Comparison of Soil Organic Carbon Methods and Carbon Sequestration and Soil Characteristics in Louisiana Crawfish Ponds

by

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Dedication

To Elizabeth, who walked me to the door.

To the homeless puppies, who found their home and granted me the opportunity.

To mum, who is not able to read English or Agronomy, but has worked on soil all her life.

To both dads, who fell when I was 3 and 21, now lying down peacefully on the top of the mountain. Hope the work will make them proud.

Abstract

Two studies were conducted for this thesis research. In the first study, nine soil organic carbon (SOC) methods were evaluated using 336 soil samples from different regions, including Alabama, Louisiana, and Arkansas, as well as sites in Ecuador, Kenya, and Tanzania. Comparisons were made between nine methods, including dry combustion, wet combustion (Walkley-Black), loss on ignition, photometry, near-infrared spectroscopy (NIRS), the Solvita® method, color, texture, and visualization, with dry combustion serving as the control method. The following variables were considered as the basis of comparison: equipment and reagents, accuracy, efficiency, complexity, cost and environmental effect. The results show that: Walkley-Black (r^2=0.89), loss on ignition (r^2=0.86), and Solvita® (r^2=0.64) correlates better with dry combustion than others; photometry has modest accuracy (r^2=0.31); other methods such as NIRS, color, texture, and visualization have limited accuracy. Loss on ignition is recommended as a routine test by soil testing laboratories, as equipment is commonly found in laboratories and no reagents are required. Further, good accuracy was achieved, and the method is simple with low cost, and no hazardous wastes.

In the second study, carbon (C) sequestration potential and soil characteristics were studied in soils under Louisiana crawfish ponds. Two crawfish culture systems, monocropping and rotational, were compared with adjacent agriculture fields. Only the upper depth of soil from crawfish monocropping systems (25.8 g kg^{-1}) had SOC concentrations that were greater than the adjacent agricultural control (20.8 g kg^{-1}). In other horizons of monocropping systems and all

horizons of rotational systems, no differences were found in either C concentration or C mass. The results indicate crawfish ponds do not sequester more C than upland cropping systems. Other soil characteristics such as bulk density, nitrogen (N) concentration and mass, particle size, pH, and carbon to nitrogen ratio (C:N), were examined. Bulk density helped explain the SOC concentration and mass distribution difference. Nitrogen concentration and mass had similar depth distribution as SOC. The average pH of monocropping and rotational system is 7.1 and 7.0 respectively, which was within the normal range. The average C:N of monocropping and rotational system is 11.4 and 9.7, which was close to a natural system of 10:1.

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In order to keep my thesis from being 100 pages longer, my list must end here. The applause, praises, gratitude, and love are the innermost for other faculty, staff, graduate students and undergraduates, for friends and hosting families standing behind me, and for family and friends overseas. If there is one thing that always makes me proud, it is not this thesis, it is them.

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Comparison of Soil Organic Carbon Methods

Introduction

Soil organic carbon (SOC) is essential for soil and environmental quality. It is a key component of soil organic matter (SOM), and it affects soil physical, chemical and biological properties. Soil organic carbon plays a critical role in the global C balance, thought to be a major factor in global climate change (Brady and Weil, 2008). Better understanding of SOC can help evaluate and classify soils, and assist in application of best management practices for irrigation, fertilization and pesticide application (Mehlich, 1984). In order to estimate C sequestration, it is important to assess soil C storage (McCarty et al., 2002). In addition, the assessment of SOC is important for the development of proposed C taxes and cap-and-trade programs within the USA, as well as worldwide (Drexler, 2008; King, 2004; Lippke and Perez-Garcia, 2008; Schimmoller, 2007).

There are many methods available for quantifying SOC, each of which has advantages and disadvantages. In this study, nine methods were assessed for equipment, accuracy, efficiency, complexity, cost, and environmental effects. These methods included dry combustion, wet combustion (Walkley-Black), loss on ignition, photometry, near-infrared spectroscopy (NIRS), Solvita®, color, texture, and visualization. More than 300 soil samples were analyzed from diverse environments, including crawfish ponds and agricultural fields from Louisiana, different soils archived at the Auburn University Soil Testing Laboratory,

and soils collected in Arkansas, Ecuador, Tanzania, and Kenya. This research provided information concerning the most suitable method for assessing SOC for laboratories depending on the desired level of accuracy, budget, and hazardous laboratory wastes generated.

Carbon, Soil Organic Matter, and Soil Organic Carbon

Carbon

The element C is the foundation of life. It is common to all life forms on earth including microorganisms, plants and animals. The cycle of C on earth is the story of life on the planet. Imbalance in the C cycle is thought to cause problems on a global scale. For example, increasing CO_2 in atmosphere has been considered a major cause of climate change, which was identified over 100 years ago (Harvey, 2010).

Soil Organic Matter

Soil organic matter (SOM), by definition, includes plant, animal, and microbial residues, fresh, and at all stages of decomposition, and the relatively resistant soil humus (SSSA, 1979). Though it is a small portion of the soil, SOM can have a large influence on soil physical, chemical and biological properties. For example, soil physical properties are affected by SOM through increased soil plasticity and cohesion of clayey soils, which improves water retention, and increases infiltration rate. Soil organic matter has high cation exchange capacity (CEC) and contributes to the pH buffering capacity of soil. Soil biological properties are also influenced by SOM, which serves as a food source for fauna and microbes. Soil organic matter further affects the production of humic substances, polysaccharides, and other non-humic compounds (Brady

and Weil, 2008).

Soil organic matter plays an important role in agricultural and environmental management practices such as irrigation, fertilization, pesticide application, and pollution management. For example, soils with a relatively high SOM have higher surface infiltration, reducing soil erosion and increasing water availability to plants. This reduces water needed for irrigation. Soil organic matter increases retention and availability of nutrients like nitrogen (N), phosphorus (P), and sulfur (S), and transforms micronutrients like iron (Fe), zinc (Zn), and manganese (Mn) into soluble and chelated forms. This nutrient storage capacity can reduce dependence on inorganic fertilizers (Baldock and Nelson, 2000; Brady and Weil, 2008). The application of pesticides is related to the quality and quantity of SOM as well. Many herbicide labels note the need to vary application rates according to SOC concentrations (Konen et al., 2003). In addition, because SOM can absorb pollutants such as lead (Pb), cadmium (Cd) and copper (Cu), it reduces water pollution. The adsorption capacity further reduces the phytotoxicity of aluminum (Al) (Brady and Weil, 2008).

Soil Organic Carbon

Soil organic C comprises more than 50% of SOM (Baldock and Nelson, 2000). It is contained in the soil organic fraction, which consists of cells of microorganisms, plant and animal residues at various stages of decomposition (Nelson and Sommers, 1996). Nelson and Sommers concluded that the conversion factor between SOC and SOM varies from 1.724 to 2.5 depending on soil type, soil horizon, and other factors. They strongly suggested scientists to determine and report SOC rather than convert it to SOM.

Soil Organic Carbon Determination Methods

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There are many methods for analyzing SOC. This study was restricted to comparison among the following methods: dry combustion, Walkley-Black, loss on ignition, photometry, NIRS, Solvita®, color, texture, and visualization.

Dry Combustion

Dry combustion is conducted by heating a soil-catalyst mixture to 1000 $^{\circ}$C in a resistance or induction furnace in a stream of O_2 or CO_2-free air, followed by quantification of evolved CO_2 (Nelson and Sommers, 1996). Automated C analyzers have been widely used for dry combustion. The majority of instruments are automated versions of dry combustion procedures (Nelson and Sommers, 1996). The steps of an automated C analyzer are: (i) soil C conversion to CO_2 in a high-temperature chamber by oxidation; (ii) CO_2 transmission by a carrier gas which is separated from other gases; and (iii) concentration of CO_2 is determined by thermal conductivity, mass spectrometry or infrared gas analyzing methods (Chatterjee et al., 2009; Smith and Tabatabai, 2004). Dry combustion has been used as a standard method (Chatterjee et al., 2009) because it is precise, rapid and does not produce hazardous wastes. Nonetheless, it is relatively expensive due to cost of equipment (Chatterjee et al., 2009) and increased level of training required.

Walkley-Black Method

The Walkley-Black method (Walkley, 1947; Walkley and Black, 1934) is a rapid dichromate oxidation-reduction technique that proceeds according to the following general equations:

$$2Cr_2O_7^{2-} + 3C^0 + 16H^+ = 4Cr^{3-} + 3CO_2 + 8H_2O \qquad [1]$$

$$Cr_2O_7^{2-}+6Fe^{2+}+14H^+=2Cr^{3+}+6Fe^{3+}+7H_2O \qquad [2]$$
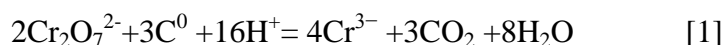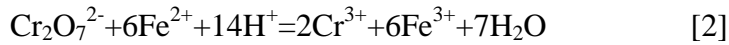
Carbon is assumed to be present in soil with an average valence of zero. The Cr_2O_7 reduced during this reaction is assumed to be a measure of the amount of organic C in the sample. The remaining Cr_2O_7 is titrated with ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$) to determine the amount of dichromate reduced (Chatterjee et al., 2009).

Walkley-Black is a classical method that was once commonly used. From its original form, there have been many modifications designed to address problems associated with the method (Chatterjee et al., 2009).

A recovery factor was introduced to calculate SOC because the Walkley-Black method does not give complete oxidation of SOC. Many studies found that the recovery factor of 1.32 used by Walkley and Black (1934) is not practical for soils coming from different regions with diverse soil depth, type, and mineralogy. Since there are many factors that affect oxidation of SOC, the recovery factor varies (Kamara et al., 2007; Nelson and Sommers, 1996). Chloride and ferrous iron in soil can lead to erroneously high values of SOC, while higher oxides of manganese result in lower values (Kamara et al., 2007). The presence of carbonized materials like charcoal, coal, and coke in soils also affect recovery (Chatterjee, 2009). Organic C in some soil types is more stable than others, lowering the recovery factor as well. Some C forms may not be oxidized or recovered completely with the heat of dilution generated by sulfuric acid (Diaz-Zorita, 1999; Hussain and Olson, 2000). Rhodes et al. (1981) showed that organic matter in tropical Ultisols was more easily oxidized during the Walkley-Black digestion than that in soils of temperate-zone countries due to the different nature of organic matter present. There are variations for recovery as stated above, but according to Kamara et al. (2007), the average recovery factor in each group is similar, which makes it possible to report the value of SOC of a

certain type of soil using the same recovery factor.

One significant drawback of the Walkley-Black method is the production of hazardous chemicals, such as dichromate, a carcinogen. Despite known disadvantages, this method is widely used for its low cost, minimum equipment requirement, simplicity, and availability. Studies have shown that external heat can increase recovery of SOC. However, for convenience, simplicity, and rapidity, the original Walkley-Black method with its heat of dilution of H_2SO_4 is more widely used (Nelson and Sommers, 1996), and was also used in this study.

Loss on Ignition

Loss on ignition is another method used to quantify SOC (Ball, 1964). After drying soil, it is heated at a specified temperature in a muffle furnace. Numerous studies (Konare et al., 2010; Sutherland, 1998; Szava-Kovats, 2009) have demonstrated the efficacy of this method using different temperatures and duration of ignition. In these studies, temperature ranged from $300^{\circ}C$ to $550^{\circ}C$, and the duration of ignition varied from 2 hours to 17 hours. Most studies (Wang et al., 2011b; Wright et al., 2008) draw the conclusion that loss on ignition has a good correlation with dry combustion. In the research of Kamara et al. (2007), both temperature and sample weight had significant effects on loss on ignition, with temperature having more effect than sample weight. However, the interactions were relatively small. In that research (Kamara et al., 2007), a one gram soil sample was ignited at 375 $^{\circ}C$ for 2 hours. The results showed the loss on ignition method correlated well with dry combustion (R^2 = 0.89-0.93). However, overestimation of organic C can occur because minerals in soil lose structural water at a certain temperature. For example, gibbsite loses water at 300 $^{\circ}C$ to 350 $^{\circ}C$ (Kamara et al., 2007). Overall, it has been concluded that loss on ignition is a practical and reliable method for quantifying SOC (Kamara et al., 2007), and is a rapid, simple, low-cost, and environmentally friendly method (Wang et al.,

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2011b).

Photometry

The photometric method involves using alkali (NaOH, diethylenetriaiminepentaacetic acid and alcohol) to extract humic acid to determine SOM. A colorimeter is used to determine absorbance (Mehlich, 1984), which is used to quantify SOC. This method is advantageous because it is rapid (500 samples per technician per day), produces no hazardous wastes, and any laboratory capable of soil testing can perform the analysis (Mehlich, 1984). However, this method is not commonly used and little research addressing the method could be found in the literature.

Near-infrared Spectroscopy (NIRS) Method

The NIRS method quantifies SOC based on reflectance spectra of illuminated soil (McCarty et al., 2002). Soils are scanned by spectra and readings are adjusted to a calibration curve for quantification (McCarty et al., 2002; Reeves et al., 2006; Reeves et al., 2002). This method was studied because current methodologies for monitoring soil C sequestration in terrestrial ecosystems are not cost effective, or depend on intensive chemical methods (McCarty et al., 2002). The method is advantageous in that it is simple, inexpensive, and rapid (McCarty et al., 2010) as well as nondestructive, consumes no reagents, produces no wastes, and is adaptable to automated and in situ measurements (McCarty et al., 2002).

McCarty (2010) conducted research on West African soils and found that both the Walkley-Black method and loss on ignition can lead to substantial errors for soils that have low organic matter content; better results were obtained using the NIRS method. A study in China (He et al., 2005) showed the NIRS method is a nondestructive analytical technique that has the

potential to accurately predict SOM in the soil and at the same time estimate soil N.

One disadvantage of the method is the need for constant calibration. Since using NIRS is an indirect method of measuring SOC, there is a need to build and maintain valid local and regional calibrations. For example, the calibration set properties are quantified by an available standard method such as dry combustion (McCarty et al., 2010). From previous research (McCarty et al., 2002), analyses of NIRS were performed by partial least squares regression (PLS) by Grams/32 (Galactic Industries, Salem, NH). After the optimal number of PLS factors were determined, a final calibration was developed and Chemometrics (Massart et al., 1998) analysis was conducted to extract information from spectral data that relates to a property measured within population of samples with a given domain of properties (McCarty et al., 2002).

Solvita® Method

The Solvita® method is used for testing soil respiration and soil health developed by the Solvita® Company (Solvita, Mt. Vernon, Maine). Soils are placed in an incubator at 25 $^{\circ}$C. The CO_2 is trapped for 24 h using the Solvita® soil test paddle. The amount of CO_2 released is determined using a digital-color reader (DCR). The Solvita® system for estimating CO_2 released from soils that have been dried and rewetted for 24 h has been shown to well-correlate with the commonly used titration method and CO_2 IRGA method (Haney and Haney, 2010; Haney et al., 2008a).

The Solvita® method is a new tool listed as an alternate soil respiration procedure in the national soil quality test kit program administered by the USDA Soil Quality Institute. Being without reagent handling and standardization, the Solvita® approach is simple and efficient (Haney et al., 2008a). The Solvita® respiration test is relatively fast, taking approximately 10 min to analyze 40 soil samples by Solvita® DCR (Haney et al., 2008b). The Solvita® approach

presents an accurate and fairly easy measurement (Haney et al., 2008b). No additional studies have been performed to date concerning comparison of this method with other more established SOC methods. The present study evaluated this commercial method in comparison with others.

<u>Color Method</u>

The color method involves the use of soil color to assess SOC content. Soil color is widely used for soil characterization in the field and soil classification (Gunal et al., 2008). Soil color can be determined by both chroma meter (Minolta Corp, Ramsey, NJ) and Munsell soil color charts (Munsell Color, 2000). Standardized soil color notation is usually achieved by comparison of samples with Munsell color charts. However, lighting conditions, observer experience, and surface characteristics of soils are major limitations (Barret, 2002; Melville and Atkinson, 1985; Post et al., 1993) which is why the chroma meter was used in this study for soil color.

Previous studies (Dematte et al., 2011) have quantified SOM by the Munsell color system using the chroma meter. In the research of Konen (2003), a significant relationship was observed between SOC by dry combustion using the automated analyzer (Model CHN 600, LECO, St. Joseph, MI) and the reflectance (r^2=0.74), Munsell value (r^2=0.74), and Munsell chroma (r^2=0.77) using a Minolta CR-310 chroma meter (Minolta Corp, Ramsey, NJ). According to previous research (Ibarra-F et al., 1995; Konen et al., 2003; Linbo et al., 1998), a chroma meter has numerous advantages for quantifying soil color properties. The technique is non-destructive so the same sample can be reused for other analyses. The instrument is portable and is convenient for both laboratory and field analysis, with color parameters determined in a few seconds. Sample preparation is minimal and the method is more consistent, accurate and precise than color quantification by the human eye using Munsell soil color charts. Soil color

determination with the human eye is subjective, and variability between soil scientists exists as demonstrated by Post et al. (1993).

<u>Texture Method</u>

Soil texture shows correlation with SOC as well as soil color. The percentage of clay content was found to be the best predictor (r = 0.86) of organic C in some studies (Nichols, 1984). Franzmeier (1988) noted that organic matter concentrations generally increased for Indiana Ap horizon samples with increasing clay content, but did not present any quantitative equations for SOM-texture relationships. The research of Konen (2003) showed a similar trend (r^2=0.71 for SOC with clay; r^2=0.75 for SOC with sand) to that described by Nichols (1984), although the slope and intercept were different. These findings show that no universal equation exists for soils and that regional differences should be considered due to variations in SOC composition and mineralogy, and that quantitative relationships should be investigated for individual soil-geographic regions (Konen, 2003).

Comparison of SOC methods

Chatterjee (2009) compared *ex situ* with *in situ* methods of determining SOC. *Ex situ* methods included wet combustion (Walkley-Black) and dry combustion (loss on ignition and C analyzer). For *in situ* methods, NIRS, Laser-Induced Breakdown Spectroscopy (LIBS), Inelastic Neutron Scattering and Remote Sensing were used. Comparison of advantages and disadvantages of these methods resulted in the conclusion that automated dry combustion, LIBS and NIRS have higher precision and lower detection limits than other methods. Automated dry combustion, as concluded by Chatterjee (2009), is the only reliable, comprehensive method to determine soil C concentration. NIRS has excellent performance (r^2=0.961 to 0.975) compared to

dry combustion when applied to calibration samples of similar particle size and as long as continual calibration and quality control are maintained (Madari et al., 2005). Analyses with LIBS measurements resulted in a high correlation of 0.96 with dry combustion for soils with similar morphology (Cremers et al., 2001). Chatterjee (2009) concluded that *in situ* analytic methods have many merits over traditional *ex situ* methods. However, since *in situ* methods are still being developed and involve relatively complicated calibrations due to spatial variability (Chatterjee et al., 2009), most studies use *ex situ* methods for analysis.

As dry combustion (C analyzer) is considered a standard method, many studies have compared it with other methods. Walkley-Black and loss on ignition are most frequently used for such comparisons (Kamara et al., 2007; Meersmans et al., 2009). Strong correlations in these studies were found and different correction factors estimated.

Although loss on ignition and C analyzer techniques are both considered to be dry combustion, the methods differ somewhat. Loss on ignition involves igniting soil samples in a muffle furnace at a temperature ranging from $300°C$ to $550°C$, while LECO and other C analyzers combust soil samples up to $950°C$ -$1800°C$ (Chatterjee et al., 2009). Although there are many types of C analyzers, they yield similar results. Wang compared two brands of C analyzers and found that, although they have different operating procedures and temperature settings, they provide comparable results (Wang et al., 2011a).

McCarty (2002) compared MIRS (Mid-Infrared Reflectance Spectroscopy) and NIRS with dry combustion, and found that MIRS has better correlation with the standard method, which indicates that although most people use NIRS, MIRS may be a more promising instrument. Research (McCarty et al., 2010) using west African soils, where SOC is generally low, showed that, compared to loss on ignition and the Walkley-Black method, NIRS and MIRS

methods demonstrate greater accuracy if calibrations are well maintained.

Deming Regression

In studies for methods comparison, regression analysis is often used to estimate the difference between method x and method y. Deming regression assumes measurement errors are present for both x and y measurements, whereas ordinary linear regression assumes only y measurements are associated with random measurement errors (Linnet, 1997).

In the statistical literature, ordinary linear regression, orthogonal regression, Passing-Bablok method, and Deming regression were compared. The results showed that Deming regression is the only method that can be applied without precautions while other methods require restrictions (Haeckel et al., 2013). The most frequently used ordinary linear regression is subject to several shortcomings, including, an erroneous testing of hypothesis, a low efficiency in cases with proportional errors, a downward bias of 12% of slope, a large Root Mean Squared Error (RMSE) (Linnet, 1997), and unreliable confidence intervals of estimated bias (Martin, 2000). Deming regression performs much better (Linnet, 1997). Deming regression, applied widely in clinical chemistry and requested by the U.S. Food and Drug Administration (FDA) in medical device submissions, is generally a preferable and applicable method for methods comparison.

Rationale and Objectives

Soil organic C is an essential soil property to quantify for agricultural and environmental purposes. Management practices including irrigation, fertilization, pesticide application, rotation, and tillage method are all influenced by SOC. Further, the C trade industry could benefit from research determining the most suitable way of testing SOC. In this study, accuracy of nine

methods were compared using a number of soils from around the world. Accuracy is not the only consideration, however, for various applications of quantifying SOC. Other factors for determining the most suitable method can include speed, level of training required, instrumentation availability, budgetary restrictions, and production of hazardous wastes. Thus, we assessed the equipment/reagents, efficiency, complexity, cost, and environmental effects in addition to the accuracy of each method.

The objectives of this research include:

1. Compare methods of quantification of SOC, such as dry combustion (C analyzer), wet combustion (Walkley-Black method), loss on ignition, photometry, NIRS, Solvita®, color, texture and visualization using a variety of soils from around the world.

2. Assess the equipment, efficiency, complexity, cost, and environmental effects of each method, and provide recommendations of method suitability.

<center>**Carbon Sequestration of Louisiana Crawfish Ponds**</center>

Introduction

Climate change is a critical issue, and increasing atmospheric CO_2 concentration has been considered by scientists to be the leading cause. Soils contain twice the amount of C as the atmosphere and vegetation combined, making them important C sinks (Brady and Weil, 2008). Thus, soil scientists have searched for a solution that decreases atmospheric CO_2 levels through soil C sequestration. Many studies (Lal et al., 2003; Lal et al., 1999; Smith, 2004) have examined agricultural soils for their ability to sequester C, and effective management strategies have been put into practice. However, few studies have investigated aquaculture sediments.

Aquaculture is on the rise, and in Louisiana, crawfish farming has grown to include more than 1,200 farms occupying more than 50,000 ha land (McClain et al., 2007). Therefore, it is vital to examine the C sequestration capacity of crawfish aquaculture pond sediments. Thus, a study on C sequestration in crawfish pond sediments in Louisiana was conducted.

Carbon Sequestration

<u>Climate change</u>

Global temperature has increased 0.6 $^{\circ}$C in the past century, and is projected to increase 1.4 $^{\circ}$C to 5.8 $^{\circ}$C by 2100 compared to 1990. Eleven out of the twelve warmest years on record have occurred since 1995 (IPCC, 2007). The evidence that climate change has occurred in past

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decades is overwhelming, and the scientific community has suggested that the consequences are more severe than first estimated (Harvey, 2010). Climate change may alter the amount, distribution, and intensity of precipitation (Lal, 2003), and may lead to climatic extremes like flooding, heavy rainfall, cyclones, extreme temperatures, landfalls, hurricanes, and drought conditions (Hallegatte and Corfee-Morlot, 2011). Climate change has been shown to increase sea level, decrease Arctic sea ice cover, and affect polar organisms and ecosystems (Robinson, 2009). It could also have an impact on soil quality, growing season duration, and biomass productivity (Lal, 2003), creating a serious threat to world food security (Downing and Parry, 1994).

Carbon Pools

There are five global C pools, oceanic (38,000Pg), geologic (5000Pg), pedologic (2300Pg), biotic (560Pg), and atmospheric (760Pg) (Lal, 2003). In the pedologic C pool, SOC comprises more than half the amount (Fig. 1).

The atmospheric concentration of CO_2 increased from 280 ppmv in 1975 to 367 ppmv in 1999 (Lal, 2003), and is currently 390 ppmv (Keeling et al., 2005). Schrag (2007) has suggested three strategies of lowering CO_2 emissions to mitigate climate change: (i) reducing fossil fuel use, (ii) developing low or no-C fuel, and (iii) sequestering CO_2 from point sources or atmosphere through natural and engineering techniques.

Carbon Sequestration

Soil C sequestration is the process of transferring CO_2 from the atmosphere into the soil through crop residues and other organic solids in a form that is not immediately reemitted

(Sundermeier et al., 2005). Soil C sequestration implies that the total soil C pool is increased through managerial interventions aimed at transferring atmospheric CO_2 to the soil C pool by moderating either organic or inorganic transformations (Lal and Follett, 2009). The practices associated with increasing the SOC pool, such as restoration of eroded or degraded soils, conversion of plow tillage to no-till farming with crop residue mulch and cover cropping, integrated nutrient management with manure, and use of complex cropping or farming systems (Lal and Follett, 2009) have been considered as approaches to mitigate greenhouse gas emissions. Carbon sequestration in soils belongs to the third strategy of Schrag (2007) discussed above, which has been considered as a natural, cost-effective, and environmentally friendly process (Sundermeier et al., 2005). Some studies (Smith, 2004) have concluded that C sequestration has finite potential, is non-permanent and may be riskier than direct emission reduction. Nonetheless, management practices that increase sequestration of C can improve soil sustainability, soil quality (Smith, 2004), environmental quality and food security (Lal, 2004). Thus, C sequestration has been described as a "win-win" option (Sundermeier et al., 2005) and a "no regrets" objective (Smith, 2004).

World Aquaculture Growth

Fish provides almost 20% of global animal protein consumed by humans (FAO, 2003). As human population continues to increase, it is likely that aquaculture will contribute more to the global food supply (Deutsch et al., 2007). Aquaculture production is increasing, covering a larger area and providing higher quantities of fish. Further, fish produced is of higher value than in the past (Fig. 2) (FAO, 2012).

Crawfish Production in Louisiana

Most crawfish production in the United States is located in Louisiana, where 52,000 ha land is used in crawfish farming (Lutz et al., 2004), compared to less than 3,000 ha outside of Louisiana (McClain and Romaire, 2004). Crawfish harvested in Louisiana are mainly two species, the red swamp crawfish (*Procambarus clarkii*) and the white river crawfish (*Procambarus zonangulus*) (McClain et al., 2007).

Crawfish production is interesting for several reasons. Crawfish are not fed on formulated feeds as in other aquaculture production. Instead, ponds are drained in summer to grow rice, sorghum-sudangrass, or other vegetation to serve as the base of a natural food chain for crawfish (McClain et al., 2007). Crawfish ponds are not stocked with hatchery-reared young as in other forms of aquaculture. Instead, unharvested crawfish produce young the next year (McClain et al., 2007). In addition, compared to common batch harvests of fish, crawfish harvests are done using baited traps (McClain and Romaire, 2004) (Fig. 3).

There are two common ways in Louisiana to culture crawfish: (i) monocropping systems, and (ii) crop rotational systems. Farmers harvest only crawfish in monocropping systems while they harvest other crops in crop rotational systems (Eversole and McClain, 2000). In monocropping systems, rice is usually planted, but not harvested (Fig. 4). In crop rotational systems, rice, soybean or other commodities are harvested. Rice-crawfish-rice, rice-crawfish-soybean, and rice-crawfish-fallow are the major strategies used for the rotational approach (McClain and Romaire, 2004).

Rational and Objectives

There have been many studies on C sequestration potential of cropland (West and Post, 2002), but few studies on the potential in aquaculture, and no studies on crawfish production to

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date. As the industry is growing, an understanding of the C pool, C cycle, and C sequestration under crawfish production is imperative. Additionally, knowledge of this aspect of C sequestration and other soil characteristics could guide decisions on best management practices in crawfish production. The objectives for this study were:

1. Compare SOC concentration and mass under monocropped crawfish ponds and rotational crawfish ponds with adjacent agricultural fields.
2. Characterize other soil properties such as N concentration and mass, particle size, bulk density, pH, C:N under crawfish ponds.

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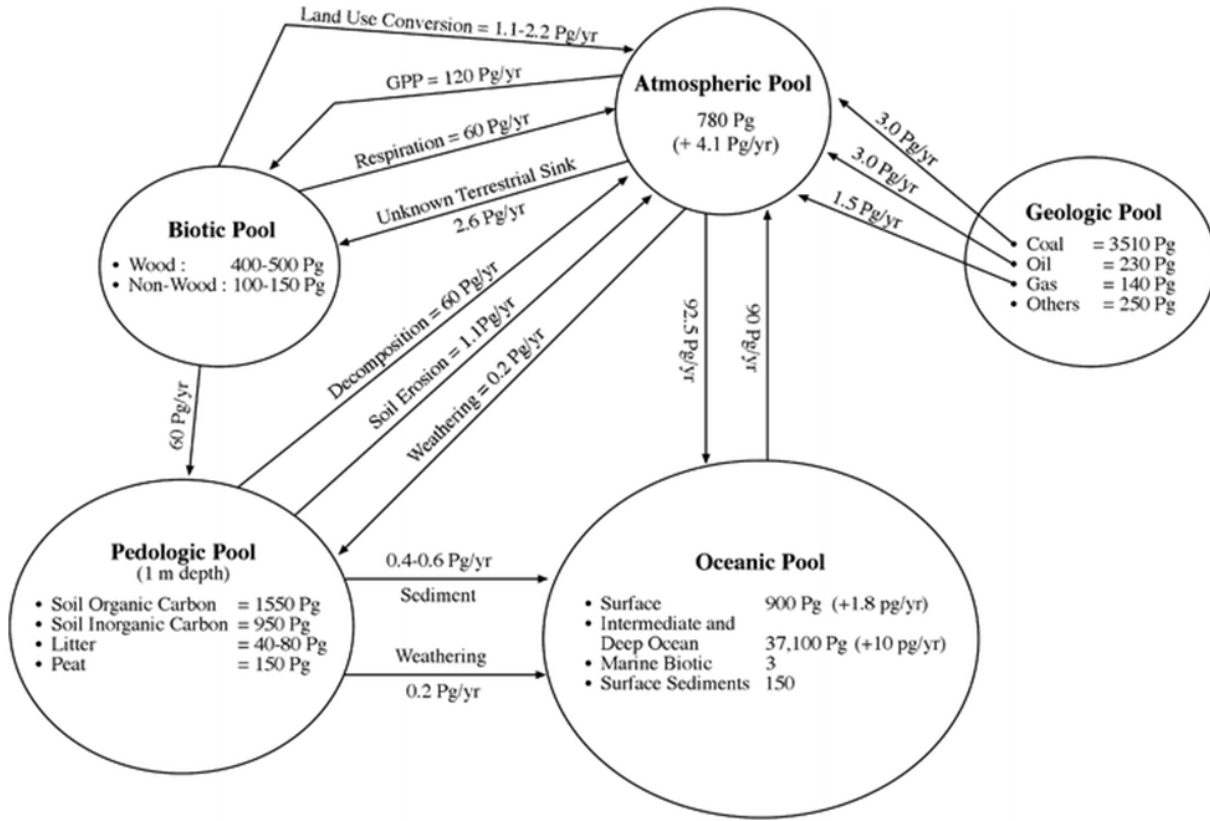
Figures

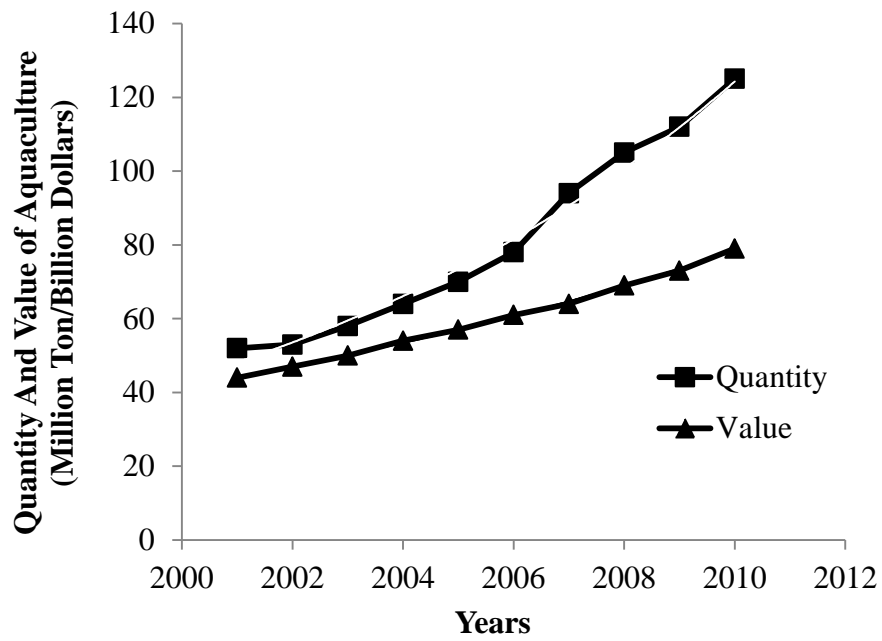Figure 1. Global C pool estimation (Lal, 2008).

Figure 2. Quantity and value of world's aquaculture (FAO, 2012).

Figure 3. Crawfish harvest.

Figure 4. Crawfish production.

II Comparison of Soil Organic Carbon Methods

Abstract

Soil organic carbon (SOC) is an important property in regard to soil and environmental quality. There are many methods to determine SOC, but there are no previous studies that compare many methods using a large variety of soils. In this research, nine methods were chosen, six variables were evaluated, and more than 300 samples representing a high diversity of soils were used for SOC methods comparison. The nine methods included: dry combustion, Walkley-Black, loss on ignition, photometry, NIRS, Solvita®, color, texture, and visualization. The six variables were: equipment, accuracy, efficiency, complexity, cost and environmental effect. More than 300 samples from different regions in the U.S. and other countries were analyzed. All methods were performed following standard protocols except for those specifically indicated. Deming regression was used to compare the accuracy of other methods with dry combustion. The results, using dry combustion as the standard, showed that Walkley-Black ($r^2=0.89$), loss on ignition ($r^2=0.86$), and Solvita® ($r^2=0.64$) have satisfactory correlations; photometry is a good semi-quantitative method with a modest accuracy ($r^2=0.31$); methods like NIRS, color, texture, and visualization need further research and justification. Loss on ignition was the preferred alternative to dry combustion when taking all factors into consideration.

Introduction

Carbon is the foundation for all life forms, and is a key component of soil organic matter (SOM). Soil organic matter plays an important role in soil physical, chemical, and biological characteristics. Soil organic C comprises more than 50% of SOM (Baldock and Nelson, 2000), and is considered an essential property of soils. Knowing SOC content aid in application of best

management practices. In addition, as the second largest C pool among global C pools (Lal, 2003), soil C pool influences C fluxes, cycling and the content of other pools. Because the SOC pool is the largest portion of the total soil C pool, estimating and assessing the dynamics of SOC is important.

There are many methods of determining SOC content. This study included comparisons among dry combustion, Walkley-Black, loss on ignition, photometry, NIRS, Solvita®, color, texture, and visualization. Dry combustion is a method using automated C analyzers and currently considered as a standard method (Chatterjee et al., 2009). Walkley-Black is a classical wet analytical method using the dichromate oxidation-reduction technique (Walkley, 1947; Walkley and Black, 1934) . Loss on ignition is a widely applied method based on weight loss at a certain temperature and duration of ignition (Kamara et al., 2007). Photometry is a semi-quantitative method using a colorimeter to determine absorbance after extracting SOM with alkali (Mehlich, 1984). Near-infrared spectroscopy is used to scan soils, followed by calibrating SOC content based on reflectance spectra. Solvita® is a commercial method measuring soil CO_2 respiration using the Solvita® Soil Kit. The color method uses soil color, correlating SOC content to Munsell hue, value, and chroma. The texture method uses sand, clay, and silt percent to estimate SOC. Visualization is a direct visual estimation of SOC content by trained professionals.

Several comparisons (Chatterjee et al., 2009; Kamara et al., 2007; Meersmans et al., 2009; McCarty et al., 2010) have been made between methods mentioned above. However, no comprehensive comparison for all of these methods has been reported to date. In addition, soils from only one region or one specific type of soils were used for previous methods comparisons. Further, only the accuracy of the methods was assessed and compared in existing literature,

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whereas equipment, price, availability, efficiency, complexity, and environmental effects all contribute to the method chosen for a particular study or laboratory.

The objective of this research was to compare nine different methods addressing six variables, using a variety of soils from different regions. The methods chosen for comparison were dry combustion, Walkley-Black, loss on ignition, photometry, NIRS, Solvita®, color, texture and visualization. More than 300 soil samples from within and outside of the U.S. were analyzed for the study. Along with accuracy of a method, other variables including equipment, efficiency, complexity, cost, and environmental effects were assessed and compared.

Methods and Materials

Soil Samples

More than 300 total soil samples were collected from three U.S. states (Alabama, Arkansas, and Louisiana) and Ecuador, Kenya, and Tanzania (Table 1). The 170 samples from Alabama were collected from different regions in the state, including the Appalachian Plateau, the Lower Coastal Plain, the Piedmont, the Tennessee Valley, and the Upper Coastal Plain (Table 2), covering most of the soil types within the state. Soil samples from Louisiana were from the southeastern part of the state including Acadia, Baton Rouge, Jeff Davis, LeBeau, St. Landry, St. James, and Vermillion Parishes (Table 3). Most of the soil samples were from surface or subsurface, within a depth of 20 cm. The SOC concentration as determined by dry combustion ranged from 0.31% to 5.52% among these soil samples.

Dry Combustion

Dry combustion is based on the oxidation of C in a resistance furnace and determination of trapped CO_2 titrimetrically or gravimetrically (Nelson and Sommers, 1996). In this study, samples were ground by mortar and pestle prior to analyses. A soil sample of 0.2 g was placed in a tin capsule, and combusted in a furnace at 950 °C flushed with pure oxygen, using a LECO TruSpec CN (LECO Corp., St. Joseph, MI). The process, which takes about 4 minutes per sample, has three phases: purge, combust and analyze (LECO, 2004). Samples were not acidified prior to analysis as samples were non-calcareous soils and not recently limed (Nelson and Sommers, 1996; Vohland et al., 2011). Total C content determined by the analyzer was considered to be SOC. There are various manufacturers of dry combustion equipment, with each manufacturer using similar, but not necessarily the same reagents. One reagent used in dry combustion in this study was magnesium perchlorate, which requires appropriate hazardous waste disposal after the reagent is spent. Dry combustion equipment produced by other manufacturers may require other reagents that also require appropriate disposal.

Walkley-Black

The Walkley-Black method used in this research was described by Boyd et al. (2002), and was slightly different from the procedure listed by Walkley (1947) and Nelson and Sommers (1996). No external heat was used in this procedure because it was more rapid, simpler, and no special equipment was required. Potassium dichromate waste generated from the procedure was sent to the Department of Risk Management and Safety in Auburn University for further neutralization and waste disposal.

Loss on Ignition

A modified loss on ignition method was used as described by Ball (1964), Ben-Dor and Banin (1989), and Nelson and Sommers (1996). Soils were put in an oven overnight at 105 °C to remove moisture and in a muffle furnace at 375 °C for 16 hours for ignition. The ignition time and temperature chosen in this research was based on comparing many procedures, with the Ball (1964) method appearing most appropriate. The percentage of weight loss during the ignition was calculated as SOC concentration. One important consideration is the complete drying of soil at a constant temperature to provide a consistent starting point for all soils before ignition, as was done in this study.

Photometry

The procedure for photometry used in this study was developed and described by Mehlich (1984) to determine humic matter in soils. A microplate spectrophotometer (BioTek, μQuant; Winooski, Vermont) with a 650 nm wavelength was used. There were several modifications compared to the original method: a 1 cm light path instead of 2 cm was used; one gram of soil was weighed instead of measuring 1 cm^3; the ratio used in this procedure for supernatant and water was 20:20 instead of 5:35; the humic acid reagent available at the time of the original Mehlich method is not available today, so standardization was not performed due to its unavailability. The humic matter was not calculated as the original method showed; instead, the absorbance was correlated with SOC content determined by dry combustion.

NIRS

Near-infrared spectroscopy is a spectroscopic method that uses the near-infrared region of the electromagnetic spectrum to quantify SOC. A Perstorp Analytical NIRSystems spectrometer (Hilleroed, Denmark) was used in this study. Soils were scanned first, and then the

generated spectrum was analyzed by WinISI software Upgrade 4.00. WinISI is a product of Infrasoft International, LLC. It is a chemometric package to analyze the transmission or reflectance of radiation from solid, semi-liquids, and liquids for the agriculture and food industries (WinISI, 2005). Partial least squares (PLS) were used for regression analyses that extract information from spectral data relating to a property measured by other methods such as dry combustion (Massart et al., 1998; McCarty et al., 2002).

Solvita® Method

Soil samples collected from the U.S. were sent to a laboratory that uses the Solvita® method developed by Woods End Laboratories (Mt Vernon, ME) for analyses. The procedure used to measure soil respiration, 1-day CO_2-C, is as follows: dry soils overnight at 50 $^{\circ}$C to pass a 2-mm sieve; weigh 40 grams of soil into a glass jar with 25 ml water added; place the Solvita® paddle, screw on the lid, and incubate for 24 h; after incubation, remove the paddle, place in the Digital Color Reader (DCR), press the button, and take the reading (Haney and Haney, 2010).

Color Method

In this method, a chroma meter CR-210 (Minolta Corp., Ramsey, NJ) was used on the Munsell setting. Preparing for a smooth and uniform flat surface of soil sample and placing the probe firmly in a vertical position were important in this method. A reference plate provided by Minolta was used for calibration at the beginning of measurement. A standard sample was used every seven samples to ensure the consistency of the chroma meter. Three readings of hue, value, and chroma were taken for each sample and the average was reported. An informative description of the procedure can be found in the research by Konen (2003).

Texture Method

The hydrometer method was chosen for this study (Bouyoucos, 1962). A standard hydrometer (ASTM No. 1.152H-Type with Bouyoucos scale in g L^{-1}) and sedimentation cylinders were used. Particle size determined in this study followed the standard analytical methods manual (Gavlak, 2003). Clay, sand, and silt content were calculated based on the temperature, soil mass, and hydrometer readings.

Visualization

Estimation of SOC by simple and direct visualization was conducted in the Crop, Soil, and Environmental Sciences Department at Auburn University. Forty-three people including faculty, staff, graduate students, undergraduates, and others participated in the visualization estimation for 30 random samples having a broad range of SOC, based on several reference samples provided, and their own experience. Both reference samples and test samples were placed in plastic containers in both dry and wet conditions. Six ranges appeared on the scorecard for people to choose: <0.5%, 0.5% - 1.0%, 1.0% - 1.5%, 1.5% - 2.0%, 2.0% -3.0%, and > 3.0%. Two reference samples were provided within each range.

Statistical analyses

Deming regression was used in most of the analyses to assess the strength or weakness of relationship between methods. It is a preferable approach for methods comparison because it assumes measurement errors occur in both methods used other than errors in only one method. SigmaPlot 12.0 and Microsoft Excel 2010 were used for figures.

Methods Evaluation

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To evaluate the methods, data accuracy was compared, and the apparatus, equipment, and reagents were listed for each method. The coefficient of determination (r^2) between other methods and dry combustion was used to assess the accuracy. The number of samples run in the laboratory in an 8-hour day was considered as efficiency of the procedure. The time it takes to train a technician or student to perform a test was regarded as complexity. The cost includes initial set-up cost, cost/sample with and without labor. The environmental effect was considered if hazardous wastes were produced by a method.

Cost Analyses

The cost of initial set-up includes specialized apparatus, equipment, and reagents, assuming that the lab already has basic equipment such as an oven, a grinder, sieves, glassware, a balance, scoops, etc. The cost/sample for dry combustion analyses includes technician labor, chemicals, supplies, gases, repair services, and student labor if used. The values presented here represent the average of three years of actual costs incurred in running the dry combustion equipment in our research program. The cost/sample with labor analyses for other methods in this study assume $8/hour for labor since most of the work was completed by students earning minimum wage. The cost/sample without labor only includes chemical consumption and supplies, and does not include labor, initial set-up equipment cost, power/water/other facility expense, or environmental cost.

Results and Discussion

The apparatus and reagents, accuracy, efficiency, complexity, cost, and environmental effects of each method is assessed and described below.

<u>Dry Combustion</u>

The apparatus needed for dry combustion were automatic analyzer such as LECO C analyzer, oxygen, helium, and air gas cylinders, various reagents and other consumables. Other C analyzers that are commonly used are Vario, Carlo Erba, and Teledyne-Tekmar Apollo. Dry combustion is considered the most accurate SOC technique, and automatic analyzers are considered reliable and accurate instruments for C determination (Nelson and Sommers, 1996). In this study, dry combustion with a C analyzer was set as the standard method, and other methods were compared against it (Table 4). The system uses the Dumas method of combustion, and can run a sample in less than 4 min (LECO, 2004). However, due to necessary maintenance, change of reagents and crucibles, one technician can normally perform 65-100 sample determinations in an 8-h day. A period of 3-5 days is considered the minimum training required for a technician/student to learn how to operate the automatic analyzer. The initial cost of the equipment is around $50,000. The cost of sending soil samples to different laboratories for C analysis varies. In the Crop, Soil, and Environmental Sciences Department at Auburn University, the 3-yr average is $4.22 per sample. The Auburn University Soil Testing Laboratory charges $6.00 per sample, which includes the instrument, maintenance and labor costs. Dry combustion has little environmental effects. One of the reagents, magnesium perchlorate is an oxidizer, which can form peroxides and needs to be disposed of properly. Other reagents are not harmful, and can be contained and discarded.

<u>Walkley-Black</u>

A titration set with a burette, Erlenmeyer flasks, fume hood and balance are needed for the Walkley-Black Method. The reagents used in the modified Walkley-Black procedure were

potassium dichromate, sulfuric acid, ferroin indicator, and ferrous sulfate solution. The Walkley-Black method has a good correlation with dry combustion with $r^2 = 0.89$ (Fig. 5). The equation (Table 4) has an intercept < 1, which indicates the Walkley-Black method underestimates SOC, a result that agrees with other literature (Chatterjee et al., 2009). The recovery factor varies from soil to soil, and can be easily developed for a specific region. The data can be used directly or calibrated with the correction factor depending on the accuracy desired.

Walkley-Black is a relatively simple method, and a technician/student can process 60-80 samples in an 8-h day. It takes less than a day to train a student/technician to perform the procedure. The apparatus used in this procedure are basic and present in most laboratories. It costs around $300 to purchase the chemical reagents needed for 120 samples. The cost of running one sample is roughly $2.01 with labor included.

This classic method was widely used for its simplicity, low set-up cost, and minimum requirement. However, the potential environmental impact has become a concern. Potassium dichromate used in the procedure is an oxidizer and Cr is listed as a heavy metal waste by the USEPA. It is a hazardous chemical, a possible human carcinogen, and proper disposal is required. Disposal costs increase the cost and complexity of the method when considered. The disposal costs are not included in this study because the waste disposal was performed by the Department of Risk Management and Safety at Auburn University.

Loss on Ignition

A muffle furnace, oven, beakers (or crucibles), and analytical balance are apparatus needed for loss on ignition. Chemical reagents are not used in the procedure. Loss on ignition has a good correlation with dry combustion with $r^2 = 0.86$ (Fig. 6), which is similar to previous

research with $r^2 = 0.89$-0.93 (Kamara et al., 2007). The equation (Table 4) has an intercept > 1, indicating that loss on ignition overestimates SOC as other research has shown (Wang et al., 2011). On average, 150 samples can be dried, weighed, ignited, and weighed again within 8 hours over a two-day period.

It takes less than one hour to learn how to perform the procedure for loss on ignition. The cost is low since most laboratories are already equipped with all the apparatus needed. However, it costs around $10,000 to purchase a muffle furnace. It costs $0.43/sample when labor only is considered. The environmental effects of this method are minimal since no chemicals are used or generated.

Photometry

The equipment and apparatus for the photometric method included a colorimeter, vials, microplate trays, pipette, and a diluter/dispenser. Reagents included sodium hydroxide, diethylenetriaiminepentaacetic acid (DPTA), and ethyl alcohol. The pattern of absorbance increased as SOC increased (Fig. 7). However, the correlation $r^2 = 0.31$ between dry combustion and absorbance was low, and photometry should only be used as a semi-quantitative method. From research done in this study, 200-400 samples can be processed per day by one technician. It is a relatively easy procedure to follow and takes less than half a day to learn. The cost of the colorimeter is about $25,000, reagents $114 for 3000 samples, and estimated $0.24 /sample with labor. There are no hazardous chemicals produced in this method, so environmental effects are minimal.

NIRS Method

The only apparatus needed for this method are a near-infrared spectrometer and sample cups. No reagents are needed. The correlation between NIRS and dry combustion was poor ($r^2 = 0.13$) (Fig. 8). However, other studies have shown much better correlation with r^2 ranging from 0.85 to 0.96 (McCarty et al., 2002; Vohland et al., 2011). A possible reason for poor correlation in this study could be that regional calibration was not performed due to the limited number of samples from one specific region. From McCarty's (2002) research, regional calibrations were feasible and applied.

In this method, at least 200 samples can be scanned and processed in a day. It takes no more than half a day to train a person to scan samples. However, the complexity of this method is not in processing samples, but in calibrating the data using the corresponding software. It takes a person with basic statistical training at least 1-2 days to be able to calibrate data. The cost of the pre-owned instrument used in this research was around $30,000, and a new one can cost between $50,000 to $85,000. Much of the cost is attributed to purchase of specific software for calibrations. There is no reagent cost, and analyses cost $0.32/sample if labor is counted.

Solvita® Method

The equipment needed for the Solvita® respiration test are Solvita® paddles (probes), a digital color reader (DCR), glass jars, beakers, a pipette, and a balance. Distilled water is the only reagent needed. The Solvita® 1-day CO_2-C emission has a good correlation with dry combustion with $r^2=0.64$ (Fig. 9). The method is efficient in that a technician can set up at least 400 tests in an 8-hour day to be read the following day. Up to 200 probes can be read per hour with the DCR. It takes about 30 minutes to train a technician to perform the test. It costs about $5,000 to purchase the test kit from Solvita® for the initial set-up, which enables a laboratory to

run 300 samples/day, and costs $8.46/sample. The environmental effect of this test is minimal since the probes are non-toxic and require no hazardous waste disposal. This method was the only method not performed in our research laboratory, so the scope for variables such as efficiency and complexity provided by Solvita® may be different from other methods.

Color Method

A chroma meter (Munsell Soil Color Charts are alternative) and a container to hold the soil are all that are needed for this procedure. No reagents are needed other than water if wetting samples is desired. No clear pattern was found between Munsell hue and SOC content (Fig. 10). Munsell value and chroma decreased with SOC content ; the trend is clear (Figs. 11 and 12), but correlation was poor (Table 4). In other research (Konen et al., 2003), significant relationships were observed between dry combustion and the chroma meter, with $r^2 = 0.74$ for reflectance, $r^2 = 0.74$ for value, and $r^2 = 0.77$ for chroma. The differing results between the Konen et al. (2003) research and this study may be due to the soil samples used in each study. In this research, a more diverse and a wide range of soils were used compared to other studies. If grouped into different categories of soils, our results may improve. Further research needs to be done on the accuracy of using chroma meters.

Using the chroma meter, 250 samples can be tested in an 8 hour day by a technician/student. It takes less than 1 hour to train a person to use the chroma meter. The cost is around $9,900 for a chroma meter ($179 for Munsell Color Charts), and $0.26 to process a sample with labor included. There is no environmental effect with this method.

Texture Method

The equipment needed for particle size analyses are a hydrometer, sedimentation cylinders, a stopwatch, a reciprocating horizontal mechanical shaker, an analytical balance, and shaker bottles with caps. The reagents required included deionized water, amyl alcohol, and sodium hexametaphosphate. No good correlation was found between clay, silt, or sand with SOC content (Table 4; Figs. 13-15). This result is different from that of Konen et al. (2003), with $r^2 = 0.71$, $r^2 = 0.75$, and $r^2 = 0.74$ respectively, between clay, sand, and silt with SOC. The difference might be that the present study includes soils from different regions, and a quantitative relationship might only exist in individual soil-geographic regions (Konen et al., 2003).

Thirty samples can be processed in one day over a 4-hr time period, due to the time span between readings, and the limited number of cylinders a laboratory would have because of expense and storage. A person needs about 2 hr to learn the method. The cost of 30 cylinders and 1 hydrometer is around $2,600. One sample would cost $1.07 to process including labor. There are no environmental effects associated with this method.

Visualization

Reference samples, plastic containers, labels, and scorecards, and water are needed for the visualization method. The correlation of visualization with dry combustion was poor (Table 4; Fig. 16). The accuracy of this method could perhaps be increased by using more experienced people, through additional training, and increasing the range of SOC content. This method is direct, straightforward, and fast. A technician can easily visually estimate 400 samples a day. The training time varies, and is estimated to be 2-4 hours. The cost is nearly none if labor is not included, and $0.16/sample with labor included. There are no environmental effects with this method.

A summary of equipment, accuracy, efficiency, complexity, equipment, cost, and environmental effects of the nine SOC determination methods is given in Table 5. From the standpoint of accuracy, Walkley-Black, loss on ignition, and the Lebeau method give a good correlation with dry combustion. In terms of efficiency, photometry, the Solvita® method, and visualization are the most rapid, followed by the color method, loss on ignition, and dry combustion. Among the nine methods, the Solvita® method, loss on ignition, and color method are the simplest, meaning they take the least time for training. The methods with the cheapest initial set-up are visualization and Walkley-Black. There are no reagents or additional costs for loss on ignition, NIRS, color, texture, and visualization if labor is not included. When labor is taken into consideration, based on the efficiency and minimum wage for cost of labor, visualization, photometry, color, and loss on ignition are the least expensive methods, costing less than $0.50 to process a sample. Most of the methods have no or minimal environmental effects other than Walkley-Black and dry combustion, which need proper disposal for some hazardous reagents used in the procedures. All aspects and characteristics described above should be considered before choosing a procedure to determine SOC.

Recommendation

Based on the description of each method and the summary, an investigator should be able to choose the most applicable and practical method for a laboratory or specific research. For example, if an accurate result is required, cost is not an issue, and the laboratory has a technician to get trained and perform the test, dry combustion should be the first choice. If a laboratory already has a muffle furnace, an analytical balance, and an oven or purchasing the equipment is

no issue, loss on ignition is the cheapest method with a desirable accuracy (r^2=0.86). If there is a cost issue with initial set-up, but high accuracy is required, Walkley-Black (r^2=0.89) is a reliable choice with $300 for basic set-up of chemical supplies. If cost is not an issue, efficiency, simplicity, and accuracy are desired, or if soil respiration data is needed, the Solvita® method is a good choice. One alternative is sending samples to the Solvita® Company, which costs $30/sample. When efficiency, simplicity, and cost are of more concern, photometry determination of humic acid is a promising semi-quantitative method to use. However accuracy is compromised with this method and additional research is needed. If a laboratory has near-infrared spectroscopy, and a regional calibration can be developed, NIRS may be a potential method. If other properties such as soil color and soil texture tests are available in a laboratory, correlation between SOC and soil color or texture could possibly be developed in a particular geological region. Further research needs to be done to justify use of these methods. If an investigator is particularly interested in an efficient, simple, and cheap method, which no apparatus or equipment is needed, and, only rough estimation is required, the visualization method is a possibility. However, additional training should be considered when using this method. Considering all the factors, loss on ignition is an approach combining accuracy, rapidity, simplicity, relatively cheap set-up, low cost/sample, and no hazardous wastes, which may pave the way for a standard approach of routine analyses for SOC in soil testing laboratories as well as research laboratories in universities.

Conclusion

In this study, more than 300 samples from different regions of the U.S. and across the world were analyzed. Dry combustion, Walkley-Black, loss on ignition, photometry, NIRS, Solvita®, color, texture and visualization approaches were used. Different aspects such as

equipment and reagents, accuracy, efficiency, complexity, cost, and environmental effect were evaluated. The recommendation for methods depends on different situations encountered. If the laboratory has the resources to purchase a C analyzer despite the expense and high complexity, dry combustion is still recommended. Walkley-Black, loss on ignition, and Solvita® have satisfactory correlations with dry combustion. Photometry is a simple and cheap semi-quantitative method recommended for a modest accuracy desired. Methods like NIRS, color, and texture failed to show good correlation in this study, though they have shown in other research to hold promise to determine SOC. Visualization can be an alternative for a rough estimation for SOC. In this research, loss on ignition appears to be a good choice for routine SOC analyses due to its combination of merits such as easy set-up, high accuracy, efficiency, simplicity, low cost, and no hazardous wastes.

Further research can be done to improve the accuracy and efficiency, lower the cost and complexity of each method described above and, possibly to develop other new methods. For example: the accuracy of Walkley-Black can be improved by adding external heat; loss on ignition has different accuracy due to ignition time and temperature; the accuracy of NIRS, color, and texture may be improved via regional calibration; visualization accuracy may improve with a person's training and experience. There are also new methods appearing in the current literature. Laser-induced breakdown spectroscopy, inelastic neutron scattering, and remote sensing are potential methods for SOC determination.

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Tables

Table 1. Sampling location and number of soil samples.

Location	Alabama	Arkansas	Louisiana	Ecuador	Kenya	Tanzania
Number of Samples	170	12	104	16	12	22

Table 2. Sampling location and number of soil samples from Alabama.

Location	Appalachian Plateau	Lower Coastal Plains	Piedmont	Tennessee Valley	Upper Coastal Plains
Number of Samples	35	35	31	34	35

Table 3. Sampling location and number of soil samples from Louisiana.

Location	Acadia	Arnaudville	Baton Rouge	Jeff Davis	LeBeau	St. Landry	St. James	Vermillion
Number of Samples	24	12	12	8	12	16	12	8

Table 4. Number of soil samples, correlation coefficient r, coefficient of determination r^2, and equation for methods compared with dry combustion.

Method	n	r	r^2	Equation
Walkley-Black	336	0.9447	0.8925	y= 0.7147x + 1.3379
Loss on Ignition	336	0.9258	0.8571	y = 2.3846 x - 1.6149
Photometry	336	0.5597	0.3133	y = 0.0101x - 0.0056
Near-Infrared Spectroscopy	336	0.3564	0.1270	y = 0.3306x + 7.8805
Solvita® CO_2-C	274†	0.7975	0.6360	y = 4.3092 x -20.5939
Chroma Meter, Hue	336	0.1561	0.0244	y = 0.0243x + 2.7746
Chroma Meter, Value	336	-0.4147	0.1720	y = -0.0258x + 4.8333
Chroma Meter, Chroma	336	-0.2978	0.0887	y = -0.0228x + 2.6715
Particle Size, Clay	336	0.2043	0.0417	y = 6.8706x - 83.5777
particle Size, Silt	336	-0.06	0.0036	y = -18.4539x + 329.2429
Particle Size, Sand	336	-0.1162	0.0135	y = -17.8010x + 326.0532
Visualization‡	30§	0.4111	0.1690	y = 0.4407x + 7.1649

† Due to the restricted rules and permit for international soils, only domestic soils were sent to the laboratory.

‡ This is the only method that uses the range of SOC instead of the exact SOC content, and vertical point plot instead of Deming regression.

§ Only 30 samples were randomly chosen, because it was not expedient to ask examinees to evaluate 336 samples.

Table 5. Summary of methods comparison.

Method	Main Apparatus	Accuracy (r^2)	Efficiency (samples/day)	Complexity	Initial Set-Up Cost	Cost/Sample (Without Labor)	Cost/Sample (With Labor†)	Environmental Effect
Dry Combustion	C Analyzer	Standard	65-100	5 days	$50,000	$1.88	$4.22	Magnesium Perchlorate
Walkley-Black	Burette	0.89	60-80	<1 day	$300	$1.10	$2.01	Potassium Dichromate
Loss on Ignition	Muffle Furnace	0.86	150	< 1 hour	$10,000	$0.00	$0.43	Minimum
Photometry	Colorimeter	0.31	200-400	< 4 hour	$25,000	$0.02	$0.24	Minimum
NIRS method	Near-Infrared Spectroscopy	0.13	> 200	2 days	$30,000	$0.00	$0.32	None
Solvita® Method‡	Solvita® Kit	0.64	300	< 30 min	$5,000	$8.25	$8.46	Minimum
Color Method	Chroma Meter	0.17 Value§	> 250	< 1 hour	$9,900	$0.00	$0.26	None
Texture Method	Hydrometer, Sedimentation Cylinder	0.04 Clay¶	30#	< 2 hour	$2,600	$0.00	$1.07	Minimum
Visualization	None	0.17	400	2-4 hour	$0	$0.00	$0.16	None

† Labor is calculated based on 8-hour/day, earning $8/hour.

‡ A commercial method, the only method not performed in the research laboratory this study is done, so the scope of data could be slightly different. It costs $30/sample for sending the samples to the company.

§Value is the best fit among Hue, Value, and Chroma.

¶ Clay is the best fit among Clay, Silt, and Sand.

Considering the expense and storage of cylinders, 30 samples/day is estimated, which actually only takes about 4 hours.
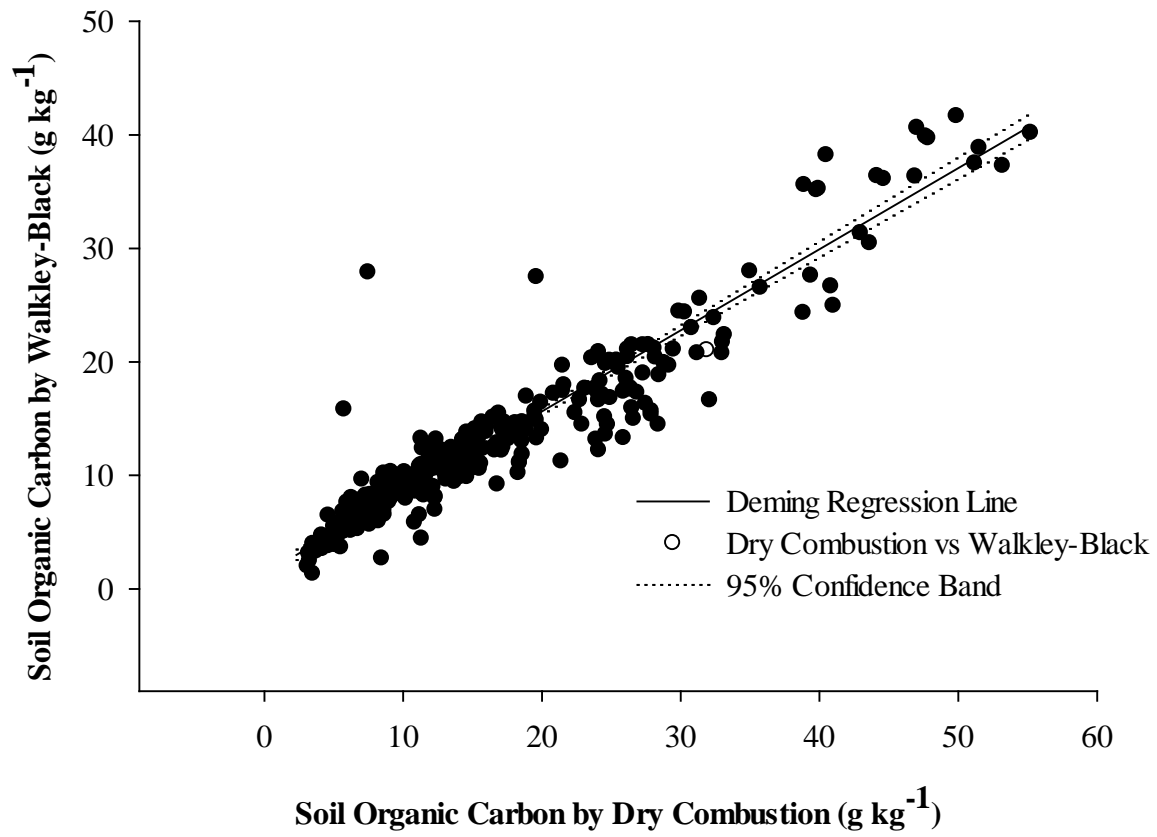
Figures

Figure 5. Soil organic carbon via the Walkley-Black method as a function
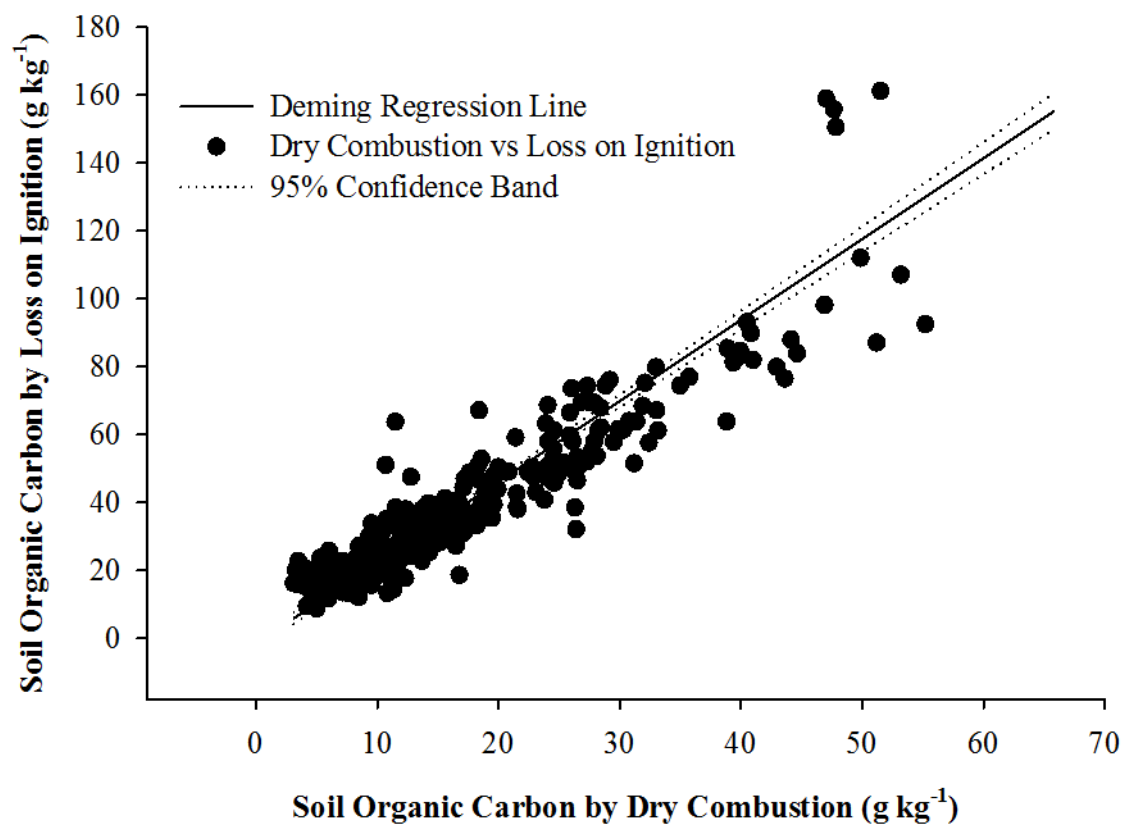of the dry combustion method.

Figure 6. Soil organic carbon via the loss on ignition method as a function
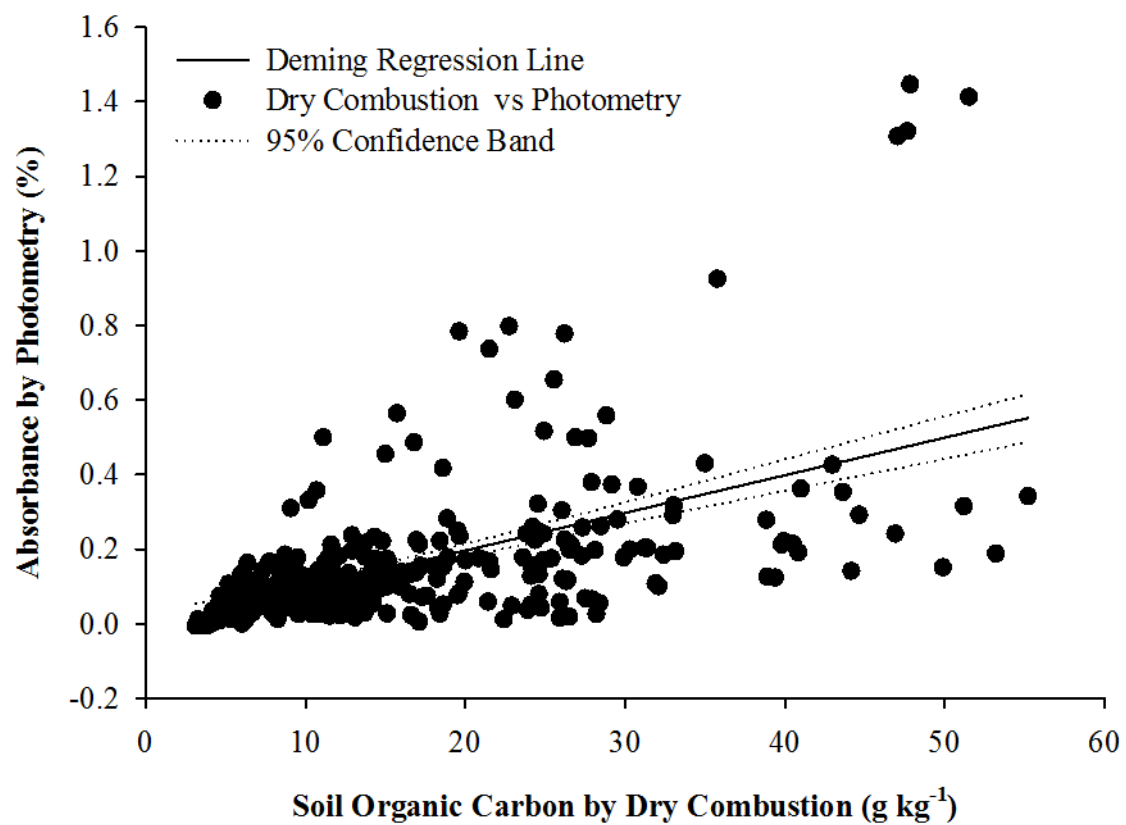of the dry combustion method.

Figure 7. Soil organic carbon via the absorbance by the photometry method as a function of the dry combustion method.
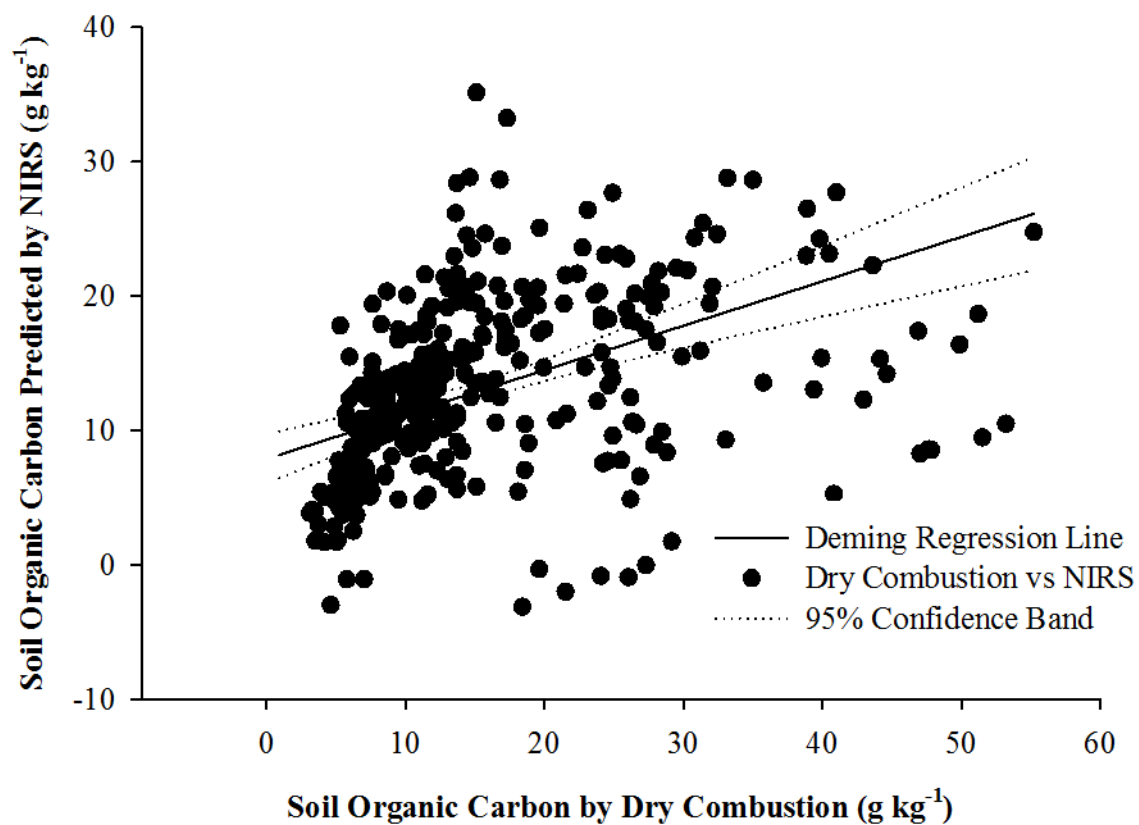
Figure 8. Soil organic carbon via the Near-infrared Spectroscopy method as a function of the dry combustion method.
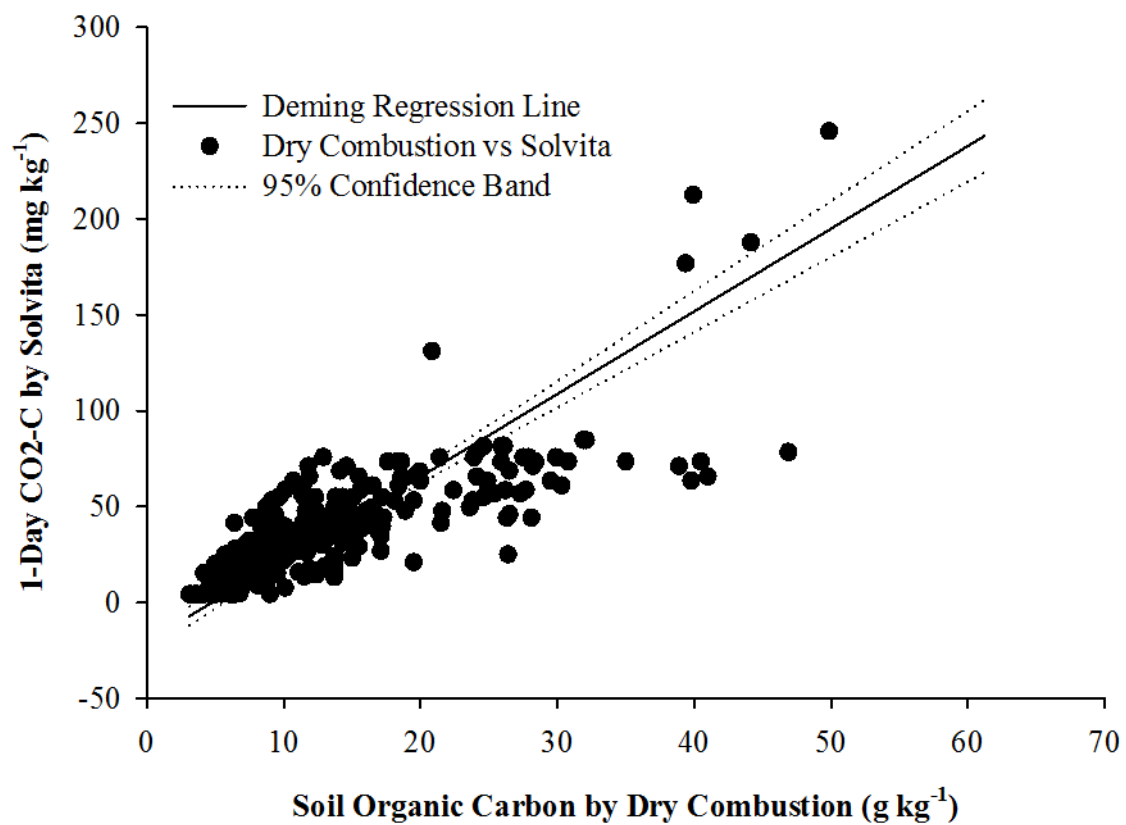
Figure 9. Soil organic carbon via the 1-day CO_2-C by Solvita® method as a function of the dry combustion method.
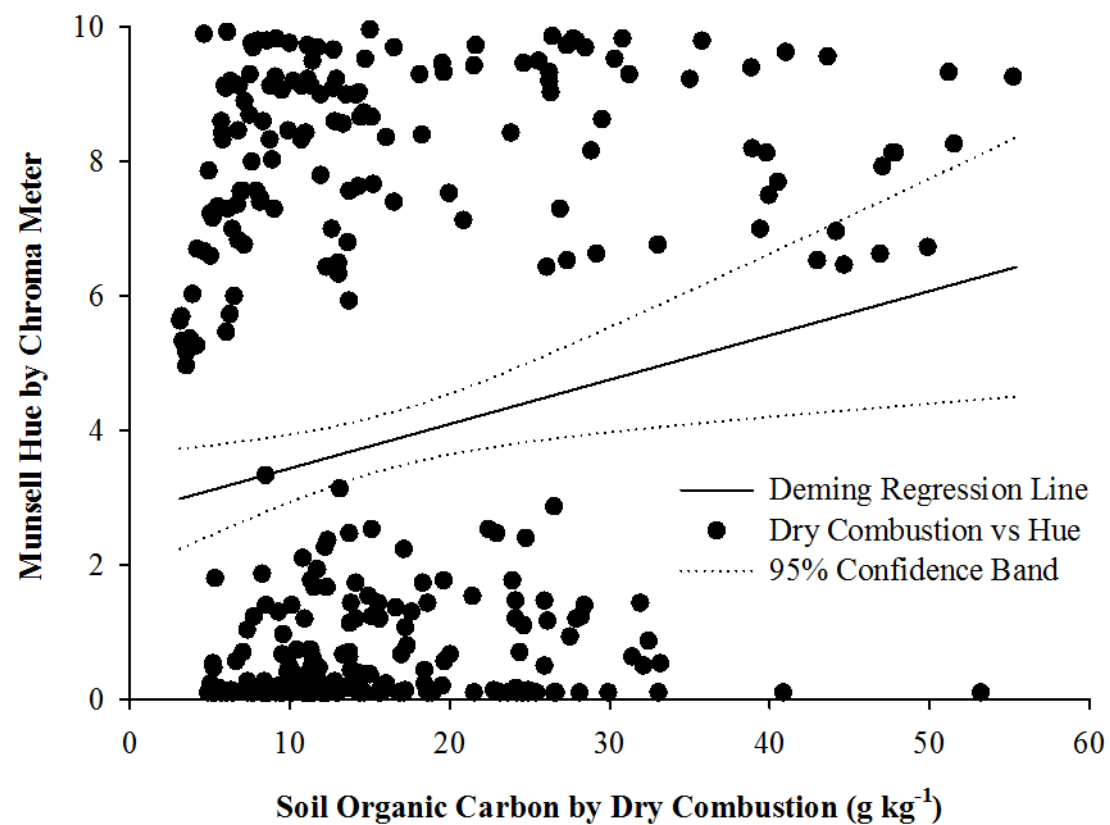
Figure 10. Soil organic carbon via the Munsell hue by chroma meter method
as a function of the dry combustion method.

Figure 11. Soil organic carbon via the Munsell value by chroma meter method
as a function of the dry combustion method.

Figure 12. Soil organic carbon via the Munsell chroma by chroma meter
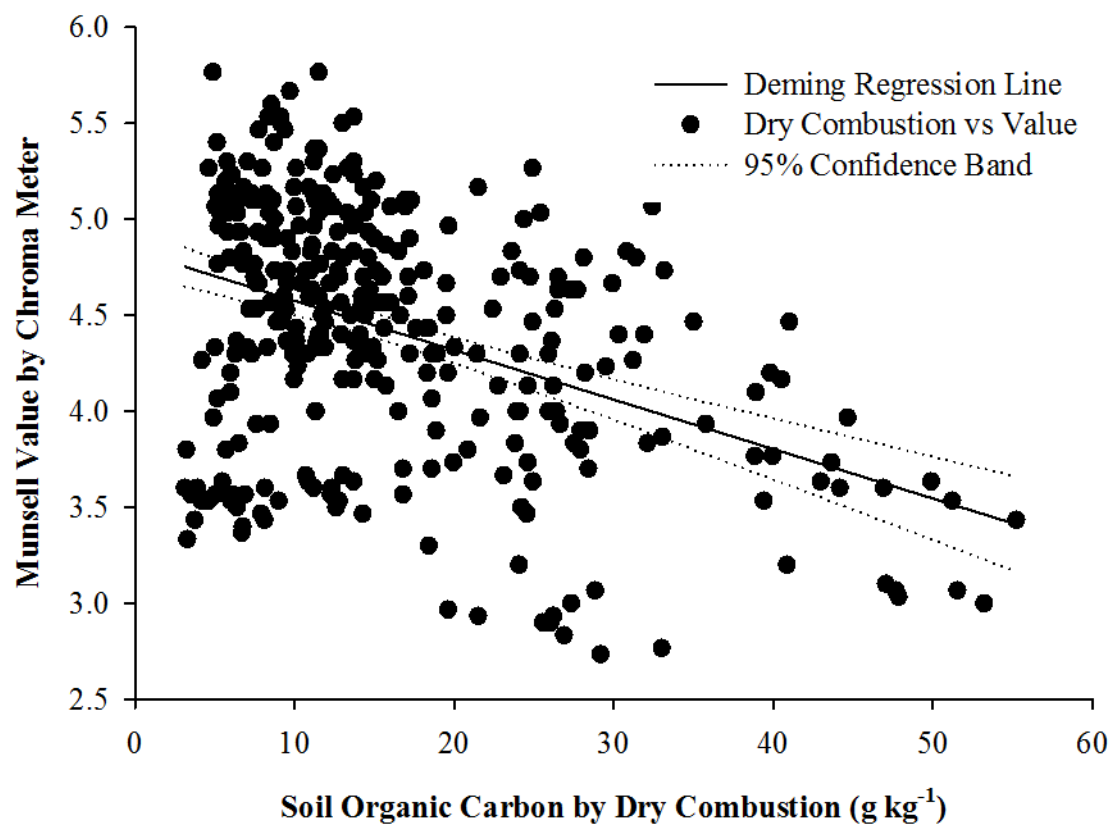method as a function of the dry combustion method.

Figure 13. Soil organic carbon via the clay content method as a function
of the dry combustion method.

Figure 14. Soil organic carbon via the silt content method as a function
of the dry combustion method.

Figure 15. Soil organic carbon via the sand content method as a function
of the dry combustion method.

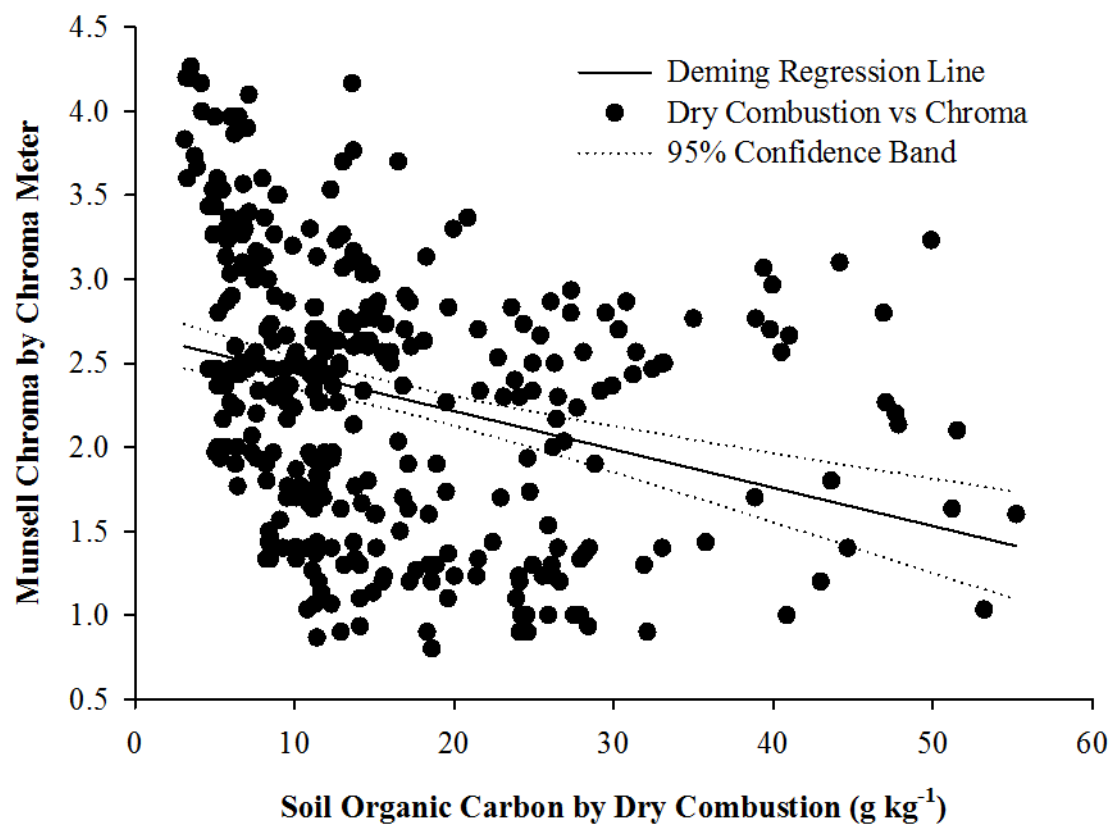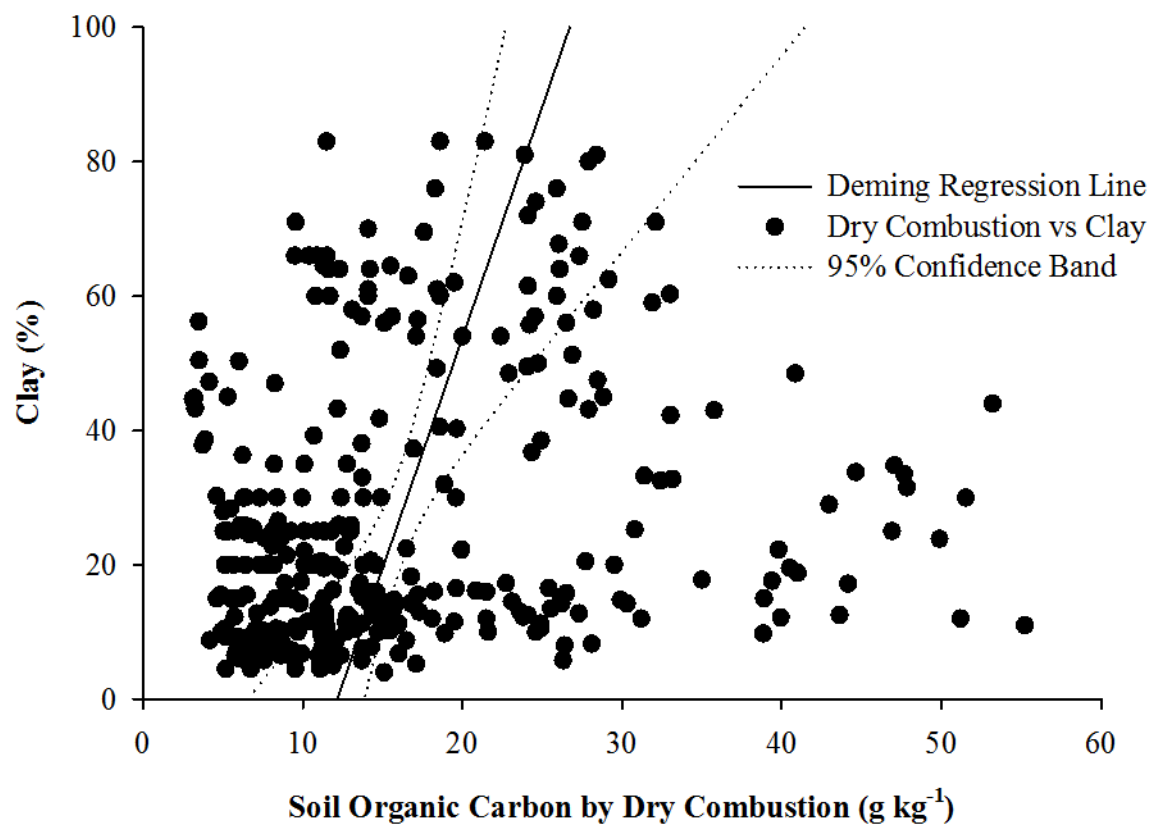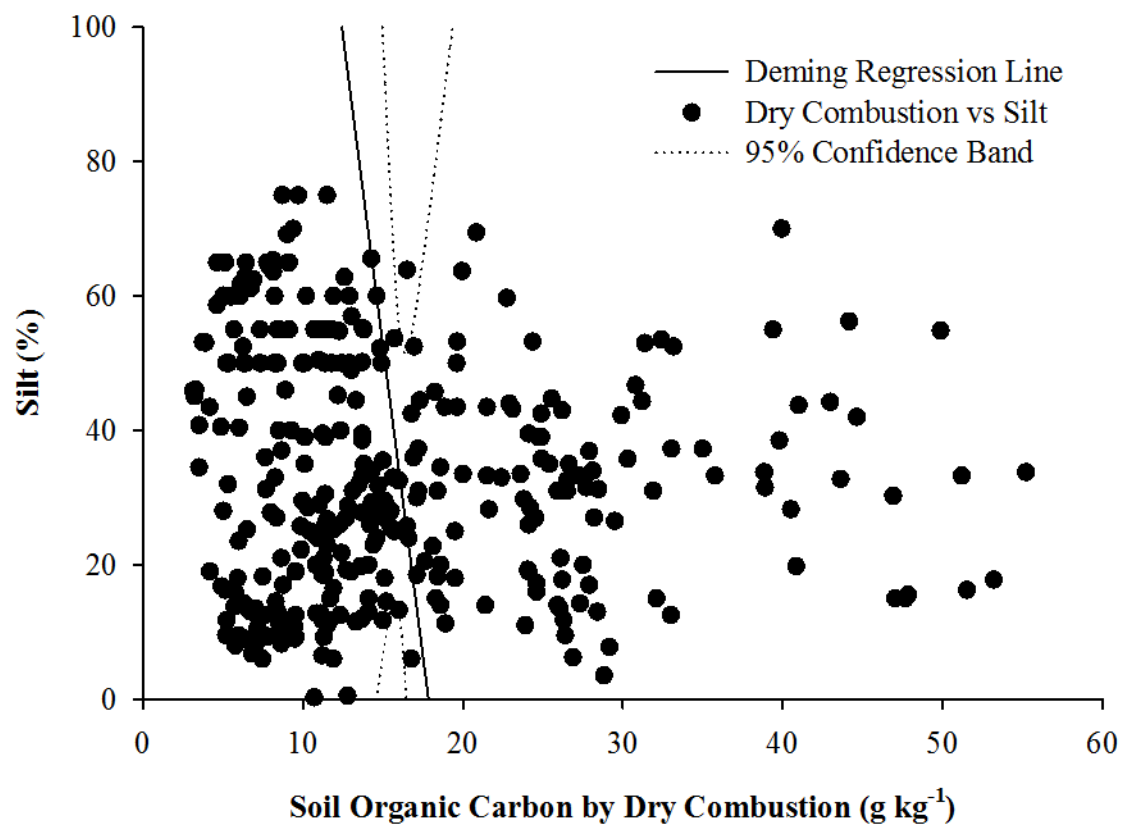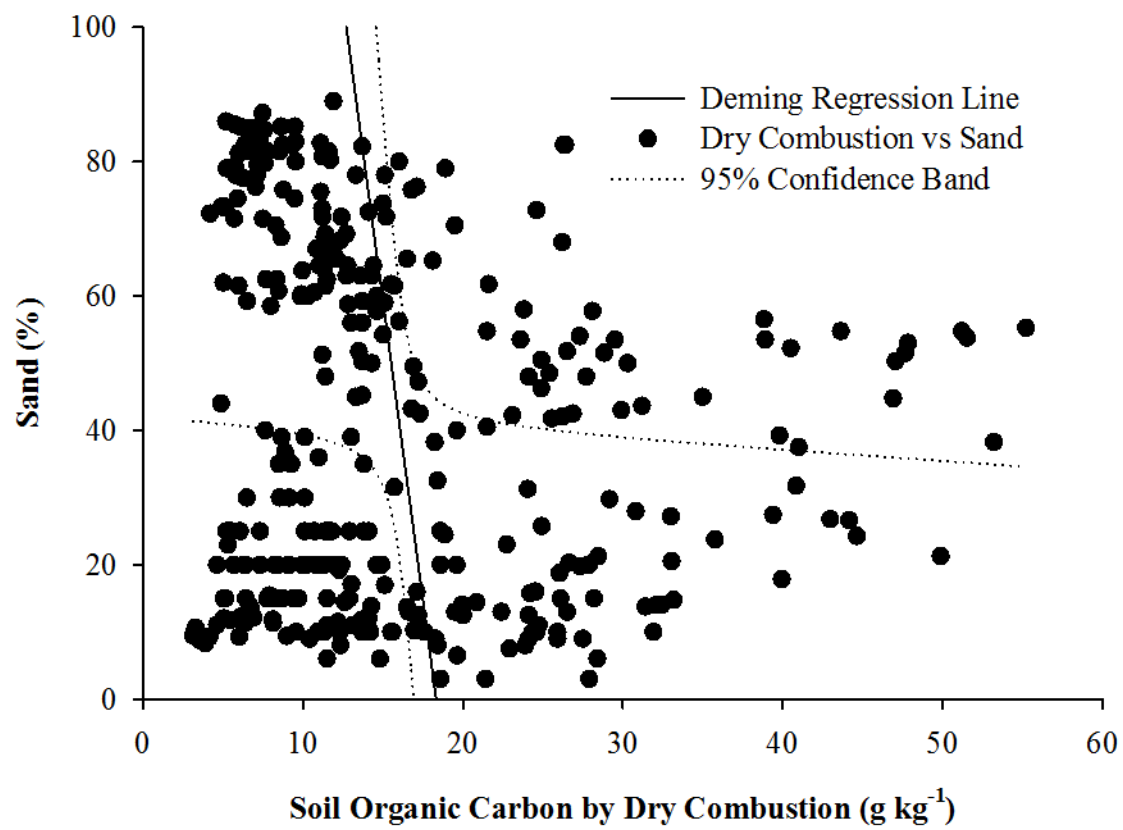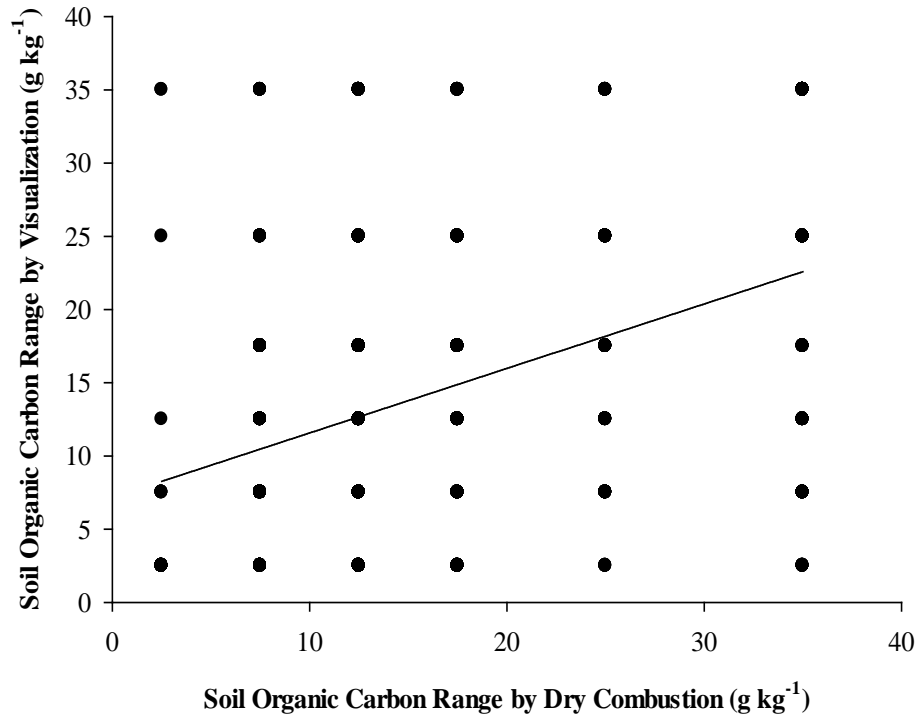Figure 16. Soil organic carbon via the visualization method as a function
         of the dry combustion method.

III. Carbon Sequestration and Soil Characteristics in Louisiana Crawfish Ponds

Abstract

Soil carbon (C) sequestration is considered an effective method to mitigate climate change and improve soil quality. Due to increased crawfish production in Louisiana and lack of pertinent research, this study examined C sequestration in crawfish pond sediments and other soil characteristics. The study compared soil organic C (SOC) concentration and mass under crawfish ponds and adjacent agriculture fields in monocropping and rotational crawfish production systems, respectively. Other characteristics such as bulk density, nitrogen (N) concentration and mass, particle size, pH, and carbon to nitrogen ratio (C:N) were also examined. A significant difference (P<0.05) was found in the top layer of SOC concentration in crawfish monocropping systems (25.8 g kg^{-1}) compared to adjacent crop fields (20.8 g kg^{-1}). However, no significant differences were found in other soil/sediment layers for C concentration or C mass in both systems. Crawfish ponds did not appear to have an advantage over upland cropping systems with regard to soil C sequestration. Nitrogen concentration and mass had a depth distribution similar to SOC. Carbon:N and pH were within a normal range for soils of the area. Bulk density helped explain the SOC and N concentration and mass distribution. The sediment properties studied provide information for future research and pond best management practices.

Introduction

Climate change has become a critical issue and increasing atmospheric CO_2 has been considered by some scientists to be the leading cause. Soils contain twice the amount of C as the atmosphere and vegetation combined, making them important C sinks (Brady and Weil, 2008).

Thus, soil scientists have been looking for a solution that decreases atmospheric CO_2 levels through soil C sequestration. Many researchers have examined agricultural soils for their ability to sequester C, and effective management strategies have been put into practice (Lal, 2003; Lal et al., 2003; Lal et al., 1999; Smith, 2004). However, few studies have investigated sediments under aquaculture. Aquacultural production is on the rise, and in Louisiana, crawfish farming has grown to include more than 1,200 farms occupying more than 50,000 ha land. Therefore, it is vital to examine the C sequestration capacity of crawfish aquaculture pond sediments.

In Louisiana, there are two common crawfish production systems: (i) monocropping systems, and (ii) crop rotational systems. In monocropping systems, farmers harvest only crawfish while harvesting other crops in crop rotational systems (Eversole and McClain, 2000). In monocropping systems, rice is usually planted, but not harvested. In crop rotation systems, rice, soybean or other commodities are harvested. Rice-crawfish-rice, rice-crawfish-soybean, and rice-crawfish-fallow are the major rotational strategies (McClain and Romaire, 2004).

Crawfish are not fed formulated feeds as in other aquaculture production systems. Instead, ponds are drained in summer to grow rice, sorghum-sudangrass or other vegetation to serve as the base of a natural food chain for crawfish (McClain et al., 2007). Crawfish ponds are not stocked with hatchery-reared young as in other forms of aquaculture and, unharvested crawfish produce young the next year (McClain et al., 2007). In addition, compared to common batch harvests of fish, crawfish harvests are done with baited traps (McClain and Romaire, 2004).

The objective of this research was to compare the SOC content of two crawfish production systems with adjacent agriculture fields. Other soil/sediment properties such as bulk density, N concentration and mass, particle size, soil pH, and C:N were also characterized.

Materials and Methods

Sampling Locations

The monocropping systems were sampled in August and September of 2011, at the beginning of the crawfish season when they were flooded. Sampling sites included Baton Rouge, St. James, St. Landry, and St. Martin Parishes (Table 6). For the crop rotational systems, soils were sampled in spring of 2012, prior to rice planting. Sampling locations were Acadia, St. Landry, Vermillion, and Jeff Davis Parishes (Table 6).

Sampling

A clear plastic core liner tube (Wildlife Supply Company, Buffalo, New York, USA) with a diameter of 5 cm and sediment core removal tool were used in pond soil sampling (Boyd et al., 2002). The plastic tube was pushed into the pond bottom, capped on the top, and removed to provide an undisturbed soil core. Twelve core samples were randomly taken to a depth of 20 cm in each pond, cut into 5 cm segments of 0-5 cm, 5-10 cm, 10-15 cm, and 15-20 cm, and stored in plastic bags. For agricultural field samples, an impact sampler with a diameter of 2 cm was used and 25 core samples were taken in each field at the same depth as pond samples. Both sets of samples were taken to the laboratory for processing and analyses.

Sample Analyses

Samples for bulk density were dried at 105°C for 72 hours and weighed afterwards to calculate dry bulk density. Other field samples were oven dried at 60°C for 72 hours, pulverized with a mechanical pulverizer (Braun, Los Angeles, USA) to pass a 2 mm screen, and stored in plastic containers. Soil pH was measured with a glass electrode inserted into a 1:1 mixture of dry, pulverized soil and distilled water. Particle size analysis was conducted by the hydrometer method using standard ASTM 152 H hydrometer and sedimentation cylinder (Bouyoucos, 1962). Carbon and N were analyzed with a LECO TruSpec CN Analyzer (Leco Corporation, St. Joseph, Michigan, USA). More detailed information on standard techniques of sample processing can be found in the methodology described by Munsiri et al. (1995).

Data Analyses

SAS (SAS Institute Inc., Cary, NC, USA) was used for data analyses. Organic C concentration, organic C mass, bulk density, total N concentration, and total N mass of two crawfish production systems were compared with adjacent agricultural fields. Differences of least squares means, using the PROC MIXED procedure in SAS were used in the data analyses. Differences were considered significant at $P < 0.10$. SigmaPlot 11.0 Statistical Software (Aspire Software International, Ashburn, VA, USA) was used to create depth distribution figures.

Results and Discussion

Soil Organic Carbon

There was no significant difference in SOC concentration between rotational crawfish ponds and adjacent agricultural fields (Fig. 17). The SOC concentration of the 0-5 and 5-10 cm

soil depths was greater in monocropping crawfish ponds than in adjacent agricultural fields (Fig. 17).

The possible explanations for greater SOC concentration in upper layers of monocropping systems instead of rotational systems include: 1) In monocropping systems, there was no crop removal, leaving the residue of plants in the ponds while in rotational systems, rice or other crops were harvested. The unharvested crops were important sources for organic matter accumulation. 2) The long flood period of crawfish ponds in monocropping systems contributed to SOC accumulation since it created an anoxic environment unfavorable for organic matter decomposition, while rotational crawfish ponds and adjacent agriculture fields were relatively less inundated. When ponds are drained, an appreciable amount of C is eroded from the surface layer of pond soils (Ayub et al., 1993). Also, when both crawfish pond systems were flooded, the depth of flood in monocropping crawfish ponds was usually deeper than the adjacent fields and rotational crawfish ponds, because these pond bottoms were often not leveled and the water was deeper in traffic lanes so the harvest boat could reach shallower areas (Dr. Ray McClain, personal communication, 2013). Furthermore, though feed was not used, a large amount of crawfish trap bait including natural fish baits and formulated baits entered the system while ponds were flooded. Lastly, the extended deep flood encourages an influx of organic material – via insect and amphibian migration, and bird droppings. 3) In crop rotational systems and adjacent agriculture fields, tillage was applied annually and the fields were laser leveled frequently, so that SOC was less likely to accumulate. 4) The monocropping crawfish ponds tended to have an abundance of permanent aquatic or semi-aquatic types of vegetation such as arrowleaf, cattail, and other such plants, that likely pulled nutrients from deep in the soil and redistributed much of that within the top layer as they decomposed.

There was no significant difference in SOC mass either between rotational crawfish ponds and adjacent fields, or between monocropping crawfish ponds and adjacent fields (Fig. 18). However, the 10-15 and 15-20 cm layers had lower SOC mass than adjacent agriculture fields under the crawfish monocropping system (Fig. 18). One reason that SOC concentration and mass showed different results in the monocropping crawfish system was the difference in soil bulk density (Fig. 19). The bulk density was significantly different in every layer of sediment between monocropping crawfish ponds and adjacent agriculture fields (Fig. 19).

Soil organic C concentration and mass decreased with depth in both systems and the adjacent agriculture fields (Figs. 17 and 18). Organic matter concentration in surficial sediments has been shown to be greater than in deeper sediment layers (Munsiri et al., 1995; Sonnenholzner and Boyd, 2000), because organic matter from the water column settles on the sediment surface. The same pattern was found in other research by Masuda and Boyd (1994), the upper layer of bottom soil typically had a higher SOC than in deeper layers of soil.

In previous research (Munsiri et al., 1995; Thunjai et al., 2004), pond soils contained more C than original pond bottom soils, but reached equilibrium with respect to C concentration (2-4%) very quickly. Numerous studies (Boyd et al., 2010; Boyd, 1995; Steeby et al., 2004) reveal that SOC concentration in ponds are seldom above 3%, which is typical of mineral soils of aquaculture pond sediments (Steeby et al., 2004), regardless of the input, season, and intensity of management.

Nitrogen

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Nitrogen is essential in pond management because it is a major component of plants and animals, and it influences pond soil quality, water quality, and crop productivity (Gross et al., 2000). Nitrogen is stored in pond sediment as organic N and NH_4 (Gross et al., 2000).

Nitrogen concentration and mass (Fig. 20 and 21) showed a similar trend as concentration and mass of SOC (Fig. 17 and 18). Nitrogen concentration was greater in the 0-5 cm depth of crawfish monocropping systems than adjacent agriculture fields (Fig. 20). In other depths there was no difference between the systems with regard to soil N concentration. Nitrogen mass under the monocropping crawfish ponds decreased rapidly with depth (Fig. 21). In the 10-15 and 15-20 cm layers, adjacent agricultural fields had greater soil N mass than monocropping crawfish ponds. Nitrogen concentration and mass decreased with soil/sediment depth, the result being different from that of Yuvanatemiya et al. (2011) who showed no clear pattern in N concentration with sediment depth under shrimp ponds.

Possible explanations for our N results include greater rice or other forage residues, dead phytoplankton, fish wastes, unused baits, and bird droppings deposited on the top layer. Also, N fertilizers were applied on the surface of pond bottom soil. Concentrations of N increased in pond soils over time in some research (Masuda and Boyd, 1994; Munsiri et al., 1995). However, in other research (Thunjai et al., 2004), the correlations between pond age and N accumulation were weak. The pond age data is not available in the present study, so the correlation between N and pond age is not clear.

In monocropping systems, the C:N ratio ranged from 10.3 to 12.5 (Table 7). The average C:N was 11.4, which is in the range of suitable C:N (10 to 15) for aquaculture (Banerjea, 1967). In rotational crawfish ponds, C:N ranged from 8.8 to 11.0 (Table 7) with an average of 9.7,

which was close to the natural ecosystem with C:N ratio at 10:1 due to the C:N of humus (Boyd, 1995). The ratio reflects dynamics of C and N, influences microbial activity and affects bottom soil-water interactions (Thunjai et al., 2004). The C:N ratio is a good indicator of pond quality, providing information for adjusting feeds and fertilizers. In this study, the sediment C:N ratio was within the normal range for aquaculture production

Bottom soil pH ranged from 6.9 to 7.2 and averaged 7.1 in monocropping crawfish ponds, and in rotational crawfish ponds, soil pH ranged from 6.7 to 7.3 and averaged 7.0 (Table 7). According to Boyd (1995), the ideal pH for aquacultural production is from 7.0 -7.5. Thus, the average pH of crawfish pond sediments in this study are within the normal range.

In some research (Yuvanatemiya et al., 2011), bottom soil pH tended to decline with increasing soil depth, but not in this study. On the contrary, there was a slight tendency of increasing pH with pond soil depth. The reason for this could be the different management practices in crawfish ponds compared to other aquaculture practices. For example, farmers do not lime crawfish ponds for the most part (although a few have put a limited amount on, likely some decades ago), but in other commercial ponds, liming is a common practice (Yuvanatemiya et al., 2011). Also, organic feed and nutrient inputs into other aquaculture ponds, compared to the natural food web in crawfish ponds, could contribute to the difference of pH pattern as well (Yuvanatemiya et al., 2011). The most likely explanation for the difference in sediment pH results from our study and that of Yuvanatemiya et al. (2011) is the inherent difference in the original soil materials.

There was no difference in soil/sediment pH between crawfish ponds and agriculture fields, suggesting that pH did not decline or increase as the pond aged in crawfish production.

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This agrees with the results of Thunjai et al (2004), refuting the idea of sediment pH declining as ponds age.

Sediment/soil bulk density averaged 1.10 g cm^{-3} in monocropping crawfish ponds compared to 1.37 g cm^{-3} in adjacent agriculture fields. Bulk density was lower in monocropping crawfish ponds and adjacent agricultural fields in every depth (Fig. 19). However, soil bulk density in rotational systems did not show a significant difference from adjacent agricultural fields. The SOC concentration was higher in monocropping crawfish ponds than adjacent fields, but this was not true in rotational systems. Organic matter is lighter in weight than mineral soil contributing to lower bulk density. Avnimelech et al. (2001) found that sediment dry bulk density was inversely related to the organic C concentration (r = - 0.84) for a diverse group of sediment samples including rivers, lakes and fish ponds. Physical processes in the ponds such as resuspension (Avnimelech et al., 2001; Munsiri et al., 1995) can decrease sediment bulk density in ponds. Conversely, cultivation, tillage, settling, and compaction may contribute to higher bulk density (Avnimelech et al., 2001). The bulk density in monocropping crawfish ponds did not vary much with depth, and the reason may be that it reached an approximate equilibrium condition (Avnimelech et al., 2001).

Conclusion

Although the upper soil layer of the crawfish monocropping systems had greater SOC concentration than adjacent agricultural fields, there was no significant difference in SOC mass. Crawfish pond systems may have limited capability to sequester C based on the data in this study. Participation in carbon emission "cap and trade" programs needs further research and evaluation for crawfish production systems.

Nitrogen concentration and mass depth distribution had trends similar to that of SOC, with the top layer significantly different, and decreasing with soil depth. The C:N ratio and pH in the study were within a suitable range for aquacultural production. The bulk density data explained the pattern of SOC and N concentration and mass depth distribution. These soil property data may provide useful information for best management practices and future research.

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Tables

Table 6. Cropping system, sampling location, soil type, and crop type under crawfish ponds and adjacent agricultural fields.

Cropping System	Sampling Location	Latitude (N)	Longitude (W)	Soil Type	Crop Type
Rotational Crawfish Ponds/Adjacent Agricultural Fields					
Crawfish pond	Acadia	$30^o14'33''$	$92^o19'59''$	Crowley Silt Loam	Crawfish/rice
Adjacent field	Acadia	$30^o14'33''$	$92^o19'59''$	Crowley Silt Loam	Soybean/rice
Crawfish pond	Acadia	$30^o14'29''$	$92^o19'28''$	Crowley Silt Loam	Crawfish/rice
Adjacent field	Acadia	$30^o14'4''$	$92^o19'20''$	Crowley Silt Loam	Soybean/rice
Crawfish pond	St. Landry	$30^o34'46''$	$92^o9'53''$	Jenerette Silt Loam	Crawfish/rice
Adjacent field	St. Landry	$30^o34'21''$	$92^o8'54''$	Jenerette Silt Loam	Soybean/milo
Crawfish pond	St. Landry	$30^o28'45''$	$91^o52'41''$	Balwin Sharkey Complex	Crawfish/rice
Adjacent field	St. Landry	$30^o28'52''$	$91^o52'34''$	Balwin Sharkey Complex	Soybean/rice
Crawfish pond	Vermillion	$30^o5'42''$	$92^o16'1''$	Kaplan Silt Loam	Crawfish/rice
Adjacent field	Vermillion	$30^o5'9''$	$92^o18'38''$	Kaplan Silt Loam	Soybean/rice
Crawfish pond	Acadia	$30^o10'39''$	$92^o20'57''$	Midland Silty Clay Loam	Crawfish/rice
Adjacent field	Acadia	$30^o10'39''$	$92^o20'57''$	Midland Silty Clay Loam	Rice
Crawfish pond	Jeff Davis	$30^o10'12''$	$92^o38'47''$	Crowley Vidrine Silt Loam	Crawfish/rice
Adjacent field	Jeff Davis	$30^o10'12''$	$92^o38'47''$	Crowley Vidrine Silt Loam	Soybean/rice
Monocropping Crawfish Ponds/Adjacent Agricultural Fields					

Cropping System	Sampling Location	Latitude (N)	Longitude (W)	Soil Type	Crop Type
Crawfish pond	Baton Rouge	30°22'12''	91°11'10''	Sharkey Clay	Crawfish
Crawfish pond	Baton Rouge	30°22'16''	91°11'10''	Sharkey Clay	Crawfish
Adjacent field	Baton Rouge	30°21'55''	91°10'22''	Sharkey Clay	Corn
Crawfish pond	Arnaudville	30°23'12''	91°51'07''	Sharkey Clay	Crawfish
Crawfish pond	Arnaudville	30°23'17''	91°50'54''	Sharkey Clay	Crawfish
Adjacent field	Arnaudville	30°23'12''	91°51'26''	Sharkey Clay	Soybeans
Crawfish pond	St. James	30°01'52''	90°55'35''	Schriever Clay	Crawfish
Crawfish pond	St. James	30°01'45''	90°55'23''	Schriever Clay	Crawfish
Adjacent field	St. James	30°02'03''	90°55'30''	Schriever Clay	Pasture
Crawfish pond	LeBeau	30°39'02''	91°55'12''	Sharkey Clay	Crawfish
Crawfish pond	LeBeau	30°39'02''	91°55'17''	Sharkey Clay	Crawfish
Adjacent field	LeBeau	30°39'02''	91°55'21''	Sharkey Clay	Sorghum

Table 7. Soil particle size, pH, and C:N ratio for different depth of two crawfish production systems and adjacent agricultural fields.

| Systems	Soil depth (cm)	Sand (%)			Clay (%)			Silt (%)			pH			C:N
Monocropping Crawfish Ponds	0-5	11.2	±	1.56	63.8	±	5.84	25.0	±	4.35	6.89	±	0.58	12.54
	5-10	10.3	±	2.59	65.7	±	5.63	24.1	±	3.18	7.01	±	0.60	11.74
	10-15	10.4	±	1.84	65.4	±	5.13	24.2	±	3.30	7.09	±	0.54	10.91
	15-20	11.1	±	2.00	66.7	±	5.76	22.2	±	3.88	7.24	±	0.43	10.32
Adjacent Field	0-5	21.1	±	9.00	52.3	±	13.68	26.6	±	5.73	6.45	±	0.49	12.96
	5-10	18.4	±	6.93	53.1	±	10.86	28.5	±	4.27	6.25	±	0.22	10.58
	10-15	17.3	±	7.25	54.8	±	10.83	28.0	±	4.60	6.24	±	0.24	10.40
	15-20	18.0	±	7.36	56.3	±	11.23	25.8	±	4.31	6.64	±	0.28	9.67
Rotational Crawfish Ponds	0-5	23.0	±	2.00	26.5	±	5.34	50.5	±	4.50	6.72	±	0.38	10.98
	5-10	22.5	±	1.94	26.0	±	5.10	51.5	±	4.85	6.76	±	0.42	10.31
	10-15	22.0	±	1.22	27.5	±	4.74	50.5	±	5.03	7.06	±	0.45	8.88
	15-20	19.5	±	1.22	28.0	±	4.57	52.5	±	5.00	7.32	±	0.46	8.75
Adjacent Field	0-5	22.0	±	2.00	20.5	±	3.00	57.5	±	4.87	6.38	±	0.27	10.67
	5-10	24.0	±	2.32	21.0	±	2.57	55.0	±	4.47	6.34	±	0.30	9.75
	10-15	22.5	±	1.58	23.5	±	2.69	54.0	±	2.32	6.93	±	0.38	7.98
	15-20	22.5	±	2.74	26.5	±	2.18	51.0	±	1.87	7.19	±	0.40	8.39

Figures
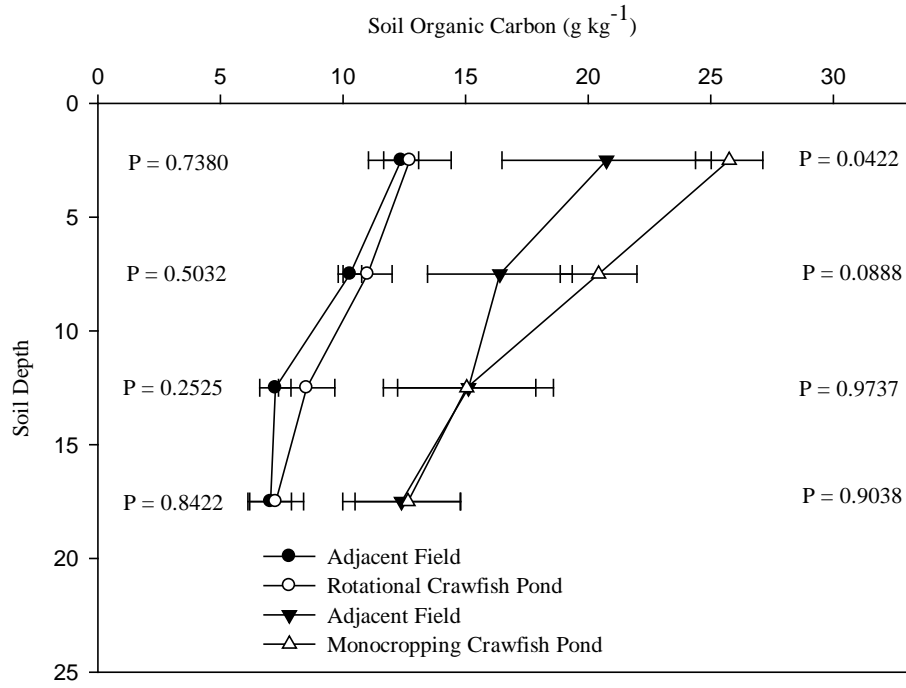
Figure 17. Depth distribution of soil organic carbon concentration in crawfish ponds and adjacent agricultural fields of monocropping systems and rotational systems.
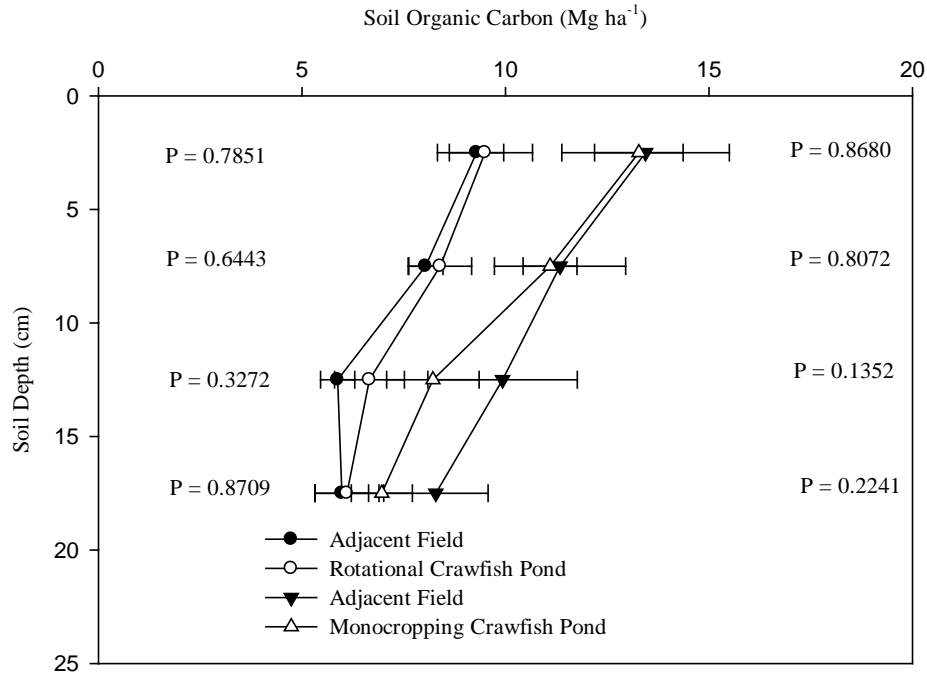
Figure 18. Depth distribution of soil organic carbon pool in crawfish ponds and adjacent agricultural fields of monocropping systems and rotational systems.
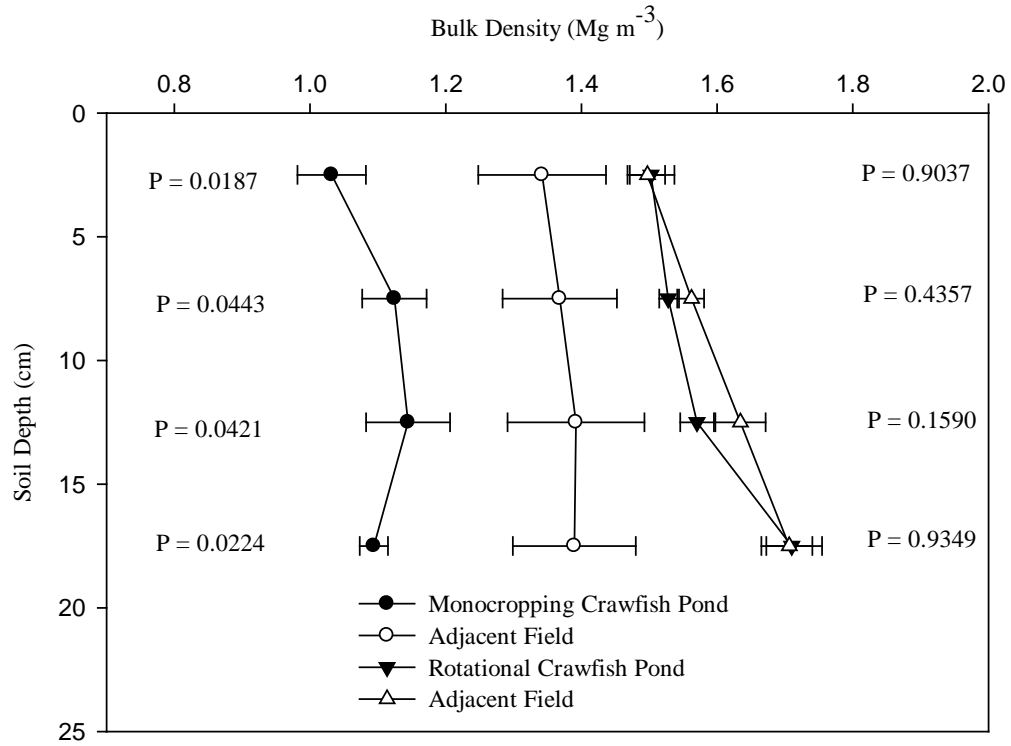
Figure 19. Depth distribution of bulk density in crawfish ponds and adjacent agricultural fields of monocropping systems and rotational systems.
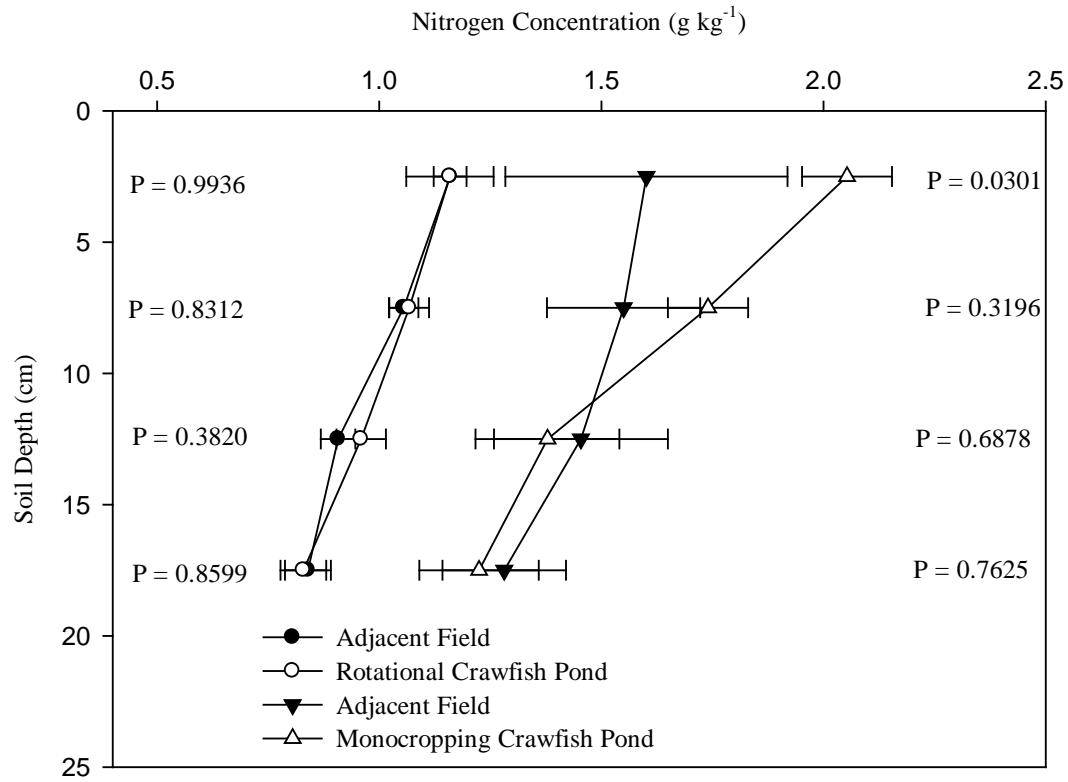
Figure 20. Depth distribution of nitrogen concentration in crawfish ponds and adjacent agricultural fields of monocropping systems and rotational systems.
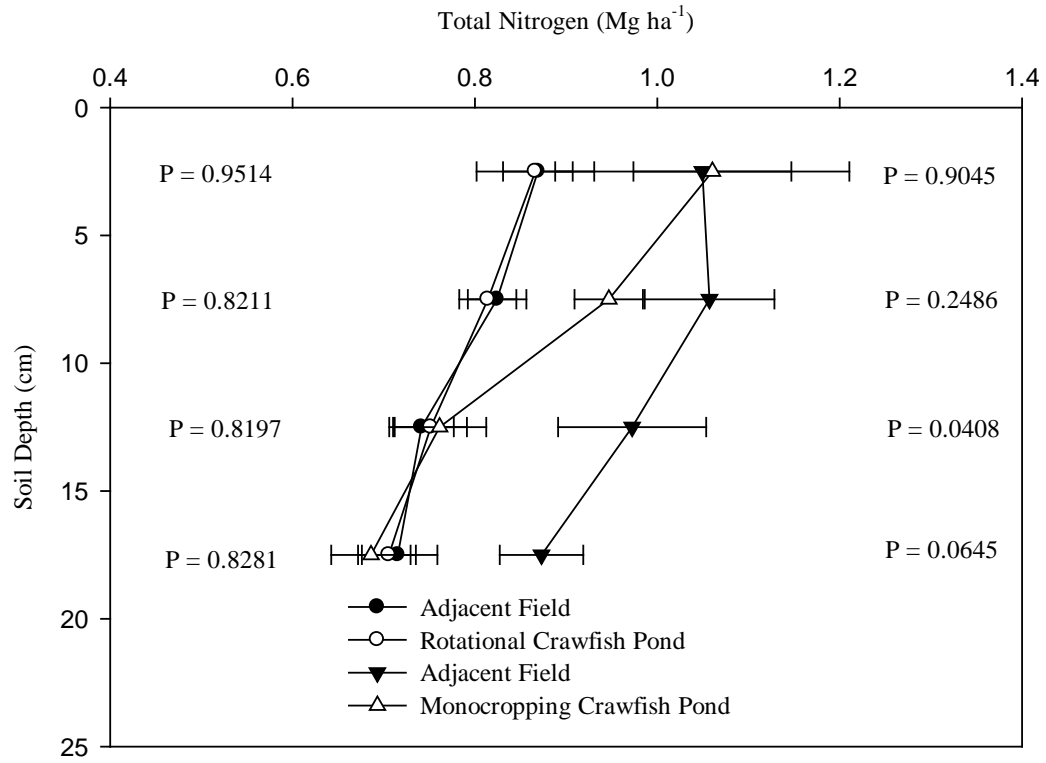
Figure 21. Depth distribution of total nitrogen in crawfish ponds and adjacent agricultural fields of monocropping systems and rotational systems.