C------			
Sample Set[§]	**r**	**Slope**	**0ffset[‡]**	**RMSEC (g kg^{-1})**	**r**	**Slope**	**0ffset**	**RMSEC (g kg^{-1})**	**r**	**Slope**	**0ffset**	**RMSEC (g kg^{-1})**
Subset 1	0.665	0.429	12.500	9.390	0.878	0.771	6.160	7.500	0.879	0.77	4.60	5.855
Subset 2	0.952	0.906	1.520	2.770	0.864	0.746	7.000	6.930	0.870	0.756	3.910	4.440
Subsets 1+2	0.902	0.813	3.230	4.240	0.806	0.650	9.300	8.450	0.848	0.719	4.880	5.380

Cross-Wise Validation												
	--------NIR Prediction of Combustion C------				---------------NIR Prediction of OM---------------				-------OM Prediction of Combustion C------			
Sample Set	**r**	**Slope**	**0ffset**	**RMSEP (g kg^{-1})**	**r**	**Slope**	**0ffset**	**RMSEP (g kg^{-1})**	**r**	**Slope**	**0ffset**	**RMSEP (g kg^{-1})**
Subset 1	0.535	0.362	14.100	10.600	0.653	0.568	12.200	12.400	0.862	0.757	5.240	6.220
Subset 2	0.933	0.882	1.850	3.250	0.839	0.729	7.060	7.500	0.846	0.748	4.030	4.530
Subsets 1+2	0.866	0.785	3.710	4.920	0.765	0.617	10.170	9.190	0.840	0.712	4.990	5.500

† Abbreviations correspond to the following: near-infrared reflectance (NIR), carbon (C), organic matter (OM), correlation (r), root mean standard error of correlation (RMSEC), and root mean standard error of prediction (RMSEP)

[‡] Offset represents the mean difference between the prediction and methods.

[§] Subset 1 consisted of soil samples taken by Tennessee landowners; Subset 2 was taken by the authors from 14 agricultural systems across Tennessee.

Table 1.2. Results of three methods of soil carbon determination employed with soils from 14 different Tennessee agricultural systems. The benchmark, combustion method was compared to, Walkley-Black, and near-infrared reflectance spectroscopy (NIR). Soil organic carbon predictions by Walkley-Black were based on linear regression against combustion while NIR predictions were based on partial least squares regression.

Sample Site	**Depth**	**Combustion SOC**	**NIR Pred SOC**	**W-B SOM SOC**	**W-B SOM Pred SOC**
	-------cm-------	**-----------------------------------g kg^{-1}-----------------------------------**			
RECG Upland Forest	0-15	22.1	23.5	35.0	24.1
RECG Pasture Sideslope	0-15	14.4	13.6	27.0	13.2
RECG Sideslope Pasture 3	0-15	17.5	17.6	32.0	17.6
RECG Depression Pasture	0-15	16.9	16.8	30.0	16.7
RECG Sideslope Tilled	0-15	9.8	7.8	19.0	6.8
RECG Upland Tilled	0-15	9.0	6.8	13.0	5.6
Ames Forest	0-15	16.1	15.8	26.0	15.6
Ames 100 yr Pasture	0-15	14.9	14.3	23.0	14.0
Ames No-till Soy-corn	0-15	8.3	5.9	14.0	4.7
Ames Tilled Soy	0-15	9.8	7.8	15.0	6.8
PREC Forest	0-15	27.4	30.2	38.0	31.5
PREC Fescue Pasture	0-15	28.3	31.3	47.0	32.7
PREC No-till Corn	0-15	18.3	18.5	33.0	18.6
PREC Tilled Potatos	0-15	11.6	10.1	18.0	9.3

† Abbreviations correspond to the following: University of Tennessee (UT) Research and Education Center at Greeneville, TN (RECG), (UT) Plateau Research and Education Center Crossville, TN (PREC), Ames Plantation Research and Education Center at Grand Junction, TN, (Ames) Walkley-Black (W-B), soil organic matter (SOM), soil organic carbon (SOC), near-infrared reflectance (NIR), and prediction (Pred).

Part II

Soil Organic Carbon Changes Under No-till Production Due to Crop Management

Abstract

The influence of various crop management practices on the rate of soil carbon storage in no-till farmland is not well defined. The objective of this research was to compare the effects of combinations of cropping sequences and soil bio-covers on soil organic carbon (SOC) under no-tillage. The experiment used a split-block design with four replications at the Milan (RECM) and Middle TN (MTREC) Research & Education Centers. The whole-block treatment was cropping sequences of corn (*Zea mays* L.), soybean (*Glycine max* L., Merr), and cotton (*Gossypium hirsutum* L., Malvaceae). The split-block treatments were bio-covers of winter wheat (*Triticum aestivum* L.), hairy vetch (*Vicia villosa* Roth), poultry litter, and fallow. Soil samples were taken at 0, 2, and 4 years of experimentation at 0-5 cm and 5 -15 cm depths. Overall, both sites showed a small but consistent loss in carbon over all treatments. The average losses of SOC at the 0-5 cm depth were 1.06 and 1.40 Mg ha^{-1} at RECM and MTREC, respectively. In the subsurface, mean SOC loss at RECM was 1.43 Mg ha^{-1}. Conversely, the subsurface SOC at MTREC was 3.14 Mg ha^{-1} higher than pre-experiment levels. Crop sequence had a significant effect on change in SOC ($P \leq 0.05$) in both surface and subsurface regions at RECM. Based on Fisher's LSD ($P \leq 0.05$), sequences with two or more years of cotton tended to lose significantly more SOC than those with two or more years of soybean. The poultry litter bio-cover lost less surface SOC (0.58 Mg ha^{-1}) than those under vetch (1.33 Mg ha^{-1}) or fallow (1.8 Mg ha^{-1}).

Sequences higher in soybean tended to lose less carbon than other sequences when combined with vetch and poultry bio-covers.

Chapter I

Introduction

Government incentives to improve environmental quality, increased fuel prices, and the economical production of herbicides, herbicide-resistant crops, and reduced-tillage equipment have led to an increase in the amount of farmland planted using conservation tillage. Farm acreage under conservation tillage in the United Sates increased from 29 million ha in 1989 to 42 million in 2000; an increase from 26% to 37% of all planted area (Towery, 2000). The trend of increasing field acreage under reduced-tillage has led to a need for knowledge about how this management strategy affects the soil environment.

Of all the conservation tillage systems, no-till systems employ practices that produce the least amount of soil disturbance. There is literally no tillage used in no-till systems, only a planter which uses a disk or coulter to cut a narrow furrow in which the seed is sown. This method maximizes the amount of crop residue left on the soil surface, which can have a variety of beneficial effects (Tolbert et al., 1999).

One benefit of crop residue is reduced erodibility (Rhoton and Tyler, 1990; Rhoton, 2000). Additionally, reduced tillage practices that have been shown to increase soil organic matter in the top 15 cm are attributable to increased surface residue (Tyler et al., 1983; Mahboubi et al., 1993; Ismail et al., 1994). As organic matter increases, so does soil aggregation (Bossuyt et al., 2002) and soil cation exchange capacity (CEC) (Hubbs et al., 1998; Ismail et al., 1994; Karanthanasis and Wells, 1989). Therefore, no-till practices may result in an overall improvement in soil fertility by increasing a soil's ability to retain nutrients.

To date, the United States is not participating in the Kyoto Treaty to reduce greenhouse emissions, but is independently developing a system of rules and guidelines for carbon sequestration. Part of these guidelines are a voluntary carbon dioxide emission and sequestration reporting system (US Department Of Energy form EIA 1605 2003). If successful, this system may ultimately lead to the creation of a "carbon credit system" which would give incentives to industry and agriculture for reducing CO_2 emission and increasing sequestration. Currently, carbon credits in the 1605(b) plan tailored to detailed agricultural soil management strategies have not been developed due to a general lack of knowledge about management effects in specific systems (Jim Hrubovcak, USDA 2003). Despite existing uncertainty, the Chicago Climate Exchange™ has developed an independent carbon trading market and enlisted the help of organizations such as the Nation Farmers Credit Union to administer a system that pays agricultural producers for sequestering greenhouse gasses to later be sold to industry. Currently, farmers can earn up to two and four dollars per acre for grass and no-tillage production, respectively (National Farmers Credit Union, 2007).

Agriculture is responsible for approximately 5% of total U.S. carbon emissions of which carbon mineralization from conventional tillage practices make up a significant part. Tillage destroys crop residue and exposes soil organic matter to oxygen, thereby promoting decomposition and mineralization of crop residue and existing soil C stocks. Agriculture can reduce CO_2 emission by shifting production methods away from conventional tillage.

Crop production without tillage has been shown to increase soil carbon as compared to conventional tillage (Campbell et al., 1999; Angers and Giroux, 1996; Karlen et al, 1994). Under no-till management, atmospheric C fixed by crops can be transferred to the undisturbed subsoil. In the subsoil, it is protected from rapid oxidation and may become part of the recalcitrant soil carbon fraction referred to as humus (Ding et al., 2002). Using no-till, agricultural systems can play a positive role in the global carbon cycle by converting arable soils from a carbon source to a carbon sink. In a review of 39 paired studies ranging in length from 5 to 20 years, Pautisian et al, (1997) concluded that no-till increased soil carbon an average of 285 g per m^2 with respect to conventional tillage. Lal et al. (1998) estimated that the total amount of C that can be sequestered by managers of US croplands following “recommended” practices to be between 2,500 and 3,500 million megatons. Land under optimized no-till production could therefore be an important resource in a carbon credit system. However, it remains unclear how specific cropping sequences and bio-covers affect soil carbon, especially in no-till production systems. Reviews of studies comparing no-tillage to conventional tillage vary in their estimations of SOC sequestration. West and Post (2002) concluded that no-till sequesters an average of 0.57 ± 0.14 Mg ha^{-1} $year^{-1}$. A later review by Franzluebbers (2005) estimated a lower rate of 0.42 ± 0.46 Mg ha^{-1} $year^{-1}$ in the southeastern United States.

A variety of factors can affect sequestration rate, including cover crop, soil amendments, and cropping system complexity. Soil organic carbon may be affected by mulch rate. Studies have found increasing crop residue input has a direct correlation with soil carbon (Duiker and Lal, 1999; Clapp et al., 2000; Duiker et al. 2002;

Franzlubbers, 2005). In addition to rate, mulch quality can also affect soil carbon. As overall system productivity increases, mulch returned to the soil surface should also increase. However, this relationship may be confounded by mulch characteristics. Drinkwater et al, (1998) suggest low carbon-to-nitrogen organic residues increased carbon retention in soil. Rotation complexity can also enhance carbon sequestration under systems recently converted from conventional to no-till (West and Post, 2002; Franzlubbers, 2005). However, West and Post observed different results for systems already under no-till. In existing no-till systems, enhancing complexity did not directly result in increased soil carbon.

A controlled study of carbon dynamics under several no-till production practices will help increase the knowledge base on how to optimize both carbon storage and productivity under different no-tillage management strategies. This study examines how soil carbon is affected by bio-cover, cropping sequence, and their interactions under no-till production.

Chapter II

Materials and Methods

Site Description

The field study began at two sites in 2002. The first site was at the MTREC near Spring Hill, TN (36.02° N, -85.13° W). This region has a mean annual temperature of 12°C and receives 135 centimeters of precipitation annually. Soil at the site is classified as a Maury silt loam (Fine, mixed, semiactive, mesic Typic Paleudalf). Prior to experiment initiation, the entire field was under a two-year corn-soybean cropping sequence, with half of the field being under corn and half under soybean. When the experiment began, those plots initially planted to soybean were planted to corn, and those in corn were planted with soybean in 2001. This site was under no tillage production for at least 15 years prior to the initiation of this experiment (Dr. Dennis Onks, personal communication, 2007).

The second site was at RECM in Milan, TN (35.54° N -88.44° W). Like MTREC, mean annual precipitation is 135 centimeters, but mean annual temperature is higher, at 16.6°C. The soil type is a Loring B2 series: (Fine-silty, mixed, thermic Typic Fragiudalf). In 2001, the site was planted in corn, soybean in 2000, and cotton in 1999, with wheat being planted each winter. The field was under no-tillage production for 16 years prior to experimentation. Differing soil types and temperature regimes between the two sites were chosen to increase the experiments' range of inference.

Prior to the study, the field was left fallow in 2001. In 2000, the entire field site was planted in corn, soybean in 1999, cotton in 1998 and 1997, and soybean in 1996 and 1995, with wheat being planted each winter. The field was under no-tillage production for over 10 years prior to initiation of this experiment.

Experiment Design

The experiment used a randomized complete split-block design in which the whole plot treatments were crop sequences, which were strip-applied across the length of the field. At MTREC, eight individual cropping sequences consisting of glyphosate resistant corn and soybean were planted (Table 1.1; Note: all tables and figures are placed in the Appendix to Part II). Four bio-cover treatments including hairy vetch, winter wheat, poultry litter, and fallow were strip-applied as a sub-plot treatment perpendicular to crop sequence strips. This created 32 sequence/bio-cover combinations applied to separate 6.1m by 12.2 m subplots. Each treatment combination was replicated four times, for a total of 128 plots. Both soybean and corn were planted on 76 cm rows.

At RECM, the plot size, and bio-cover treatments were similar to those at MTREC. However, because of the regional importance of cotton, glyphosate resistant cotton was added to some of the crop sequences. The addition of cotton brought the total number of crop sequences at RECM to thirteen (Table 1.1), for a total of 52 sequence/bio-cover combinations replicated four times, totaling 208 plots at RECM. Varieties planted were ‘PM 1218 BG/RR’ cotton, ‘DKC 6410 RR’ corn, and ‘USG 7440nRR’ soybean. Cotton was planted on 102 cm rows while corn and soybean were planted on 76 cm rows.

Fertilizer was applied as appropriate to each crop according to soil tests and the guidelines published in the Tennessee Agricultural Extension Service Guidelines to

Crop Production, and litter application rates were applied at the rate of 66.7 kg N ha^{-1} assuming 50% bioavailability (UT Extension Service, 2003). Nutrient content of the poultry litter, vetch, wheat, and winter weeds (fallow) bio-covers were tested by A&L Analytical Laboratories, Inc. (Memphis, TN). All bio-cover strips received the equivalent of 66.7 kg N ha^{-1} prior to planting. Pre-plant fertilizer application rates were reduced by the amount of nitrogen supplied by the vetch or wheat bio-covers, as appropriate (Table 2.2). Corn plots received 128.5 kg N ha^{-1} and cotton received 33.35 kg N ha^{-1} as side-dress treatments.

Sample Collection

To determine carbon and nutrient dynamics over time, background data on soil carbon and nutrients were established. Initial data were taken from eight 15-cm depth soil cores randomly collected from each plot. The cores were divided into two depth classes, 0-5 cm and 5-15 cm. After dividing, the different groups were homogenized into one composite sample. In total, 672 composite samples were analyzed for total carbon and Melich III extractable elements (Mehlich, 1984). These samples formed the baseline measurements for comparisons of subsequent years. The same sampling and analyses were repeated two (Spring 2004) and four years (Spring 2006) after the experiment began.

Additional samples were taken from similar soils that were under different agro-ecosystems. Sites were sampled at RECM that were under either no-tillage production for 25 years or forest for over 75 years. At MTREC samples were taken from sites that had been in no-tillage production for 25 years, pasture for 50 years, and forest for 75 years. These samples gave an idea of potential maximum carbon levels in stable long-term agro-ecosystems with similar soils and climates.

Total Soil Organic Carbon Measurements

Total soil organic carbon (SOC) was measured at 0-5 and 5-15 cm depths using near infrared diffuse reflectance spectroscopy (NIR). The NIR measurements were taken with a Field Spectrometer (Analytical Spectral Devices, Boulder, CO) at wavelengths between 400-2400 nm, using a rotating sample cup. The NIR method was calibrated using a Flash EA 1112 Series NC Soil Analyzer (Thermo Electron Corporation, Waltham, MA) as described in part I. Examples of measurement performance can be seen in Table 2.3. These results were used to determine overall carbon change in each plot to a depth of 15 centimeters. Additionally, dividing the samples into two depths allowed the observation of changing carbon content within the soil profile.

Statistical Analysis

Change over the four year period was analyzed using Mixed Model procedures (SAS, 1997). These procedures produce Type III F-values but do not print mean square values or error terms for each model term within the analysis. Mean separation was performed by the SAS macro "pdmix800" (Saxton, 1998) with a Fisher's Least Significant Difference test type one error rate of 0.05%. Analysis of variance tests were performed to identify differences caused by sequence and bio-cover, and the interaction of crop sequence with bio-cover.

Chapter III

Results and Discussion

Research and Experiment Station at Milan (RECM)

At the surface 0–5 cm at RECM, mean SOC dropped from 9.2 Mg ha^{-1} in 2002 to 8.12 Mg ha^{-1}, when averaged across sequences and covers in 2006 (Table 2.4). This is approximately 0.27 Mg ha^{-1} yr^{-1}. Significant differences ($P \leq 0.05$) were seen in carbon change due to cropping sequence (Fig. 2.1). The greatest losses were seen in the continuous cotton sequence (2.11 ±1.5 Mg ha^{-1}). This was significantly different ($P \leq 0.05$) from four of the five sequences containing at least two years of soybean. The lowest loss was seen in the soybean(S)-soybean(S)-corn(Cr)-cotton(Ct) (S-S-Cr-Ct) sequence, which was 0.3 ±1.5 Mg ha^{-1}. Continuous corn resulted in essentially zero carbon losses (1.19 ±1.2 Mg ha^{-1}), which were not significantly different from any other sequence. Small losses of 0.64 ±0.38 Mg ha^{-1} were observed in continuous soybean, which were significantly lower than three of the five cropping sequences containing two or more years of cotton at RECM (Fig. 2.1). Generally, sequences containing higher frequencies of cotton lost more carbon, while those with higher frequencies of soybean lost the least. Sequences containing more plantings of corn experienced median carbon losses at RECM (Fig.2.1).

In the subsurface 5–15 cm layer, losses were slightly lower than in the 0-5 cm layer, with mean SOC across all treatments falling from 10.58 ±0.87 Mg ha^{-1} to 9.77 ±0.59 Mg ha^{-1}(Fig. 2.1). This is an annual loss of approximately 0.20 Mg ha^{-1} yr^{-1}. Overall, the pattern of loss was similar to that seen at the surface, with continuous cotton losing significantly more ($P \leq 0.05$) SOC (2.53 ±1.71 Mg ha^{-1}), and S-S-Cr-Ct losing the least (0.69 ±1.53 Mg ha^{-1}). Cr-Ct-S-Cr and Cr-Cr-S-Ct averaged across all covers, lost significantly more ($P \leq 0.05$) carbon (2.31 ±1.82 Mg ha^{-1} and 2.06 ±1.77 Mg ha^{-1}) respectively, than the other sequences. The subsurface depth generally mirrored the surface layer, as the general pattern of mean carbon loss was that those sequences with two or more years in cotton were higher than those with two or more years of corn. Sequences with two or more years of soybean tended to have the lowest mean SOC loss.

Bio-cover also had a significant effect ($P \leq 0.05$) on carbon loss at the surface, with vetch and fallow losing significantly more carbon ($P \leq 0.05$) than plots amended with poultry litter (Fig. 2.1). The losses were 1.45 ±1.53 Mg ha^{-1}, 1.33 ±1.28 Mg ha^{-1}, 0.92 ±1.38 Mg ha^{-1}), and 0.6 ±1.83 Mg ha^{-1}, for vetch, fallow, wheat, and litter, respectively. The standard errors are as large, or larger than the means, therefore the losses are zero for all practical purposes. However, the general trend, with mean losses being vetch ≥ fallow ≥ wheat ≥ litter is consistent across bio-covers at RECM and MTREC.

After the initial decline in 2004, mean SOC increased slightly or stabilized in many of the sequence/bio-cover treatments. Across all treatments and both depths at RECM (Figs. 2.2 and 2.3), over half of the treatment combinations displayed gains or no change from 2004 to 2006. These sequences were typically those with higher frequencies of soybean under poultry litter or vetch bio-covers at RECM and MTREC. This indicates that the systems may be stabilizing or recovering from their initial losses at the outset of the experiment.

Middle Tennessee Research and Experiment Station (MTREC)

Mean SOC in the surface 0 – 5 cm layer fell from 8.91 ±0.93 Mg ha^{-1} in 2002 to 6.56 ±0.95 Mg ha^{-1} in 2006 at MTREC (Table 2.4). This is a loss of approximately a 0.59 Mg ha^{-1} yr^{-1}, which was twice the rate of loss than that seen at RECM.

Although no significant differences were seen amongst the treatments at either depth at MTREC, some trends were observed. In the surface, Cr-Cr-S-Cr lost the most carbon, at 2.59 ±0.96 Mg ha^{-1} which was similar to that seen in the continuous corn sequence (1.95 ± 1.41 Mg ha^{-1}). The treatment losing the least SOC was S-S-Cr-S (1.02 ± 1.46 Mg ha^{-1}) while continuous soybean lost 2.54 ± 1.46 Mg C ha^{-1}. Carbon loss at the surface by bio-cover followed a trend similar to that seen at RECM, with the SOC loss being least in plots amended with poultry litter 2.07 ± 0.87 Mg ha^{-1} (Fig. 2.4). The comparative carbon loss by bio-cover was also similar to that seen at RECM, being fallow≈vetch>wheat>litter.

Mean carbon in the subsurface fell from 12.14 ±1.52 Mg ha^{-1} to 9.00 ±1.81 Mg ha^{-1} in 2006, a rate of 0.79 Mg ha^{-1} yr^{-1}. This was the highest rate of loss seen at any depth or location, being approximately three times higher than those seen at RECM and 50% higher than those exhibited in the surface layer at MTREC.

No significant differences were seen due to crop sequences, or bio-covers in the 5-15 cm layer. However, trends in mean losses were different from those seen at RECM. Cotton was not grown at the site, and mean losses in sequences with higher frequencies of corn tended to lose less carbon than those with more plantings of soybean. The Cr-S-S-Cr sequence lost the most carbon (3.88 ±1.46 Mg ha^{-1}) while Cr-Cr-S-Cr lost the least (2.15 ±1.59 Mg ha^{-1}). Like those seen in the 0-5 cm layer, trends in SOC loss in the subsurface, according to bio-cover treatments, were also similar to RECM. Greatest losses were seen in fallow, which was approximately equal to those seen under vetch (Fig 2.4.) Plots receiving wheat as a bio-cover lost less SOC (2.99 ±1.63 Mg ha^{-1}) than those receiving vetch or fallow (3.36 ±1.69 Mg ha^{-1} and 3.37 ±1.46 Mg ha^{-1}, respectively) , but this was more than plots amended with poultry litter (2.64 ±2.13 Mg ha^{-1}).

Unlike what was observed at the RECM site, carbon levels generally did not begin to climb again after the initial drop in 2004. However, at the 0 – 5 cm depth, three sequences under vetch (S-S-S-S, S-S-Cr-S, and S-Cr-Cr-S) and one under wheat (S-S-S-S) showed higher mean SOC in 2006 (Fig. 2.5). The general pattern of losses sustained across both depths and all years at MTREC may be related to the history of the site, which has a long record of dairy manure application (Dr. Dennis Onks, personal communication). Manure application can lead to elevated SOC levels (Jiao et al., 2005). These SOC levels may not have reached a new equilibrium following cessation of manure application when this experiment began.

An interaction was significant at the $P \leq 0.10$ level in the subsurface 5-15cm (Fig. 2.6). Poultry litter affected SOC losses differently according to which cropping sequence it was applied to. Two of the three crop sequences with consecutive years of soybean were Cr-S-S-Cr and S-S-S-S. These sequences experienced some of the highest losses (4.68 ±1.76 Mg ha^{-1}), (4.4 ±1.47 Mg ha^{-1}), respectively. This differs from what was observed at RECM, where sequences with higher frequencies of soybeans tended to lose the least amount of carbon. Lower losses were seen in poultry litter plots in the following cropping sequences; S-Cr-Cr-S, Cr-Cr-S-Cr, S-S-Cr-S, and S-Cr-S-Cr.

Generally, sequences with higher frequencies of corn and rotated with soybean tended to lose less SOC that those with higher frequencies of soybean and successive years in soybean.

Others have observed small losses in SOC in fields under no-tillage production at the soil surface, (Rhoton, 2000) and in the subsurface (VandenBygaart et al., 2003). Gas exchange studies have found net carbon balances of well established no-tillage systems to be essentially zero (Verma et al., 2005), or the fields to be slight carbon accumulators.

Typically under no-tillage, the most rapid and dramatic gains in SOC are seen in the first ten years (Dick, et al 1991; West and Six, 2007). The sites chosen for this study were under no-tillage production for over 16 years, and may have experienced most of the gains possible under the previous crop systems. Studies have observed at a steady state in SOC, the residue inputs equal the total rate of degradation empirically (Polglase et al., 2000; Conant et al., 2001; West et al., 2004) and theoretically (Johnson et al. 1995; West and Six, 2007). We speculate that the saturation level is above our initial SOC values. Others have shown that surface concentrations of organic matter found in virgin soils near the area can have organic carbon contents as high as 60 to 80 Mg ha^{-1} in the top 15 centimeters, which is three to four times higher than observed in these experiments (Rhoton and Tyler, 1990). However, these sites were under hardwood forest, which can build higher SOC than adjacent row crop systems. To investigate the possibility of the experiment systems at MTREC and RECM having neared saturation prior to the onset of the experiment, SOC in comparable systems was measured.

Our survey of nearby stable agro-ecosystems showed that secondary growth hardwood forest could reach much higher soil carbon levels (see Table 2.7) than those seen under no-tillage, being 22.73 and 8.00 Mg ha^{-1} in the surface 0-5 cm and subsurface 5-15 cm, respectively at RECM. However, the SOC level of a long-term no tillage row crop production system like those used in the experiment at RECM was similar to what was observed at the experiment site. SOC in the analogous no tillage system at RECM in 2006 was roughly equal to that seen at the experiment site at the initiation of treatment, being approximately 21 Mg ha^{-1}. Long term no-till SOC levels at MTREC were 27 Mg ha^{-1}, which was 23% higher than SOC levels at the MTREC experiment site at treatment onset. A similar silt loam soil at RECM under a tilled cotton production system had a SOC value of 17.26 Mg ha^{-1}, which is 82% of what was seen at the RECM site initially. This supports the theory that the no-tillage systems at the experiment sites had elevated carbon with respect to tilled systems, and were approaching their maximum storage potential.

Considering that the plots in this experiment may have achieved much of the SOC gains possible under conversion to no tillage row crop production, the initial losses could be attributed to a priming effect (Kuzyakov et al., 2000) as fertilization and crops were somewhat altered at the outset of the experiment. These systems may be approaching a new steady state of SOC. These experiments are ongoing to determine the steady state SOC of each cropping system.

Chapter IV

Conclusions

No-tillage systems typically increase SOC (Franzluebbers, 2005; West and Post 2002) however, a small loss was seen at both experiment locations and depths. Although losses were seen at both locations and both depths after four years of no-tillage production, it was seen that specific cropping sequences and winter bio-covers can affect soil organic carbon in no-tillage production systems. Cropping sequences with higher frequencies of cotton lost the most SOC and those with higher frequencies of soybean lost the least SOC at RECM. Cropping sequences with higher frequencies of corn had an intermediate effect, losing less than those of cotton, but more than those with higher frequencies of soybean at RECM. Under no-till, cotton is less conducive to SOC buildup than corn production. Soybean production was observed to be the most favorable toward increasing SOC of these three crops at RECM.

When only soybean and corn were used, as at the MTREC site, rotations with a higher frequency of corn tended to lose less SOC than those with higher frequencies of soybean. This may be due to the environmental factors at the site, previous site history, or a result of the treatments. Corn tends to produce more biomass and residue than soybean, which could be stabilized in the soil under the proper conditions.

Bio-covers also affected SOC. Over all crop sequences investigated in this study, sequences combined with poultry litter or wheat bio-covers tended to lose less SOC than those under vetch or left fallow at both locations. If producers are managing no-tillage production systems for SOC, over all common cropping sequences, wheat or poultry litter should be chosen as bio-covers rather than hairy vetch or allowing the fields to lay fallow during the winter. However, certain sequences may perform better under vetch or wheat bio-covers.

The change in production practices, defined as a priming effect, may have been responsible for the initial loss in SOC as cropping system and fertilization were changed when the treatments were implemented. This study is unique in that it compares different no tillage production systems. Alternate cropping sequences in combination with bio-covers were investigated on sites that have previously been under long-term no-till production. These sites allow comparisons of these cropping systems in different physiographic areas. The experiments are on-going for at least four more years.

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APPENDIX

Part II

Table 2.1. Cropping sequences planted over a four-year period at two sites in Tennessee.

Middle Tennessee Research and Education Center				
	------------------------Year-------------------------			
Crop Sequence	**2002**	**2003**	**2004**	**2005**
1	corn(Cr)	corn	corn	corn
2	soybean(S)	soybean	soybean	soybean
3	soybean	soybean	corn	soybean
4	corn	soybean	soybean	corn
5	corn	soybean	corn	soybean
6	soybean	corn	soybean	corn
7	soybean	corn	corn	soybean
8	corn	corn	soybean	corn
Research and Education Center at Milan				
	------------------------Year-------------------------			
Crop Sequence	**2002**	**2003**	**2004**	**2005**
1	cotton(Ct)	cotton	cotton	cotton
2	corn(Cr)	corn	corn	corn
3	soybean(S)	soybean	soybean	soybean
4	soybean	soybean	corn	cotton
5	corn	soybean	corn	soybean
6	soybean	cotton	soybean	cotton
7	soybean	cotton	corn	soybean
8	corn	corn	soybean	cotton
9	corn	cotton	soybean	corn
10	cotton	soybean	cotton	corn
11	cotton	soybean	corn	cotton
12	cotton	corn	cotton	soybean
13	cotton	corn	cotton	corn

Table 2.2. Adjusted nitrogen application rates for bio-covers at the Research and Education Centers at Milan (RECM) and Spring Hill (MTREC) from 2002 to 2005.

	RECM				MTREC			
	Year							
	2002	2003	2004	2005	2002	2003	2004	2005
Bio-cover	kg ha^{-1}							
Fallow	67-90	62	67	67	67	62	67	67
Litter	67	67	67	67	67	67	67	67
Vetch	62	45	50	50	50	56	67	50
Wheat	67-90	50	50	50	50	56	67	50

Table 2.3. Comparison of combustion (CC) and near infrared reflectance (SOC) derived soil organic carbon measurements taken at the Milan (RECM) and Middle Tennessee (MTREC) Research and Education Centers in the years 2001, 2004, and 2006.

			Year					
			2001		2004		2006	
Sequence/bio-cover†	Site	Depth	CC	SOC	CC	SOC	CC	SOC
		---cm---	Mg ha^{-1}					
Cr-S-Cr-S/W	MTREC	0-5	10.99	10.37	12.96	10.10	6.27	6.99
Ct-Cr-Ct-S/F	RECM	0-5	11.84	10.37	9.26	8.39	9.60	7.58
Ct-S-Ct-Cr/F	RECM	0-5	7.64	6.06	6.06	8.35	8.57	7.53
S-S-Cr-S/F	MTREC	0-5	8.04	8.04	6.91	6.47	8.22	6.32
S-S-S-S/L	RECM	0-5	9.16	9.24	6.91	7.31	7.70	7.90
S-S-S-S/W	MTREC	0-5	7.79	7.08	9.93	8.18	7.64	6.48
Cr-S-Cr-S/L	MTREC	5-15	5.36	5.87	6.91	6.60	5.31	5.64
Ct-Cr-Ct-Cr/F	RECM	5-15	5.89	5.89	6.28	5.93	7.12	6.48
S-Ct-Cr-S/V	RECM	5-15	5.39	5.28	3.44	4.02	3.79	4.29
S-S-Cr-S/F	MTREC	5-15	5.15	4.65	3.95	3.95	4.54	3.26
S-S-S-S/L	RECM	5-15	5.29	5.24	3.18	3.55	3.03	4.01
S-S-S-S/W	MTREC	5-15	6.21	4.99	5.47	5.26	5.47	4.09

†Ct = Cotton; Cr = corn; S = soybean; F = fallow; L = poultry litter; V = hairy vetch; W = winter wheat.

Table 2.3. Changes in soil organic carbon at two depths after four years (2002-2006) of cropping sequences and bio-covers under no-tillage at the Milan (RECM) and Middle Tennessee (MTREC) Research and Education Centers.

	RECM†			MTREC†			RECM			MTREC		
	2002	2006	Loss	2002	2006	Loss	2002	2006	Loss	2002	2006	Loss
	Depth(cm)											
Treatment	0-5						5-15					
<u>Sequence‡</u>	$Mg\ ha^{-1}$											
Ct-Ct-Ct-Ct	9.39	7.28	2.11	-	-	-	11.10	8.57	2.53	-	-	-
Cr-Cr-Cr-Cr	9.61	8.42	1.19	9.56	7.61	1.95	10.19	8.60	1.59	13.64	10.30	3.34
S-S-S-S	8.72	8.34	0.38	8.23	5.69	2.54	10.96	9.62	1.34	10.87	7.34	3.53
S-S-Cr-S	-	-	-	8.22	5.96	2.02	-	-	-	11.14	7.84	3.3
Cr-S-S-Cr	-	-	-	9.45	7.04	2.41	-	-	-	13.25	9.37	3.88
S-S-Cr-Ct	8.37	8.07	0.29	-	-	-	10.18	9.49	0.69	-	-	-
Cr-S-Cr-S	9.35	8.83	0.52	9.88	7.57	2.31	10.54	9.62	0.92	13.06	10.39	2.67
S-Cr-S-Cr	-	-	-	7.95	5.55	2.40	-	-	-	10.98	7.97	3.01
S-Ct-S-Ct	9.04	7.72	1.32	-	-	-	10.81	9.72	1.09	-	-	-
S-Cr-Cr-S	-	-	-	7.98	5.60	2.38	-	-	-	11.16	7.97	2.84
S-Ct-Cr-S	9.13	8.30	0.83	-	-	-	10.67	9.35	1.32	-	-	-
Cr-Cr-S-Cr	-	-	-	10.04	7.45	2.59	-	-	-	13.00	10.85	2.15
Cr-Cr-S-Ct	9.13	7.94	1.19	-	-	-	11.12	9.06	2.06	-	-	-
Cr-Ct-S-Cr	9.50	8.43	1.07	-	-	-	10.91	8.60	2.31	-	-	-
Ct-S-Ct-Cr	9.35	8.21	1.14	-	-	-	10.57	9.36	1.21	-	-	-
Ct-S-Cr-Ct	9.15	7.97	1.18	-	-	-	10.30	8.78	1.52	-	-	-
Ct-Cr-Ct-S	9.44	7.99	1.45	-	-	-	10.04	9.38	0.66	-	-	-
Ct-Cr-Ct-Cr	9.38	8.24	1.14	-	-	-	10.48	9.20	1.28	-	-	-
Average	9.20	8.13	1.06 (12%)	8.91	6.56	2.33 (26%)	10.61	9.18	1.45 (13%)	12.14	9.00	3.09 (25%)
LSD (0.05)§			0.94	LSD (0.05)		NS	LSD (0.05)		1.17	LSD (0.05)		NS
<u>Bio-cover</u>												
Fallow	9.44	8.11	1.33	9.07	6.50	2.57	10.58	9.77	0.81	12.48	9.10	3.37
Litter	8.79	8.19	0.60	9.22	7.15	2.07	11.00	9.02	1.98	13.02	10.38	2.64
Vetch	9.50	8.05	1.45	8.63	6.21	2.42	10.47	8.93	1.54	11.14	7.78	3.36
Wheat	9.06	8.14	0.92	8.74	6.38	2.36	10.38	8.98	1.40	11.90	8.93	2.99
Average	9.20	8.12	1.08 (12%)	8.92	6.56	2.36 (26%)	10.61	9.18	1.44 (14%)	12.14	9.05	3.09 (25%)
LSD (0.05)§			0.66	LSD (0.05)		NS	LSD (0.05)		1.79	LSD (0.05)		NS

† n= 208 for average of sequences and bio-covers at RECM; n= 128 for average of sequences and bio-covers at MTREC.
‡ Ct = Cotton; Cr = corn; S = soybean.
§ LSD = Fishers protected least significant difference at $P \leq 0.05$.
(-) = treatment not present at that location.
(%) = decline from 2002 to 2006.

Table 2.4. Changes in soil organic carbon at two depths after four years (2002-2006) of combinations of cropping sequences and bio-covers under no-tillage at the Milan (RECM) and Middle Tennessee (MTREC) Research and Education Centers.

	RECM†			MTREC†			RECM			MTREC		
	2002	2006	Loss	2002	2006	Loss	2002	2006	Loss	2002	2006	Loss
	Depth(cm)											
Treatment	0-5						5-15					
Sequence/biocover												
	Mg ha^{-1}											
All continuous‡												
Ct-Ct-Ct-Ct/F	9.72	7.90	1.82	-	-	-	12.29	9.70	2.59	-	-	-
Ct-Ct-Ct-Ct/L	8.25	6.99	1.26	-	-	-	11.30	8.80	2.50	-	-	-
Ct-Ct-Ct-Ct/V	10.37	6.83	3.55	-	-	-	11.02	8.03	2.99	-	-	-
Ct-Ct-Ct-Ct/W	9.53	7.41	2.12	-	-	-	9.78	7.74	2.04	-	-	-
Average	9.47	7.28	2.19 (23%)	-	-	-	11.10	8.57	2.53 (23%)	-	-	-
Cr-Cr-Cr-Cr/F	9.59	8.26	1.32	9.46	7.27	2.19	10.57	9.09	1.49	14.20	10.05	4.14
Cr-Cr-Cr-Cr/L	9.61	8.57	1.04	10.19	8.54	1.66	9.99	7.53	2.47	14.08	11.74	2.33
Cr-Cr-Cr-Cr/V	10.36	8.05	2.31	9.05	7.20	1.34	9.96	8.62	1.34	12.41	8.59	2.98
Cr-Cr-Cr-Cr/W	8.90	8.79	0.11	9.56	7.44	2.12	10.22	9.17	1.06	13.89	10.82	3.07
Average	9.62	8.42	1.2 (12%)	9.56	7.61	1.83 (19%)	10.19	8.60	1.59 (16%)	13.64	10.30	3.13 (23%)
S-S-S-S/F	9.16	7.57	1.59	8.26	5.79	2.47	11.37	9.74	1.63	10.53	7.18	3.35
S-S-S-S/L	8.24	8.91	-0.67	7.99	6.24	1.75	11.83	8.81	3.02	12.94	8.54	4.40
S-S-S-S/V	8.38	8.82	-0.44	8.43	5.18	3.25	10.17	9.79	0.38	10.01	6.47	3.54
S-S-S-S/W	9.12	8.05	1.07	8.24	5.56	2.67	10.45	9.86	0.58	10.01	7.19	2.82
Average	8.73	8.34	0.39 (4%)	8.23	5.69	2.54 (31%)	10.96	9.55	0.13 1.40	10.87	7.34	3.53 (32%)
One Year of Soybean‡												
Cr-Cr-S-Cr/F	-	-	-	9.87	6.83	3.04	-	-	-	13.44	9.77	3.66
Cr-Cr-S-Cr/L	-	-	-	10.36	7.77	2.59	-	-	-	12.87	11.56	1.31
Cr-Cr-S-Cr/V	-	-	-	10.18	7.61	2.90	-	-	-	12.25	10.43	3.44
W/Cr-Cr-S-Cr	-	-	-	9.75	7.60	2.15	-	-	-	13.43	11.65	1.79
Average	-	-	-	10.04	7.45	2.67 (27%)	-	-	-	13.00	10.85	2.55 (20%)
Cr-Cr-S-Ct/F	9.49	8.36	1.14	-	-	-	10.44	9.83	0.61	-	-	-
Cr-Cr-S-Ct/L	8.82	7.55	1.26	-	-	-	10.51	8.58	1.93	-	-	-
Cr-Cr-S-Ct/V	8.83	8.10	0.73	-	-	-	12.29	8.93	3.36	-	-	-
Cr-Cr-S-Ct/W	9.38	7.75	1.63	-	-	-	11.25	8.89	2.36	-	-	-
Average	9.13	7.94	1.2 (13%)	-	-	-	11.12	9.06	2.07 (19%)	-	-	-
Cr-Ct-S-Cr/F	9.03	8.31	0.72	-	-	-	11.08	9.39	1.69	-	-	-
Cr-Ct-S-Cr/L	9.98	8.41	1.57	-	-	-	10.79	7.11	3.69	-	-	-
Cr-Ct-S-Cr/V	10.10	8.66	1.44	-	-	-	10.10	9.93	1.57	-	-	-
Cr-Ct-S-Cr/W	8.89	8.36	0.53	-	-	-	10.29	7.98	2.32	-	-	-
Average	9.50	8.44	1.07 (11%)	-	-	-	10.57	8.60	2.31 (22%)	-	-	-
Ct-S-Ct-Cr/F	9.55	8.16	1.40	-	-	-	10.09	9.66	0.42	-	-	-
Ct-S-Ct-Cr/L	8.53	8.69	-0.16	-	-	-	11.59	10.06	1.53	-	-	-
Ct-S-Ct-Cr/V	9.89	7.62	2.27	-	-	-	10.43	8.11	2.32	-	-	-
Ct-S-Ct-Cr/W	9.43	8.36	1.06	-	-	-	10.20	9.59	0.61	-	-	-
Average	9.35	8.21	1.14 (12%)	-	-	-	10.58	9.36	1.22 (12%)	-	-	-
Ct-S-Cr-Ct/F	9.17	8.19	0.98	-	-	-	10.77	10.10	0.67	-	-	-
Ct-S-Cr-Ct/L	8.49	7.62	0.87	-	-	-	11.23	8.59	2.64	-	-	-
Ct-S-Cr-Ct/V	9.62	7.60	2.02	-	-	-	10.00	8.13	1.88	-	-	-
Ct-S-Cr-Ct/W	9.32	8.47	0.85	-	-	-	9.21	8.29	2.04	-	-	-
Average	9.15	7.97	1.18 (13%)	-	-	-	10.30	8.78	1.81 (18%)	-	-	-
Ct-Cr-Ct-S/F	10.01	7.96	2.06	-	-	-	9.86	8.67	1.19	-	-	-
Ct-Cr-Ct-S/L	9.23	6.68	2.55	-	-	-	10.91	11.05	-0.14	-	-	-
Ct-Cr-Ct-S/V	10.00	7.46	2.53	-	-	-	10.23	8.05	2.18	-	-	-
Ct-Cr-Ct-S/W	8.51	8.83	-0.32	-	-	-	9.18	9.74	-0.56	-	-	-
Average	9.44	7.73	1.70 (18%)	-	-	-	10.05	9.38	0.67 (7%)	-	-	-
Ct-Cr-Ct-Cr/F	9.40	8.69	0.70	-	-	-	8.72	10.72	-2.00	-	-	-
Ct-Cr-Ct-Cr/L	8.88	8.19	0.69	-	-	-	11.50	8.30	3.20	-	-	-
Ct-Cr-Ct-Cr/L	9.64	7.99	1.66	-	-	-	10.88	8.96	1.92	-	-	-
Ct-Cr-Ct-Cr/W	9.60	8.07	1.54	-	-	-	10.82	8.83	1.99	-	-	-
Average	9.38	8.24	1.15 (12%)	-	-	-	10.48	9.20	1.28 (12%)	-	-	-
LSD (0.05)¶			1.67	LSD (0.05)		NS	LSD (0.05)		2.64	LSD (0.05)		NS

† n= 208 for average of sequences and bio-covers at RECM; n= 128 for average of sequences and bio-covers at MTREC.

‡ Ct = Cotton; Cr = corn; S = soybean.

§ F = fallow; L = poultry litter; V = hairy vetch; W = winter wheat.

¶ LSD = Fisher's protected least significant difference at $P \leq 0.05$.

(-) = treatment not present at that location.

(%) = decline from 2002 to 2006.

Table 5. Changes in soil organic carbon at two depths after four years (2002-2006) of combinations of cropping sequences and bio-covers under no-tillage at the Milan (RECM) and Middle Tennessee (MTREC) Research and Education Centers.

	RECM†			MTREC†			RECM			MTREC		
	2002	2006	Loss	2002	2006	Loss	2002	2006	Loss	2002	2006	Loss
	Depth(cm)											
	0-5						5-15					
Sequence/biocover												
	Mg ha^{-1}											
Three Years of Soybean‡												
S-S-Cr-S/F	-	-	-	8.71	6.32	2.39	-	-	-	11.95	9.30	2.65
S-S-Cr-S/L	-	-	-	8.46	6.52	1.87	-	-	-	11.65	9.48	1.83
S-S-Cr-S/V	-	-	-	8.01	5.30	2.71	-	-	-	9.66	6.16	3.51
S-S-Cr-S/W	-	-	-	7.70	5.70	2.36	-	-	-	11.29	6.43	2.99
Average	-	-	-	8.22	5.96	2.33 (28%)	-	-	-	11.14	7.84	2.75 (25%)
Two years of Soybean‡												
Cr-S-S-Cr/F	-	-	-	9.67	7.18	2.48	-	-	-	13.80	9.63	4.17
Cr-S-S-Cr/L	-	-	-	10.16	7.98	2.17	-	-	-	15.07	10.39	4.68
Cr-S-S-Cr/V	-	-	-	8.51	6.49	2.01	-	-	-	11.84	7.83	4.01
Cr-S-S-Cr/W	-	-	-	9.48	6.49	2.99	-	-	-	12.30	9.64	2.66
Average	-	-	-	9.45	7.04	2.41 (26%)	-	-	-	13.25	9.37	3.88 (0.29%)
S-S-Cr-Ct/F	8.91	8.10	0.82	-	-	-	-	-	-	10.02	9.86	0.16
S-S-Cr-CtL	7.87	8.18	-0.31	-	-	-	-	-	-	10.83	9.66	1.17
S-S-Cr-Ct/V	8.53	8.16	0.37	-	-	-	-	-	-	9.51	9.16	0.35
S-S-Cr-Ct/W	8.16	7.84	0.33	-	-	-	-	-	-	10.37	9.29	1.08
Average	8.37	8.07	0.30 (05%)	-	-	-	-	-	-	10.18	9.49	0.69 (0.07%)
Cr-S-Cr-S/F	10.48	8.54	1.94	9.77	6.87	2.90	10.39	9.33	1.07	12.59	10.36	2.22
Cr-S-Cr-S/L	8.72	8.80	-0.08	10.08	7.92	2.16	10.90	8.70	2.20	13.24	10.25	3.00
Cr-S-Cr-S/V	9.40	9.50	-0.10	10.20	8.13	2.47	10.56	10.73	-0.17	13.24	10.84	3.35
Cr-S-Cr-S/W	8.81	8.50	0.31	9.46	7.36	2.10	10.31	9.70	0.61	13.19	10.12	3.07
Average	9.35	8.84	0.52 (6%)	9.88	7.57	2.41 (24%)	10.54	9.62	0.93 (9%)	13.06	10.39	2.91 (22%)
S-Cr-S-Cr/F	-	-	-	8.44	5.54	2.90	-	-	-	11.38	7.94	3.44
S-Cr-S-Cr/L	-	-	-	8.47	6.43	2.05	-	-	-	12.04	10.18	1.86
S-Cr-S-Cr/V	-	-	-	7.04	4.93	2.11	-	-	-	9.87	6.05	3.83
S-Cr-S-Cr/W	-	-	-	7.85	5.30	2.55	-	-	-	10.64	7.72	2.92
Average	-	-	-	7.95	5.55	2.40 (30%)	-	-	-	10.98	7.97	3.01 (27%)
S-Ct-S-Ct/F	9.35	7.34	2.01	-	-	-	11.41	10.63	0.77	-	-	-
S-Ct-S-Ct/L	8.73	7.84	0.89	-	-	-	10.41	10.08	0.32	-	-	-
S-Ct-S-Ct/V	8.93	7.99	0.94	-	-	-	9.97	9.46	0.51	-	-	-
S-Ct-S-Ct/W	9.15	7.71	1.44	-	-	-	11.47	8.72	2.75	-	-	-
Average	9.04	7.72	1.32 (15%)	-	-	-	10.82	9.72	1.09 (10%)	-	-	-
S-Cr-Cr-S/F	-	-	-	8.37	6.16	2.21	-	-	-	11.96	8.61	3.36
S-Cr-Cr-S/L	-	-	-	8.04	5.83	2.21	-	-	-	12.30	10.93	0.74
S-Cr-Cr-S/V	-	-	-	7.61	4.83	2.78	-	-	-	9.83	5.90	3.93
S-Cr-Cr-S/W	-	-	-	7.91	5.59	2.32	-	-	-	10.55	7.84	2.71
Average	-	-	-	7.98	5.60	2.38 (30%)	-	-	-	11.16	8.32	2.69 (24%)
S-Ct-Cr-S/F	8.87	8.01	0.87	-	-	-	10.52	10.34	0.18	-	-	-
S-Ct-Cr-S/L	8.90	9.01	-0.11	-	-	-	11.19	9.94	1.25	-	-	-
S-Ct-Cr-S/V	9.42	7.88	1.54	-	-	-	9.60	8.21	1.38	-	-	-
S-Ct-Cr-S/W	9.34	8.32	1.02	-	-	-	11.36	8.91	2.46	-	-	-
Average	9.13	8.31	0.83 (9%)	-	-	-	10.67	9.35	1.32 (12%)	-	-	-
LSD (0.05)¶			1.67	LSD (0.05)			LSD (0.05)		2.64	LSD (0.05)		

† n= 208 for average of sequences and bio-covers at RECM; n= 128 for average of sequences and bio-covers at MTREC.
‡ Ct = Cotton; Cr = corn; S = soybean.
§ F = fallow; L = poultry litter; V = hairy vetch; W = winter wheat.
¶ LSD = Fisher's protected least significant difference at $P \leq 0.05$.
(-) = treatment not present at that location.
(%) = decline from 2002 to 2006.

Table 6. Soil organic carbon (SOC) in agro-ecosystems near the Research and Education Centers at Middle Tennessee (MTREC) and Milan (RECM).

Sample Site	**----Depth----**	**----Soil Organic Carbon----**	
	(cm)	(%)	(Mg ha^{-1})
MTREC 25 Year No-till	0-5	2.47	17.43
MTREC 25 Year No-till	5-15	1.35	9.55
MTREC Forest	0-5	5.63	39.73
MTREC Forest	5-15	4.07	28.69
MTREC Pasture	0-5	3.92	27.61
MTREC Pasture	5-15	1.80	12.68
RECM 25 Year No-till	0-5	2.10	14.79
RECM 25 Year No-till	5-15	0.85	6.00
RECM Tilled	0-5	1.41	9.91
RECM Tilled	5-15	1.04	7.35
RECM Forest	0-5	3.22	22.73
RECM Forest	5-15	1.14	8.00

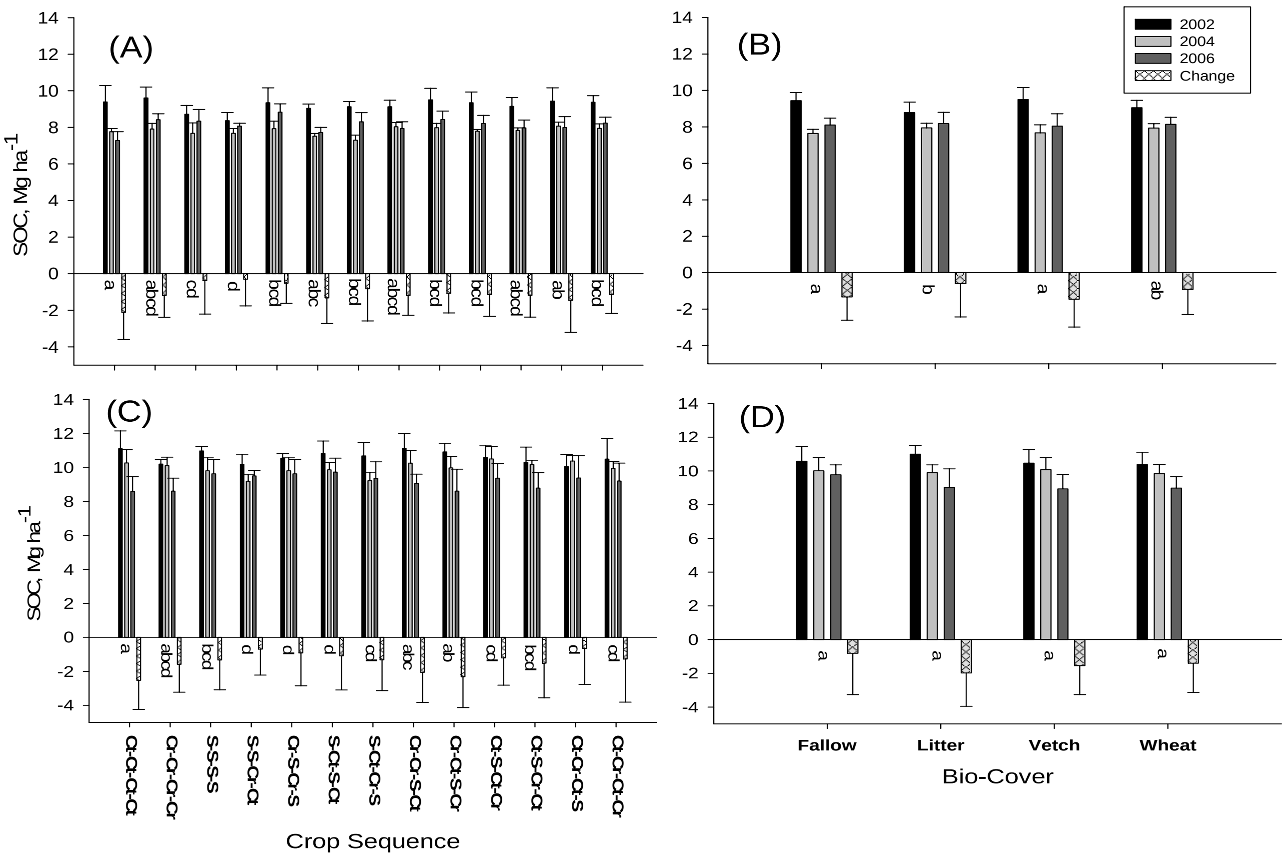

Fig. 1. Changes in total soil organic carbon (SOC) over time (2002-2006) at the Research & Education Center at Milan by crop sequence (Ct, cotton; Cr, corn; S, soybean) averaged across bio-covers at 0-5 cm depth (A) and 5–15 cm depth (C); and by bio-cover averaged across crop sequence at 0-5 cm depth (B) and 5-15 cm depth (D). Within a chart, SOC losses that are followed by the same letter are not significantly different, based on Fisher's Protected LSD ($P \leq 0.05$). Whiskers denote 95% confidence interval.

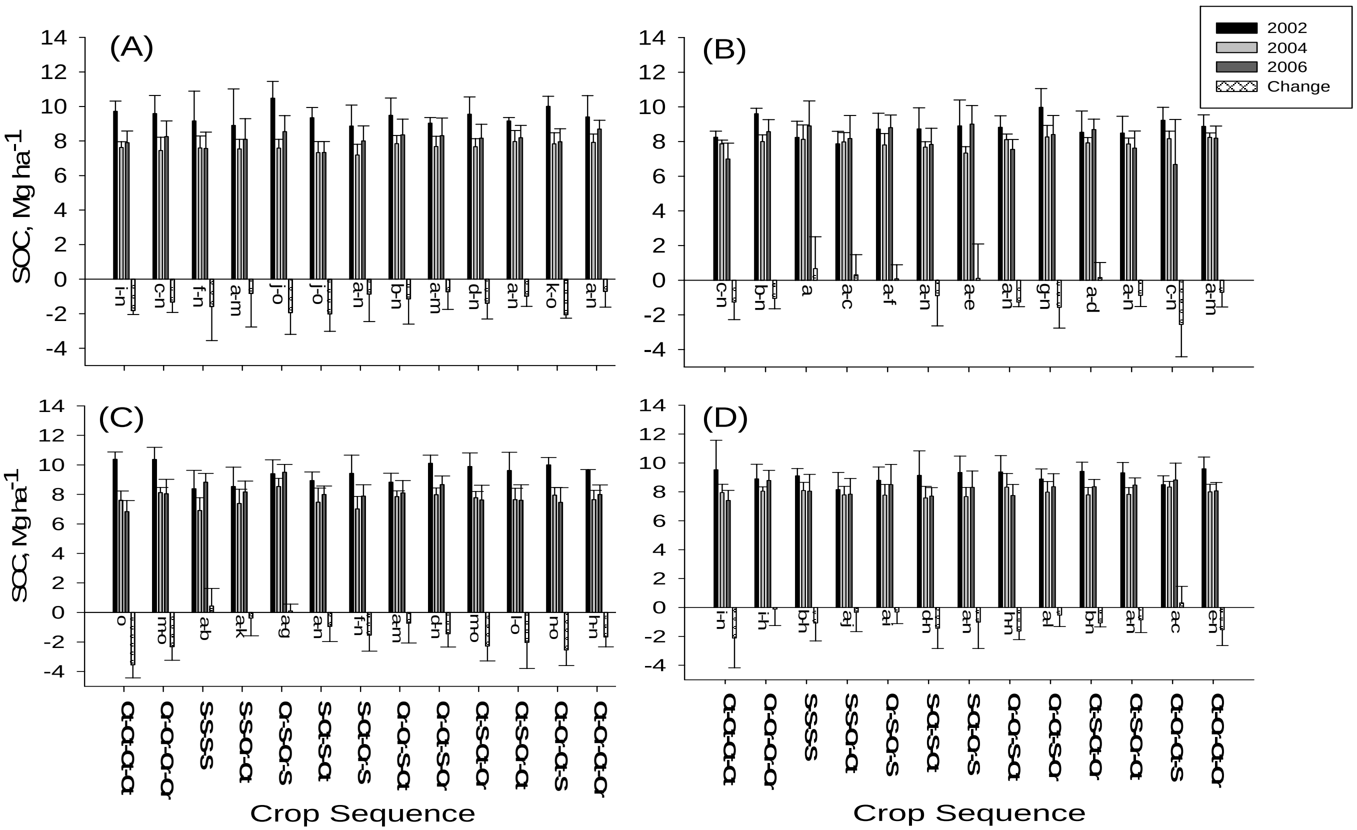

Fig 2. Changes in total soil organic carbon (SOC) over time (2002-2006) at the Research & Education Center at Milan resulting from the combination of crop sequence (Ct, cotton; Cr, corn; S, soybean) and the bio-cover fallow (A), vetch (B) poultry litter (C), and wheat (D) at 0 – 5 cm depth. Across all four charts, losses that are followed by the same letter are not significantly different, based on Fisher's Protected LSD (P≤0.05). Whiskers denote 95% confidence interval.

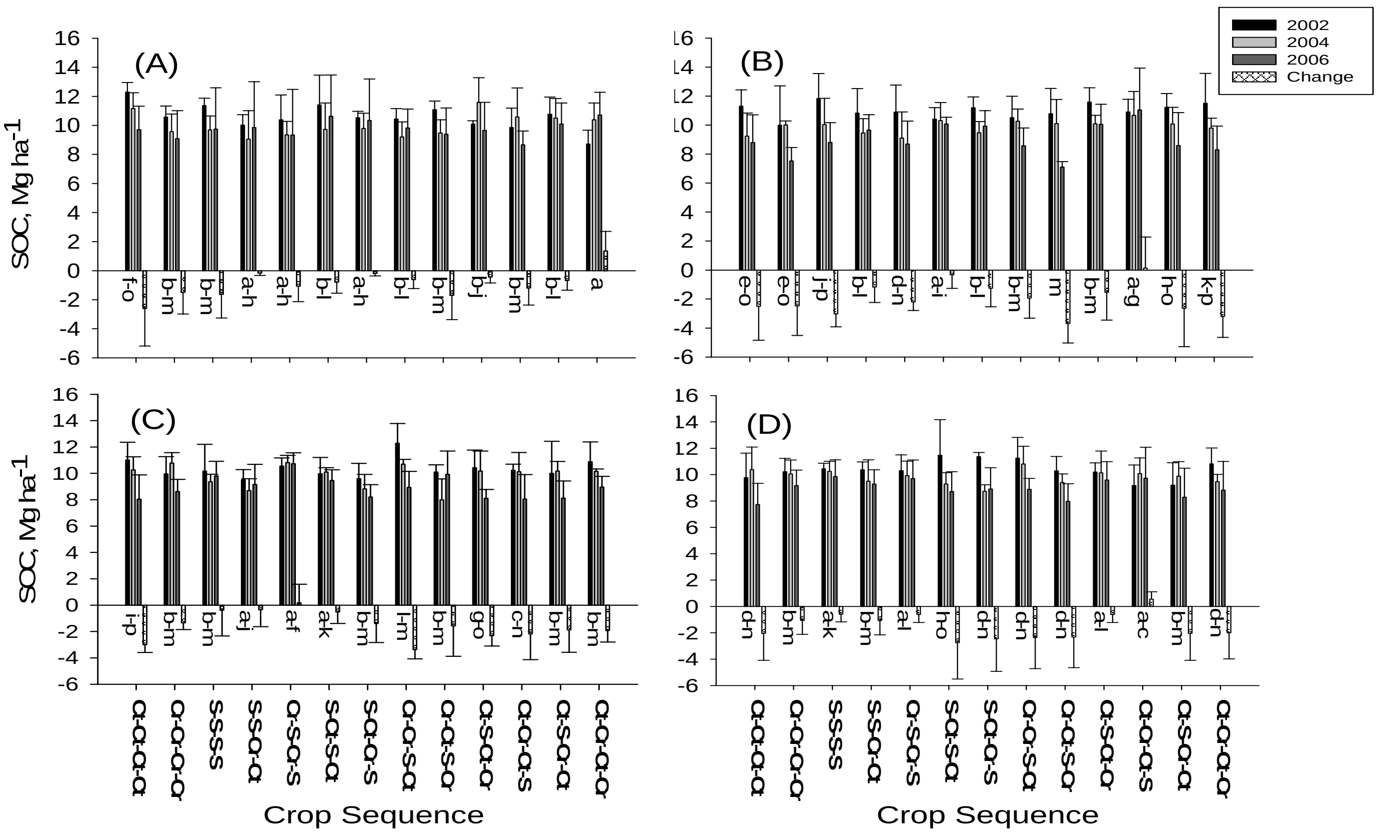

Fig 3. Changes in total soil organic carbon (SOC) over time (2002-2006) at the Research & Education Center at Milan resulting from the combination of crop sequence (Ct, cotton; Cr, corn; S, soybean) and the bio-cover fallow (A), poultry litter (B) vetch (C), and wheat (D) at 5 – 15 cm depth. Across all four charts, losses that are followed by the same letter are not significantly different, based on Fisher’s Protected LSD (P≤0.05). Whiskers denote 95% confidence interval.

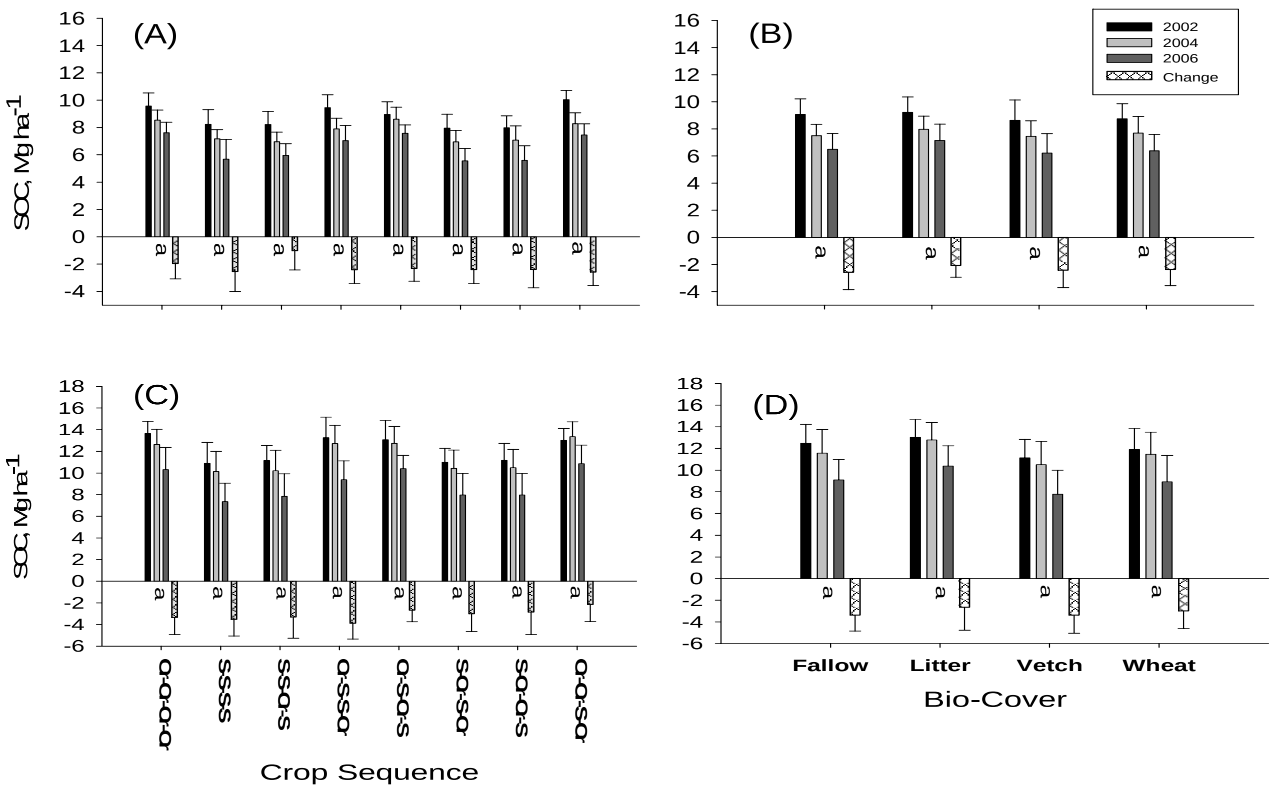

Fig. 4. Changes in total soil organic carbon (SOC) over time (2002-2006) at the Middle Tennessee Research & Education Center by crop sequence (Cr, corn; S, soybean) averaged across bio-covers at 0-5 cm depth (A) and 5–15 cm depth (B); and by bio-cover averaged across crop sequence at 0-5 cm depth (C) and 5-15 cm depth (D). Within a chart, SOC losses that are followed by the same letter are not significantly different, based on Fisher's Protected LSD (P≤0.05). Whiskers denote 95% confidence interval.

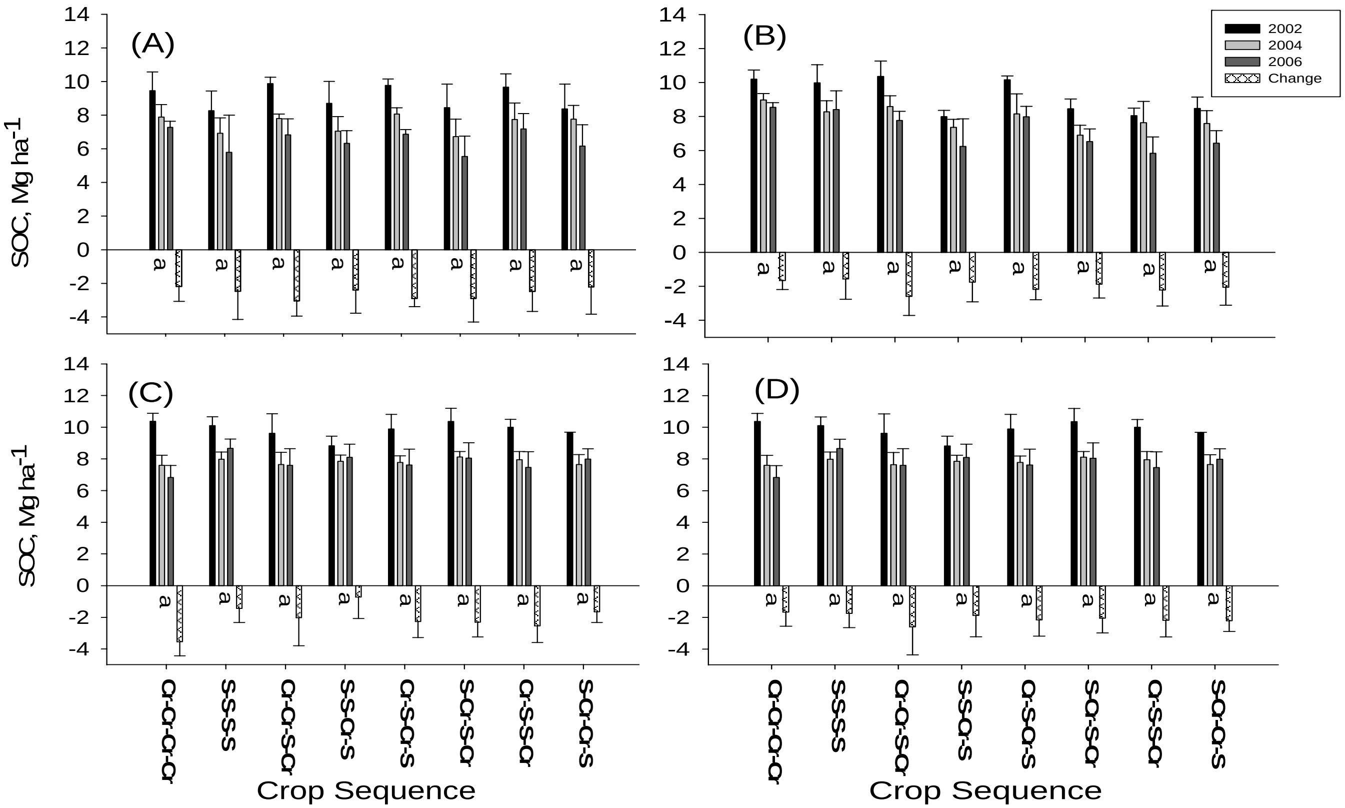

Fig 5. Changes in total soil organic carbon (SOC) over time (2002-2006) at the Middle Tennessee Research & Education Center resulting from the combination of crop sequence (Cr, corn; S, soybean) and the bio-cover fallow (A), poultry litter (B), vetch (C), and wheat (D) at 0 – 5 cm depth. Across all four charts, losses that are followed by the same letter are not significantly different, based on Fisher's Protected LSD (P≤0.05). Whiskers denote 95% confidence interval.

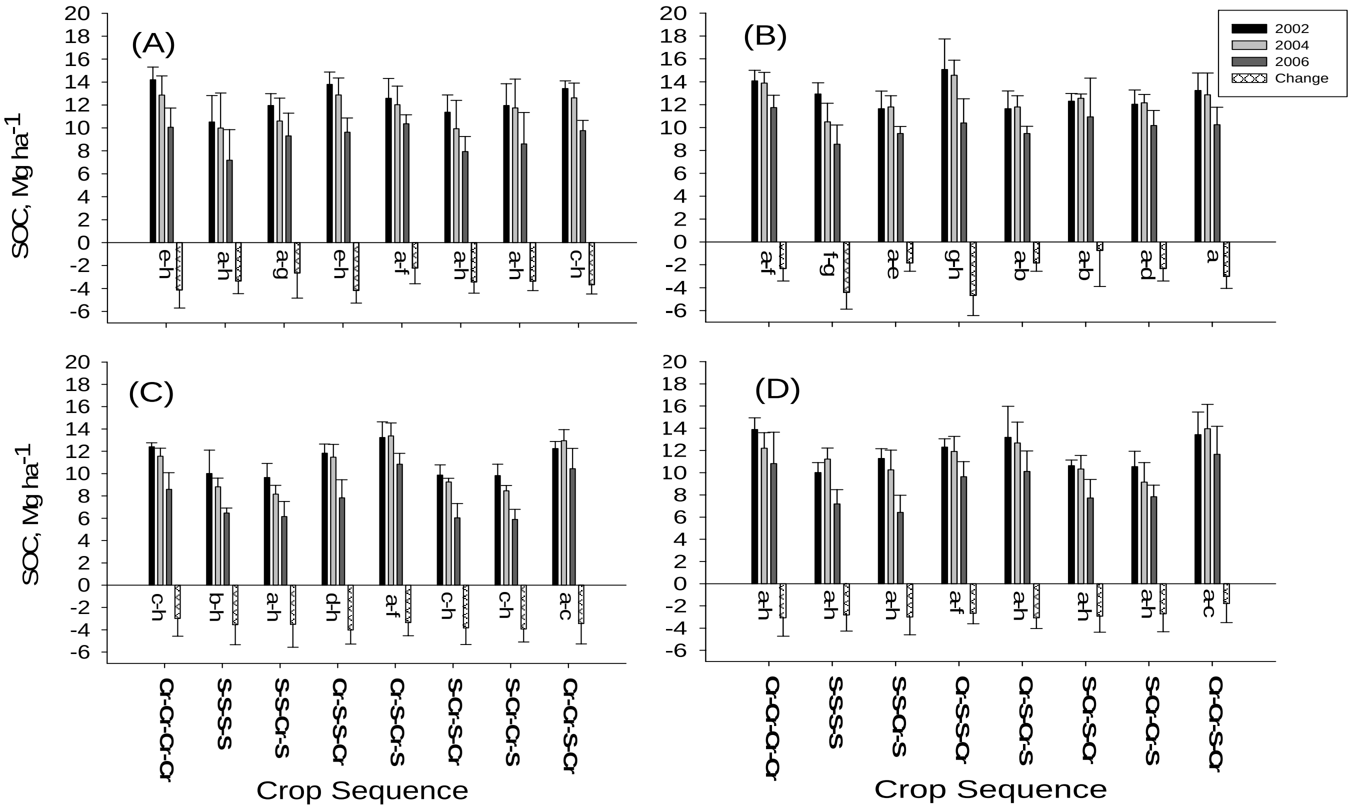

Fig 6. Changes in total soil organic carbon (SOC) over time (2002-2006) at the Middle Tennessee Research & Education Center resulting from the combination of crop sequence (Cr, corn; S, soybean) and the bio-cover fallow (A) poultry litter (B), vetch (C), and wheat (D) at 5 – 15 cm depth. Across all four charts, losses that are followed by the same letter are not significantly different, based on Fisher's Protected LSD (P≤0.05). Whiskers denote 95% confidence interval.

Part III

Impact of cropping sequence and bio-covers on the soybean cyst nematode, *Heterodera glycines*

Abstract

The influence of various crop management practices on soybean cyst nematode (SCN) varies with environmental and cultural practices. The objective of this research was to examine the effects of combinations of cropping sequences and soil bio-covers on SCN egg population density under no-tillage. The experiment used a split-block design with four replications at the Milan Research & Education Center (RECM) in western Tennessee. The whole-block treatments were cropping sequences of corn (*Zea mays* L.), soybean (*Glycine max* L., Merr), and cotton (*Gossypium hirsutum* L., Malvaceae). The split-block treatments were bio-covers of winter wheat (*Triticum aestivum* L.), hairy vetch (*Vicia villosa* Roth), poultry litter, and fallow. Soil samples were taken after 2, 3, 4 years of experimentation at 0-15 cm depth. Overall, SCN egg population density was well below the economic threshold in all treatments. Mean SCN egg population density across all treatments was 146 ± 116 eggs per 100 cm^3 at the onset of the experiment and highest after three years of experimentation, being 195 ± 95 eggs per 100 cm^3 soil in 2005. For the other years, mean SCN egg population density was 146 ± 109 and 140 ± 121 eggs per 100 cm^3 SCN in 2004 and 2006, respectively. Eggs were found in sequences containing no soybean throughout the experiment, indicating survival of over seven years without soybean. Sequences with higher frequencies of soybean in the sequence had significantly higher ($P \leq 0.01$) SCN egg population density than others, with continuous soybean having the greatest egg population density. One year of rotation with a non-host crop reduced SCN egg population density. Although variability obscured treatment significance, poultry litter bio-cover numerically had the lowest mean SCN egg population density while vetch resulted in the highest.

Chapter II

Introduction

Nematodes are a diverse, complex group of microscopic roundworms that can be found in all soils. Plant-parasitic species can severely impact agricultural productivity. An estimated $100 billion in annual agricultural yield losses are due to nematode damage (Sasser and Freckman, 1987). Soybean cyst nematode (*Heterodera glycines*) was first described in 1952 (Ichinohe, 1952). It first appeared in North America in North Carolina in 1954 (Winstead et al., 1955). Currently, this species is known to occur in 29 states ranging from Florida to Minnesota (NAPIS Annual Survey, 2007) and is the most damaging pathogen to soybean (*Glycine max* L. Merr.) in the United States (Pratt and Wrather, 1998; Wrather et al., 2001). Soybean cyst nematode (SCN) is responsible for an estimated 3% to 7% soybean yield losses in North America (Riggs, 1992), roughly 5 million metric tons of yield losses annually (Wrather et al., 2001). Nematodes can play such a significant role that when other limiting factors are taken into account, their populations can be used as predictors of yield losses in annual crops (Barker et al., 1985). Therefore, improvements in alternative methods of nematode control are critical to maintain agricultural productivity and profitability.

Nematodes can cause up to 30% yield loss with no visible above ground symptoms (Noel, 1992; Noel and Edwards, 1996; Wang et al., 2003). This makes soil testing of SCN populations critical to prevent crop damage. There are two key levels of infestation, the action threshold and the economic threshold. The action threshold is the SCN egg or cyst population density at which it is recommended that producers alter their practices to manage a SCN infestation. The economic threshold is the infestation level at which yield loss is predicted to occur. These two levels vary by soil type and fertility, climate, and soybean cultivar (Niblack, 2005). These site specific conditions should be considered in determining the yield impact of SCN.

There has been extensive research devoted to minimizing the effect of these nematodes on crop yields (Kinloch, 1998). For centuries, crop rotation and cover crops have been used to minimize crop loss due to nematode predation. This control strategy involves alternating susceptible crops with non-host species. When eggs hatch and no host plant is present, nematodes cannot reproduce, resulting in a population reduction. The amount of time that critical plant-parasitic species can survive in soil with no host is dependent on a variety of factors, with annual temperature pattern being a major one.

Cool temperatures promote survival of SCN eggs within cysts (Kinloch, 1998). Eggs encapsulated within a cyst go dormant during cooler temperatures, allowing them to survive harsh winter conditions (Yen et al., 1995). They hatch when temperatures warm to between 16 and 20^{o}C (Alston and Schmitt, 1987) or upon exposure to root exudates (Riga et al., 2001).

The length of rotations with non-host crops required to reduce SCN to sub-critical levels is dependant on regional weather conditions. In the United States, SCN survival rate is generally higher in northern regions and lower in southern regions (Riggs et al., 2001). Eggs can remain viable in soil for more than three years in Mississippi with no host plant present (Moore et al., 1991), and over five years in Minnesota (Porter et al., 2002).

Regional differences in SCN survival has lead to rotation designs that are tailored to site-specific conditions in order to minimize economic impact. Slack et al. (1981) observed population declines of 75% in 1-year rotations of soybean with a non-host and as much as 92% in 2-year rotations in Arkansas. In Minnesota it was observed that corn was the least effective of 16 non-host species for reducing nematode populations, and a single year was not effective in controlling SCN before planting susceptible soybean (Miller et al., 2006). However, another Minnesota study found that one year of corn followed by on year of growing a resistant soybean cultivar in sequences with susceptible soybeans was sufficient to significantly reduce SCN egg population density (Chen, 2007). Because cyst nematode eggs can remain viable in Tennessee soil for over three years, it is recommended that severely infested fields must have lengthy rotations of non-host species to allow for natural dieback (Noe, 1998).

Winter covers, or bio-covers, are an important part of no-till production; reducing erosion and promoting soil and water quality. They can also affect nematode populations. Bio-covers that are host alternative host plants, such as hairy vetch (*Vicia villosa* Roth) (Riggs, 1992), can increase nematode populations during winter (Creech et al., 2005). Cover crops such as wheat (*Triticum aestivum* L.) can also reduce SCN populations. When exposed to wheat root exudates their winter dormancy is broken, causing juveniles to be exposed to harsh conditions and killed (Hershman and Bachi, 1995). Some covers, such as red clover (*Trifolium pratense* L.) act as trap crops; nematodes enter their roots, but do not leave or reproduce poorly (Kushida et al., 2002). Weeds that commonly arise during winter fallow, including henbit (*Lamium amplexicaule*), chickweed (*Stellaria media*), and purple deadnettle (*Lamium purpureum*) can serve as SCN hosts (Riggs, 1992, Venkatesh et al., 2000). It is uncertain what the effect of host winter weeds is on SCN numbers. It has been reported that these hosts, combined with soil temperatures in the range required for SCN development can increase SCN numbers (Creech et al., 2005). However, under western Tennessee field conditions, no indication of SCN reproduction was observed during three winter fallow periods on weeds or crimson clover in soybean production fields (Donald et al., 2007).

Poultry litter, a bio-cover used as a soil amendment, can also affect nematode communities (Koenning and Barker, 2004). While supplying plant nutrients, poultry litter may encourage the growth of nematode parasites such as the *Fusarium oxysporum* and *Verticillium chlamydosporium*, and may be used as an alternative or supplemental control method. Morant et al. (1997) found that poultry litter applied at a rate of 5 tons per acre can reduce SCN cyst numbers and increase soybean yields.

Conservation tillage systems may not have the same beneficial effects on reducing nematode populations that disking or plowing have. These mechanical means cause root material to be brought to the surface, causing it to be exposed to potentially harsh environmental conditions. This typically reduces overall nematode survival. Additionally, intact crop residue can affect egg hatch by increasing water infiltration and reducing surface soil temperatures. Stubble can also provide habitat for nematode antagonists, negatively impacting nematode species. The effects of tillage on *H. glycines* reproduction have been mixed. Koenning et al., 1993; Tyler et al., 1987; Baird and Bernard, 1984; and Workneh et al., 1999 all found that no tillage decreased the population denisty of SCN. Chen et al., 2001; Noel and Wax, 2003; and Hershman and Bachi, 1995 found no effect of tillage systems on SCN population density. Others have found differing tillage effects on SCN. Tyler et al. (1987) found that no-tillage production reduced SCN cyst numbers compared to disk, chisel, moldboard plow, or subsoiling consistently over a 6 year period. The objective of this study was to evaluate the effects of cropping sequence and winter bio-cover combinations on SCN populations under no-till production.

Chapter II

Materials and Methods

Site Description

The field study began in 2002 at the Research and Education Center at Milan (RECM) in Milan, TN (35.54^{o} N -88.44^{o} W). Mean annual precipitation is 135 centimeters and mean annual temperature is 16.6^{o}C. The soil type is a Loring B2 series: Fine-silty, mixed, thermic Typic Fragiudalf.

Prior to the study, the field was left fallow in 2001. In 2000, the entire field site was planted in corn, soybean in 1999, cotton in 1998 and 1997, and soybean in 1996 and 1995, with wheat being planted each winter (Table 3.2). The field was under no-tillage production for over 16 years prior to initiation of this experiment.

Experiment Design

The experiment used a split-block design with treatments arranged in randomized complete blocks. The whole plot treatments were crop sequences, which were strip-applied across the length of the field. Thirteen individual cropping sequences consisting of glyphosate resistant corn, soybean, and cotton were planted (Table 3.1; Note: all tables and figures are placed in the Appendix to Part III). Four bio-cover treatments, including hairy vetch, winter wheat, poultry litter, and fallow were strip-applied as a sub-plot treatment perpendicular to crop sequence strips. This created 52 sequence bio-cover combinations applied to separate 6.1m by 12.2m subplots. Each treatment combination was replicated four times, for a total of 208 plots. Varieties planted were ‘PM 1218 BG/RR’ cotton, ‘DKC 6410 RR’ corn, and ‘USG 7440nRR’ soybean which is moderately resistant to races 3 and 14 of SCN (UniSouth Genetics Inc., 2006). Cotton was planted on 102 cm rows while corn and soybean were planted on 76 cm.

Fertilizer was applied as appropriate to each crop according to soil tests and the guidelines published in the Tennessee Agricultural Extension Service Guidelines to Crop Production, and litter application rates were applied at the rate of 66.7 kg N ha^{-1} assuming 50% bioavailability (UT Extension Service, 2003). Nutrient content of the poultry litter, vetch, wheat, and winter weeds (fallow) bio-covers were tested by A&L Analytical Laboratories, Inc. (Memphis, TN). To determine nutrient content of vetch, wheat, and winter weeds, a 0.5 by 1 m sample of the living material was taken just prior to burndown. All bio-cover strips received the equivalent of 66.7 kg N ha^{-1} prior to planting. Pre-plant fertilizer application rates were reduced by the amount of nitrogen supplied by the vetch or other bio-covers, as appropriate. Corn plots received an additional 128.5 kg N ha^{-1} and cotton received 33.35 kg N ha^{-1} as side-dress treatments in late May or early June. Corn, soybean, and cotton plots were planted at the recommended seeding rates of 64,200 seeds $hectacre^{-1}$, 344,500 seed $hectacre^{-1}$, and 64,500 seeds $hectacre^{-1}$, respectively (UT Extension, 2003).

Herbicides were applied as field conditions necessitated. Prior to planting, burn down herbicides were used. Either paraquat (1,1-Dimethyl-4,4-bipyridinium) or glyphosate (N-phosphonomethyl-glycine) was applied in April. Post-emergence, one or two applications of glyphosate were applied to the soybean and corn plots in either May or June of each year. For the cotton plots, additional pesticide use was required and application dates ranged from June through September.

Glyphosate and clethodim (RS)-2-9[(E)-1-[(E)-3-chloroallyloxyimino] propyl]-5-[2-(ethylthio) propyli]-3-hydroxycyclohex-2-en-1-l-one) were used all four years. Def (S,S,S-Tributyl phosphorotrithioate), Bidrin (Dimethyl phosphate of 3-Hydroxy-N,N-dimethyl-cis-crotonamide) were used as defoliants and Pix (1,1-dimethylpiperidinium chloride) was applied to regulate cotton growth. A list of the pesticides and application rates used at the site can be found in Appendix A.

Sample Collection

SCN egg population density was determined in 2003, 2004 and 2005, the final three years of the four-year cropping sequence. To determine SCN egg population density, a composite sample of twelve 2.5 cm cores to a 15 cm depth were collected from the within and between row sections of each plot. Samples were collected approximately three weeks prior to spring bio-cover burndown, during the third week of March.

Soil samples were stored in a refrigeration chamber at 4.5^oC before the analysis. Samples were first thoroughly mixed and large aggregates were crushed. Cysts were extracted from a 120 cm^3 subsample with a semiautomatic elutriator (Byrd et al., 1976). Eggs were released from cysts mechanically (Faghihi and Ferris, 2000), sieved through a 250 µm pore size sieve, collected, and counted. Nematode population density was expressed as eggs 100 cm^3 of soil.

Data Analysis

SCN egg population densities were rank transformed to achieve a normal distribution and residual normality. The data were analyzed using Mixed Model procedures (SAS, 1997). These procedures produce Type III F-values but do not provide mean square values or error terms for each model term in the analysis. Mean separation was performed with the SAS macro “pdmix800” (Saxton, 1998) and Fisher’s Protected Least Significant Difference with a Type I rate of less than 0.05% . Analysis of variance tests were performed to identify differences caused by crop sequence and bio-covers, and their interaction.

Chapter III

Results and Discussion

Mean pre-plant SCN egg population density over all treatments rose from 146 ± 4 eggs 100 cm^3 of soil to 195 ± 3 eggs per 100 cm^3 in 2005, and fell to 140 ± 4 eggs per 100 cm^3 soil in 2006 (Fig.3.1). SCN infection and development is inhibited in soil with high moisture content (Young and Heatherly, 1988). The late spring and summer are typically high in precipitation at Milan, but in 2005, June, July, and September were below average in precipitation, with July and September receiving less than 25% of the historical average precipitation (National Climate Data Center, 2007). Temperatures in March, April, May, June, and July of 2005 were higher than historical averages, which may have stimulated an early egg hatch and rapid development (Ross, 1964; Alston and Schmitt, 1987).

Cropping sequence had a highly significant ($P<0.01$) effect on SCN egg population density for each of the three observed years (Table 3.2). Mean SCN density was typically significantly ($P\leq 0.05$) higher in sequences consisting of higher frequencies of soybeans. Additionally, sequences in which the previous crop was soybean had higher mean SCN eggs densities than those planted in cotton or corn. This indicates that SCN was able to infect and reproduce on the soybean variety USG 7440nRR, even though it is moderately resistant to races 3 and 14 (UniSouth Genetics Inc., web source). Higher SCN egg densities were seen consistently throughout the 4-year sequence in the continuous soybean treatment. The highest density was 927 ± 582 eggs per 100 cm^3 of soil being seen after two years of the agronomic crop (Fig. 3.1). SCN egg densities were relatively constant in subsequent years, being 672 ± 313 eggs and 909 ± 737 eggs per 100 cm^3 of soil in 2005 and 2006, respectively. Assuming an average of 200 eggs per cyst (Niblack, 2005), these egg densities are equivalent to approximately 3 to 4 cysts per 100 cm^3 of soil. These levels are well below the Tennessee economic threshold value of 40 cysts per 100 cm^3 of soil (Newman, 2003).

SCN egg population density dropped after one year of rotation with a non-host crop in all treatments. However, some carryover between years was observed in that sequences that had two years of soybeans in the four-year sequence, such as (S)-soybean(S)-corn(Cr)-cotton(Ct) had higher mean SCN egg population density (78 ± 10 per 100 cm^3) than those that had one, like Cr-Ct-S-Cr (63 ± 13 per 100 cm^3) and Ct-S-Ct-Cr (36 ± 4 per 100 cm^3) after the four year sequence was completed in 2006 (Table 3.4).

These results are dissimilar to those other work done in Minnesota (Miller et al., 2001;) and Missouri (Jackson et al., 2005) who observed that one year of non-host crop rotation and winter fallow did not consistently negatively affect SCN survival. The discrepancy may be due to different climatic conditions, as regions with warmer winters are less favorable for SCN survival than those that are cool (Riggs et al., 2001). Another factor that may be allowing SCN management with brief non-host rotation is that that USG 7440nRR is moderately resistant to races 3 and 14. Others have seen that corn annually rotated with resistant soybean cultivars provide adequate SCN control (Chen, 2007).

Residual SCN population densities were also seen throughout the experiment, as SCN eggs were found in all treatments combinations, even those that had not been planted with soybean at any time during the 4-year cropping sequence. Continuous cotton and an annual corn-cotton rotation both had mean SCN egg population densities greater than zero in all three years while continuous corn had mean SCN egg population density of 105 ± 43 and 39 ± 35 eggs per 100 cm^3 soil in 2005 and 2006, respectively. SCN eggs found in sequences without soybean do not appear to be experimental error for five reasons reasons. First, cysts and eggs can be moved by anything moves soil (Chen, et al., 2001), and may have been carried into the site or across the plots. Second, the site was planted in soybean three, six, and nine years prior to initiation of the experiment, and may have developed a well established SCN population. SCN cysts are known to persist in similar environments for over four years. SCN egg dormancy can be dependant on climatic conditions, root exudates, and time (Yen et al., 1995). Third, eggs were found consistently in most bio-covers and treatment repetitions of these entirely non-host cropping sequences each year. Fourth, those plots exhibiting SCN infestation in any single year continued to have elevated egg densities throughout the course of the experiment. A fifth possibility is that there may have been some reproduction on weeds that grew on the plots over the winter. Although no reproduction was seen on winter weeds by Donald et al. (2007), given the proper weather conditions, SCN can reproduce on chickweed (*Stellaria media*), and henbit (*Lamium amplexicaule*) (Riggs, 1992) which grew on soybean sites during the non-growing period. SCN was also found in winter fallow plots that had not been planted in soybean. No SCN was found in the fallow bio-cover in continuous corn or cotton sequences in 2004 and 2006. However, 2005 when SCN density was highest throughout the experiment, egg population densities were 133 ± 82 and 58 ± 42 eggs per 100 cm^3 in continuous cotton and continuous corn, respectively (Table 3.4).

Although, variability obscured treatment significance, there were some trends amongst the mean SCN population density under winter bio-covers (Fig. 3.2). Fallow, poultry litter, and wheat were in the lowest 50th percentile of mean SCN density two out of the three years (Table 3.3). This observations seen amongst the fallow bio-cover is in agreement with research performed by Donald et al. (2007) who found no SCN reproduction on weeds in winter fallowed soybean fields in southwestern Tennessee.

The wheat bio-cover may have had a suppressive effect on *H. glycines*. Jennings and Bernard (1987) noted that residual wheat roots appeared to reduce the ability of *H. glycines* to locate and enter soybean roots while Hershman and Bachi (1995) concluded that wheat reduced SCN numbers in no-till wheat-soybean cropping systems.

Across all crop sequences, SCN egg population density under poultry litter was lowest of all bio-covers in two of the three years sampled, being 36 ± 2 eggs cm^{-1} and 103 ± 7 eggs cm^{-1} in 2004 and 2006, respectively (Table 3.3). Others have found that poultry litter can promote the growth of bacteria and fungi (Riegel and Noe, 2000), and may increase the population of nematophagous fungi such as *Fusarium solani, F. oxysporum, Paecilomyces lilacinus and Verticillium chlamydosporium*, which parasitize SCN (Bernard et al., 1996). This phenomena may explain the slightly lower mean SCN egg densities observed under poultry litter, which has been shown to suppress populations of SCN (Morant et al., 1997) and other nematode species, including the root lesion nematode (*Pratylenchus penetrans)* (Everts et al., 2006).

Vetch performed the poorest of the four bio-covers, being in the lower 50th percentile mean SCN density all three years. The highest egg population density across all bio-covers and years was seen under vetch in 2004, which was 224 ± 8 eggs 100 cm^3.

Under proper conditions, hairy vetch can serve as a host to SCN (Riggs, 1992). This property may be responsible for the effect observed amongst the cover crops. During the historically warm winter of 2005 SCN egg densities were highest in continuous soybean with vetch bio-cover (1068 ± 108 eggs 100 cm^3) with the next highest being under fallow (576 ± 80 eggs 100 cm^3), followed by poultry litter (552 ± 36 eggs 100 cm^3) and wheat (492 ± 38 eggs 100 cm^3). Conversely, in sequences consisting only of corn and cotton, vetch had the lowest SCN density in two of the three years. High variability precluded significance of the bio-cover treatment or interactions with cropping sequence, so it is difficult to make strong inferences about these systems.

Chapter IV

Conclusions

In spring, 2006 SCN eggs were found in plots that had not been planted with soybean for seven years. This indicates that *H. glycines* may have a prolonged persistence in western Tennessee. SCN was able to establish and maintain an infective population on the cultivar USG 7440nRR, which is rated as moderately resistant to SCN races 3 and 14. However, egg population density remained well below the economic threshold throughout the experiment. Rotating SCN resistant soybean with non-host agronomic crops can reduce SCN egg population density, with as little as one year being enough to dramatically reduce SCN density. The effects seen were cumulative, with egg population density declining with decreasing frequency of soybean in four year cropping sequences. Mean egg population density was generally lowest under the poultry litter bio-cover and highest under hairy vetch, with wheat and fallow being intermediate.

Acknowledgements

Trade names or commercial products were mentioned solely for the purpose of providing specific information and does not constitute an endorsement or recommendation by the University of Tennessee.

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APPENDIX

Part III

Table 3.1. Cropping sequences planted over a four-year period.

Research and Education Center at Milan				
	---------------------------Year---------------------------			
Crop Sequence	**2002**	**2003**	**2004**	**2005**
1	cotton(Ct)	cotton	cotton	cotton
2	corn(Cr)	corn	corn	corn
3	soybean(S)	soybean	soybean	soybean
4	soybean	soybean	corn	cotton
5	corn	soybean	corn	soybean
6	soybean	cotton	soybean	cotton
7	soybean	cotton	corn	soybean
8	corn	corn	soybean	cotton
9	corn	cotton	soybean	corn
10	cotton	soybean	cotton	corn
11	cotton	soybean	corn	cotton
12	cotton	corn	cotton	soybean
13	cotton	corn	cotton	corn

Table 3.2 Agronomic crops grown at experiment site prior treatment initiation.

Year	Crop
2001	fallow
2000	corn
1999	soybean
1998	cotton
1997	cotton
1996	soybean

Table 3.2. Means and confidence intervals (CI) for soybean cyst nematode numbers for three years of cropping sequences under no-tillage at the Milan Research and Education Center.

	Year					
	2004		2005		2006	
Sequence‡	Mean	95% CI	Mean	95% CI	Mean	95% CI
	-----------------------------Eggs per 100 cm^3-----------------------------					
Ct-Ct-Ct-Ct	6ef§	11	84bc	52	12de	10
Cr-Cr-Cr-Cr	3ef	6	105bc	43	39cde	35
S-S-S-S	927a	582	672a	313	909a	737
S-S-Cr-Ct	429ab	325	222ab	75	78bcde	78
Cr-S-Cr-S	21de	17	138bc	65	132bc	174
S-Ct-S-Ct	114cd	100	549a	234	114bcd	89
S-Ct-Cr-S	204bcd	166	153bc	85	318b	217
Cr-Cr-S-Ct	*f	0	93cd	47	42bcde	35
Cr-Ct-S-Cr	3ef	6	132bc	62	63cde	102
Ct-S-Ct-Cr	111abc	83	81cd	61	36cde	31
Ct-S-Cr-Ct	69def	113	51d	29	12e	13
Ct-Cr-Ct-S	3ef	6	138bc	58	30bcde	20
Ct-Cr-Ct-Cr	3ef	6	117cd	59	33cde	26
Average†	**146**	**109**	**195**	**91**	**140**	**121**

† n= 208 for average of sequences at RECM; each mean is an average across all bio--covers of that crop sequence.

‡ Ct = Cotton; Cr = corn; S = soybean. Cropping sequences were started in 2003.

§ Fishers protected least significant difference at $P \leq 0.05$; performed on rank transformed data (not shown). Values in a column followed by a common letter are not significantly different.

* SCN eggs were not detected.

Table 3.3. Means and confidence intervals (CI) for soybean cyst nematode numbers for three years of bio-covers under no-tillage at the Milan Research and Education Center.

	Year					
	2004		2005		2006	
Cover	Mean	95% CI	Mean	95% CI	Mean	95% CI
	Eggs per 100 cm^3					
Fallow	188a‡	167	169a	74	105a	74
Litter	36a	24	198a	66	103a	99
Vetch	184a	137	224a	115	162a	118
Wheat	175a	135	189a	68	189a	220
Average†	**146**	**116**	**195**	**81**	**140**	**128**

† n= 208 for average of sequences at RECM; each mean is an average across all bio-covers of that crop sequence.

§ Fishers protected least significant difference at $P \leq 0.05$; performed on rank transformed data (not shown). Values in a column followed by a common letter are not significantly different.

Table 3.4. Means and confidence intervals (CI) for soybean cyst nematode numbers for three years of combinations of cropping sequences and bio-covers under no-tillage at the Milan Research and Education Center.

	Year					
	2004		2005		2006	
Sequence‡	Mean	95% CI	Mean	95% CI	Mean	95% CI
	Eggs per 100 cm^3					
Ct-Ct-Ct-Ct/F	*	0	192	120	*	0
Ct-Ct-Ct-Ct/L	24	41	84	96	24	24
Ct-Ct-Ct-Ct/V	*	0	36	39	12	20
Ct-Ct-Ct-Ct/W	*	0	24	24	12	20
Average	**6**	**10**	**84**	**70**	**12**	**16**
Cr-Cr-Cr-Cr/F	*	0	84	61	*	0
Cr-Cr-Cr-Cr/L	*	0	156	70	96	88
Cr-Cr-Cr-Cr/V	12	20	60	77	*	0
Cr-Cr-Cr-Cr/W	*	0	120	97	60	77
Average	**3**	**5**	**105**	**76**	**39**	**41**
S-S-S-S/F	1764	1232	576	642	300	256
S-S-S-S/L	192	200	552	295	804	1043
S-S-S-S/V	672	1033	1068	876	912	968
S-S-S-S/W	1080	1233	492	304	1620	2398
Average	**927**	**924**	**672**	**529**	**909**	**1166**
S-S-Cr-Ct/F	372	631	108	84	*	0
S-S-Cr-Ct/L	108	107	204	126	108	157
S-S-Cr-Ct/V	924	912	312	181	156	238
S-S-Cr-Ct/W	312	306	264	108	48	58
Average	**429**	**489**	**222**	**125**	**78**	**113**
Cr-S-Cr-S/F	36	39	108	51	408	611
Cr-S-Cr-S/L	12	20	156	186	36	39
Cr-S-Cr-S/V	*	0	108	51	12	20
Cr-S-Cr-S/W	16	20	124	96	152	223
Average						
S-Ct-S-Ct/F	*	0	324	369	36	39
S-Ct-S-Ct/L	12	20	492	496	*	0
S-Ct-S-Ct/V	300	289	708	453	168	158
S-Ct-S-Ct/W	144	141	672	446	252	248
Average	**114**	**113**	**549**	**441**	**114**	**111**
S-Ct-Cr-S/F	24	41	84	143	252	328
S-Ct-Cr-S/L	48	81	156	130	72	78
S-Ct-Cr-S/V	444	285	312	195	720	535
S-Ct-Cr-S/W	300	482	60	61	228	360
Average	**204**	**222**	**153**	**132**	**318**	**325**
Cr-Cr-S-Ct/F	*	0	72	71	96	115
Cr-Cr-S-Ct/L	*	0	72	53	36	39
Cr-Cr-S-Ct/V	*	0	60	77	12	20
Cr-Cr-S-Ct/W	*	0	168	122	24	24
Average	*****	**0**	**93**	**81**	**42**	**50**
Cr-Ct-S-Cr/F	*	0	204	171	216	367
Cr-Ct-S-Cr/L	12	20	132	96	12	20
Cr-Ct-S-Cr/V	*	0	84	90	*	0
Cr-Ct-S-Cr/W	*	0	108	84	24	24
Average	**3**	**5**	**132**	**110**	**63**	**103**
Ct-S-Ct-Cr/F	228	231	12	20	*	0
Ct-S-Ct-Cr/L	36	61	180	177	24	24
Ct-S-Ct-Cr/V	36	39	12	20	36	61
Ct-S-Ct-Cr/W	144	166	120	85	84	84
Average	**111**	**124**	**81**	**76**	**36**	**42**
Ct-S-Cr-Ct/F	12	20	36	61	12	20
Ct-S-Cr-Ct/L	12	20	72	78	*	0
Ct-S-Cr-Ct/V	*	0	24	41	24	41
Ct-S-Cr-Ct/W	252	401	72	24	12	20
Average	**69**	**110**	**51**	**51**	**12**	**20**
Ct-Cr-Ct-S/F	*	0	180	146	12	20
Ct-Cr-Ct-S/L	12	20	180	112	72	53
Ct-Cr-Ct-S/V	*	0	108	84	12	20
Ct-Cr-Ct-S/W	*	0	84	70	24	24
Average	**3**	**5**	**138**	**103**	**30**	**29**
Ct-Cr-Ct-Cr/F	12	20	216	135	36	61
Ct-Cr-Ct-Cr/L	*	0	132	96	60	61
Ct-Cr-Ct-Cr/V	*	0	24	41	36	39
Ct-Cr-Ct-Cr/W	*	0	96	88	*	0
Average	**3**	**5**	**117**	**90**	**33**	**40**

† n= 16 for average of sequence, bio-cover interactions at RECM.

‡ Ct = Cotton; Cr = corn; S = soybean; F = fallow; L = poultry litter; V = hairy vetch; W = winter wheat. Cropping Sequences were started in 2003.

* SCN eggs were not detected.

Fig. 1. Changes in soybean cyst nematode (SCN) over time (2004-2006) at the Research & Education Center at Milan by crop sequence (Ct, cotton; Cr, corn; S, soybean) averaged across bio-covers at 0-15 cm depth (A); and by bio-cover averaged across crop sequence at 0-15 cm depth (B). Within a chart, SCN counts in a specific year that are followed by a common letter are not significantly different, based on Fishers Protected LSD (P≤0.05). Whiskers denote 95% confidence interval.

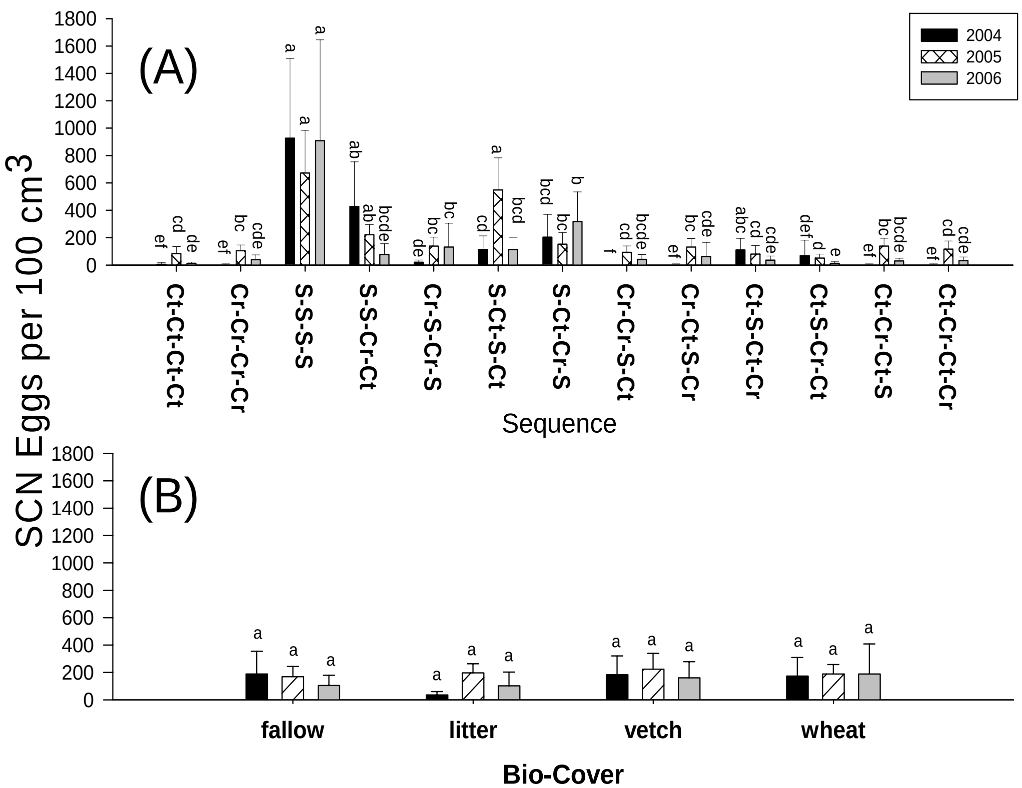
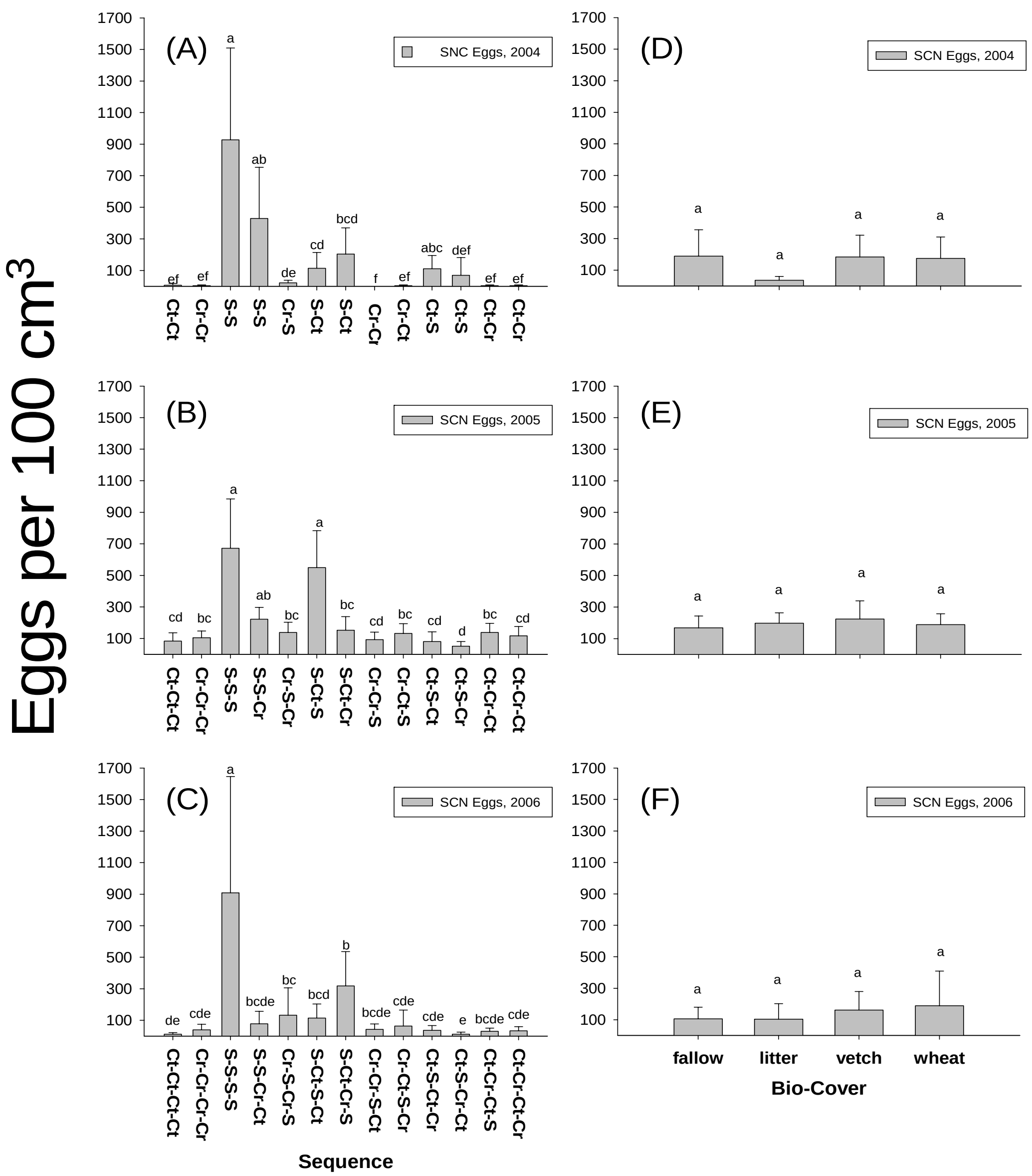


Fig. 2. Changes in soybean cyst nematode (SCN) over time (2004-2006) at the Research & Education Center at Milan by crop sequence (Ct, cotton; Cr, corn; S, soybean) averaged across bio-covers in 2004 (A), 2005 (B) and 2006 (C); and by bio-cover averaged across crop sequence at 0-15 cm depth in 2004 (D), 2005 (E), and 2006 (F). Within a chart, SCN counts that are followed by the same letter are not significantly different, based on Fishers Protected LSD (P≤0.05). Whiskers denote 95% confidence interval.



SUMMARY

As concern about climate change, environmental quality, and rising fuel costs grows, the number or acres under no-tillage production will increase. This necessitates the long-term evaluation of these cropping systems. In this study, 13 cropping sequences at the Research and Education Center at Milan and eight crop sequences at the Middle Tennessee Research and Education Center were combined with four winter bio-covers and evaluated for their effect on soil organic carbon (SOC) and to determine their impact on the plant parasite soybean cyst nematode (SCN). Due to the scope of the experiment, a third objective was developed, to create a rapid and inexpensive system of soil carbon analysis.

Near-infrared reflectance spectroscopy (NIR) was developed to determine SOC. Soil samples were collected from the site and across Tennessee. These were used to calibrate NIR measurements with the benchmark combustion detection method. Ultimately, a viable alternative to combustion was developed, that allowed the rapid and cost-effective analysis of the soils for SOC. Others have shown that the method can be used to measure other soil properties, including nitrogen, phosphorous, potassium, heavy metals, texture, and moisture. The method is non-destructive, consumes no reagents, is portable, and adaptable to automated and in-situ measurements. NIR may be able to replace many routine soil analytical techniques and deployed in-field to make real-time site-specific crop management decisions for precision agriculture. Further development should be performed to realize this instruments' full potential.

Agricultural ecosystems are highly complex, relatively minor site characteristics can alter the overall a system. This was observed in the lack of SCN at MTREC, lack of treatment significance in SOC change analysis, and difference in general trends due to crop sequence seen at both sites. At RECM, sequences with high frequencies of soybean had the best effect on SOC while those high in cotton had the worst with corn being intermediate. Conversely, at MTREC, sequences with higher frequencies of corn performed better than those with higher frequencies of soybean. However, differences observed at MTREC were not statistically significant.

The effect of bio-cover on SOC was similar at both sites, though only statistically significant at RECM. Sequences in combination with poultry litter and wheat lost less SOC than those involving vetch or fallow at both locations. If producers are managing no-tillage production systems for SOC, over all common cropping sequences, wheat or poultry litter should be chosen as bio-covers rather than hairy vetch or allowing the fields to lay fallow during the winter.

Soybean cyst nematode was seen to persist in Western Tennessee soils for over seven years though it was not found at MTREC, which is in the central portion of the state. Crop rotation is the oldest nematode control method available, and is still used currently. Sequences with higher frequencies of soybean had significantly higher SCN density than those with fewer soybean plantings. In this study, it was seen that one year of planting a field with a non-host crop was sufficient to dramatically reduce nematode populations. Genetic resistance, a modern innovation in SCN control, was also effective. The cultivar used in this study, 'USG 7440nRR', is moderately resistant to races 3 and 14. The crop sequences that were continuously planted with this cultivar exhibited the highest SCN density throughout the experiment, indicating that SCN could maintain a reproductive population on these plants. However, SCN populations were controlled, with SCN densities being well below the economic threshold.

Agricultural ecosystems are intricate and their behavior is difficult to predict. In this study, even though the productions systems were similar, the history of each site was different, with differing weather, pest, and productivity. Long-term cropping system experiments are important tools to observe the effect of management decisions. Lengthy experiments may allow time dependant variables such as site history and weather variation to equilibrate. Repetition across multiple sites allows the determination how site characteristics such as soil type and climate interact with cropping system to determine carbon dynamics. These types of experiments can provide information about potentially harmful long-term trends while also yielding short-term contributions to agricultural science. No-tillage agricultural production is rapidly adopted by produces in the United States and around the world, which will insure the future importance of the cropping systems study at MTREC and RECM.

Vita

Jason P. Wight is a PhD student and graduate research assistant for the Agronomic Crop Variety Testing Program in the Department of Plant Sciences at the University of Tennessee. He is a native of Louisiana and holds two degrees from Louisiana State University. He has a Bachelor of Science degree in Environmental Management Systems from the Department of Agronomy and a Master of Science degree in Environmental Toxicology from the Department of Environmental Studies. Jason began his doctoral studies in Plant Sciences at the University of Tennessee in 2003, and completed his doctorate in December 2007.