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Soil organic carbon accumulation in organic cropping systems

James Littrell
University of Tennessee, jlittre1@vols.utk.edu

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I am submitting herewith a thesis written by James Littrell entitled "Soil organic carbon accumulation in organic cropping systems." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Environmental and Soil Sciences.

Sindhu Jagadamma, Major Professor

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Sean Schaeffer, Jaehoon Lee

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

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

Soil organic carbon accumulation in organic cropping systems

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James Julius Littrell

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ABSTRACT

How organic and conventional grain production systems under different tillage regimes affect total and biologically active soil organic carbon (SOC) accumulation is poorly understood as organic no-till systems were only developed in the past two decades. In this study, how long-term conventional and organic row-crop management under different tillage regimes influence SOC dynamics and aggregation was examined at the Rodale Institute's Farming Systems Trial (FST) in Pennsylvania. The experiment includes three cropping system treatments which were initiated in 1981: (i) conventional (cropping systems which uses synthetic fertilizers and plant protection chemicals), (ii) organic-legume (organic cropping systems that use legumes as nitrogen sources), (iii) organic-manure (organic cropping systems that use manure plus leguminous cover crops as nitrogen sources); and two tillage treatments which were initiated in 2008: (i) conventionally-tilled (tilled) and (ii) no-till. Organic-manure treatments were expected to maximize total and biologically active SOC accumulation. Soil samples were collected from three depth increments (0-10, 10-20, and 20-30 cm) in mid-June, 2018, and measured for total SOC and biologically active microbial biomass carbon, permanganate oxidizable carbon, and water-extractable organic carbon concentrations. Soils under organic-manure management had the highest and conventionally managed soils the lowest concentrations of total and biologically active SOC regardless of tillage regime. Additionally, no-till and organic management, especially organic-manure management, increased the proportion of large macroaggregates, wet aggregate stability, and SOC protection within macroaggregates. Therefore, organic and/or no-till management regenerates soil health by enhancing SOC accumulation and aggregate stability.

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CHAPTER I: INTRODUCTION

Soil organic matter (SOM) content can improve soil quality by retaining water and nutrients, improving soil structure, increasing soil microbial activity, and filtering and degrading pollutants (Kladivko *et al.,* 2014; Blanco-Canqui and Benjamin, 2013). With 13.3% of US land under harvested cropland (USDA, 2017), cultivation practices play a major role in SOM storage. Therefore, it is essential to investigate the ability of different cropping systems to promote SOM accumulation and the associated ecosystem services to support air, water, and soil quality and to mitigate climate change. Soil organic carbon (SOC), the major component of SOM consists of biologically active and stable C fractions. Biologically active SOC fractions including water-extractable organic carbon (WEOC), microbial biomass carbon (MBC), and permanganate-oxidizable carbon (POXC) respond rapidly to soil and crop management practices and are important for nutrient cycling and supply (Jastrow *et al.,* 2007). Differently, stable SOC fractions are resistant to biodegradation and respond slowly to management change, contributing to long-term soil C sequestration. Carbon inputs entering the soil undergo microbial transformation to stable fractions through (bio)chemical alteration and/or physical stabilization (Jastrow *et al.,* 2007).

Soil aggregate structure and stability are important soil health indicators closely related to increased SOC concentration, the ability of soil to retain water during droughts, increased infiltration/percolation of water during wet periods, and reduced erosion (Blanco-Canqui and Francis 2016). Soil aggregates protect organic C, restricting access to enzymes while reducing the availability of oxygen necessary to mineralize the C (Six *et al.,* 2000). According to Six et al. (2000), soil aggregates are classified into four size classes: large macroaggregates (>2 mm), small macroaggregates (0.25-2.0 mm), microaggregates (0.053-0.250 mm) and silt- and clay-sized aggregates (<0.053 mm). Microaggregates are more stable than macroaggregates and generally protect greater quantities of organic C than macroaggregates, allowing otherwise active carbohydrates and amino acids to be preserved for a longer period by a combination of clay adsorption and occlusion mechanisms (Dungait *et al.,* 2012). Aggregate stability can be enhanced by increasing C inputs through organic amendments and cover crops and minimizing disturbance through no-till farming.

Organic cropping systems are expected to increase SOC accumulation and improve aggregation through increased organic matter inputs by manure application and cover cropping. However, organic systems typically undergo frequent tillage operations to control weeds and to incorporate cover crops. Such intensive tillage activities adversely affect SOC accumulation by disrupting soil aggregates and accelerating soil C mineralization through increased soil aeration (Beare *et al.,* 1994). However, little is understood about how conventional and organic management interacts with tillage to affect SOC accumulation in row-crop production systems because such long-term field experiments to enable side-by-side comparison are rare. In addition, most organic row-crop systems follow a multi-year rotation of grain crops with cover crops. This rotational cropping is important to consider as the residue C:N ratios and root architecture of different crops may greatly influence aggregate formation and C accumulation as a function of soil depth.

The Farming Systems Trial (FST) established in 1981 at the Rodale Institute in Eastern Pennsylvania provides a unique opportunity to investigate the long-term effects of transitioning from conventional to rotational organic cropping systems on crop yields and soil quality. The original study initiated in 1981 consists of conventionally managed systems amended with inorganic fertilizers and other plant protection chemicals, and organically managed systems amended with dairy manure and/or leguminous cover crops. All treatments were tilled until 2008, when no-till plots were added by splitting the tilled plots in two. The main cash crops grown in this trial are corn (*Zea mays*), wheat (*Triticum aestivum*), and soybean (*Glycine max*); the most extensively grown grain crops in the United States.

The goal of this study is to obtain quantitative information on how organic crop management influence SOC accumulation compared to conventional management. The specific objective is to determine how different cropping systems and tillage regimes interactively affect total SOC concentration, biologically active SOC concentrations, and aggregation in the long term.

CHAPTER II: LITERATURE REVIEW

2.1. Organic and conservation agriculture: benefits and challenges

Conventional agriculture is plagued by numerous environmental and public health problems through its heavy dependence on tillage and synthetic chemical inputs such as herbicides, pesticides, and inorganic fertilizers to maintain high crop yields. However, herbicides and pesticides are not only toxic to non-target organisms and humans but are driving the evolution of resistant weeds and pests which are expected to become a greater problem in the future. Mineral fertilizers are more nutrient-dense and transportable than manures but accounts for over 50% of energy usage associated with commercial agriculture (Woods *et al.,* 2010) and are implicated in groundwater contamination of nutrients and plant protection chemicals, and eutrophication of rivers, lakes, and oceans. Tillage, a common practice for loosening the soil, controlling weeds, and preparing seedbeds increases aggregate disruption and SOC decomposition and leaves the soil vulnerable to wind and water erosion. Various organic and conservation agriculture management systems were developed to address these problems. Organic agriculture relies on animal manures and/or leguminous green manures for maintaining soil fertility and avoids synthetic herbicide and pesticide applications. However, organic agriculture typically relies on tillage for weed and cover crop management, and for maintaining a healthy environment for predatory insects to control insect pests. Conservation agriculture, on the other hand, relies on minimizing or eliminating tillage, especially moldboard-plowing, to protect against soil erosion but typically relies on herbicides and pesticides for weed and pest management. Most organic and conservation management options are partial solutions to the problems associated with conventional tilled agriculture because of tillage dependence for organic management and herbicide- and pesticide- dependence for conservation agriculture.

Organic management has numerous benefits. The absence of pesticide and herbicide inputs combined with heavy organic matter inputs benefit soil microbial communities, including arbuscular mycorrhizal fungal associations, important for maintaining soil and crop health (Wander *et al.,* 1995; Douds and Milner, 1999). However, one of the most important benefits of organic management is enhanced SOC accumulation (e.g. Lorenz and Lal, 2016; Bai *et al.,* 2018) with its attendant benefits of enhanced soil water and nutrient retention, reduced nitrate leaching (Michalak, 2004), and enhanced drought tolerance, which enables organic cropping systems to out-yield their conventionally-managed counterparts during drought years (Pimental *et al.,* 2005).

Despite its benefits, organic agriculture is practiced on only ~1% of global agricultural land (Lorenz and Lal 2016). The most important challenge facing wider adoption of organic farming is the yield reduction compared to conventional farming. Ponisio et al. (2015) reported that the average yields of organic cereals were 15.5-22.9% lower than their conventionally managed counterparts. Reasons for this yield reduction in organic systems include lower nitrogen availability and greater weed pressure and pest abundance. Moreover, most cereal varieties grown commercially were selectively bred to respond optimally to mineral fertilizer applications (Ponisio *et al.,* 2015). Corn responds especially poorly to organic management due to high nitrogen demand. Larsen et al. (2014) reported in a 15-year experiment comparing how corn yields respond to organic fertilization (poultry manure) vs. conventional management (ammonium nitrate), corn yields in organic plots were only half that of conventional plots.

Heavy tillage dependence also limits the wider adoption of organic management by increasing labor requirements and making the soil more vulnerable to erosion. To help address these shortcomings, organic rotational cropping systems managed with reduced- or no-tillage were developed.

Even though organic cropping systems typically have lower yields than their conventionally managed counterparts, the yield gap may be a transitory effect, especially where crop rotations are utilized. During the first five years of the Rodale FST, it was reported that in organic corn-wheat-soybean rotation system, corn yields were only 75% of that of conventionally managed plots in Year 1 but the yield gap was closed in year 5. In addition, no yield gap for soybean and wheat were observed throughout the study period (Liebhardt *et al.,* 1989). Similarly, Schrama et al. (2018) reported that the yield gap between potato-peas-leek-barley-sugar beet-corn rotation under conventional and organic management disappeared 13 years after transitioning to organic management even though organic plots receive N than conventional plots. These benefits may be the result of improvements to soil structure (i.e. aggregation, earthworm burrows), increased SOC accumulation, and overall increase in microbial activity associated with organic management.

Organic no-till systems show enormous promise in combining the benefits of organic and conservation agriculture but how they affect crop yields depend on several factors including the cover crops grown, the method of cover crop termination, cash crops grown, and whether or not the soil received organic-manure. Corn tends to respond poorly to strictly no-till organic management, yielding 50-70% less per unit land compared to conventional management (e.g. Vincent-Caboud *et al.,* 2017; Drinkwater *et al.,* 2000) which could be attributed to the high nitrogen demand of corn crops (Wallace *et al.,* 2017). Soybean and small grain yields under no-till organic management can be comparable to that of their conventional counterparts if the cover crops in organic no-till cropping systems form an effective weed-suppressing mulch (e.g. Vincent-Caboud *et al.,* 2017; Wallace *et al.,* 2017) and are provided comparable quantities of nitrogen through cover crops and/or manure (e.g. Ponisio *et al.,* 2015).

2.2. Soil organic carbon

The soil C pool is the largest terrestrial C pool. Soil contains an estimated 2157-2293 Pg C, which exceeds the size of the global biotic (550 Pg) and atmospheric (750 PG) C pools (Batjes, 1996). While soil also contains inorganic C, typically carbonate minerals which are more abundant in drylands, the bulk of soil C is organic C. Soil organic C mediates numerous essential soil functions. Roughly 5% of SOC consists of living organisms including plant roots, earthworms, insects, bacteria, fungi, and viruses (Bot, 2005). Soil organisms are responsible for residue decomposition, nutrient cycling, and improving soil structure by facilitating aggregate formation, which enhances soil water infiltration and aeration. Non-humic compounds, including simple carbohydrates, amino acids, starches, cellulose, hemicellulose, lignin, waxes and lipids make up 5-25% of SOC. Except hemicellulose, lignin, and waxes, non-humic compounds are biologically active and easily degraded unless protected via aggregate occlusion or complexation with clay minerals (Jastrow *et al.,* 2007). Non-humic substances are important food sources for soil organisms and help bind soil aggregates. Humic C, which make up 35-55% of SOC, consists of biochemically stable components of C inputs and by-products of microbial decomposition of residues (Bot, 2005). Besides enhancing soil water and nutrient retention, humic compounds also buffers pH, protects against salinity stress, chelates trace metal nutrients (and enhancing plant uptake), helps enhance further SOC accumulation, and helps immobilize pollutants and toxic substances (Bot, 2005).

Despite the importance of SOC for maintaining essential soil functions, humans drastically altered the SOC pool through conventional intensive agricultural practices. Using a machine-learning based model, Sanderman et al. (2017) reported that 116 Pg of SOC was lost from the top two meters of soil worldwide through conversion of native vegetation to cropland or rangeland/pasture for grazing. In this model, the results were compared to a projected world with no agriculture. Though twice as much land formerly covered with native vegetation was converted grazing lands compared to croplands, the contribution of grazing and croplands to SOC loss was roughly equal (Sanderman *et al.,* 2017). In addition, it was reported by Sanderman et al. (2017) that tilled cropland may have lost 75% of their original SOC.

Conversion of native forests and grasslands to agricultural land accelerates SOC losses through multiple mechanisms. Native forests and grasslands typically consist of perennials which maintain permanent soil cover, protecting soil from erosion while their roots and associated mycorrhizae help stabilize soil aggregates, which protects SOC from microbial decomposition (Jastrow *et al.,* 2007). Soils under native grasslands and forests also receive continuous SOC inputs from litter and rhizodeposition. However, many of these benefits are lost upon conversion to rangeland/pasture or cropland. Overgrazing accelerates erosive SOC losses from rangelands and pastures while reducing net primary productivity and SOC inputs. However, croplands experience far higher SOC losses in comparison. Most crops grown are annuals, which have shallower and less extensive root systems than native perennial vegetation, which combined with C removals from croplands via grain harvests entails lower C inputs and reduced C protection compared to native vegetation. Additionally, tillage is typically used to suppress weeds and prepare seedbeds, and fallow periods utilized to allow the soil to rest—both practices enhance erosive SOC losses though tillage also accelerates SOC mineralization.

2.3. Factors affecting SOC accumulation

Organic C is preserved through both biochemical recalcitrance, chemical bonding with clay minerals, and physical protection in aggregates. Based on these preservation mechanisms, SOC is classified as fast, slow, and passive pools based on the residence time of the organic compounds making up these pools (Dungait *et al.,* 2012). Fast pools consist largely of plant litter, which mainly consists of polysaccharides, proteins, sugars, and amino acids with a mean residence time of 0-4 years while slow pools consist of polyphenols, waxy compounds, and lignified materials with mean residence time of 15-100 years. Lignified materials are degraded into quinones, which contribute to humification by polymerizing with other organic compounds (Jastrow *et al.,* 2007). However, the estimated mean residence times of the aforementioned organic compounds does not consider physical protection. Even though sugars and amino acids are highly labile, these compounds may be preserved by mineral adsorption and/or aggregate occlusion. Von Lutzow et al. (2006) reported that microaggregates (<250 µm) protect SOC on the order of decades to centuries while macroaggregates (>250 µm) protect SOC on the order of years to decades. However, stable macroaggregates also promote the formation of microaggregates with stabilized SOC (Six *et al.,* 2000). Similarly, Jagadamma et al. (2014) reported that 12-46 % of C from particulate SOC but only 3-10 % of C from mineral-adsorbed

SOC was respired during a 150-day laboratory incubation experiment, further demonstrating that mineral-adsorbed SOC is more stable than particulate SOC.

Quality of plant roots and litter greatly influence SOC dynamics. Root matter represent roughly 70 % of total plant biomass (Poorter *et al.,* 2012) and root exudates also contribute to C addition to soil. Extensive fibrous roots and roots which form symbiotic relationship with mycorrhizae are associated with enhanced SOC accumulation compared to taproots. High root length density and branching intensity is associated with enhanced micro- and macro-aggregation through entanglement and enmeshment of soil particles and production of aggregate-binding exudates (Poirier et al, 2018). Legume root nodules also enhance SOC accumulation by stimulating microbial activity and therefore the production of microbial necromass through N fixation. Microbial necromass and decomposition by-products are more readily adsorbed to minerals or humified compared to particulate SOC (Martins and Angers, 2015). While roots rich in biochemically recalcitrant lignin with high C:N ratios increase short-term SOC stabilization through biochemical recalcitrance, they decrease long-term SOC stabilization as root-derived particulate SOC is less well protected than aggregate-occluded and/or mineral-adsorbed decomposition by-products (Poirier et al, 2018).

Similar to roots, plant litter with lower C:N ratios are associated with enhanced SOC stabilization compared to plant litter with high C:N ratios. While low C:N ratios favors decomposition, decomposition by-products are generally more readily protected by mineral adsorption and aggregate occlusion than plant residues (e.g. Cordova et al. 2018; Poirier et al. 2018). It was reported in a 4-day laboratory incubation study by Cordova et al. (2018) that while soils amended with plant litter with low C:N ratios such as Oat and Alfalfa litter exhibited higher rates of litter decomposition compared to soils amended with high C:N ratio litter such as corn and soybean litter. However, there is evidence that plant roots contribute more to SOC accumulation than plant litter. This was demonstrated in a study by Puget and Drinkwater (2001) at the Rodale Institute where they found that after a six-month growing season, roughly half of the original root-derived SOC but only 13% of shoot-derived SOC remained in the soil. Reasons include the continuous nature of root SOC inputs through exudation and turnover, the greater biochemical recalcitrance of root SOC, and that root SOC is more readily protected by mineral adsorption and aggregate occlusion than above-ground litter SOC (Puget and Drinkwater, 2001).

Soil organic C losses in croplands can be reversed by adopting conservation management practices, including replacing inorganic N fertilizers with livestock manures or leguminous green manures, leaving crop residues in the fields. Likewise, erosive SOC losses can be minimized through adopting reduced or no-till management, cover cropping, crop rotation, and adding perennial grass or forage rotations. In a study comparing the SOC dynamics of continuous corn plots to those incorporating perennial orchardgrass or mixed cereal rye and legume (red or white clover or hairy vetch), Nunes et al. (2018) reported that no-till cover cropped treatments had the highest total and active SOC followed by corn-orchardgrass treatments while continuous corn treatments had the lowest. Similarly, Larsen et al. (2014) reported that organic no-till sweetcorn plots amended with poultry manure and with mixed winter wheat/clover cover had twice the total SOC concentration as conventionally tilled continuous sweetcorn plots amended with ammonium nitrate. Additionally, adopting crop rotation alongside organic management showed reduction in the organic/conventional yield gap (Ponisio *et al.,* 2015).

2.4. Soil aggregates

Besides mineral adsorption and biochemical recalcitrance, aggregate occlusion in aggregates is another important SOC preservation mechanism. The interiors of aggregates could be anaerobic, slowing SOC decomposition within aggregates. In addition, aggregates create physical barriers between occluded SOC and soil microbes (Tisdall and Oades, 1982). Moreover, water-stable aggregates protect SOC from erosive losses. While all aggregate size classes protect SOC, microaggregates (0.053-0.250 mm) generally protect a significantly higher proportion of SOC for a longer time than macroaggregates (>0.250 mm) (Beare *et al.,* 1994). This is because of the higher stability of microaggregates, which are bound by the link between polyvalent metal cations and charged organic aromatic species compared to the more transient nature of macroaggregates that are bound by the plant roots and fungal hyphae (Tisdall and Oades, 1982). While several studies showed that microaggregates contain greater SOC concentration per unit mass than macroaggregates (e.g. Jastrow *et al.,* 2007; von Lutzow *et al.,* 2006), treatments which enhance macroaggregation also enhance SOC preservation by microaggregates within macroaggregates (e.g. Six *et al.,* 2000; Six and Paustian, 2014). Mucilage, a microbial by-product consisting mostly of polysaccharides is an important transient binding agent for both micro- and macroaggregates (Tisdall and Oades, 1982). Additionally, fungi contribute disproportionately to both SOC preservation and aggregate stabilization through biochemically recalcitrant cell walls and macroaggregate binding by fungal hyphae

(Murugan *et al.,* 2019) and in the case of arbuscular mycorrhizal fungi, the secretion of aggregate-binding glomalin (Rillig *et al.,* 2004).

Both organic amendments and reduced and/or no tillage enhance aggregate stability. Organic amendments promote the growth of microorganisms which secrete aggregate-binding compounds which enhance aggregate stability (Jastrow *et al.,* 2007; Long *et al.,* 2014). Likewise, reduced-till management minimizes mechanical disruption of plant roots and fungal hyphal networks. Arshad et al. (1999), Angers et al. (1993), and Kasper et al. (2009) reported that transitioning from tilled to no-till management significantly increased wet aggregate stability. Similarly, Lynch et al. (2014) reported that organically managed soils exhibited greater wet aggregate stability than conventionally managed soils throughout the temperate region. Additionally, no-till management enhances SOC protection within macroaggregates according to Beare et al. (1994). This may be because no-till management enhances both the proportion of microaggregates-within-macroaggregates and the amount of C occluded by microaggregates-within- macroaggregates (Six and Paustian, 2014). Nonetheless, aggregate stability, SOC protection within aggregates, and favorable hydraulic properties are closely linked. Shukla et al. (2003) reported that manured and no-tilled continuous corn maximized wet aggregate stability, aggregate protected C, and soil water infiltration followed by no-till continuous corn without manure application and no-till corn-soybean rotations without manure application in a study in Northern Ohio. However, this study contains only one rotational treatment—no-till corn-soybean rotation and all other cropped treatments were continuous corn under different soil amendments and tillage regimes.

2.5. Soil organic C fractions

Different SOC fractions exist depending on their turnover rates and are often classified based on the means of laboratory separation of the total SOC. Total SOC, representing all SOC in a soil sample is typically determined by combustion analysis. Three common SOC fractions to describe active fractions of SOC are permanganate-oxidizable C (POXC), microbial biomass C (MBC), and water-extractable organic C (WEOC). Permanganate oxidizable C is considered a useful measure of biologically-active SOC, which represents active and decomposed SOC with a turnover rate of 2-5 years (Weil *et al.,* 2003). However, POXC corresponds also closely to small particulate (0.053-0.250 mm) and heavy (i.e. occluded and mineral-adsorbed) SOC, which may be biochemically labile but physically protected (Culman *et al.,* 2012). Microbial biomass carbon (MBC) represents organic C compounds in microbial biomass, which indicates how management affects soil microbial populations. water-extractable organic carbon (WEOC) represents water-soluble root exudates and decomposition by-products, typically amino acids and carbohydrates and are the most active SOC fractions with a turnover rate of 0.1-0.5 years (Dungait *et al.,* 2012).

2.6. Organic farming systems trial at the Rodale Institute

The Rodale farming systems trial (FST) is one of the most extensive studies of how different combinations of cropping systems and tillage regimes affect crop yields and soil fertility and health. The Rodale FST was initiated in 1981 on land which was once conventionally tilled with moldboard plow for seedbed preparation, and applied herbicides for weed management and inorganic fertilizers (mostly urea ammonium nitrate) for soil fertility. The Rodale FST included three cropping systems treatments: conventional, organic-legume, and organic-manure, all of which were originally tilled. In 2008, each of these plots were split to add no-till treatments. In the conventional treatment, corn (*Zea mays*) and soybean (*Glycine max*) are grown as cash crops and cereal rye (*Secale cereal*) is grown as a winter cover crop to scavenge fertilizer N. Weeds are controlled with applications of atrazine and pendimethalin to the corn rotations, metolachlor to both corn and soybean rotations, and metribuzin to soybean rotations in conventionally managed treatments. Both organic-legume and organic-manure cropping systems have more diverse rotations. In both organic treatments, wheat (*Triticum aestivum*) and oats *Avena sativa*) in addition to corn and soybean are grown as cash crops. Legume cover crops including red clover (*Trifolium pratense*) and hairy vetch (*Vicia villosa*) are used as nitrogen (N) sources for the cash crop in organic-legume system while both composted cattle manure and rye/hairy vetch cover crops are used as N sources for organic-manure system. Organic-manure treatments also contain hay rotations during which mixed orchardgrass (*Dactylis glomerata*)/alfalfa (*Camelina sativa*) hay is grown to provide both perennial soil cover and cattle feed. Since 2008, when plots were split into tilled and no-till replicates, roller-crimped cover crop mulching is utilized in organic no-till plots.

Prior studies from the Rodale FST and similar studies elsewhere demonstrated that both legume- and manure- based cropping systems increased SOC accumulation. Early in the FST study, Wander et al. (1994) reported that at the time, organic-legume plots accumulated the most total SOC, conventionally managed plots accumulated no SOC, and organic manure plots accumulated the most active (mainly water-dispersible and particulate) SOC after 10 years. Later, Marriott and Wander (2006) reported that both organic cropping systems accumulated 30-40% greater particulate SOC than conventional cropping systems. Similarly, studies by

Maeder *et al*. (2003) in Switzerland and Bloem *et al*. (1994) in the Netherlands reported enhanced SOC accumulation in organic manure- or legume- based cropping systems compared to conventional systems. However, none of these studies explored how combining organic management with no-tillage affected total and biologically active SOC dynamics.

Organic no-till farming research at the Rodale FST focused largely on the viability of using roller-crimpers to terminate cover crop mixtures as a means to suppress weeds and enable cash crop yields comparable to tilled conventional and tilled organic systems. A rolled cover-crop stand must contain at least 8000 kg ha^{-1} biomass to adequately suppress weeds. Rye and hairy vetch monocultures produce roughly 6000 kg ha^{-1} biomass, bi-cultures of these crops are more likely to produce the required biomass for weed suppression (Mirsky *et al.,* 2012). It was also reported in the Reduced-tillage Organic Systems Experiment (ROSE), an inter-disciplinary, multi-institutional effort between 2010 and 2013 at Pennsylvania State University, the USDA Agricultural Research Service (ARS), Sustainable Agricultural Systems Laboratory at Beltsville, Maryland, and the University of Delaware that different crops respond differently to organic-no-till management (Wallace *et al.,* 2017). Average soybean yields from organic no-till plots were not significantly different from tilled organic plots, but organic no-till corn yields remained significantly lower than tilled organic plots after a 3-year transition period (Wallace *et al.,* 2017). Teasdale and Coffman (2007) studied how tilled and no-tilled organic management affects SOC accumulation in Beltsville, Maryland, a region with a climate and topography similar to the Rodale FST study site and reported that chisel-plowed organic corn and soybean rotations amended with dairy manure and cover crops exhibited significantly higher total SOC accumulation than no-till cover cropped systems utilizing rye/vetch mixtures terminated using herbicides. This study, however lacked side-by-side comparisons of tilled conventional systems and no-till organic systems. Therefore, knowledge of the response of SOC to long-term conventional and organic farming management in tandem with different tillage regimes is essential to gain a holistic understanding of how the interaction of cropping system management and tillage differences affects SOC accumulation and distribution.

Prior Rodale FST studies (e.g. Wander et al. 1994; Marriott and Wander 2006) considered particulate SOC as an indicator for biologically active SOC. Many other studies also relied on particulate SOC (e.g., Culman *et al.,* 2012) and soil basal respiration (e.g., McNally *et al.,* 2018) for measuring biologically active SOC concentrations. However, particulate SOC is not entirely active as it also contains compounds that are not easily decomposable by microbes including hemicellulose, tannins, and lignin in addition to highly bioavailable compounds such as sugars and proteins (Dungait *et al.,* 2012). While soil basal respiration represents direct measurement of active SOC concentrations, both respiration and particulate SOC measurements are extremely labor-intensive and time-consuming compared to other measures of active SOC including POXC, MBC, and WEOC.

2.7. Objectives and hypotheses

The overall goal of this study is to improve our understanding of how the interactive effects of tillage and cropping system management influences SOC dynamics and soil aggregation. The specific objectives of this study are to determine how different organic and conventional grain cropping systems under tilled and no-till management affect 1) total and active SOC accumulation, and 2) aggregate stability and associated C protection. The hypotheses are 1) no-till organic-manure cropping systems will maximize total, biologically-active and aggregate-protected SOC accumulation by maximizing biomass inputs while minimizing soil disturbance compared to tilled organic-manure systems and 2) no-till conventional and organic-legume treatments will exhibit enhanced total, active, and aggregate-protected SOC accumulation, and aggregate stability compared to their tilled counterparts.

2.8. Rationale

There is evidence that organic management utilizing manures or composts promotes SOC accumulation (e.g. Nicoloso *et al.,* 2018). However, there are concerns in operating and sustaining these systems due to the limited availability and high transportation costs of manures and composts. Additionally, manures and composts have higher decomposition rates and may increase greenhouse gas emissions (e.g. Oertel *et al.,* 2016; Lorenz and Lal, 2016). As an alternative, incorporation of legume cover crops was shown to be a promising practice for sustaining organic systems with enhanced SOC sequestration (e.g. Wander *et al.,* 1994). On the other hand, practices which minimize soil disturbance including reduced- or no-tillage can enhance both total SOC concentration and microbial activity (e.g. Nunes *et al.,* 2018) Nonetheless, what is not done in the past is a side-by-side evaluation of SOC dynamics in response to long term conventional or organic management utilizing manure and/or legume incorporation under both intensive- and no-tillage regimes.

To understand how conventional and organic cropping systems and different tillage regimes affect SOC formation and preservation, this study was conducted to analyze SOC dynamics and aggregation at different soil depths under various management regimes by leveraging the Rodale FST. In addition to total SOC, this study investigated active SOC fractions, including MBC, POXC, and WEOC, which respond faster than total SOC to changes in soil management.

CHAPTER III: MATERIALS AND METHODS

3.1. Field experiment

This study was conducted at the Rodale Institute' Farming System's Trial in southeastern Pennsylvania (40°31′ N, 75°47′ W), based on a long-term experiment of conventional and organic grain cropping systems initiated in 1981. The soil type is Comly silt loam (fine-loamy, mixed, active, mesic Oxyaquic Fragiudalfs). Three cropping system treatments, 8 replicates each, were included: 1) conventional 3-year rotation systems amended with urea ammonium nitrate (conventional), 2) organic 4-year rotation systems incorporated with legume cover crops (organic-legume), and 3) organic 8-year rotation systems incorporated with a combination of legume cover crops and composted dairy manure (organic-manure). The rotation sequences, biomass inputs, and nutrient sources for each cropping system are shown in Table 1.

All plots were conventionally tilled with annual moldboard plowing and field harrowing since the cropping rotational systems were initiated in 1981. In 2008, four plots from each cropping system were placed under no-till management. The no-till conventional plots were completely exclusive from tillage, while no-till organic plots were moldboard-plowed every other year to loosen the soil. Weeds are managed with herbicides in conventional treatments, moldboard and harrow tillage in tilled organic treatments, and rolling and crimping cover crop mulches in no-till organic treatments. The experimental design is a randomized complete block design with split-plots arrangement of treatments. Main-plots are two tillage regimes (tilled and no-tilled) and sub-plots are three cropping systems (conventional, organic legume, and organic-manure). Overall, there are four field replications for each combination of tillage regime and cropping system.

Table 1: Cropping systems in the Rodale Institute Farming Systems Trial

Cropping system	Cropping sequence	Biomass inputs	N inputs
Conventional	corn/rye[*] ➔ corn/rye[*] ➔ soybean/rye[*ǂ]	8,200-10,800 kg ha^{-1} yr^{-1} from cover crops (Mirsky *et al.,* 2012)	146 kg ha^{-1} from mineral fertilizers (mostly urea ammonium nitrate) (Pimental and Burgess 2014)
Organic-legume	wheat/vetch[*] ➔ corn/rye[*] ➔ oats/clover[*]/rye[*] ➔ soybean/wheat	8,200-10,800 kg ha^{-1} yr^{-1} from cover crops (Mirsky *et al.,* 2012)	176 kg ha^{-1} from hairy vetch, 102 kg ha^{-1} from red clover, 140 kg ha^{-1} from mixed legume cover (Pimental and Burgess 2014)
Organic-manure	wheat/hay[§] -➔hay[§] ➔ hay[§] ➔ silage[¶]/wheat ➔ wheat/vetch[*] ➔ corn[¶]/rye[*] ➔ oat/rye[*] ➔ soybean/wheat	8,200-10,800 kg ha^{-1} yr^{-1} from cover crops; 18,000 kg ha^{-1} manure before corn grain and 27,000 kg ha^{-1} manure before silage (Mirsky *et al.,* 2012)	169-188 kg ha^{-1} from manure, 39 kg ha^{-1} from hay plow-down, 176 kg ha^{-1} from hairy vetch (Pimental and Burgess 2014)

Notes:

[*] cover crop

[§] mixture of orchardgrass and alfalfa

[¶] composted cattle manure applied before this rotation

[ǂ] wheat instead of rye was planted after the soybean crop in 2018

3.2. Soil sampling

The soils were sampled in May 2018. Since the crop planted at sampling time may influence SOC, especially active SOC measurements, we selected the plots planted with the same type of cash crops (soybean/ wheat) to minimize variation. Then soil cores were collected from 0 to 30 cm at each treatment plot. Each soil was separated into three samples by depth (0-10 cm, 10-20 cm and 20-30 cm) and all samples from each plot at the same depth were combined and homogenized (n = 72). Fresh soil samples were sieved through 8 mm mesh and air-dried after setting aside 50 g of fresh sieved sample for gravimetric soil moisture and microbial biomass C measurements. Air-dried soil was sieved through 2 mm mesh for total and biologically active SOC measurements except for the 250 g from each sample that is set aside for aggregate analysis.

3.3. Gravimetric soil moisture measurement

Gravimetric soil moisture was determined by weighing out roughly 10 g of field-moist soil from each sample, drying at 105 ^{o}C in a Thermo Scientific HERATHERM oven for two days, then dividing the mass of soil moisture by the mass of dry soil. The gravimetric soil moisture data was used to calculate microbial biomass C concentrations in dry weight basis.

3.4. Total SOC

Total SOC concentrations were measured on pulverized air-dried samples following the dry combustion method using an Elementar vario TOC cube (Elementar Americas Inc., Ronkonkoma, NY, USA). Air-dried soil samples were ground using a mortar and pestle to a powder-like consistency prior to analysis.

3.5. Microbial biomass C

Microbial biomass C concentrations were measured using the chloroform fumigation and extraction method modified from Vance *et al*. (1987). Two 10 g field-moist sub-samples of each sample were used; one was extracted immediately with 45 ml 0.5M potassium sulfate (K_2SO_4) and the other fumigated for 48 hours with chloroform ($CHCl_3$) then extracted with 45 ml 0.5M K_2SO_4 after $CHCl_3$ was evacuated from these samples. The extracts were filtered through Whatman #2 filter papers, and then analyzed on an Elementar vario TOC cube (Elementar Americas Inc., Ronkonkoma, NY, USA). Microbial biomass C was calculated as the difference between the total carbon concentrations of the fumigated and unfumigated extracts. The estimated extraction efficiency factor of 0.45 (Beck *et al.,* 1997) was used.

3.6. Permanganate-oxidizable C

Permanganate-oxidizable C concentrations were measured using the method developed by Weil *et al*. (2003). Each air-dried soil sample (2.5 g) was taken in 50 mL polypropylene tubes. Then 18 mL of milliQ water and 2 mL of 0.2 M potassium permanganate (K_2MnO_4) solution were added to each tube and the tubes were shaken for exactly 2 minutes at 240 oscillations/minute on an oscillating shaker, then left to incubate at room temperature in the dark for exactly 10 minutes, as the reaction was time sensitive. After 10 minutes, 0.5 mL of the supernatant from each sample were transferred to new 50 mL centrifuge tubes containing 49.5 mL milliQ water. An aliquot (300µL) of each sample was transferred to a 96-well microplate containing a milliQ water blank and seven standard stock solutions: 0.002 M, 0.004 M, 0.008 M, 0.011 M, 0.015 M, 0.017 M, and 0.02 M K_2MnO_4. The absorbance of the samples was read at

550 nm using a BioTek Synergy H1 microplate reader. Permanganate-oxidizable C concentrations were calculated with the equation:

$$Mg\ POXC/kg\ dry\ soil = [0.02\ mol/L-(a + b \times absorbance)] \times (9000\ mg\ C/mol) \times (0.02\ L\ solution / 0.0025\ kg\ soil)$$

where 0.02 mol/L represents the initial concentration of K_2MnO_4 solution , *a* is the slope and *b* is the intercept of the standard solution curve, 9000 mg C/mol is the amount of C oxidized from 1 mol of $KMnO_4$ when Mn^{7+} is reduced to Mn^{4+}, and *0.02L* is the volume of K_2MnO_4 solution reacted.

3.7. Water-extractable organic C

Water-extractable organic C concentrations were measured by adding 25 mL of milli-Q water to 5 g air-dried soil, shaking for 10 minutes at 200 revolutions/min, centrifuging for 5 minutes at 3500 rpm, and then filtering the supernatants. The supernatants were analyzed by combustion analysis using the Elementar vario TOC cube.

3.8. Dry aggregate fractionation

One hundred grams of air-dried 8 mm-sieved samples were placed on a stack of sieves with openings of 2 mm, 1 mm, 0.25 mm, and 0.053 mm and sieved for 5 minutes on a vertical sieving apparatus (Model 18480, CSC Scientific, Fairfax, VA, USA). Aggregates collected on each sieve were weighed and compared to the total sample mass to determine aggregate size distribution. Mean weight diameter (MWD) was calculated with the following equation (Youker and McGuiness, 1957):

$$MWD = \sum_{i=0}^{n} \bar{x}_i w_i$$

where x_i is the mean diameter (mm) of the size class, and w_i is the soil mass fraction remaining on the sieve (g g^{-1}).

3.9. Wet aggregate stability

Wet aggregate stability (WAS) was determined using a commercial wet sieving apparatus (Eijkelkamp Agrisearch Equipment, Giesbeck, The Netherlands). Four grams of air-dried 1-2 mm sized aggregates were placed on 0.25 mm mesh sieves and immersed in distilled water for 20 minutes. The sieves were placed within the apparatus and raised and lowered in distilled water at 35 oscillations/minute for five minutes to remove the unstable aggregate fraction. This is modified from the standard wet aggregate sieving method from Kemper and Rosenau (1986) in which the aggregates are wetted from the bottom through capillary rewetting for 10 minutes then sieved for 3 minutes. As the Rodale FST samples all exhibited high WAS, the new method was used to ensure greater variation between the treatments. The stable fraction represented by the material remaining on the sieve was oven-dried and weighed. The stable fraction was washed under running tap water through the 0.25 mm sieve with the remaining aggregates broken up using a rubber policeman until only sand remains and the sand was oven-dried and weighed to correct for sand content within the aggregates. The water-stable aggregate fraction was calculated as:

WAS% = (soil remaining on sieve-sand)/(total sample-sand) X 100

3.10. Aggregate-protected C

Total SOC concentration of each aggregate size class for each sample was measured by grinding the aggregate fraction obtained in the dry aggregate size fractionation step by to a powdery consistency, then measuring their SOC concentrations by dry combustion analysis using an Elementar vario MAX TOC cube (Elementar Americas Inc., Ronkonkoma, NY, USA). The aggregate-protected SOC concentrations of each aggregate size class and the weight percentage of each aggregate size class were used to calculate the proportion of SOC within each aggregate size class per unit soil mass.

3.11. Statistical analysis

Statistical analysis was conducted based on the Proc GLIMMIX procedure in SAS (SAS v9.4, Cary, NC) using the DandA.sas macro (Saxton 2013). Least square means were separated using Fisher's protected least significant differences (LSD) test at $P < 0.05$ significance level. To test the differences in total SOC, MBC, WEOC, POXC, and wet aggregate stability between treatments, tillage regimes, cropping systems, depths, and interactions between the three factors were considered as fixed effects, and replication and its interaction with tillage regimes and cropping systems were considered as random effects. For analyzing aggregate size distribution and aggregate-protected SOC, two-way ANOVA analysis of tillage regime and cropping system was conducted for each aggregate size at individual depths separately. Pearson correlation tests between total SOC, biologically-active SOC, aggregate-protected SOC, and wet aggregate stability were conducted using the PROC CORR procedure in SAS (SAS v9.4, Cary, NC).

CHAPTER IV: RESULTS

4.1. Total soil organic C

Total soil organic C concentrations varied significantly by cropping systems ($P < 0.05$) and soil depth ($P < 0.05$) but was unaffected by tillage and interactions between cropping system, tillage, and depth. The SOC concentrations for organic-legume (20.4 g C kg^{-1} soil) and organic-manure (23.2 g C kg^{-1} soil) systems were 31% and 48% higher than conventional plots (15.7 g C kg^{-1} soil) respectively (Fig. 1) . Additionally, total SOC concentrations at 0-10 and 10-20 cm depth (~22-23 g C kg^{-1} soil) were 35-36% higher than in the 20-30 cm layer (Fig. 1).

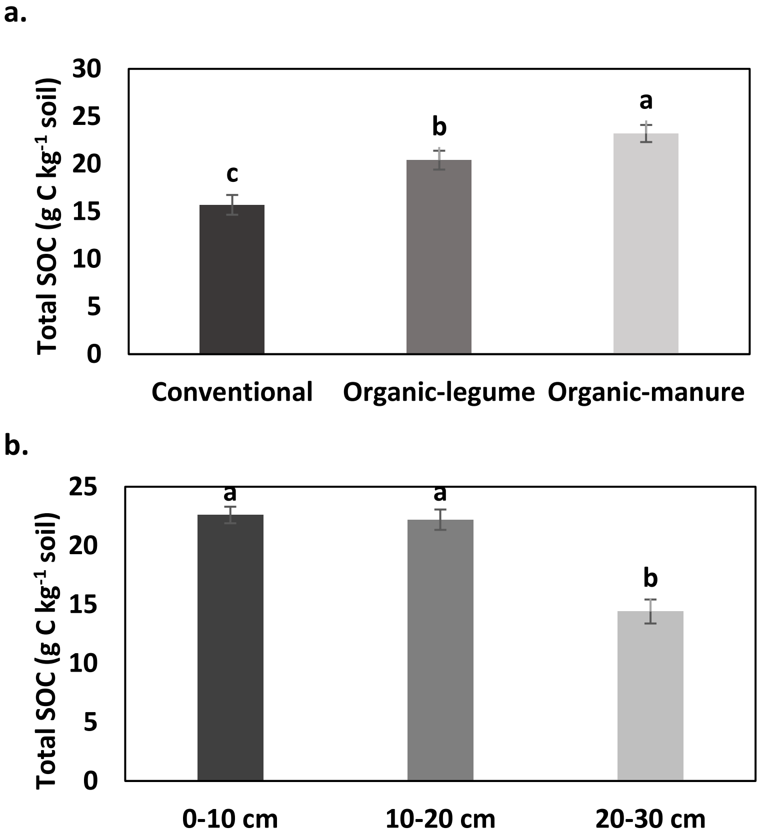

Fig. 1. Total SOC concentrations as affected by cropping systems (a) and soil depth (b). Different letters represent statistically different means at P < 0.05.

4.2. Microbial biomass C

Microbial biomass C concentrations were affected by the interactive effects of cropping system and depth (*P < 0.05*) (Fig. 2). At 0-10 cm, organic-manure plots had a higher MBC concentration (278 mg C kg^{-1} soil) than organic-legume plots (203 mg C kg^{-1} soil) while the MBC concentrations of organic-legume plots were not significantly different from that of conventionally-managed plots (226 mg C kg^{-1} soil) (Fig. 2). At 10-20 cm depth, MBC from organic-legume (227 mg C kg^{-1} soil) and organic-manure plots (272 mg C kg^{-1} soil) were not significantly different from one another and it was 97-132% higher than that under conventional management (115 mg C kg^{-1} soil). At 20-30 cm depth, MBC was the highest in organic-manure plots (172 mg C kg^{-1} soil), followed by organic-legume plots (112 mg C kg^{-1} soil), and the lowest in conventionally managed plots (36 mg C kg^{-1} soil). The MBC from conventional plots decreased from the top to the lowest soil layers. The MBC under both organic-manure and organic-legume management, however, were not significantly different between the top two layers, and only decreased at 20- 30 cm deep.

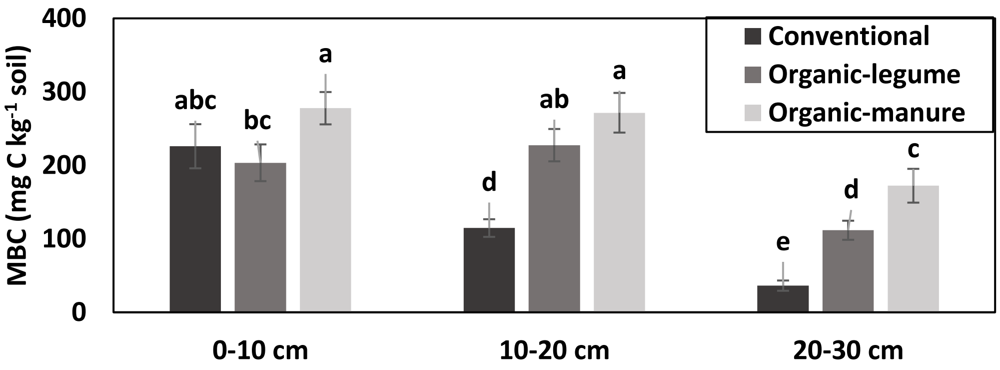

Fig. 2. Microbial biomass carbon concentrations as affected by cropping systems and soil depth. Different letters represent statistically different means at P < 0.05.

4.3. Permanganate-oxidizable C

Permanganate-oxidizable C concentrations were significantly affected by cropping system management and soil depth (*$P < 0.05$*) (Fig. 3) while the effects of tillage and its interaction with cropping system management or depth were not significant. Overall, organic-manure treatments had the highest POXC concentrations (~675 mg C kg^{-1} soil), which were 16-29% higher than that from conventional (~528 mg C kg^{-1} soil) and organic-legume plots (~581 mg C kg^{-1} soil), which were not significantly different from each other. Additionally, POXC concentrations in the 0-10 and 10-20 cm layers were not significantly different and were 28-37% higher than that at 20-30 cm depth. Additionally, the POXC concentrations were weakly but significantly correlated with total SOC (*$P = 0.0018$*, R = 0.36) and MBC (*$P = 0.0094$*, R = 0.30) and strongly correlated with WEOC (*$P < 0.0001$*, R = 0.48) (Fig. 5).

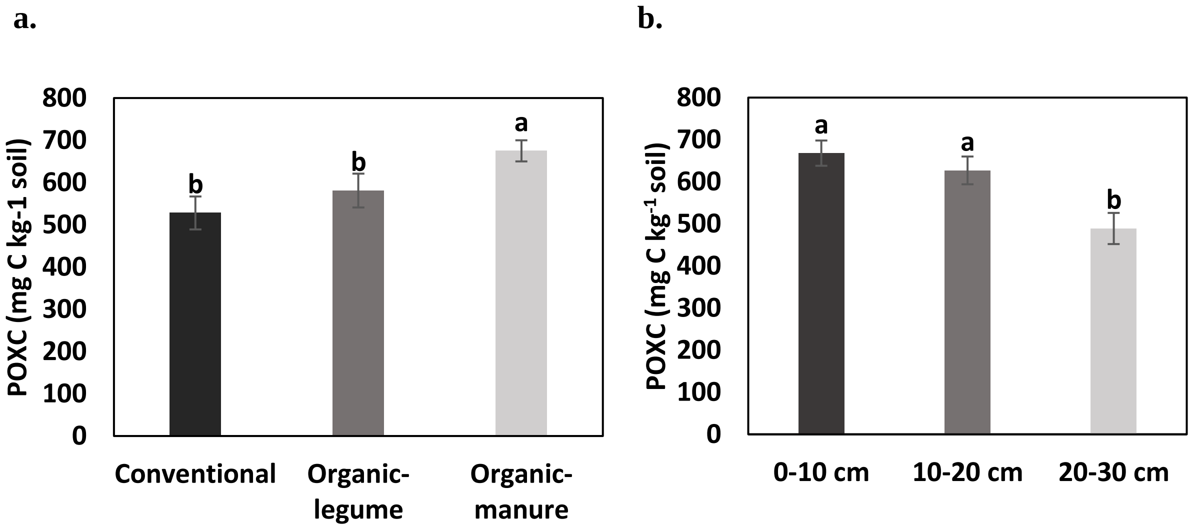

Fig. 3. Permanganate-oxidizable carbon concentrations as affected by cropping systems (a) and soil depth (b). Different letters represent statistically different means at P <0.05.

4.4. Water-extractable organic C

Similar to other SOC fractions, WEOC concentrations were only affected by cropping systems ($P < 0.05$) and soil depth ($P < 0.05$) (Fig. 4). Consistently with POXC, the conventional and organic-legume plots had roughly equal WEOC concentrations of 89-90 mg C kg^{-1} soil, which was lower than that of organic-manure plots (124 mg C kg^{-1} soil) (Fig. 4). Water-extractable organic C concentrations are depth-stratified as the top 10 cm of the soil profile had ~25% higher WEOC concentrations than at 10-20 cm depth, which in turn had ~45% higher WEOC concentrations than at 20-30 cm depth. Unlike POXC, WEOC concentration was strongly correlated to MBC ($P < 0.0001$, R = 0.73) and total SOC ($P < 0.0001$, R = 0.67) (Fig. 5).

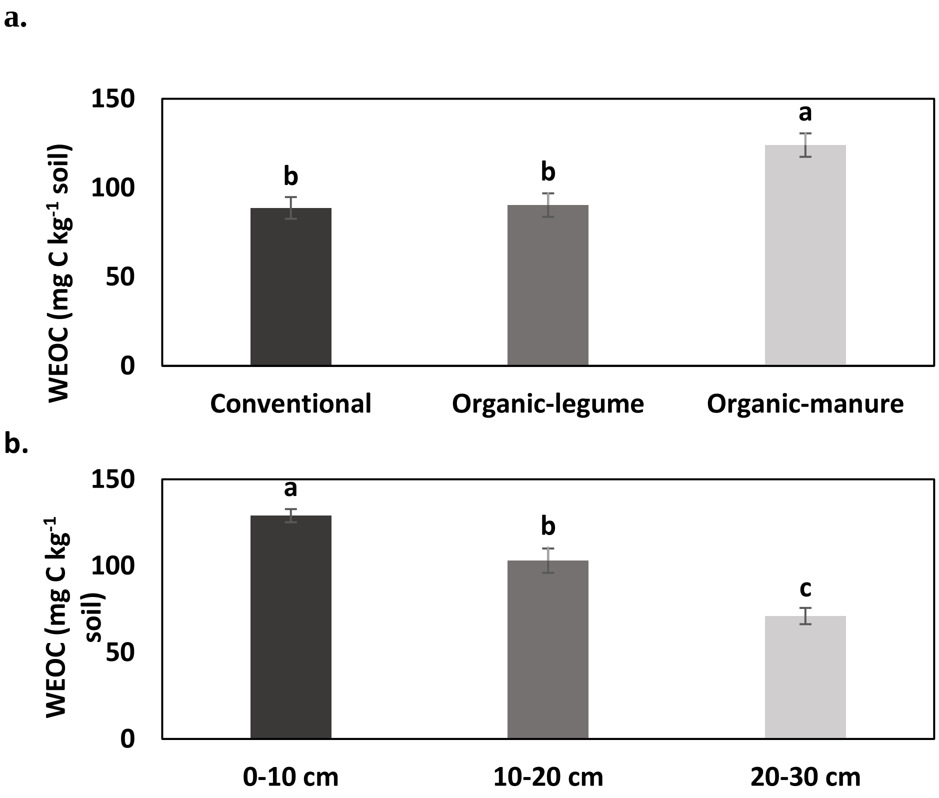

Fig. 4. WEOC concentrations as affected by cropping systems (a) and soil depth (b). Different letters represent statistically different means at P<0.05.

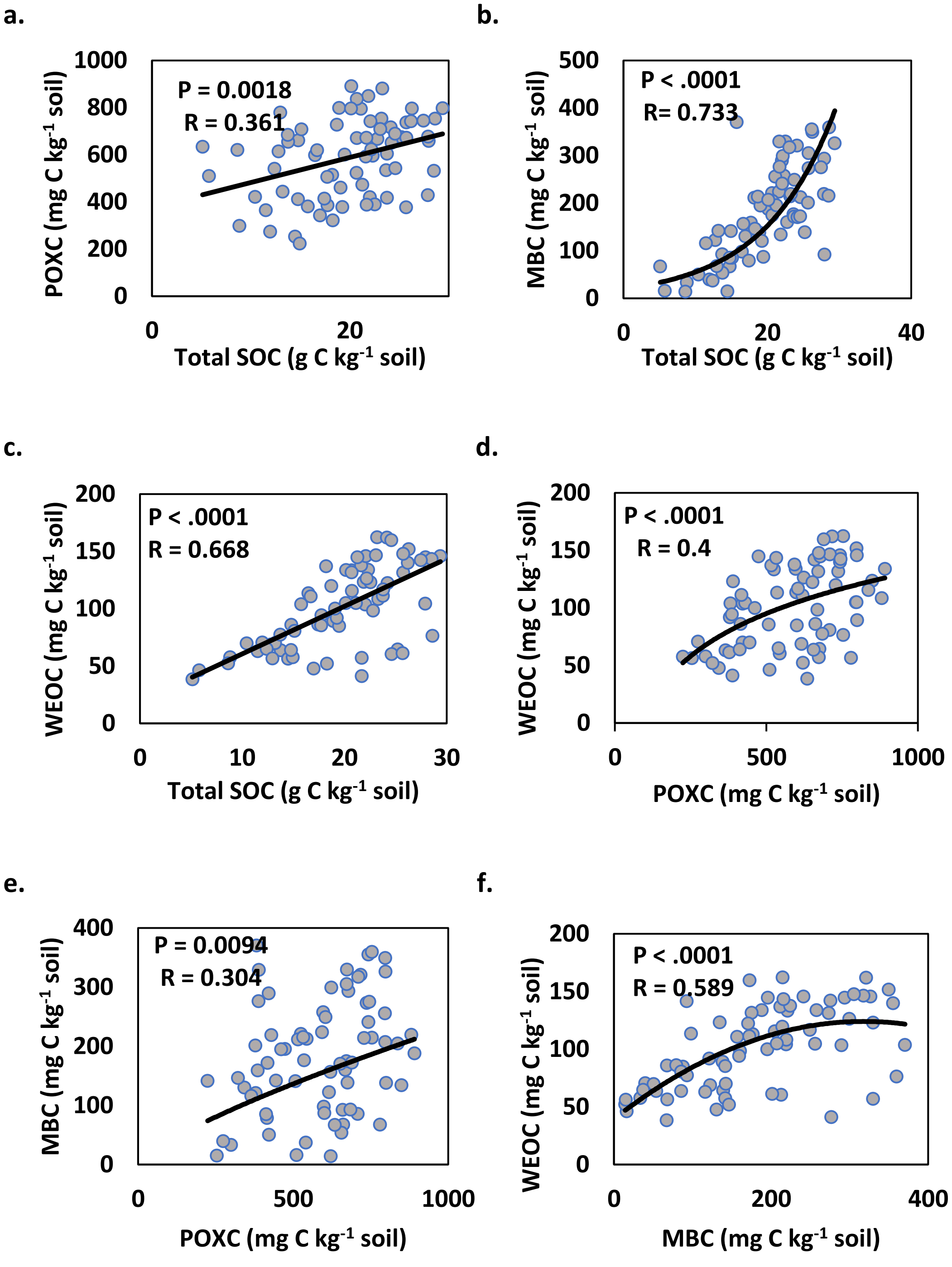

Fig. 5. Relationship between total SOC and POXC (a), total SOC and MBC (b), total SOC and WEOC (c), POXC and WEOC (d), POXC and MBC (e), and MBC and WEOC (f).

4.5. Aggregate size distribution

Dry aggregate mean weight diameter (MWD) was not influenced by tillage, cropping system, or their interactions at all three soil depths (*$P > 0.05$*). Averaged by depth, the MWD for all treatments were 2.2 mm, 2.3 mm, and 2.5 mm for 0-10 cm, 10-20 cm, and 20-30 cm, respectively. However, the relative aggregate size proportions were significantly affected by tillage regime at 0-10 cm and 20-30 cm depths. No-till management increased the proportion of 1-2 mm sized aggregates by 32% and 14% at 0-10 cm and 20-30 cm depths respectively, and it decreased the proportion of 0.25-1.0 mm sized aggregates by 25% and 23% (Table 3). At 10-20 and 20-30 cm depths organic (both organic-legume and organic-manure) management increased the proportion of 1-2 mm aggregates by 20-30% and decreased the proportion of the smaller aggregates by 16-63% compared to conventional management. Neither cropping systems nor tillage regime affected the proportion of 2-8 mm- sized macroaggregates at any depth.

Table 2: Mean weight diameter (cm) under each tillage regime and cropping system. Numbers in parentheses are (±) standard error of the mean.

	Tilled			No-till		
Depth	**Conventional**	**Organic-legume**	**Organic-manure**	**Conventional**	**Organic-legume**	**Organic-manure**
0-10 cm	2.15 (0.070) ns	2.15 (0.070) ns	2.15 (0.070) ns	2.27 (0.070) ns	2.15 (0.070) ns	2.23 (0.070) ns
10-20cm	2.17 (0.085) ns	2.27 (0.085) ns	2.27 (0.085) ns	2.38 (0.085) ns	2.34 (0.085) ns	2.39 (0.085) ns
20-30cm	2.34 (0.11) ns	2.53 (0.11) ns	2.42 (0.11) ns	2.53 (0.11) ns	2.51 (0.11) ns	2.49 (0.11) ns

Table 3: Aggregate size proportion (%) influenced by tillage regime and cropping system. Numbers in parentheses are (±) standard errors of the means. Different lower-case letters within each depth indicate statistical significance across cropping systems at P < 0.05, different upper-case letters within each depth indicate statistical significance between tillage regimes at P < 0.05, and ns means the difference between treatments is not significant (P > 0.05).

Aggregate Size (mm)					
Treatment	**2-8**	**1-2**	**0.25-1.0**	**0.053-0.250**	**<0.053**
0-10 cm					
Cropping system					
Conventional	30.1 (1.0) ns	34.2 (1.9) ns	29.7 (2.7) ns	5.05 (0.36) ns	0.99 (0.15) ns
Organic-legume	28.2 (1.4) ns	30.9 (2.1) ns	35.9 (2.4) ns	4.02 (0.31) ns	0.84 (0.21) ns
Organic-manure	29.2 (0.66) ns	33.2 (2.4) ns	32.0 (2.8) ns	4.94 (0.29) ns	0.83 (0.27) ns
Tillage regime					
Tilled	28.9 (0.75) ns	27.4 (1.3) B	38.4 (2.0) A	4.22 (0.19) B	1.12 (0.18) ns
No-till	29.4 (0.80) ns	38.2 (0.79) A	26.6 (1.3) B	5.12 (0.10) A	0.65 (0.10) ns
10-20 cm					
Cropping system					
Conventional	32.5 (1.8) ns	30.2 (1.1) b	30.4 (2.3) a	5.92 (0.58) a	1.07 (0.14) a
Organic-legume	31.4 (0.92) ns	41.5 (2.2) a	23.4 (2.3) b	3.39 (0.35) b	0.35 (0.10) b
Organic-manure	32.5 (0.95) ns	39.4 (1.4) a	23.8 (2.1) b	3.92 (0.50) b	0.50 (0.12) b
Tillage regime					
Tilled	31.1 (1.0) ns	37.2 (2.1) ns	27.1 (2.1) ns	3.98 (0.45) ns	0.57 (0.13) ns
No-till	33.1 (0.87) ns	36.8 (1.2) ns	24.6 (1.2) ns	4.84 (0.51) ns	0.70 (0.13) ns
20-30 cm					
Cropping system					
Conventional	35.7 (2.2) ns	31.7 (2.7) b	26.4 (2.7) a	5.30 (0.40) a	1.04 (0.14) a
Organic-legume	36.1 (1.7) ns	27.9 (1.6) b	21.3 (1.6) b	3.94 (0.58) b	0.57 (0.13) b
Organic-manure	34.1 (0.69) ns	40.3 (2.2) a	21.8 (2.0) b	3.22 (0.65) b	0.48 (0.11) b
Tillage regime					
Tilled	34.9 (1.4) ns	34.3 (1.9) B	26.1 (2.0) A	4.07 (0.41) ns	0.73 (0.13) ns
No-till	35.8 (1.3) ns	39.1 (1.5) A	20.2 (0.98) B	4.24 (0.59) ns	0.66 (0.12) ns

4.6. Aggregate-protected SOC per kg aggregates

In general, organic-manure cropping systems exhibited the highest aggregate-protected SOC concentrations regardless of aggregate size class, which ranged from 19.2-29.2 g C kg^{-1} aggregates at 0- 10 cm, 20.5-29.5 g C kg^{-1} aggregates at 10-20 cm, and 15.2-25.8 g C kg^{-1} aggregates at 20-30 cm (Fig. 8). Organic-legume cropping systems also increased the aggregate-protected SOC concentrations (17.2-25.4 g C kg^{-1} aggregates, 17.6-24.0 g C kg^{-1} aggregates, and 10.2-21.9 g C kg^{-1} aggregates for 0-10, 10-20, and 20-30 cm layers, respectively) compared to conventional plots (17.2-23.4 g C kg^{-1} aggregates, 14.2-19.7 g C kg^{-1} aggregates, and 4.82-21.6 g C kg^{-1} aggregates for 0-10, 10-20, and 20-30 cm layers respectively), but the differences were significant only at 10-20 and 20-30 cm depths. Across all treatments and depths, aggregate-protected SOC concentration was generally the highest in 0.053-0.250 mm aggregates (23.4-29.2 g C kg^{-1} aggregates, 19.7-29.5 g C kg^{-1} aggregates, and 17.4-25.8 g C kg^{-1} aggregates for 0-10, 10-20, and 20-30 cm layers, respectively), and the lowest in 2-8 mm aggregates (17.2-19.2 g C kg^{-1} aggregates, 14.2-20.5 g C kg^{-1} aggregates, and 4.8-15.2 g C kg^{-1} aggregates for 0-10, 10-20, and 20-30 cm layers, respectively). The tillage effect was only detected in 2-8 mm aggregates at 20-30 cm, which was higher under conventional tillage (11.8 g C kg^{-1} aggregates) compared to no-till (8.4 g C kg^{-1} aggregates) management.

Table 4: Aggregate-protected SOC concentration (g C kg^{-1} aggregate basis) influenced by tillage regime and cropping system. Numbers in parentheses are (±) standard errors of the means. Different lower-case letters within each depth indicate statistical significance across cropping systems at $P < 0.05$, different upper-case letters within each depth indicate statistical significance between tillage regimes at $P < 0.05$, and ns means the difference between treatments is not significant ($P > 0.05$).

Aggregate size class (mm)					
Treatment	**2-8**	**1-2**	**0.25-1.0**	**0.053-0.250**	**<0.053**
0-10 cm					
Cropping system					
Conventional	17.2 (1.3) ns	18.5 (1.0) b	20.6 (1.3) b	23.4 (1.4) b	20.7 (1.6) b
Organic- legume	17.4 (0.78) ns	18.8 (0.66) b	22.2 (0.89) ab	25.4 (1.5) ab	22.4 (1.3) ab
Organic- manure	19.7 (1.1) ns	21.3 (0.34) a	24.6 (0.78) a	29.2 (0.81) a	24.5 (1.8) a
Tillage regime					
Tilled	19.1 (0.92) ns	20.1 (0.70) ns	22.6 (1.1) ns	26.8 (1.1) ns	22.9 (0.88) ns
No-till	17.1 (0.80) ns	19.0 (0.65) ns	22.3 (0.75) ns	25.2 (1.4) ns	22.2 (1.7) ns
10-20 cm					
Cropping system					
Conventional	14.2 (1.3) c	15.6 (1.3) b	15.4 (1.6) c	19.7 (1.6) c	18.7 (1.6) b
Organic legume	17.6 (0.63) b	19.6 (0.72) a	19.4 (1.7) b	24.0 (1.3) b	22.2 (1.3) a
Organic manure	20.5 (0.80) a	21.7 (0.75) a	23.1 (1.2) a	29.5 (0.73) a	23.4 (1.8) a
Tillage regime					
Tilled	18.3 (1.8) ns	19.8 (1.1) ns	18.3 (1.6) ns	25.4 (1.6) ns	21.9 (0.96) ns
No-till	16.5 (1.4) ns	18.1 (1.1) ns	20.3 (1.3) ns	23.4 (1.5) ns	21.0 (1.6) ns
20-30 cm					
Cropping system					
Conventional	4.82 (0.74) c	7.06 (0.74) c	21.6 (3.2) ns	17.4 (2.9) b	14.8 (1.6) b
Organic legume	10.2 (0.86) b	12.7 (0.86) b	18.7 (1.5) ns	21.9 (1.5) ab	15.8 (1.4) ab
Organic manure	15.2 (1.2) a	17.0 (1.2) a	21.1 (0.92) ns	25.8 (1.9) a	19.2 (1.4) a
Tillage regime					
Tilled	11.8 (1.6) A	13.1 (1.9) ns	22.5 (1.9) ns	21.6 (1.7) ns	16.8 (1.1) ns
No-till	8.39 (1.3) B	11.4 (1.2) ns	18.5 (1.2) ns	21.8 (2.3) ns	16.4 (1.1) ns

4.7. Aggregate-protected SOC per kg soil

Aggregate-protected SOC content (g C kg^{-1} soil) was largely affected by cropping system management and to a lesser extent by the tillage treatments (Table 5). In general, among three cropping systems, organic-manure management had the highest aggregate-protected SOC content in 2-8 mm (5.61 g C kg^{-1} soil), 1-2 mm (7.33 g C kg^{-1} soil) and 0.25-1 mm (7.61 g C kg^{-1} soil) aggregates at 0-10 cm depth. The same trend was also followed at 10-20 cm (6.53 g C kg^{-1} soil, 8.21 g C kg^{-1} soil, and 6.00 g C kg^{-1} soil for 2-8 mm, 1-2mm, and 0.25-1 mm aggregates, respectively) and 20-30 cm depths (4.63 g C kg^{-1} soil, 6.78 g C kg^{-1} soil, and 4.59 g C kg^{-1} soil for 2-8 mm, 1-2 mm, and 0.25-1 mm aggregates, respectively). Organic-legume management had similar aggregate-protected SOC content in all aggregate sizes at 0-10 cm compared to conventional management, but also increased the SOC content in 2-8 mm and 1-2 mm aggregates at 10-20 and 20-30 cm depths. Conventional managed systems, on the other hand, had the highest SOC content in the <0.053 mm size among three treatments at 10-20 and 20-30 cm.

Table 5: Aggregate-protected SOC concentration (g C kg^{-1} soil basis) influenced by tillage regime and cropping system. Numbers in parentheses are (±) standard errors of the means. Different lower-case letters within each depth indicate statistical significance across cropping systems at $P < 0.05$, different upper-case letters within each depth indicate statistical significance between tillage regimes at $P < 0.05$, and ns means the difference between treatments is not significant ($P > 0.05$).

Aggregate size class (mm)					
Treatment factors	**2-8 mm**	**1-2 mm**	**0.25-1.0 mm**	**0.053-0.250 mm**	**<0.053 mm**
0-10 cm					
Cropping system					
Conventional	5.20 (0.41) ns	6.25 (0.39) ab	5.82 (0.81) ns	1.17 (0.08) b	0.20 (0.03) ns
Organic-legume	5.08 (0.35) ns	6.08 (0.48) b	7.51 (0.61) ns	1.05 (0.06) b	0.18 (0.05) ns
Organic-manure	5.61 (0.29) ns	7.33 (0.44) a	7.61 (0.92) ns	1.46 (0.08) a	0.24 (0.06) ns
Tillage regime					
Tilled	5.66 (0.23) ns	5.84 (0.30) B	8.10 (0.66) A	1.18 (0.09) ns	0.28 (0.03) ns
No-till	5.06 (0.32) ns	7.27 (0.34) A	5.87 (0.39) B	1.28 (0.06) ns	0.14 (0.02) ns
10-20 cm					
Cropping system					
Conventional	4.53 (0.36) c	4.67 (0.39) b	4.89 (0.66) ns	1.14 (0.11) ns	0.20 (0.02) a
Organic-legume	5.67 (0.40) b	7.78 (0.55) a	4.85 (0.60) ns	0.89 (0.09) ns	0.09 (0.02) b
Organic-manure	6.53 (0.31) a	8.21 (0.32) a	6.00 (0.71) ns	1.20 (0.14) ns	0.13(0.03) b
Tillage regime					
Tilled	5.57 (0.34) ns	6.99 (0.57) ns	5.40 (0.62) ns	1.07 (0.10) ns	0.14 (0.03) ns
No-till	5.58 (0.42) ns	6.79 (0.60) ns	5.09 (0.37) ns	1.09 (0.10) ns	0.14 (0.02) ns
20-30 cm					
Cropping system					
Conventional	2.02 (0.35) b	1.91 (0.18) c	6.04 (1.4) a	0.92 (0.16) ns	0.20 (0.03) a
Organic-legume	3.71 (0.33) a	4.67 (0.45) b	4.13 (0.65) b	0.86 (0.13) ns	0.09 (0.02) b
Organic-manure	4.63 (0.66) a	6.78 (0.46) a	4.59 (0.51) ab	0.78 (0.12) ns	0.13 (0.03) b
Tillage regime					
Tilled	3.73 (0.55) ns	4.53 (0.67) ns	6.18 (0.96) ns	0.86 (0.10) ns	0.12 (0.02) ns
No-till	3.17 (0.42) ns	4.50 (0.64) ns	3.66 (0.22) ns	0.85 (0.12) ns	0.11 (0.02) ns

4.8. Wet aggregate stability

Wet aggregate stability was significantly affected by the interactions between cropping systems, tillage regime, and soil depth ($P < 0.05$) (Fig. 6). Overall, WAS was significantly higher in the top 20 cm than the lower depth. No-till increased WAS by 17% in conventional plots at 0-10 cm and by 15% in organic-legume plots at 20-30 cm. At 0-10 cm, WAS in no-till organic-manure plots (86.5%) was higher than tilled organic-legume plots (75.2%) and no-till conventional plots (74.7%) and was the lowest for tilled conventional plots (63.9%). At 10-20 cm, organic-manure plots had higher WAS (79.6-86.5%) than conventional plots (67-69%), but neither of them was significantly different from that of organic-legume plots. Similar to 0-10 and 10-20 cm depths, the WAS at 20-30 cm was the lowest for the conventional plots.

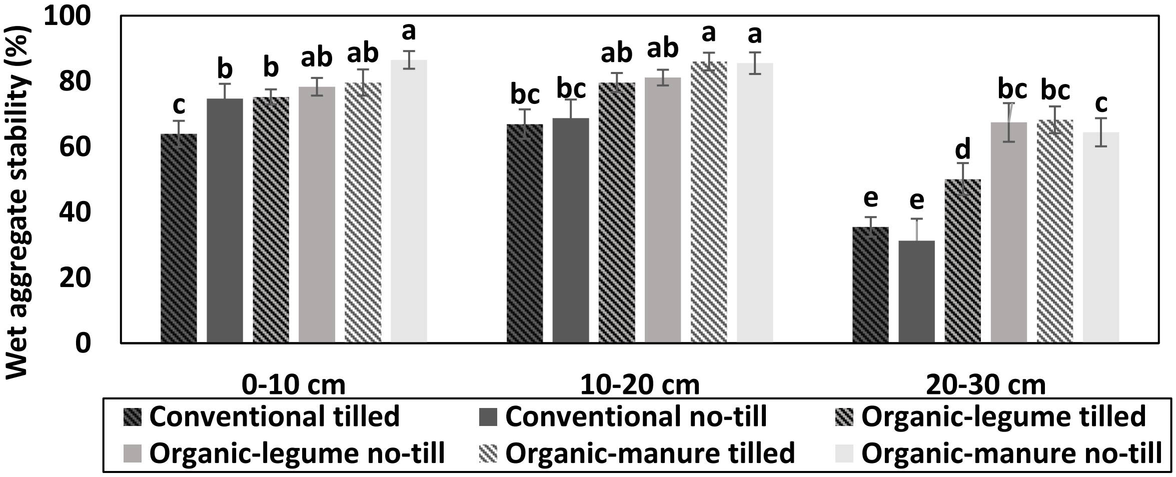

Fig. 6. Wet aggregate stability influenced by tillage regime, cropping system, and soil depth. Different letters represent statistically different means at $P < 0.05$.

CHAPTER V: DISCUSSION

5.1. Total and active SOC

Our finding of increased SOC under organic-manure management was consistent with other studies where similar cropping systems also increased organic C inputs through composted cattle and poultry manure (Pimentel and Burgess 2014; Teasdale and Coffman 2007). Besides directly increasing SOC inputs, manure amendments in organic-manure systems also benefits SOC accumulation by providing greater quantities of decomposed biomass, which is far more readily preserved by mineral adsorption, aggregate occlusion, or humification than plant litter (e.g. Bot 2005; Martins and Angers 2015; Murugan *et al.,* 2019). Organic-manure management in the present study also minimized soil disturbance through the cultivation of mixed perennial orchardgrass/alfalfa forage for four out of every eight years, during which the soil was not disturbed by tillage. Perennial mixed forages and/or hay can also enhance SOC accumulation and protection because of their deep and extensive root system (Christensen, 2001; Franzluebbers, 2005; Franzluebbers *et al.,* 2012; Fan *et al.,* 2016: Poirier *et al.,* 2018). Additionally, high-quality (low C:N ratio) plant C inputs such as alfalfa and oat (Cordova *et al.,* 2018), and legume root nodules (Poirier *et al.,* 2018) are associated with higher SOC concentrations, despite their increased bioavailability, as decomposition by-products are more readily preserved by physical protection within aggregates and/or mineral adsorption than undecomposed or partially decomposed plant litter. In fact, bacterial-derived mucilage together with fungal hyphae are among the most important aggregate, particularly macroaggregate, binders, and aggregate occlusion is one of the most important SOC preservation mechanisms (e.g. Tisdall and Oades 1982; Jastrow *et al.,* 2007; Murugan *et al.,* 2019). Therefore, even though the biomass inputs in conventional and organic-legume cropping systems are roughly similar, the greater bioavailability of legume litter and root residues translates to enhanced production of aggregate-binding organic compounds and with it, increased SOC preservation within aggregates. This is demonstrated by increased macroaggregation (Table 3), aggregate-protected SOC per unit aggregate mass (Table 4), and WAS (Fig. 6) in organic-legume plots compared to conventional plots, especially in the subsoil, where the effects of root bioavailability and transport of microbial-derived SOC from the surface to subsoil are especially important.

Organic-legume plots had lower SOC concentrations than organic-manure plots. This can be explained by the absence of C inputs from manure applications and the lack of perennial alfalfa and orchardgrass in organic-legume cropping systems. Nevertheless, organic-legume plots had higher total SOC concentrations than conventional plots despite almost similar organic C inputs in both management plots. Root nodules from clover and hairy vetch are more bioavailable than the grass roots such as rye root, which favors enhanced SOC preservation via enhanced microbial biomass C concentrations (Fig. 2) and with it, aggregate occlusion and mineral adsorption of decomposition by-products (Poirier *et al.,* 2018; Martins and Angers, 2015). Similarly, increase in soil N is often correlated with increase in SOC accumulation since even though lower C:N ratios enhances crop residue decomposition, it also increases crop productivity (and with it biomass inputs) and formation of organo-mineral complexes, especially if the soil is fertilized with an organic fertilizer such as composted manure (e.g. Maillard *et al.,* 2015; He *et al.,* 2018). Furthermore, a significant proportion of mineral-adsorbed organic compounds contain amine groups, which adsorb strongly to clay minerals (Yan *et al.,* 2012).

Our results also indicate an increase in MBC due to organic-manure and organic-legume management compared to conventional management. The organic manure application can provide the substrates and nutrients for microbial activities which can significantly enhance microbial biomass (e.g. Wander *et al.,* 1994; Min *et al.,* 2003). The incorporation of perennials such as orchardgrass and alfalfa into crop rotations can further increase MBC by enhancing belowground C inputs through deeper roots (Fan *et al.,* 2016) and enhanced root biomass production (Loges *et al.,* 2018), and by promoting the formation of arbuscular mycorrhizal associations (Poirier *et al.,* 2018). Further, legumes such as alfalfa, hairy vetch, or clover can support enhanced microbial growth with easily accessible nutrient sources through nodule shedding and legume root and litter residues, which have lower C:N ratios than that of grasses (e.g. Sievers and Cook 2018; Poirier *et al.,* 2018).

In this study, the increased MBC in both organic cropping systems were detected at lower soil depths, not at 0-10 cm. Different from the conventional plots in which MBC was heavily stratified by soil depth, the MBC under both organic treatments were not significantly different between 0-10 cm and 10-20 cm. These results suggest that the organic management can increase MBC in deeper soil layers. While conventional plots may have sufficient organic matter and nutrients for sustaining high microbial biomass in the top 10 cm, the lack of manure inputs and/or legume nodule shedding can limit microbial growth in deeper layers. Likewise, vetch roots have significantly lower C:N ratios than cereal rye roots (Sievers and Cook 2017) and therefore support more microbial activity than cereal roots. Additionally, organic-manure treatments maximized subsoil MBC concentrations by maximizing total biomass inputs, which is exported to deeper layers through leaching following decomposition, as demonstrated by the fact that organic-manure management maximized WEOC at all depths (Fig. 4). Similar to total SOC, the effect of tillage on MBC was insignificant.

Similar to total SOC and MBC, POXC concentrations were not significantly different between 0-10 cm and 10-20 cm. This may be because the majority of corn, wheat, and cereal rye root biomass are in the top 20 cm rather than just the top 10 cm (Fan *et al.,* 2016; Plumer 2012). Additionally, the SOC fractions which are the most strongly correlated with POXC are fine particulate (0.053-0.250 mm) and heavy fraction (i.e. microaggregate-occluded and/or mineral-adsorbed) SOC (Culman *et al.,* 2012). These SOC fractions correspond closely to root-derived SOC (e.g., Puget and Drinkwater 2001; Jastrow *et al.,* 2007; Poirier *et al.,* 2018).

Our results of enhanced POXC concentrations in organic-manure plots were consistent with previous studies which demonstrated that manure amendments can increase active SOC fractions in soils (e.g., Wander *et al.,* 1994; Min *et al.,* 2003; Larsen *et al.,* 2014). However, unlike the total SOC and MBC, the organic-legume treatment didn't improve POXC concentrations compared to conventional plots. Additionally, the method used for POXC measurements in this study, the one developed by Weil et al. (2003), leaves soil microaggregates intact and therefore, much of the active SOC may have been protected from oxidation by potassium permanganate within soil aggregates (Gruver, 2015). In this study, the correlations between POXC and SOC, and POXC and MBC were weak. This may be because POXC contains a large amount of biochemically recalcitrant aromatic compounds including lignin and phenolic compounds in addition to more active organic compounds (Gruver 2015).

Just as with POXC, organic-legume treatments did not significantly increase WEOC concentrations compared to conventional treatments. This may be because the SOC from cover crops is better protected than SOC from cattle manure, as was reported by Wander et al. (1994). Different from the study by Wander et al. (1994) which found lower WEOC in organic-legume plots than conventional managed plots, the organic-legume treatment has similar WEOC concentrations to conventional treatments. Organic-legume treatments in this study include mixed rye/legume cover crops, which may increase WEOC concentrations compared to pure legume cover cropping due to enhanced cover crop biomass (Mirsky *et al.,* 2012; Pimental and Burgess 2014; Wallace *et al.,* 2017), which in turn provides greater quantities of biomass for decomposers. Additionally, the strong correlation of WEOC with MBC and total SOC is consistent with past studies that demonstrated close links between WEOC, microbial activity, and SOC accumulation (e.g., Wander *et al.,* 1994; McNally *et al.,* 2018; Nunes *et al.,* 2018).

Overall, our results demonstrated that organic-manure cropping systems, which maximized both total and decomposed biomass maximized total SOC along with MBC, POXC, and WEOC concentrations just as hypothesized. However, while it was hypothesized that no-till organic-manure management would maximize total and biologically-active SOC concentrations, this was not the case as there were no significant differences between the total and biologically-active SOC concentrations of tilled and no-till organic-manure treatments. This may be because, the effects of tillage may have been masked by both high biomass inputs and low overall disturbance as both tilled and no-till organic manure plots as three out of eight years had perennial orchardgrass/alfalfa hay rotations during which the plots were not tilled.

Tillage effect on total and active SOC was not detected in any of the three cropping systems. While the different cropping system treatments at the Rodale FST were around for 37 years, tilled and no-till treatments were around for only 10 years, which may not be enough time to result in significant differences in bulk SOC accumulation (Sheehy *et al.,* 2015). Additionally, no-till management, during the initial several years, may reduce crop yields and therefore biomass inputs (Ogle *et al.,* 2012). While tillage may stimulate crop residue decomposition, it may also enhance humification and SOC preservation within the suboxic subsoil while no-till management may reduce humification rates by not incorporating surface residues into the soil (e.g. Martins *et al.,* 2011; Sheehy *et al.,* 2015. This is evidenced by the enhanced macroaggregate-protected SOC concentrations within tilled plots than in no-till plots at 20-30 cm deep (Table 4).

5.2. Aggregates

Neither cropping system nor tillage management affected dry aggregate MWD. Macroaggregates are also susceptible to breakage during sieving (Marquez *et al.,* 2004). Additionally, while tillage disrupts macroaggregates, it promotes the formation of soil clods (>10mm aggregates) while reducing the proportion of smaller macroaggregates in fine-textured soils (Wiesmeyer *et al.,* 2012; Ciric *et al.,* 2012). Therefore, it is important to use other aggregate stability measures, including aggregate size distribution and wet aggregate stability in addition to dry aggregate MWD. Unlike dry aggregate MWD, dry aggregate size distribution, aggregate-protected SOC concentrations, and WAS were all influenced by differences in tillage and cropping system.

Similar to several past studies (e.g., Yoo and Wander 2008; Kasper *et al.,* 2009; Sheehy *et al.,* 2015: Nouri *et al.,* 2018), we observed that no-till management increased the proportion of macroaggregates while tillage increased the proportion of aggregates less than 1 mm in diameter. At 0-10 cm depth, tilled plots were heavily disturbed by moldboard plowing and field harrowing, reducing the proportion of macroaggregates. In contrast, no-till soils are under continuous cover, which in combination with less disturbance minimizes macroaggregate disruption. In addition, no-till management typically promotes arbuscular mycorrhizal fungal colonization, which enhances aggregate stability (Jastrow *et al.,* 2007). At 10-20 cm depth, aggregate size distribution was not significantly different between tilled and no-till plots, which may be attributed to the combined effects of soil inversion, stimulation of microbial activity, and soil disturbance.

Our results are consistent with previous studies demonstrating that soil under legume- and organic-manure management contains higher concentrations of SOC, which helps stabilize aggregates (Min *et al.,* 2003; Larsen *et al.,* 2014; Nunes *et al.,* 2018) than conventionally-managed soils. Despite this, the positive effect of aggregate stabilization by SOC inputs may be less important than soil disturbance by tillage and soil exposure, resulting in no significant effect of cropping systems on aggregate distribution at 0-10 cm. At 20-30 cm deep, organic-manure but not organic-legume treatments increased the macroaggregate proportion compared to conventional plots. The organic-manure system may have outperformed other systems in enhancing macroaggregation due to the effects of perennial grasses included in the organic-manure systems. Perennial grasses have more extensive root systems than annual crops, which together with the minimal disturbance associated with perennial rotations and high organic matter inputs enhance macroaggregate stability, particularly WAS (e.g. Shukla *et al.,* 2003; Jastrow *et al.,* 2006; Poirier *et al.,* 2018).

Aggregate-protected SOC concentrations per unit aggregate mass showed similar trends to total SOC, as the organic (especially organic-manure) treatments increased SOC concentrations in all aggregate size classes. These results are consistent with previous studies which showed that organic management strategies such as manure application, legume cover crops, or perennial grass incorporation can benefit the SOC accumulation in all aggregate sizes (e.g., Yoo and Wander 2008; Kasper et al. 2019; Six and Paustian 2014; Sheehy et al. 2015). Differences in aggregate-protected SOC concentrations among aggregate size classes is attributed to the differences in the formation of separate aggregate size classes. The low SOC concentrations of large (>1.0 mm) macroaggregates compared to microaggregates (<0.250mm) may be because large aggregates contain sand particles which are associated with very little organic compounds compared to finer silt and clay particles (Hassink 1997; Six et al, 2002; Jastrow *et al.,* 2007). Moreover, macroaggregate-occluded SOC is still accessible to decomposers and extracellular enzymes and therefore have a much shorter residence time than microaggregate-protected SOC (e.g. Beare et al. 1994; Von Lutzow et al. 2006; Jastrow et al. 2007). In contrast, microaggregate-protected SOC is better protected as it is more tightly adsorbed to soil particles and microaggregates are far more resistant to soil disturbance than macroaggregates. Additionally, tilled organic-manure cropping systems has higher macroaggregate-protected SOC concentrations than their no-till counterparts at 20-30 cm. Organic matter enriched soils from the surface were inverted to this depth by tillage, which is evidenced by the increased SOC concentrations within macroaggregates within tilled plots compared to no-till plots (Table 4).

Similar to the aggregate size distribution results, aggregate-protected SOC content per unit mass of soil from 2-8 mm, 0.25-1 mm, and < 0.053 mm-sized aggregates were not affected by differences in cropping systems at 0-10 cm depth. However, organic-manure cropping systems exhibited elevated aggregate-protected SOC content in 1-2 mm and 0.053-0.250 mm aggregate size classes, which is attributed to the significantly higher aggregate-protected SOC concentration per unit mass of soil in these two sizes, especially in the 0.053-0.25 mm size which was 25% higher than that from the conventional plots (Table 4). Similarly, the increased 2-8 mm aggregate-protected SOC content per unit mass of soil in organic-legume and organic-manure plots at 10-20 and 20-30 cm was attributed to the increased SOC concentration in this fraction as no change was detected in its weight proportion. This suggests that the two organic treatments improved SOC sequestration through accumulating more SOC in large macroaggregates at lower soil depths. The increased 1-2 mm aggregate associated SOC content under organic treatments, on the other hand, was attributed to the increase in both weight proportions and SOC concentrations of these aggregates. However, no cropping system effect was observed on the SOC concentrations per unit mass of soil within 0.25-1 mm and 0.053-0.25 mm aggregates because organic management increased the SOC concentration within these aggregates (Table 4) but decreased the weight proportion (Table 3) of these two aggregate size classes. This is consistent with other studies which indicated that organic management enhances both aggregate stability and SOC accumulation (e.g. Jastrow et al. 2007; Larsen et al. 2014; Sheehy et al. 2015). Differently, conventional treatments contained a higher SOC content within the silt- and clay-sized fraction because of the increased proportion of silt- and- clay-sized particles from the breakdown of larger aggregates and silt- and clay- adsorbed SOC is a relatively stable SOC fraction (Dungait *et al.,* 2012), although the SOC concentration associated with this size in conventional systems was lower than that from organically managed systems.

Our results of no-till management enhancing macroaggregate-protected SOC content in the topsoil are consistent with other studies which demonstrated that no-till systems accumulate more macroaggregate-protected SOC than tilled systems in the topsoil. This could be due to the higher weight proportion of macroaggregates and greater SOC protection within macroaggregates under minimal disturbance (e.g. Beare et al. 1994; Six and Paustian 2014; Sheehy et al. 2015). However, higher macroaggregate-protected SOC concentrations per unit soil mass in no-till plots compared to till plots represents a redistribution of SOC from small macroaggregates and microaggregates to large macroaggregates rather than an increase in total SOC concentrations. This is because increases in large macroaggregate-protected SOC per unit soil mass are counterbalanced by decreases in small macroaggregate- and microaggregate-protected SOC per unit soil mass as no-till management increases the weight proportion of large macroaggregates while reducing the weight proportion of small macroaggregates.

Similar to aggregate size distribution and aggregate-protected SOC, WAS is also influenced by differences in cropping systems and/or tillage. At 0-10 cm, WAS is influenced by the combined effects of cropping systems and tillage. Wet aggregate stability was lower in tilled conventional plots than in tilled organic-legume and organic-manure plots, while the wet aggregate stability of no-till conventional plots was only lower than that of no-till organic-legume plots. These results suggest that no-till management combined with organic, especially organic-manure, cropping systems maximizes wet aggregate stability. Heavy disturbance from a combination of moldboard plowing and field harrowing in tilled plots may have reduced wet aggregate stability in the top 10 cm by disrupting the roots and fungal hyphal networks which bind macroaggregates. This is consistent with past research that showed decreased wet aggregate stability from conventional tillage in the top 10 or 20 cm of the soil profile (e.g. Angers *et al.,* 1993; Beare *et al.,* 1994; Six and Paustian 2014). Organic-manure cropping systems exhibited the greatest wet aggregate stability because perennial grass/legume rotations like the hay rotations in organic-manure plots are more effective at enhancing both aggregate stability and SOC accumulation than annual cover crops as their roots are more extensive, branching, and fibrous than annual roots (Poirier *et al.,* 2018). Also, perennial grass/legume rotations involve minimal soil management/disturbance compared to annual crop rotations. Moreover, the utilization of leguminous cover crops in both organic-legume and organic-manure cropping systems is more conducive for enhancing both mineral-adsorbed and aggregate-protected SOC concentrations (Poirier *et al.,* 2018) and therefore better aggregate stability.

Unlike at 0-10 cm deep, wet aggregate stability was only affected by cropping system management, not by tillage at the 10-20 cm layer. Soil at this depth is largely unaffected by raindrop impact or field harrowing even in tilled plots, enhancing the relative importance of different SOC inputs associated with different cropping systems. While wet aggregate stability in organic-manure plots exceeded that of conventional plots by 36%, organic-legume management did not significantly improve wet aggregate stability compared to conventional management.

Wet aggregate stability was maximized by organic-manure (both tilled and no-till) and no-till organic-legume management at 20-30 cm deep. Interestingly, the tillage effect on WAS was observed at this depth despite being beneath the plow depth. No-till organic-legume plots may have enhanced WAS compared to their tilled counterparts at 20-30 cm deep because legume crops, particularly if no-tilled, favor enhanced soil water infiltration and with it, SOC export to deeper layers. Legume root channels are wider than grass root channels and legumes support higher populations of earthworms than grasses (Fischer *et al.,* 2014). Additionally, no-till management enhances soil water infiltration by leaving macroaggregates and worm and insect burrows intact and reducing fatalities to earthworms and burrowing insects normally caused by tillage, thereby enhancing macroporosity (e.g. Shukla *et al.,* 2003; De Almeida *et al.,* 2018; Liu *et al.,* 2018).

Overall, organic-manure management maximized macroaggregation, the proportion of SOC protected within macroaggregates, and wet aggregate stability similar to what was hypothesized. This is consistent with past studies which demonstrated that manure applications (i.e. Shukla *et al.,* 2003; Long *et al.,* 2015: Bai *et al.,* 2018) and/or perennial forage or hay rotations (i.e. McNally *et al.,* 2018; Loges *et al.,* 2018; Nunes *et al.,* 2019) enhances aggregate stability as decomposed and readily humified SOC found within livestock manures help bind aggregates just as minimal disturbance in perennial rotations preserves existing macroaggregates. However, the effects of tillage in organic-manure treatments did not significantly affect aggregate-protected SOC concentrations and WAS, unlike as hypothesized, as the combined effects of heavy decomposed biomass inputs and lack of tillage disturbance during the four out of eight years spent in perennial hay rotations may have masked the effects of tillage. However, no-till management significantly increased the share of SOC protected within large macroaggregates within the topsoil without improving bulk SOC concentrations by increasing the proportion of large macroaggregates compared to other aggregate size classes in all size classes while enhancing topsoil WAS in conventional plots and subsoil WAS in organic-legume plots.

CHAPTER VI: CONCLUSION

After 37 years, both legume- and manure-based organic management of grain cropping systems improved SOC accumulation, macroaggregation, and WAS compared to conventional grain cropping utilizing chemical inputs. Treatments which increased biologically active SOC concentrations also increased total SOC concentrations in the long term. Organic-legume and organic-manure treatments added C inputs with low C:N ratio (legumes and manure, respectively), which are more readily converted to microbial-derived compounds that are protected better by aggregate occlusion and mineral adsorption. Organic-manure treatments in particular maximized SOC accumulation through combined crop residue, cover crop biomass inputs, and manure applications.

Compared to the effect of cropping system management, tillage treatments had no significant effect on total and biologically active SOC accumulation with the exception of macroaggregate-protected SOC. However, there were indications that no-till management will enhance SOC accumulation over time, particularly in the topsoil as no-tillage increased aggregate stability, the proportion of large macroaggregates, and macroaggregate-protected SOC in the top 10 cm. The benefits of no-till management were especially evident in conventional systems, where no-tillage enhanced WAS in the topsoil and in organic-legume plots, where no-tillage enhanced WAS in the subsoil. This study revealed that while organic-manure management, regardless of tillage regime, is most suitable for maximizing SOC sequestration, organic-legume management is also an effective option when easy access to livestock manures is limited.

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VITA

James Littrell was born on March 5, 1992 in Shady Grove, Maryland. He attended Boston University, where he got a bachelor's degree in Marine Science in 2014. After that, James got a master's degree in Marine Science at the University of Massachusetts- Dartmouth in 2016. In 2017, James joined the Department of Biosystems Engineering and Soil Science at the University of Tennessee as an MS student in the Department of Biosystems Engineering and Soil Science at the University of Tennessee under the supervision of Dr. Sindhu Jagadamma. James received the Hazlewood and Rehabilitation Corporation of Tennessee graduate scholarships and the Spring 2019 Access and Diversity Fellowship.