Long-term Impacts of Conservation Management Practices on Soil Carbon Storage, Stability, and Utilization under Cotton Production in West Tennessee

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ABSTRACT

Biogeochemical cycling of soil carbon (C) is heavily influenced by conservation agricultural (CA) practices. This study examined SOC stability under three CA practices: reduced nitrogen (N) fertilizer application rate, cover cropping, and zero-tillage implemented for 31 years. Respiration rates measured from a 602-day incubation period were fitted to a double-pool first order exponential model of SOC decomposition. The active [respired] SOC pool showed distinct differences between applications of reduced (34N kg ha^{-1} [-1]) and high fertilization rates (101N kg ha^{-1}) combined with tillage, and suggest that high fertilizer applications with conventional tillage allocated more C into a rapidly turning over C-pool. The slow SOC pool dynamics revealed that given more time, soil C losses would stabilize under reduced N-rates and no-tillage conservation practices.

More detailed biological controls on broad nutrient acquisition efforts were indicated by extracellular enzyme potential activities of whole soil, and reflected differences between N availability and N form under organic or inorganic additions to this cotton system. The effects on soil physical properties showed that less soil disturbance had a clear effect on the distribution of dry aggregate fractions, where no-tillage had greater abundance of large macroaggregates (> 2 mm) and persisted when combined with cover crop management.

Macro- (> 0.25 mm) and microaggregates (< 0.25 mm) within conventional tillage systems were distinct from those in no-tillage systems due to greater enzyme activity in total C, N, and phosphorus (P), and could indicate faster decomposition rates in

conventional tillage compared to no-tillage management. To determine where soil C was most vulnerable to decomposition across the aggregate fractions, respired C was measured after a short-term incubation study and showed greater mean respired C under small macroaggregates (0.25-1 mm) and microaggregates compared to large and medium (1-2 mm) macroaggregates. However, future research must measure total C from each aggregate fraction to determine if soil aggregates can act as a physical protection barrier to sequester SOC.

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CHAPTER I INTRODUCTION AND GENERAL INFORMATION

Conservation soil management practices in agroecosystems can greatly influence the biogeochemical cycling of carbon (C). Biological, physical, and chemical soil processes are integral to soil microbial community function and reflect emergent properties capable of transforming and stabilizing soil C. Specifically, the microbial decomposition of soil organic C (SOC) can be a major source of C-loss via respiration, as well as long-term C storage in terrestrial ecosystems. Soil microbial decomposition is driven, in part, by extracellular enzymes produced by the microbial community (Burns and Wallenstein, 2010; Schimel and Weintraub, 2003). These enzymes are released by the microbial community to catalyze depolymerization of organic C substrates that vary in chemical lability or recalcitrance, which in turn influence the stability of dynamic SOC pools (Wang et al., 2013). Another factor that indirectly influences C cycling is the physical protection of SOC within soil aggregates (Paul, 2014; Six et al., 2006). Stable aggregate formation primarily occurs after decomposition where microorganisms have produced chemical breakdown products (USDA, 1996). These breakdown products can include polysaccharides, calcium carbonate, and iron oxides that are reactive with the soil's clay mineralogy to help form protective aggregates, and are further stabilized by fungal hyphae and rooting systems (Hillel et al., 1998). Soils with higher organic matter (OM) content have shown relatively greater aggregate stability (Plante and McGill, 2002).

As soils are disturbed through cultivation, OM previously protected decomposes more rapidly and leads to greater rates of soil respiration (Schlesinger and Andrews,

2000). Estimates show that a quarter of original C existing in uncultivated soils is lost as atmospheric CO_2 once cultivated (Cole et al., 1997) and even further C loss occurs once intensive management practices are implemented (Conant et al., 2007). Estimates like these become relevant in the global C-cycle, as we are very concerned with the effects of rising CO_2 on our climate, and how these climate-driven changes will affect our ability to feed a growing population. The Intergovernmental Panel on Climate Change (IPCC) 2014 synthesis report concluded that negative impacts of climate change on crops yields have been more common than positive impacts, and that feedback between climate change and C-cycling will amplify global warming.

There is a critical need to examine the effects of agriculture management practices on SOC storage, stability, and utilization. Practices like nitrogen (N) fertilizing, cover cropping, and tillage are commonly implemented in cropping systems to maximize yields and prevent soil erosion (Causarano et al., 2006). However, research in conservation efforts of such practices shows greater productivity and sustainability in cropland soils (Franzluebbers and Stuedemann, 2005; Salon, 2006; Six and Jastrow, 2002), and my study sought to investigate whether soil biological and physical effects on soil C-cycling are driven by long-term conservation management practices. A first order decay function was used to distinguish relative pool sizes of respired SOC in the bulk soil, and physical fractionation procedures were used to investigate the vulnerability of soil C to degradation by microbial action, and specifically by enzymatic activity. A better understanding of SOC stability and the role of microbial community

structure and function on SOC stability are needed, and will be useful to predict future impacts of climate change on SOC dynamics.

Literature Review

Conservation and stability of soil organic carbon in agroecosystems

SOC sequestration in agroecosystems has the potential to mitigate climate change while also improving long-term soil fertility (Cole et al., 1997; Wieder et al., 2013). The increase or decrease of atmospheric CO_2 emissions can be a direct result of SOC residence time and decomposition rates, which are influenced by management practices. Practices that disturb the soil surface, such as tillage, can negatively affect soil structure by decreasing aggregate formation (Tisdall and Oades, 1982). Changes in soil structure can affect soil water movement, erosion, biodiversity, nutrient cycling, and impact climate (Bronick and Lal, 2005; Dukes and Hungate, 2002; Niklaus et al., 2001). Sequestered C, operationally defined, is a form of stable soil C obtained when atmospheric CO_2 is negated by conservative land use where SOC and soil inorganic C (SIC) stocks are transferred into persisting terrestrial pools that allow long-term storage without immediate remittance (Lal, 2004). Yearly total C sequestration potentials range from 400 to 1200 teragram C worldwide (Sauerbeck, 2001; Lal, 2004), and although these potentials are limited in capacity and duration (~50 years) the implementation of effective strategies to meet these potentials will promote conservation while reductions in fossil fuel and alternative energy sources take effect (Lal, 2005).

The need for increased production from cultivated land will continue to rise with population, and thus there is a heavy incentive to increase and stabilize SOC in cropland soils as a means to maintain long-term productivity. Levels of SOC in agroecosystems are dependent on the combined effects of management practices, climatic conditions, soil type and properties, and the initial soil C level of the site (Chan, 2008). Management practices that influence SOC storage potential include tillage, cover cropping practices, and fertilization. Conservative management approaches, known collectively as conservation agriculture (CA) practices, help promote soil C sequestration by balancing C inputs relative to C outputs (Franzluebbers, 2007).

When conventional tillage is used to cultivate land (mouldboard or disc plough), OM decomposition is stimulated and OM previously protected in aggregates is redistributed to areas in the soil profile that are vulnerable to wet-dry and freeze-thaw cycles and even raindrop impacts (Beare et al., 1994; Paustian et al., 1997). A single conventional tillage event can reduce soil C content by 11%, with C losses increasing as tillage frequency increases when compared to no-tillage systems (Conant et al., 2007). These estimates give rationale for the implementation of more no-tillage systems in croplands. No-tillage management is a CA practice that minimizes soil disturbance and allows crop residue to be left at the soil surface, aiding in erosion control and building soil OM. A global dataset of long-term (15 years) agricultural experiments by West and Post (2002) showed that conversion of conventional tillage systems to no-tillage systems resulted in an average increase in SOC sequestration rate of 57 ± 14 g C $m^{-2}\,yr^{-1}$. In addition, they also that there might be little to no increase in SOC in the first

2-5 years after a change in management practice, but a large increase will ensue in the next 5-10 years (West and Post, 2002). After 23 years of no-tillage management on a continuous corn silt loam soil, near-equilibrium conditions of SOC and N inputs were reached (Dolan et al., 2006). However, estimates like these are highly variable based on several soil properties and management practices that create new equilibrium levels of SOC; tillage intensity, soil type, bulk C, aggregation, cropping system, and length of management practices contribute to equilibrium status (Mishra et al., 2010; Dick et al., 1998; Lal and Kimble, 1997).

The southeastern United States (U.S.) has a long history of extensive tillage from 1860-1920, largely for cotton cultivation, resulting in severe soil erosion rates that depleted SOC in the region (Causarano et al., 2006). Despite lower amounts of SOC, it appears that once no-tillage management was implemented greater SOC sequestration occurred in these heavily degraded soils compared to more fertile soil in other regions. Franzluebbers (2007) showed the ratio of SOC under no-tillage to conventional tillage was greater in soils with inherently lower SOC compared to soils with inherently higher SOC content (Figure 1). Overall, the sequestration of SOC was relatively greater in soils that were previously heavily degraded. A meta-data analysis of cotton production systems throughout the southeast region estimated that mean SOC sequestration rates under no-tillage were greater by 0.48 ± 0.56 mg C ha^{-1} yr^{-1} compared to conventional tillage management (Causarano et al., 2006; Franzluebbers, 2007). No-tillage management in this region was largely implemented for erosion control, but it seems that greater C storage potential is an additional benefit with this practice. Other CA

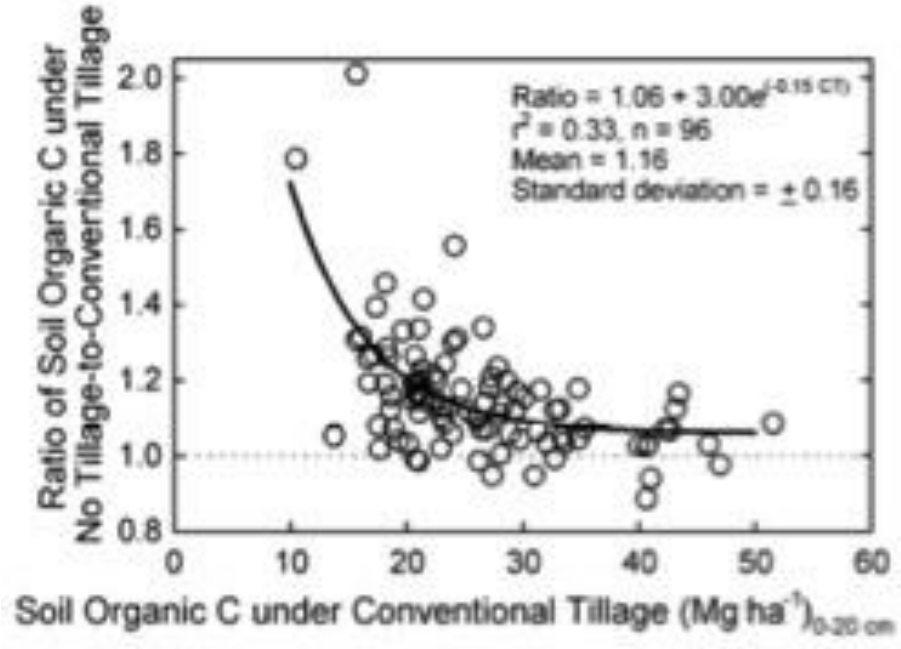


Figure 1. The ratio of $SOC_{No\text{-}Tillage}$: $SOC_{Conventional\ Tillage}$ when related to initial SOC under conventional tillage results in a non-linear relationship (Franzluebbers, 2005)

management practices, like cover cropping, can influence the effects of SOC storage and stability when coupled with no-tillage systems. Cover cropping primarily protects soil from erosion during the off-season, but also affects the quantity and quality of C inputs to the soil in terms of crop residues (Denef et al., 2011).

Cover-cropping practices create diversity in residues, depths and patterns of roots, and thus add greater C inputs to the soil (Franzluebbers, 2007). It is suggested that SOC sequestration would be twice as high in cotton production systems by combining no-tillage management with cover cropping (0.67 mg C ha^{-1} yr^{-1}) than simply no-tillage management without a cover crop (0.34 mg C ha^{-1} yr^{-1}) (Causarano et al., 2006). Meta-data analyses show that SOC storage under management practices like no-tillage and cover cropping can differ widely across field studies as a function of climate, with more SOC storage occurring in tropical, moist regions versus temperate, dry regions (Ogle et al., 2005; Denef et al., 2011).

Fertilization is an extensive component of land cultivation, as it is needed to stimulate crop biomass production and overcome soil nutrient deficiencies when intensive disturbances and continuous harvesting of crop products occur (Franzluebbers, 2007). Recommended N-rates for cotton production in Tennessee generally range from 67 to 90 kg N ha^{-1} for upland soils and 50 to 67 kg N ha^{-1} for lowland soils. Conventional tillage requires slightly less N fertilizer (~13 kg N ha^{-1}) compared to no-tillage practices, and when cover crops high in C:N content are implemented (i.e. winter wheat), recommended N applications range from ~56 to 78 kg N ha^{-1} under conventional and no-tillage, respectively. However, management systems

incorporating leguminous cover (i.e. hairy vetch) can have low (34 kg N ha^{-1}) to zero recommended N fertilizer application (Larson et al., 2001). The average N fertilizer rate needed to achieve maximum SOC sequestration was reported at 171 kg N ha^{-1} yr^{-1} in the southeastern U.S. (Franzluebbers and Stuedemann, 2005). However, increases in SOC from such fertilizing rates can be negated when other greenhouse emissions, like nitrous oxide (N_2O) losses via denitrification, and C production emissions, like fertilizer production itself, are accounted for (Denef et al., 2011). Franzluebbers and Stuedemann (2005) concluded that net C offset from N fertilization would be maximized at 0.07 mg C ha^{-1} yr^{-1} with the application of 24 kg N ha^{-1} yr^{-1}. These calculations are positive, but also present a diminishing return of investment when applications of N fertilizer continue to increase (Causarano et al., 2006).

Mechanistic drivers for greater soil C in response to high inputs of inorganic N include increased primary plant productivity in which above ground and belowground biomass increase soil C and N storage and biologic activity (Haynes and Naidu, 1998; Halvorson et al., 2002; Alvarez, 2005; Jagadamma et al., 2007; Mbuthia et al., 2015). Further research in SOC sequestration under N fertilizing regimes has shown that microbial communities with fast growth rates, requiring labile C sources, can increase in abundance under inorganic nutrient inputs and replace slower growing communities able to breakdown more recalcitrant forms of soil C (Fierer et al., 2007; Fontaine et al., 2003). If N-fertilizing applications yield more communities less capable of recalcitrant C decomposition, there will be greater potential for SOC sequestration (Ramirez et al., 2012). Previous research in the same experimental site showed more soil C under high

inorganic N fertilizer rates (101N kg ha^{-1}) or when cover crops were implemented under no-tillage (Table 1) (Mbuthia et al., 2015). These results require further investigation to determine whether the form or degradability of bulk soil C differs between integrated CA practices, and my study seeks to fill these gaps by evaluating the long-term, mechanistic response of microbial activity within distinguished pools of SOC varying in decomposability.

Organic matter decomposition

Löhnis and Fred (1923) illustrated the first 'cycling of matter' diagram and explained that all organic residues are processed via mineralization, and without such a process, we would still see the earth littered with organic residues. The formation and decay processes of OM found in soils link residual, organic compounds to the production of greenhouse atmospheric gases like CO_2, N_2O, and CH_4 largely driven by land-use changes (Paul, 2014). Formation of soil OM (SOM) is primarily controlled by decomposition and transformation of above- and below-ground plant litter (Cotrufo et al., 2013), while its degradation is a function of microbial decomposition, recalcitrance of C compounds, and aggregate protection (Six et al., 2006).

Two important groups of compounds that make up SOM include nonhumic and humic substances; nonhumic substances are commonly utilized as substrates (simple carbohydrates and polysaccharides, amino sugars and acids, peptides and proteins, lipids and phospholipids, lignin) by microorganisms and decomposed to inorganic components like CO_2 and H_2O (Essington, 2004). Humic substances make up a distinctive class of SOM extracted from decaying biomatter; biomatter refers to

Table 1. Bulk soil C (mg g^{-1}) under continuous cotton system with N fertilizer rates (N-rate), cover cropping, and tillage treatments. Numbers inside parentheses are (±1) standard error of the mean, and lettering differences denote statistical significance at $P < 0.05$. Bolded ANOVA numbers show statistical significance at $P < 0.05$ (Analysis and table created from raw data set collected by Mbuthia et al. (2015).

Treatment factors	Bulk soil C (mg g^{-1})
N-rate (kg ha^{-1})	
0N	11.1(0.9) b
34N	12.4(0.9) b
67N	12.1(0.7) b
101N	14.2(1.4) a
Cover Crop	
No Cover	11.8(0.9) b
Vetch	13.3(1.1) a
Wheat	12.3(0.9) ab
Tillage	
No-tillage	13.2(1.1) a
Conventional tillage	11.7(0.8)b
Sources of Variation	ANOVA (*P* values)
N-rate	**0.0029**
Cover Crop	**0.0143**
Tillage	**0.0006**
N-rate*Cover Crop	0.1949
N-rate*Tillage	0.1543
Cover Crop*Tillage	0.4566
N-rate*Cover Crop*Tillage	0.6647

biologically synthesized matter that is not living, or a component of a living cell (MacCarthy, 2001). In a broad scope, both humic and nonhumic substances play major roles in SOM maintenance and stabilization (Paul, 2014).

Nonhumic substances are the source for living soil organisms to obtain biomolecules used for function, structure, and reproduction (Essington, 2004). Thus, microbial biomass C constitutes a significant portion of SOC (Simpson et al., 2007). C isotopic labeling research by Voroney et al. (1988) compared labeled ^{14}C-glucose from microbial products and wheat straw, and found slower mineralization of labeled C in glucose-amended soil compared to wheat-straw amended soil possibly due to a small portion of residual C present in undecomposed plant constituents, like lignin. Non-proliferating microbial biomass, known as necromass, could also be a contributor to SOM stability by accounting for 80% of soil organic C (Liang and Balser, 2011). The efficiency of microbial groups, like bacteria and fungi, to utilize C substrates and differences in the chemical makeup of their byproducts further affect the stability of SOM (Six et al., 2006). For example, bacterial cell wall structure consists of peptidoglycan, where greater peptidoglycan content can slow decomposition (Paul, 2014). It seems that many factors that include biomass abundance, necromass, and substrate use efficiency of microbes attribute to SOM stability.

The stabilization of SOM is also associated with a chemically recalcitrant OM pool that is responsible for long-term soil C residence time and stability (Adair et al., 2008). A review by Lutzow et al. (2006) explains that selective preservation, interactions with surfaces and metal ions, and spatial inaccessibility are mechanisms controlling

SOM stabilization. Residues of plant litter, microbial products, humic polymers, and charcoal can be selectively preserved by the soil matrix due to their recalcitrant nature (Kalbitz et al., 2003; Kögel-Knabner, 2002; Swift, 2001). The complexation of OM on mineral surfaces by ligand exchange between inorganic hydroxyl groups of iron and aluminum oxides increases in soils with lower pH, and forms strong organo-mineral associations (Essington, 2004). One of the strongest factors of SOM stabilization results from cropping management practices, where soil aggregation can act as a physical constraint to the transfer of enzymes, substrates, water, oxygen, and microoganisms (Lutzow et al., 2006; Tisdall and Oades, 1982). The formation and occlusion of particulate OM (POM) unbound to minerals is protected against decomposition within aggregates and can have residence times ranging from 400 to 1000 years dependent on soil silt and clay content (Paul, 2014.)

The implementation of CA management strategies adds crop residues and thus can help increase SOM mean residence time (Six and Jastrow, 2002). Conversely, conventional tillage practices can remove or bury crop residues and in turn decrease SOM at the soil surface, and reduce soil quality processes in the long-term (USDA, 1996). Previous research at my study site by Stearman et al. (1989) showed conventional tillage was composed of more aromatic compounds in the humic acid fraction (a component of humic substances) of SOM, indicating soil C was in an advanced decomposition state compared to no-tillage. The addition of cover cropping with low C:N input, such as vetch, to active C decomposition under conventional tillage would further advance decomposition relative to a cover crop residue with high C:N

input, such as wheat (Salon, 2006). N fertilizer application can increase rates of SOM decomposition by incorporating enriched inorganic nutrients and crop residue for breakdown by microorganisms (Haynes and Naidu, 1998). Studies do not often measure the combined effects of multiple CA practices (N fertilizer, cover cropping, and tillage) on long-term managed monoculture crops to gain a mechanistic understanding of OM decomposition and its associated stability with different soil C-pools. However, there should also be continued efforts to explore complex management schemes utilized for our increasing need for food production.

Soil organic carbon decay rates

The mean residence times of C compounds within the soil can range from days to millennia (Davidson and Janssens, 2006; Paul, 2014) and are a function of OM quantity, quality, and the structure of the decomposer community (Cotrufo et al., 2013). Mineral soil and its associated C is often operationally divided into multiple C pools as a function of its degradability (Adair et al., 2008): the single pool model represents easily decomposable (active) C with fast turnover rates, the double pool model represents active and slowly degrading C, and the triple pool model includes active, slow, and recalcitrant rates of C turnover (Schädel et al., 2013; Davidson and Janssens, 2006; Trumbore, 1997). Fast cycling OM in the active C-pool consists of fresh residue plant material, living organisms, and hydrolysable components associated with mineral surfaces that promote rapid C turnover times ranging from less than 20 or greater than 60 years dependent on low or high altitude climate conditions (Parton et al., 1993; Trumbore, 1997). Slower-cycling OM, like lignin components, make up the slow and

recalcitrant C pools, and is strongly associated with mineral surfaces (Parton et al., 1993). Their turnover times range from centuries to millennia and represent long-term climate conditions based on soil maturity and mineralogy (Trumbore, 1997). These slow decomposition rates occur when there are substrates available with complex structure that make them resistant to decomposition, or when environmental processes, such as soil aggregate formation, restrict enzyme access to break down simple molecules (McLauchlan and Hobbie, 2004). Subsequently, a situation could occur where a simple C compound, like glucose, protected in soil aggregates could be pooled together with a complex C compound that is not protected in the soil (Davidson and Janssens, 2006).

Long-term incubations allow for a direct measurement of soil respiration to understand decomposition kinetics of different C-pools. Incubation periods ranging from 200 to 300 days typically utilize a double C-pool model with a first order decay function to deconvolute soil C efflux (Paul, 2014; Schädel et al., 2013). Incubation data from such periods are not long enough to model significant changes in the decay rate of a third C-pool, thus longer incubation periods would be needed to constrain parameters of a recalcitrant pool (Paul et al., 2006). All C-pool parameters were optimally constrained by Scharnagl et al. (2010) when using the RothC model, a model that partitions soil OM into five pools (Schädel et al., 2013), but the experiment was conducted with an incubation time period that lasted 1200 days.

C-pool models can be used to help identify management practices in agroecosystems that control C and nutrient cycling by generating data on SOM pool sizes and rates of nutrient transformation (Paul, 2014). Intensive tillage practices are

known to decrease surface C stocks, and research often shows greater labile C in the surface soil of no-tillage management compared to conventional tillage (Collins et al., 2010; Hu et al., 1997). However, the inverse of this trend has occurred in soil subsurface (20 to 25 cm depth) where greater SOC and N concentrations resulted under conventional tillage practices from buried residue by soil inversion management (Dolan et al., 2006). SOC pool sizes and turnover rates determined by Collins et al. (2010) used a constrained double pool, first-order model from a Corn Belt system. The concentration of C in both the labile and intermediate C-pools in the surface (20 cm) was greatest under no-tillage compared to conventional tillage, and SOC turnover rates ranged from 18 to 100 years. This directly related to the time period in which the experimental sites had been cultivated for corn monoculture and with the soil type and texture at the site. Jagadamma et al. (2014) also showed soil type and substrate quality effects on SOC mineralization. They incorporated four different soil types that spanned a range of native soil C content, organic C quality, and weathering properties. Contrary to expected results, recalcitrant substrates isotopically labeled with ^{13}C induced the highest mineralization rates of native SOC compared to labile substrates at the end of the incubation period. It was concluded that the increase in SOC mineralization by recalcitrant substrates was largely due to the activation of specialized, slow-growing microorganism populations capable of decomposing more recalcitrant components of SOC dependent on soil type. It is evident that the persisting microbial community plays a crucial role in the mineralization of SOC compounds. The analysis of such microbial activity through C-pooling models in agriculture should be further researched to

investigate effects of different soil types under various management practices commonly used in food production on soil C substrate utilization and stability.

Soil enzyme activities

Biological controls on SOC dynamics correspond with C substrate chemistry or substrate quality available to the soil microbial community. Microbial substrates in soil vary in structural complexity and are metabolized at different rates by different kinds of microbes, which then yield complex interactions that affect decomposition (Schimel and Schaeffer, 2012). Extracellular enzymes produced by bacteria and fungi also facilitate nutrient cycling in terrestrial ecosystems (Six et al., 2006). When activated by microbes in the soil, extracellular enzymes catalyze large OM molecules (polymers and particulate OM) and release smaller molecules that are absorbed for energy or nutrient acquisition by the microbial community (Bandick and Dick, 1999; Tabatabai, 1994) (Figure 2).

Soil enzyme activities have become a prevalent indicator of soil quality because of their capacity to reflect rapid changes in the soil environment (Dick, 1994). Such analyses provide an index of substrate utilization by enzymes that integrate physical, chemical, and biological effects on OM degradation and nutrient mineralization from soil management strategies (Doran and Parkin, 1994). Potential enzyme activity measurements are associated with all forms of soil enzymes, from viable proliferating cells or as an excretion from these cells, as well as from stabilized enzymes in the soil matrix that were excreted from dead cells and then stabilized on clay surfaces and OM (Boyd and Mortland, 1990; Burns, 1982).

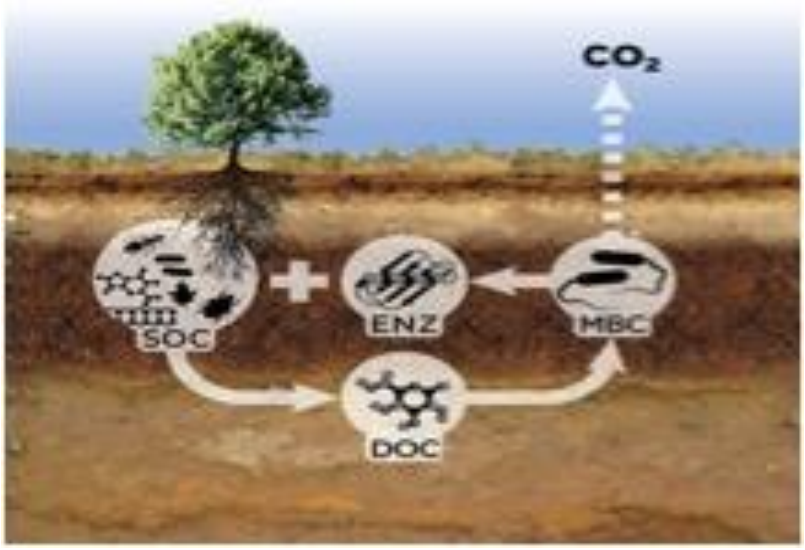


Figure 2. Microbial Enzyme Decomposition (MEND) model for soil C cycling developed at Oak Ridge National Laboratory (Wang et al., 2012). The microbial community releases extracellular enzymes (ENZ) to break down complex SOC into simpler forms like dissolved organic C (DOC). Portions of C in microbial biomass (MBC) is respired as atmosphere CO_2.

Also known as ‘free’ enzymes, their accumulation in the soil is independent of the proliferating microbial population and has been experimentally shown to persist for long periods, for example, over 5000 years in a permafrost soil (Skujiņš and Burns, 1976). Thus, it is important to understand the limitations of laboratory extracellular enzyme assays that measure a potential activity rather than an activity reflective of *in-situ* conditions. The injection of synthetic substrates to soil samples induce activity of all enzymes present, whether they are stabilized as ‘free’ or excreted from proliferating cells.

Agricultural management practices like N fertilizer additions, cover cropping, and tillage each influence soil OM inputs, and can act as a primary controls on the response of extracellular enzymes to breakdown SOC (Alvear et al., 2005; Bandick and Dick, 1999; Bergstrom et al., 1998; Mathew et al., 2012). Thus far, investigations of enzyme activity on such agricultural practices have only been done on a small number of enzymes (Dick, 1994), and are commonly used to interpret broad patterns in nutrient cycling when there are many enzymes yet to be systematically investigated (Bandick and Dick, 1999). Soil enzymatic laboratory methodologies are still quite variable between studies (Bach and Hofmockel, 2014), and results should not be taken beyond context in relation to soil quality.

However, the potential activities of extracellular enzymes can link microbial metabolic rates (Fontaine et al., 2003; Ramirez et al., 2012) to assess nutrient demands in the scope of principal C, N, and P sources (Saiya-Cork et al., 2002). Glucosidases and cellulase degrade low molecular weight sugars for microbial energy and hydrolyze

plant inputs like cellulose to the soil (Bandick and Dick, 1999). Glucosaminidase and aminopeptidase are important N-acquiring enzymes involved in the breakdown of chitin and peptidoglycan of cell walls, and with hydrolyzing leucine and other hydrophobic amino acids from polypeptides (Sinsabaugh et al., 2009). Phosphatase enzyme activity is an important indicator for the release of inorganic phosphate from OM (Allison and Jastrow, 2006).

The research of extracellular enzymes within agroecosystems extends beyond bulk soil analyses (Matthew et al., 2012) to investigate management effects at the soil aggregate scale (Allison and Jastrow, 2006; Bach and Hofmockel, 2014). Aggregation results in improved soil structure and it physically protects soil C (Six and Jastrow, 2002), but as soil disruption events increase during cultivation, aggregates are broken up and soil C is easily degraded (Elliott, 1986; Six et al., 1998). Soil intra-aggregate pore spaces can act as a physical constraint to the function of microbial communities (McLauchlan and Hobbie, 2004). If easily decomposable compounds, such as glucose, were protected within aggregates from microbial decomposition, the microbial community might instead act on more complex compounds for decomposition to meet metabolic needs. This could result in slower decomposition rates, high activation energy requirements, and a higher temperature sensitivity compared to labile C decomposition (Davidson and Janssens, 2006). Schimel and Weintraub (2003) describe these circumstances as a ‘negative return on investment’ because less C and energy would come back to the microbes than the costs to produce extracellular enzymes. More research is needed to investigate whether soil C is spatially occluded from

degradative enzymes under integrated field management practices (Allison and Jastrow, 2006).

Soil aggregation

Soil structure is a primary factor controlling biogeochemical processes that contribute to soil health and fertility (Yang and Wander, 1998). Thus, long-term agroecosystem management emphasizes sustainable practices that lessen degradation disturbances and advance soil structure. Soil aggregates serve as a basic, physical structural unit and indicate resistance to disruptive forces associated with conventional tillage management and water or wind erosion in agroecosystems (Balesdent et al., 2000; Kemper and Rosenau, 1986). The size and abundance of soil aggregates influence root growth and crop yield, soil porosity, water movement and retention, and greenhouse gas exchanges (Kasper et al., 2009). Thus, by influencing water, air, heat, and nutrient transfer rates, aggregate sizes directly affect soil function.

The processes that form and stabilize soil aggregates are not the same, and often occur as a function of abiotic and biotic factors. In loam and clay textured soils, structure and aggregate formation is mostly associated with abiotic processes such as shrink-swell capacity and cohesion of colloidal particles during wetting and drying cycles. In more coarse textured soil, aggregate formation is less associated with these abiotic influences due to low or minimal shrink-swell capacities, so biotic influences like plant root systems and macrofauna presence are more important (Oades, 1992). Following formation, biotic stabilization of soil microaggregates is associated with microorganisms and occurs via a complex array of mechanisms that can include

adsorption, physical entanglement and envelopment, and cementation by excreted mucilaginous products (Hillel et al., 1998). Stabilization by physical entanglement occurs with the growth of fungal hyphae while adsorption involves the attachment of microbes to cell surfaces via cell surface hydrophobicity and deposit organic polymers that can encapsulate around aggregates (Hillel, 1998; Krasowska and Sigler, 2014; Oades, 1992). Common microbially-produced mucilaginous products that bind soil aggregates include polysaccharides, hemicelluloses, calcium carbonate, and iron and aluminum oxides; these materials attach to clay surfaces by cation bridges, hydrogen bonding, van der Waals forces, and anion adsorption mechanisms (Hillel et al., 1998).

Aggregates in cultivated land form due to OM content, crop roots, and management practices (Balesdent, 2000; Jastrow, 1996; Tisdall and Oades, 1982; Elliott, 1986). They are grouped by size where macroaggregate (> 0.25 mm) stability is associated with agricultural practices while microaggregates (< 0.25 mm) are strongly stabilized by clay associations with organic material (Tisdall and Oades, 1982). Macroaggregates may be further divided into sub-classes that include: large macroaggregates (> 2 mm), medium macroaggregates (1-2 mm), and small macroaggregates (0.25-1 mm) (Tisdall and Oades, 1982; Bach and Hofmockel, 2014). Aggregate hierarchy is a concept widely accepted in literature and explains that macroaggregates are collections of microaggregates held together by temporary (i.e. roots and hyphae) and transient (i.e. polysaccharies) organic binding agents (Bronick and Lal, 2005; Tisdall and Oades, 1982).

Studies show that greater aggregate stability is achieved when CA practices are

implemented, and also promote greater sustainability due to decreased losses of soil C and nutrients (Bronick and Lal, 2005; Castro Filho et al., 2002; Linquist et al., 1997; Mikha and Rice, 2004). Intensive tillage systems have been found to reduce aggregate stability, and aggregate C and N content (Kasper et al., 2009; Spiegel and Mentler, 2003). Macroaggregates are able to form and degrade more slowly in no-tillage systems compared to conventional tillage, making the microaggregates collected within the macroaggregates more stable and able to effectively stabilize and sequester soil C for a relatively longer period of time (Six et al., 2000). N applications to agricultural systems have resulted in variable effects on soil aggregates that are dependent on certain conditions. Chemical fertilizers often indirectly improve soil structure in comparison to unfertilized soils due to increased plant reside return, greater SOC accumulation and microbial activity (Munkholm et al., 2002). Although research also shows that ammonium fertilizers may decrease SOC, reduce microbial biomass, and decrease aggregate formation due to clay dispersion by NH_4^+ (Bronick and Lal, 2005; Haynes and Naidu, 1998). However, this adverse effect is noted to diminish as NH_4^+ nitrifies to NO_3^- (Haynes and Naidu, 1998). The influence of N fertilization on soil aggregation requires additional data to better understand this fine scale process.

CHAPTER II OBJECTIVES, APPROACH, AND RATIONALE

Objectives

The objective of this study was to examine soil biological and physical properties and their effect on microbial C-dynamics in a continuous cotton system treated with various conservation agriculture practices in West Tennessee. The stability of SOC, microbial functional diversity, and the aggregate scale effects on biological activity were determined by a comparative analysis of a long-term management experiment which included a gradient of nitrogen fertilizing regimes, cover cropping strategies, and conventional and no-tillage systems.

Specific objectives and hypotheses

1. Determine the effect of CA practices on stability of SOC and microbial activity during the cotton-growing season.
   - $H_{1.1}$ High N rate, tillage, and cover cropping will increase rapid cycling (active) carbon relative to more slowly cycling (recalcitrant) C compared to controls.
   - $H_{1.2}$ The slow pool will generate a much larger pool of respired C over a longer time period relative to the active pool.
   - $H_{1.3}$ Microbial functional [enzyme] activity will be greater in cover cropping and N rate treatments compared to controls.
2. Assess the soil aggregate-scale effects on biological activity

$H_{2.1}$ No-tilled plots will have a broader range of soil aggregate size fractions compared to tilled plots.

$H_{2.2}$ There will be greater C respiration from soil microaggregates compared to larger soil macroaggregates.

$H_{2.3}$ Greater potential enzyme activity will occur within soil macroaggregates compared to soil microaggregates.

Approach

CO_2 respiration rates from long-term incubations were measured and the data fit to standard models of SOC decomposition to calculate whether the size and turnover time of labile, intermediate, and recalcitrant pools of SOC are affected by the establishment of long-term CA practices on cotton production. To further understand which C substrates (varying in chemical lability and recalcitrance) are more or less utilized by the microbial community, potential activity of extracellular enzymes was measured on bulk soil samples with a fluorometric assay. A physical fractionation procedure was then used to determine if CA practices, like no-tillage, would result in more macroaggregate mass compared to conventional tillage. Aggregate-scale effects on biological activity were the final component of this study. Extracellular enzyme assays of the aggregate size fractions investigated whether soil C substrates are spatially isolated from degradative enzyme catalysis, and a short-term incubation measuring respired C was conducted to determine if a physical protection barrier exists for C, and to help identify where soil C is most vulnerable.

Rationale

Incubation studies measuring soil respiration provide a great opportunity to understand mineralization kinetics of different fractions of C-pools because each pool contributes to the total soil CO_2 efflux differently, with substrate availability to microbes shifting during the incubation course (Schädel et al., 2013). Most studies show that CA practices, like no-tillage systems, increase microbial activity by having more SOM for microorganisms to utilize as energy (Six and Jastrow, 2002). Other CA practices, like cover cropping, alter the C:N of OM inputs to the soil where more, or less substrates are available to microbes. Additional N fertilizer rate management can further aid or limit microbial activity by increasing SOC, but simultaneously limiting communities to labile C sources and encouraging faster growth rates less able to breakdown recalcitrant C forms (Ramirez et al., 2012). The microbial functional diversity (i.e. substrate utilization) in response to the interactions of different CA practices can play a critical role in determining rates of C loss from soil (Six et al., 2006). Microbial activity has a quick and sensitive response to changes in agricultural management practices, and thus soil enzyme activities are indicative of rapid nutrient cycling and transformations (Dick et al., 1996; Gianfreda et al., 2005). This study used bulk soil enzyme assays to provide an index of substrate utilization and demand, and to provide insight to differences in microbial functional diversity.

In conjunction with these biological processes, the effects of soil physical properties and structure on soil C stability were evaluated. Soil intra-aggregate pore spaces can act as a physical constraint to the function of microbial communities

(McLauchlan and Hobbie, 2004); movements of water and gas through these pore spaces shape and determine the habitat through which microbes utilize substrates and colonize (Bach and Hofmockel, 2014). This final portion examined SOC stability and microbial functional diversity within soil aggregate fractions to tell us if there are any positive or negative feedbacks associated with both soil biological and physical components. My research provides distinctive data to represent the effects of long-term (31 years) conservation agriculture on C storage, stability, and utilization.

CHAPTER III MATERIALS AND METHODS

Study Design

Site description and experimental design

The study site was located at the West Tennessee Research and Education Center (WTREC) in Jackson, TN. Research was conducted under continuous cotton (*Gossypium hirsutum*) plots with applications of CA practices implemented since 1981. The soil is classified as a Lexington silt loam *(fine-silty, mixed, thermic, Ultic Hapludalf)*, well drained, with a 0 to 2% slope. The WTREC site has been compiling climate data since 1981; high and low mean average temperatures were 26.8°C and -0.72°C, respectively, and mean total precipitation was 10.9 cm yr^{-1}.

The experimental design is a randomized complete block (RCB) design with split-split plots and includes four replications. This design allowed to investigate interactions between all treatments, and the blocking accounted for any variations, like soil texture for example, within the soil. Experimental CA treatments in this study were originally chosen to determine whether cotton yield was affected by tillage systems under different cover cropping with various nitrogen fertilizing rates. The whole plot was represented by nitrogen rates (Factor A) with four levels: 0, 34, 67, 101 N kg ha^{-1}. Cover cropping was the split-plot (Factor B) with three levels: hairy vetch (*Vicia villosa*), winter wheat (*Triticum aestivum*), and no cover (control). Tillage was the split-split plot (Factor C) with two levels: conventional tillage and no-tillage plots. Sampling was collected from 96 of the total 128 plots. Each plot was 8 x 12m in size.

Soil sampling

Soil samples were collected in July of 2013, during the cotton flowering stage, using stainless steel probes (2.5 cm in diameter) to a depth of 7.5 cm. Composite samples of each plot were made by homogenizing (~30) random grab samples within each plot located 10 to 15 cm from the crop root zone. Plot to plot contamination was avoided by disinfecting sampling gear (probes, buckets, gloves) with 70% ethanol. Composite samples were stored and sealed in plastic bags (Whirl-pak™, Nasco) and placed in a cooler under dry ice during transport back to the laboratory where they were then stored in 4°C. A subset of the homogenized composite samples was stored and sealed in plastic bags (Whirl-pak™, Nasco) bags, and then were immediately flash frozen in the field with liquid nitrogen. Once in the laboratory, the samples were placed in a freezer at -80°C for future extracellular enzyme analysis.

A second sampling trip was made the following year to collect soil core samples during the cotton flowering stage in July of 2014. Metal cores (7.62 cm diameter and 7.62 cm height) were used to collect intact core samples to a depth of 7.5-cm. Two sample cores per plot were taken randomly, and metal cores were kept sterile with 70% ethanol between sampling. Soil cores were gently removed from the metal cores, and placed in heavy-paper lined sample bags (Fisherbrand™ 6.4 x 24 x 8.6 cm) to keep their form during transportation to the laboratory. The samples were then air-dried for future aggregate fractionation analysis.

Moisture Content

Gravimetric moisture content was measured by weighing (10 g) soil sample into aluminum weigh dishes, and then transferred to an oven heated at 105°C for a 48 h time period. Samples were removed, cooled, and weighed to determine the dry soil weight. Soil moisture content was calculated using equation 1:

$$\frac{Weight_{wet\ soil} - Weight_{dry\ soil}}{Weight_{dry\ soil}}$$

Equation 1. Soil Moisture Content

Microbial respiration

Soil microbial respiration was measured from batch mesocosms (0.5 L Ball® Mason Jar) fitted with butyl rubber septa (Kimble™ Stoppers for Headspace Vials) using a CO_2 infrared gas analyzer (IRGA), (LiCOR-820, LiCor Inc., Lincoln NE). Each 50 g field moist soil sample was placed in a mason jar. Septa ports were constructed at the top of each aluminum seal in order to draw gas samples with a syringe (0.5 mL). The jars were stored in a dark incubator at 25°C for a 602-day period. Gravimetric soil moisture content was measured prior to respiration measurements and was maintained throughout the experiment by adding sterile water, drop-wise, in a spiral pattern to balance moisture losses via evaporation.

Initial IRGA CO_2 measurements were taken three days per week for two weeks, and then were taken twice per week for two weeks. After this period measurements were only taken once per week for two weeks, and then monthly CO_2 IRGA measurements were taken for the rest of the incubation period. After each monthly CO_2 respiration measurement, the mason jars were fanned with ambient air to obtain a ‘zero’

starting point and to avoid anaerobic conditions. This continued for 602 days and cumulative amount of C respired (unit defined as mg C g^{-1} dry soil) was determined. In addition, amount of different SOC pools were determined by exponential decay modeling. Equations 2, 3, and 4 were used to determine SOC pool sizes based from a first order decay function:

$$C_{tot} = C_1(e^{-k_1 t}) \quad \text{Equation 2. Single Pool Model}$$

$$C_{tot} = C_1(e^{-k_1 t}) + C_2(e^{-k_2 t}) \quad \text{Equation 3. Double Pool Model}$$

$$C_{tot} = C_1(e^{-k_1 t}) + C_2(e^{-k_2 t}) + C_3(e^{-k_3 t}) \quad \text{Equation 4. Triple Pool Model}$$

Where:

C_{tot} is total carbon, and C_1, C_2, and C_3 represent the labile, intermediate, and recalcitrant C pools being measured. The constant, *k*, determines the rate of mineralization; it encompasses all the physical and biological factors that control microbial decomposition. The variable, *t*, serves as the time period at which each pool is measured. The parameters were estimated using nonlinear regression (SigmaPlot 13.0).

Soil carbon concentration

Soil C concentration was examined by subtracting the accumulated respired C (mg g^{-1} dry soil) after 602 incubation days from bulk soil C (mg g^{-1}) that had been previously determined by Mbuthia et al. (2015) (Table 1) in the same samples collected in 2013. Soil bulk density, also determined by Mbuthia et al. (2015), was used to define soil C concentration units as g m^{-2} dry soil.

$$\text{Total soil C} - \text{Respired C} = \text{Soil C concentration} \qquad \text{Equation 5}$$

Whole soil microplate extracellular enzyme assay

To determine the microbial functional diversity of whole soil, extracellular enzyme assays using fluorescence were conducted by following protocol and calculations by Steinweg and McMahon (2012). Enzymatic activity was determined by the release of a fluorescent label bound to substrates during an enzyme-catalyzed reaction. Higher fluorescence was indicative of more substrate degradation by the microbial community while lower fluorescence was indicative of less substrate degradation (Steinweg and McMahon, 2012). A mean value of soil pH for the experimental plots (mean equal to 5.5) was used from Mbuthia et al. (2015) (Table A 1). For extracellular enzyme analysis, sodium acetate buffer solution pH was adjusted to match soil pH using glacial acetic acid. Gravimetric soil moisture content was measured prior to the enzyme analysis for use in data calculations. The synthetic substrates used in this experiment were bound with one of two fluorescent indicators: 4-methylumbelliferone (MUB) and 7-amino-4-methylcoumarin (MUC) (Sigma-Aldrich Co.). Table 2 lists the substrates assayed for in this experiment along with their general function. A set of 18 samples with 2.75 g soil was blended into a soil slurry and injected with the synthetic substrates within a one-hour period, in which a standard curve of MUB and MUC were prepared for each sample separately. A negative control was prepared for each sample with sodium acetate buffer solution. After three hours of incubation at 25°C, samples were centrifuged (Thermo Scientific Sorvall STR16 Centrifuge) for three minutes at 1500 rpm

and transferred from deep 96-well plates (2 ml Deep 96-Well PlateOne Polypropylene Plate) to flat-bottomed black 96-well plates (Corning™ 96-Well Polypropylene Assay Plates). Fluorescence was measured using a Synergy HT microplate reader (BioTek, Winooski, VT) at the following wavelengths: excitation 365 nm, emission 450 nm. Activity from each sample was performed in duplicate, and units of enzyme activity are defined as the nanomole (nmol) of substrate hydrolyzed at 25°C per gram dry soil per hour (nmol gram^{-1} dry soil h^{-1}).

Table 2. Fluorescent labeled substrates used in extracellular enzyme assays and their general function (Steinweg & McMahon, 2012).

FLUOROMETRIC METHODS		
Enzyme Assayed	**Substrate**	**General Function**
β-D-Cellubiosidase (CB)	4-MUB-β-D-Cellubiosidase	Cellulose degradation
α-Glucosidase (AG)	4-MUB-α-D-Glucopyranoside	Sugar degradation
β-Glucosidase (BG)	4-MUB-β-D-Glucopyranoside	Sugar degradation
Leucine aminopeptidase (LAP)	L-leucine-7-amido-4-methylcoumarin hydrochloride	Protein degradation
N-actyl-β-D-Glucosaminidase (NAG)	4-MUB-N-actyl-β-D-Glucosaminide	Chitin and peptidoglycan degradation
Phosphatase (PHOS)	4-MUB Phosphate	Phosphorus mineralization
β-Xylosidase (XYL)	4-MUB-β-D-Xylopyranoside	Hemicellulose degradation

Aggregate size fractionation

A dry-sieving approach was used for aggregate isolation by placing 200 g of air-dried soil (sample) on a stack of sieves that included 2, 1, and 0.25 mm mesh openings with one bottom catch-sieve to determine an aggregates size less than 0.25 mm. The sieves were shaken both horizontally and vertically with a sieve shaker (CSC Scientific, catalog no. 18480) for 3 minutes on a 5 of 10-power setting (50 to 60-hz). Each sieve size was then weighed to calculate the mass of each aggregate size (unit defined as g

g^{-1} dry soil). A dry-sieving method was chosen over a traditional, wet-sieving method due to results from Bach and Hofmockel (2014) that explain wet-sieving overestimated potential extracellular enzyme rates by 100% of activity. This indicated that additional activity was generated during the wet-sieving process and simulated a rapid rewetting event; a dry-sieving approach was chosen to more closely mimic *in situ* interactions between soil enzymes and the substrates.

Microplate extracellular enzyme essay of aggregate fractions

A subset of the resulting dry aggregate fractions were removed from sieves, stored and sealed in plastic bags (Whirl-pak™, Nasco), and then frozen (-20°C) for future extracellular enzyme analysis (Wang et al., 2012; Bach and Hofmockel, 2014). The method and calculations from Steinweg and McMahon (2012) were repeated to measure enzyme activity within the different soil aggregate size fractions measured via dry aggregate distribution. A set of 36 samples with 2.75 g soil was blended into a soil slurry and injected with the synthetic substrates within a one-hour period, in which a standard curve of MUB and MUC (Sigma-Aldrich Co.) were prepared for each sample separately. A negative control was prepared for each sample with sodium acetate buffer solution. After three hours of incubation at 25°C, samples were centrifuged (Thermo Scientific Sorvall STR16 Centrifuge) for three minutes at 1500 rpm and transferred from deep 96-well plates (2 ml Deep 96-Well PlateOne Polypropylene Plate) to flat-bottomed black 96-well plates (Corning™ 96-Well Polypropylene Assay Plate). Fluorescence was measured using a Synergy HT microplate reader (BioTek, Winooski, VT) at the following wavelengths: excitation 365 nm, emission 450 nm. Replications were not

made in this analysis due to time constraints from such a large sample size (n = 384). It is also important to note that this extracellular enzyme analysis was performed on *dry* aggregate fractions, and thus the values cannot be directly compared to whole (bulk) soil samples performed from thawed, *field moist* samples. The analysis was specific to activity within dry aggregate fraction mass, in which the data were normalized for the mass of soil in each fraction. Units of enzyme activity are defined as the nanomole (nmol) of substrate hydrolyzed at 25°C per gram dry soil per hour (nmol $gram^{-1}$ dry soil h^{-1}).

Microbial respiration of aggregate fractions

The other subset of the resulting dry aggregate fractions were sealed in plastic bags (Whirl-pak™, Nasco) and left in dry storage. These dry-aggregate samples were each weighed to 5 g and placed in 60-mL Wheaton™ serum bottle vials. Prior to the dry storage of the aggregate fractions, gravimetric soil moisture content was measured. For respiration measurements, each sample was rewetted to 15% of the moisture content by adding sterile water, drop-wise, in a spiral pattern to induce mineralization. Butyl rubber septa ports (Kimble™ Stoppers for Headspace Vials) were constructed at the top of each serum bottle and were sealed with 20-mm crimp seals by a Wheaton ® E-Z Crimper™. Initial gas samples of CO_2 were drawn with a syringe at 0.5-mL from the headspace of each serum bottle vial and injected into an IRGA (LiCOR-820, LiCor Inc., Lincoln NE) to measure CO_2 and capture the 'burst' of microbial activity induced by rewetting the soil samples. The serum bottles were stored in a dark incubator at 25°C

for a 24-h period, and then gas samples were drawn again with a syringe at 0.5-mL and injected into a CO_2 IRGA (LiCOR-820, LiCor Inc., Lincoln NE). A short-term, 24-h incubation period was chosen due to time constraints from such a large sample size (n = 384).

Data analysis

The effects of long-term CA management on soil C stability in biological processes and physical structure were evaluated by analysis of variance (ANOVA) based from the Generalized Linear Mixed Model (GLIMMix). Fisher's protected least significant difference (LSD) in SAS 9.3 (SAS Institute, Cary, NC, 2012) was used to determine significant differences among treatment means at $P < 0.05$ for aggregate fractionation, and for whole soil- and aggregate-respired C analyses. Mean significant difference for whole soil- and aggregate-extracellular enzyme activities was considered significant at $P < 0.1$. Data were log-transformed where necessary to improve normality.

CHAPTER IV RESULTS

Soil Organic Carbon Stability

Cumulative data of respired SOC after incubation for 602 days were fit to a nonlinear regression, double-pool exponential model (Equation 3) of SOC decomposition where the active (easily decomposable C) and slow degrading C-pool sizes were estimated by best fit (significance at $P < 0.05$) of respired C over time. Similar to other soil C-pooling studies, a triple pool model did not improve the total modeled respiration over the incubation period (Collins et al., 2000; Schädel et al., 2013). Under reduced N fertilization (34N kg ha^{-1}), no-tillage treatment had greater respired C in the active pool compared to conventional tillage; means were 0.98±0.09 and 0.72±0.11 mg respired C g^{-1} dry soil, respectively (Figure 3A, Table A 2). Conversely, under the highest N fertilizer rate (101N kg ha^{-1}), no-tillage had lower respired C in the active pool compared to conventional tillage; means equal to 0.67±0.16 and 0.96±0.11 mg respired C g^{-1} dry soil, respectively (Figure 3A, Table A 2). The slow SOC pool showed the greatest respired C under the highest N fertilization rate (101N kg ha^{-1}) compared to all other fertilizing rates (67, 34 or 0N kg ha^{-1}) at 4.07±0.50 mg respired C g^{-1} dry soil (Figure 4A, Table A 2). No-tillage treatment also had greater C respired in the slow pool at 3.55±0.59 mg respired C g^{-1} dry soil compared to conventional tillage treatment means at 2.72±0.37 mg respired C g^{-1} dry soil (Figure 4B, Table A 2). The combined effects of N fertilization and tillage treatment showed similar differences among respired C as seen in the active SOC pool; under reduced N

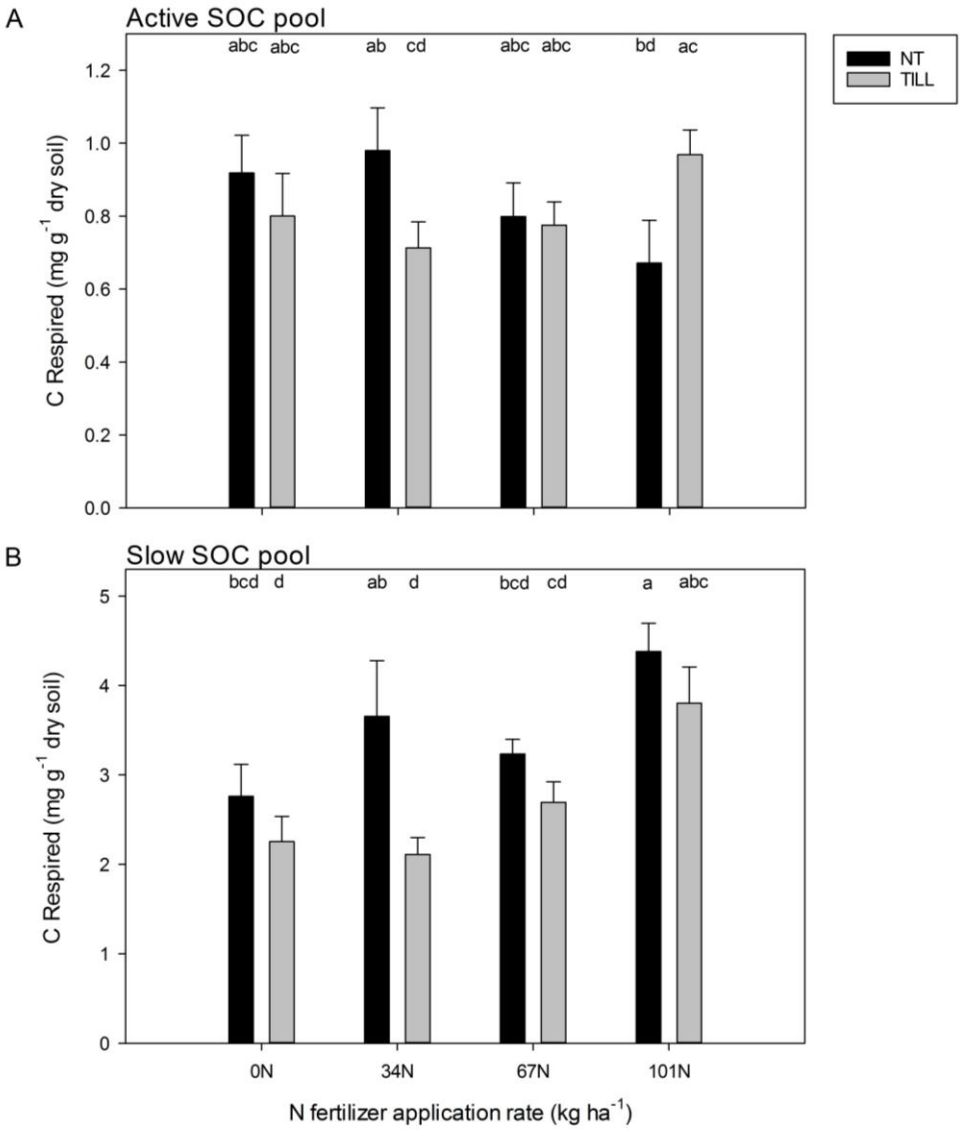


Figure 3. Active and slow SOC pools under N fertilizer application separated by no-tillage (NT) and conventional tillage (TILL) treatments. (A) Mean respired C in the active pool. (B) Mean respired C in the slow pool. Different letters denote statistically different means at $P < 0.05$ and error bars represent standard error of the mean.

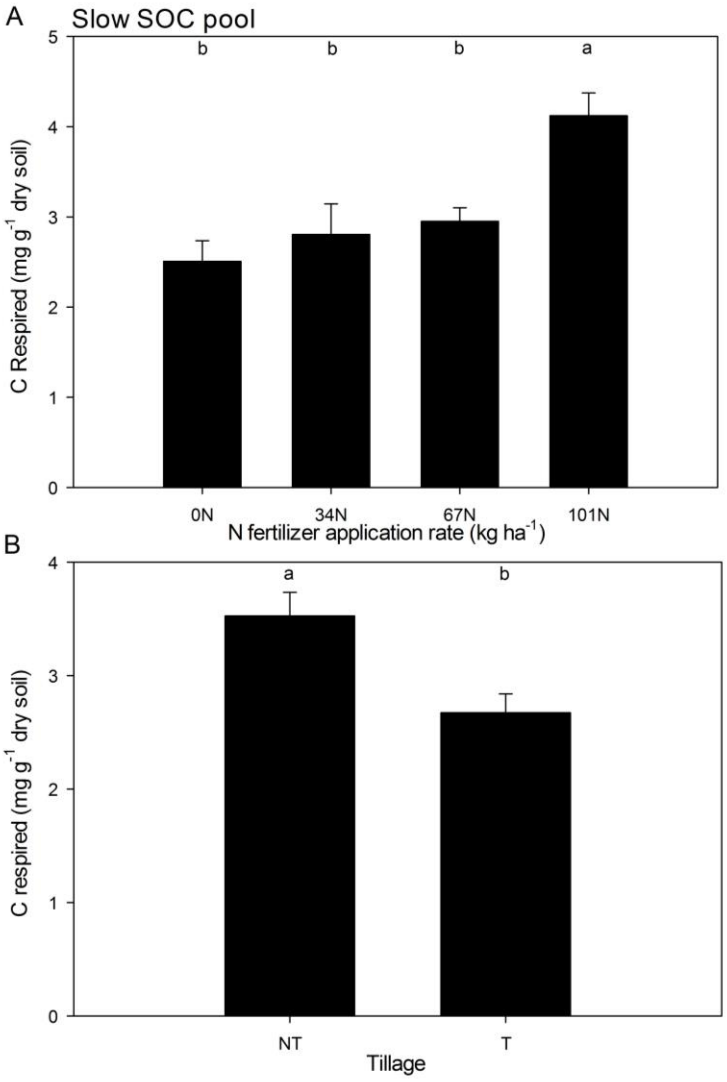


Figure 4. Slow SOC pool under N fertilizer and tillage treatments. (A) Mean respired C of the slow pool under N fertilizer application. (B) Mean respired C of the slow pool under no-tillage (NT) and conventional tillage (T). Different letters denote statistically different means at $P < 0.05$ and error bars represent standard error of the mean.

fertilization (34 kg ha^{-1}), no-tillage resulted in greater C respired in the slow pool compared to conventional tillage (means equal to 3.83±0.95 and 2.19±0.23 mg respired C g^{-1} dry soil, respectively) (Figure 3B, Table A 2). A separate statistical analysis compared N fertilizer, cover cropping, and tillage treatments between the active and slow SOC pools. The slow pool showed a 383% increase in respired C under high N-rates (Figure 5A), and a 312% increase in respired C under no-tillage (Figure 5B) compared to the active pool. The fast turning over, active C-pool, did not show any differences among the treatments, but the increase of respired C in the more slowly degrading C-pool was effected by N fertilizer and tillage, which were the two treatments that also showed greater bulk C storage from previous research (Mbuthia et al., 2015). There were not any significant effects of respired C under cover cropping treatments for either the active or slow SOC pool.

Soil Carbon Concentration

To examine the remaining soil C concentrated over the 602-day incubation period cumulative respired C was subtracted from bulk soil C data previously collected by Mbuthia et al. (2015) (Table 1). The no-tillage treatment showed 20% greater SOC concentration compared to conventional tillage treatment (means equal to 1117.2±40.8 and 933.0±28.2 g C m^{-2} dry soil, respectively) (Figure 6B, Table A 3). Zero to high N fertilizing (0 to 101N kg ha^{-1}) resulted in 21% greater SOC concentration with means ranging from 949.5±46.1 to 1145.4±56.6 g C m^{-2} dry soil (Figure 6A, Table A 3).

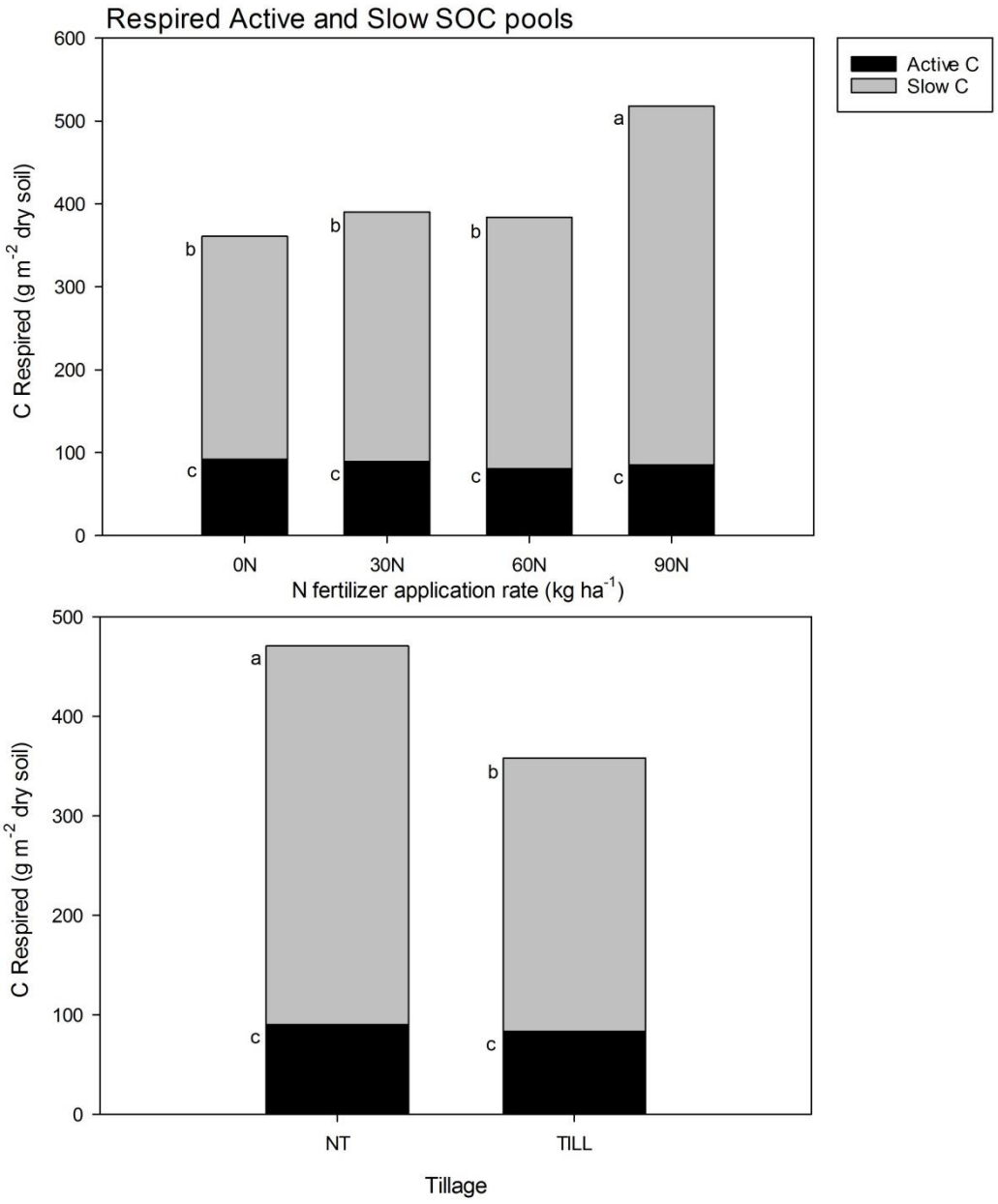


Figure 5. Size comparison of active and slow SOC pools. (A) Mean respired C of active and slow pools under N fertilizer application. (B) Mean respired C of active and slow pools under no-tillage (NT) and conventional tillage (T). Different letters denote statistically different means at $P < 0.05$ and error bars represent standard error of the mean.

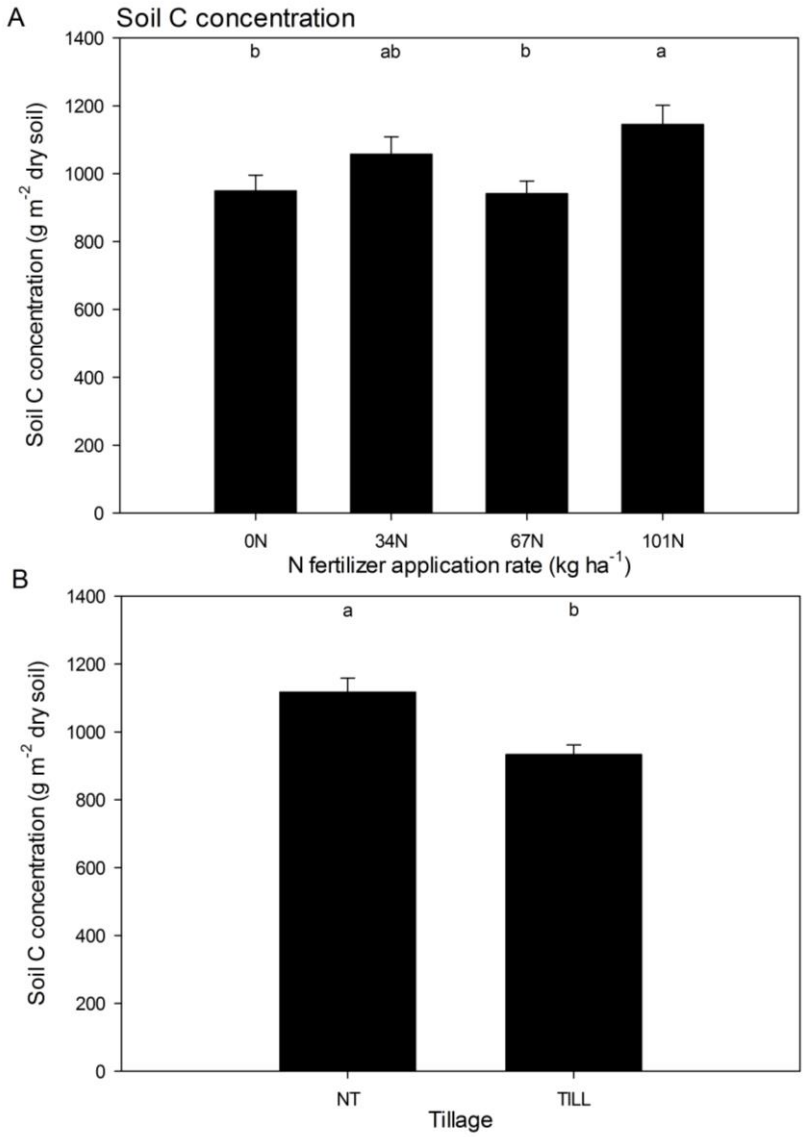


Figure 6. Amount of soil C concentrated under N fertilizer application and tillage treatments. (A) Mean C concentration under N fertilizer application. (B) Mean C concentration under no-tillage (NT) and conventional tillage (TILL). Different letters denote statistically different means at $P < 0.05$ and error bars represent standard error of the mean.

Bulk Soil Extracellular Enzyme Activity

Microplate assays measuring extracellular enzyme activity (nmol g^{-1} dry soil hr^{-1}) were used to determine how microbes utilize various substrates within the bulk soil. Microbial functional differences occurred under the combination of cover crop and tillage treatments, where the following extracellular enzymes showed a pattern in potential activities: sugar degrading β-glucosidase (BG), cellulose degrading β-D-cellubiosidase (CB), protein degrading leucine aminopeptidase (LAP), and P acquiring Phosphatase (PHOS). BG, LAP, and PHOS potential activities were 10%, 21%, and 11% greater under conventional tillage combined with wheat cover crops compared to no-tillage with wheat cover (Figure 7A, D, and B, Table A 4). Conversely, CB activity was 18% greater under no-tillage combined with vetch cover crops compared to conventional tillage with vetch cover (Figure 7E, Table A 4).

The BG, CB, LAP, and PHOS enzyme activities varied with cover crop treatment, while N fertilizing effects were observed for starch degrading α-glucosidase (AG), and with chitin and peptidoglycan degrading N-acetyl-β-glucosaminidase (NAG) enzyme activity. Mean AG activity ranged from 4.63±0.52 to 9.78±1.0 nmol g^{-1} dry soil, with greater activity under 34N and 101N kg ha^{-1} compared to 0N and 60N kg ha^{-1} (Figure 8A, Table A 4). NAG enzyme activity means ranged from 143.60±0.13 to 193.42±0.12 nmol g^{-1} dry soil, and were lowest under N fertilizing controls (0N kg ha^{-1}) compared to all other N fertilizer inputs (Figure 8B, Table A 4).

To investigate large-scale patterns in agroecosystem C decomposition and nutrient cycling, the potential activities of the seven enzymes assayed were categorized

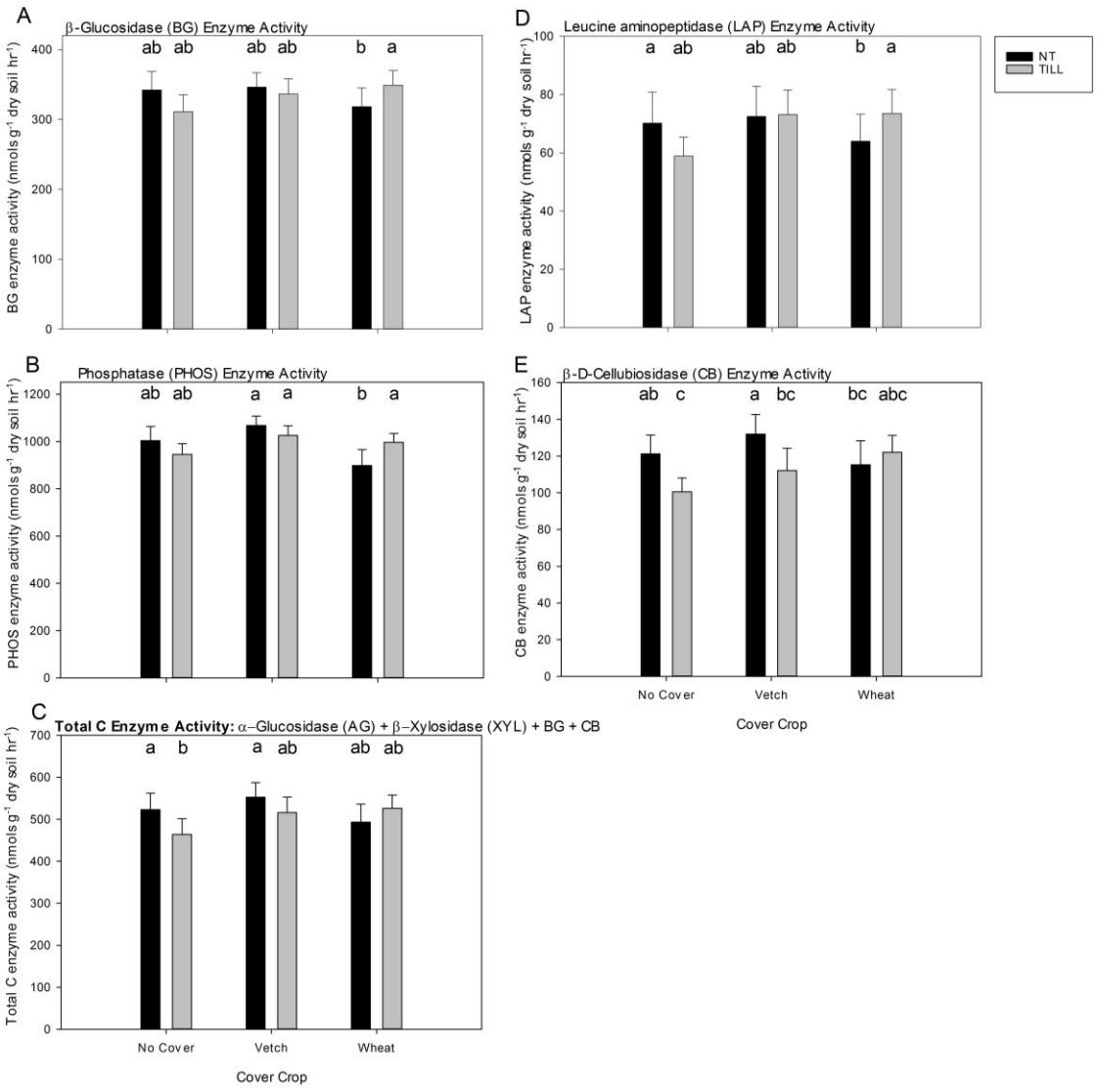


Figure 7. Potential extracellular enzyme activities under cover cropping separated by no-tillage (NT) and conventional tillage (TILL) treatments. (A) BG activity, (B) PHOS activity, and (D) LAP activity are greater under wheat cover with TILL compared to NT. (E) CB potential activity is greater under vetch cover with NT compared to TILL. (C) Total C potential activity is greater under no cover with NT compared to TILL. Different letters denote statistically different means at $P < 0.1$ and error bars represent standard error of the mean.

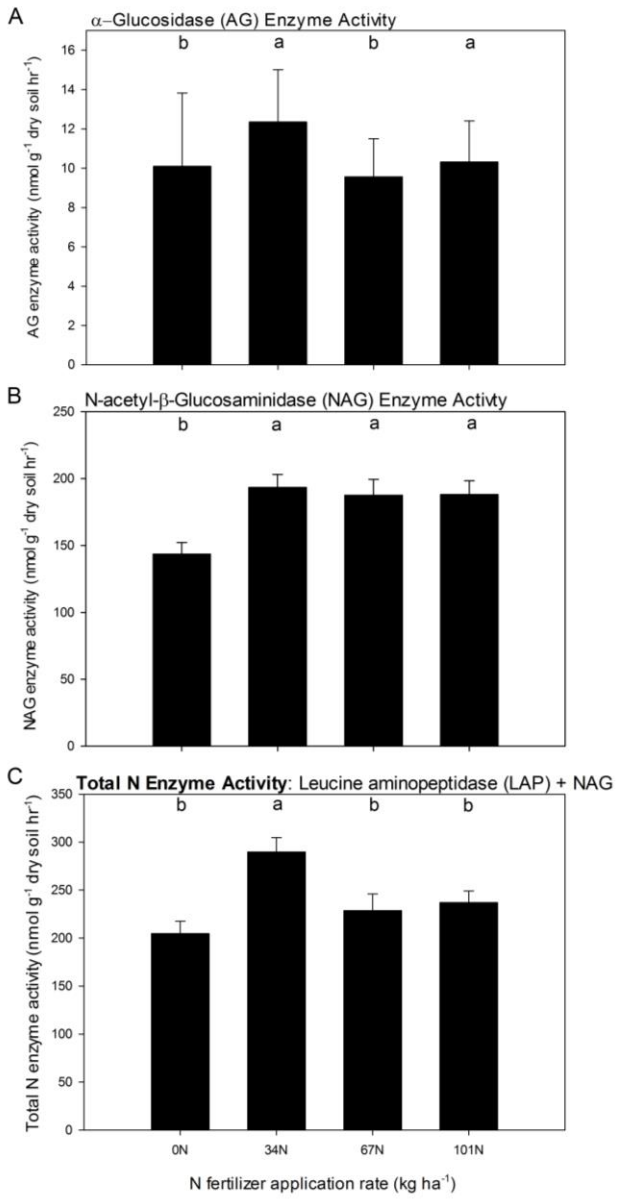


Figure 8. Potential extracellular enzyme activities under N fertilizing treatment. (A) AG activity is greatest under 34N and 101N kg ha^{-1}. (B) NAG activity is less under N fertilizer controls (0N kg ha^{-1}). (C) Total N activity is greatest under 34N kg ha^{-1} fertilizer. Different letters denote statistically different means at $P < 0.1$ and error bars represent standard error of the mean.

into broadly defined groups based on major biogeochemical functions: Total C (BG+AG+XYL+CB), N (NAG+LAP), and P (PHOS) acquisition enzymes. Tillage and cover crop treatments did not show clear patterns for total C, N, or P nutrient acquisition, and N fertilization only showed differences in total N enzyme activity. Mean total N enzyme activity ranged from 204.7±27.6 to 289.8±35.9 nmol g^{-1} dry soil hr^{-1}, with the greatest activity resulting from reduced N fertilizer treatment (34 kg ha^{-1}) (Figure 8C, Table A 4). Interactive effects were observed for total C and P enzyme activity under cover cropping by tillage treatment. In the no cover treatments, total C activity was 13% greater under no-tillage compared to conventional tillage (mean of 523.15±73.51 and 464.02±69.56 nmol g^{-1} dry soil, respectively) (Figure 7C, Table A 4). PHOS enzyme activity alone represents P mineralization, and showed 11% greater activity under wheat cover with conventional tillage compared to no-tillage with wheat cover (Figure 7B, Table A 4).

Aggregate Size Distribution

The distribution of dry aggregate size fractions (g g^{-1} dry soil) was skewed toward large macroaggregates (> 2 mm) in tillage, cover crop, and N fertilizing treatments (Table 3). Vetch and wheat cover crops under no-tillage had 12% and 23% more macroaggregates compared to conventional tillage with vetch and wheat cover (Figure 9A). The mean mass of large macroaggregates under N fertilizing treatments ranged from 116.6±8.84 to 140.0±8.15 g g^{-1} dry soil, with greater mass under high N-rate application (101N kg ha^{-1}) compared to reduced N (34N kg ha^{-1}) and N fertilizing controls (0N kg ha^{-1}) (Table 3). The mean mass of medium (1-2 mm) and small (0.25-1

Table 3. N fertilizing rate (N-rate), cover crop, and tillage effects on soil aggregate (dry) distribution. Column numbers are means (g g^{-1} dry soil) of the corresponding treatment effect in four aggregate size classes: large macroaggregates (> 2 mm), medium macroaggregates (1-2 mm), small macroaggregates (0.25-1 mm), and microaggregates (< 0.25 mm). Numbers inside parentheses are (±1) standard error of the mean, and lettering denotes statistical difference at $P < 0.05$. Bolded ANOVA numbers show statistical significance at $P < 0.05$.

	Soil Aggregate Size Distribution (g g^{-1} dry soil)			
Treatment factors	Large Macroaggregate > 2 mm	Medium Macroaggregate 1-2 mm	Small Macroaggregate 0.25-1 mm	Microaggregate < 0.25 mm
N-rate (kg ha^{-1})				
0N	0.61(0.03)b	0.094(0.009)b	0.119(0.017)ab	0.165(0.021)a
34N	0.58(0.04)b	0.108(0.013)a	0.142(0.022)a	0.161(0.018)a
67N	0.65(0.03)ab	0.085(0.012)b	0.107(0.017)b	0.150(0.021)a
101N	0.70(0.04)a	0.080(0.010)b	0.093(0.015)b	0.116(0.019)a
Cover Crop				
No cover	0.61(0.045)b	0.097(0.014)a	0.128(0.021)a	0.157(0.022)a
Wheat	0.63(0.039)ab	0.088(0.012)a	0.114(0.019)a	0.155(0.018)a
Vetch	0.668(0.035)a	0.091(0.008)a	0.104(0.014)a	0.132(0.019)b
Tillage				
No-tillage	0.675(0.043)a	0.086(0.012)b	0.105(0.018)b	0.129(0.019)b
Conventional tillage	0.599(0.037)b	0.098(0.010)a	0.125(0.018)a	0.167(0.020)a
Sources of Variation		ANOVA (LSD protected, $P < 0.05$)		
N-rate	**0.0275**	**0.0014**	**0.0173**	0.0626
Cover crop	**0.0129**	0.3524	0.0533	**0.0318**
Tillage	**< 0.0001**	**0.0178**	**0.0137**	**< 0.0001**
N-rate*Cover crop	0.6181	0.4011	0.8037	0.2688
N-rate*Tillage	0.9602	0.7704	0.5501	0.5141
Cover crop*Tillage	**0.0265**	0.4163	0.0701	**0.0306**
N-rate*Cover crop*Tillage	0.1503	0.2786	0.2087	0.4993

mm) macroaggregates was greater under conventional tillage by 14% and 19% compared to no-tillage treatments (Table 3). Reduced N fertilizing (34N kg ha^{-1}) means for medium and small macroaggregates were higher than all other N-rates at 21.62±2.55 and 28.39±4.45 g g^{-1} dry soil, respectively (Table 3). The mass of microaggregates (< 0.25 mm) was 54% greater under conventional tillage paired with wheat cover compared to no-tillage with wheat cover (Figure 9B).

Extracellular Enzyme Assay of Aggregate Fractions

Extracellular enzyme activity within dry aggregates was normalized for the mass of soil in each fraction. Activity (nmol h^{-1} g^{-1} dry aggregate) was variable between most size fractions and experimental treatments (Tables A 5-8). BG (sugar degrading), LAP (protein degrading), and PHOS (P acquisition) enzyme activities were the only substrates to show similar treatment utilization patterns across multiple aggregate sizes. BG enzyme activity was greater under high N fertilizer rates (101N kg ha^{-1}) combined with conventional tillage compared to high N-rates with no-tillage treatments in large (> 2 mm), medium (1-2 mm), and small macroaggregate (0.25-1 mm) size fractions by 60%, 108%, and 122%, respectively (Figure 10, Tables A 5-7). LAP enzyme activity was greater under conventional tillage combined with no-cover crop compared to no-tillage paired with no-cover in large and medium macroaggregate sizes by 29% and 50%, respectively (Figure 11A and B, Tables A 5-6). PHOS enzyme activity was greater in small macroaggregates (0.25-1 mm) and microaggregates (< 0.25 mm) by 55% and 53% under conventional tillage combined with wheat cover compared to no-tillage with a wheat cover crop (Figure 11C and D, Tables A 7-8). Enzyme activities for XYL and

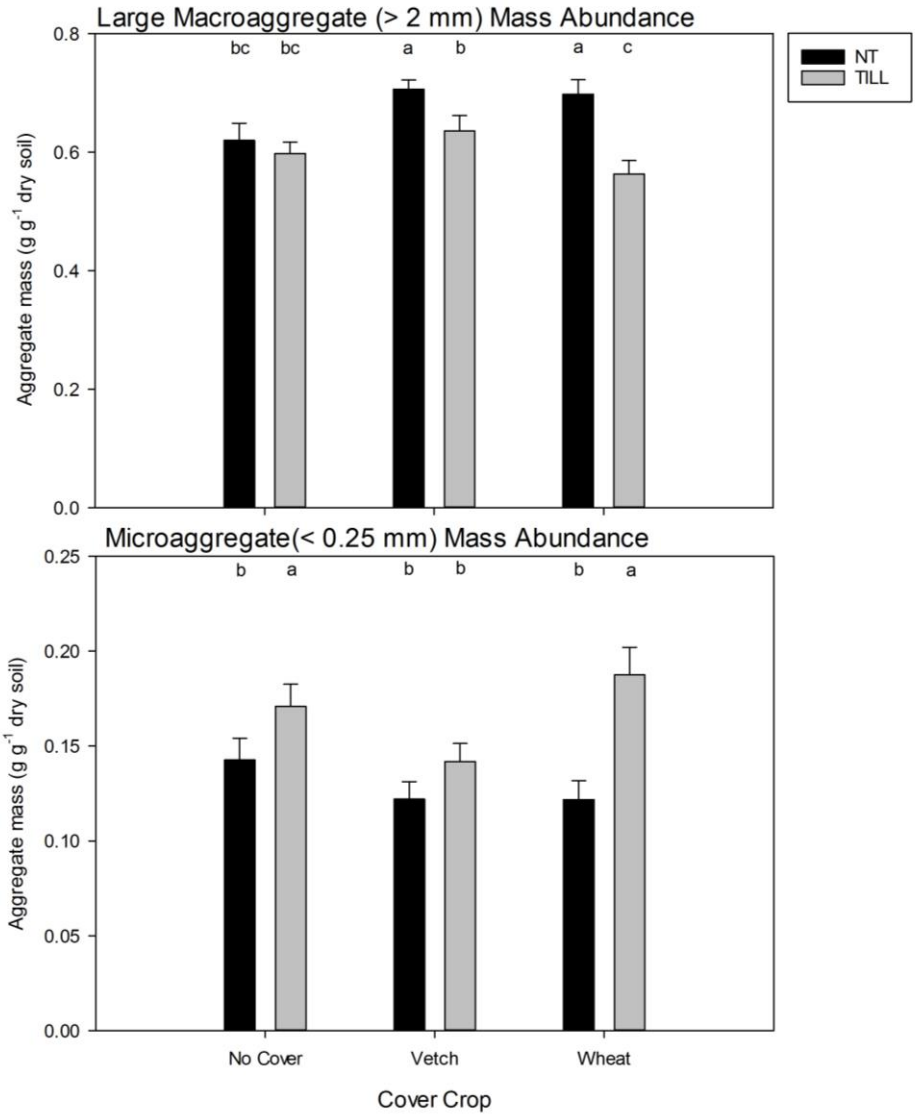


Figure 9. Dry aggregate distribution under cover cropping separated by no-tillage (NT) and conventional tillage (TILL) treatments. (A) Large macroaggregates (> 2 mm) and (B) microaggregates (< 0.25 mm) under no-tillage (NT) and conventional tillage (TILL) combined with cover crop treatments. Different letters denote statistically different means at $P < 0.05$ and error bars represent standard error of the mean.

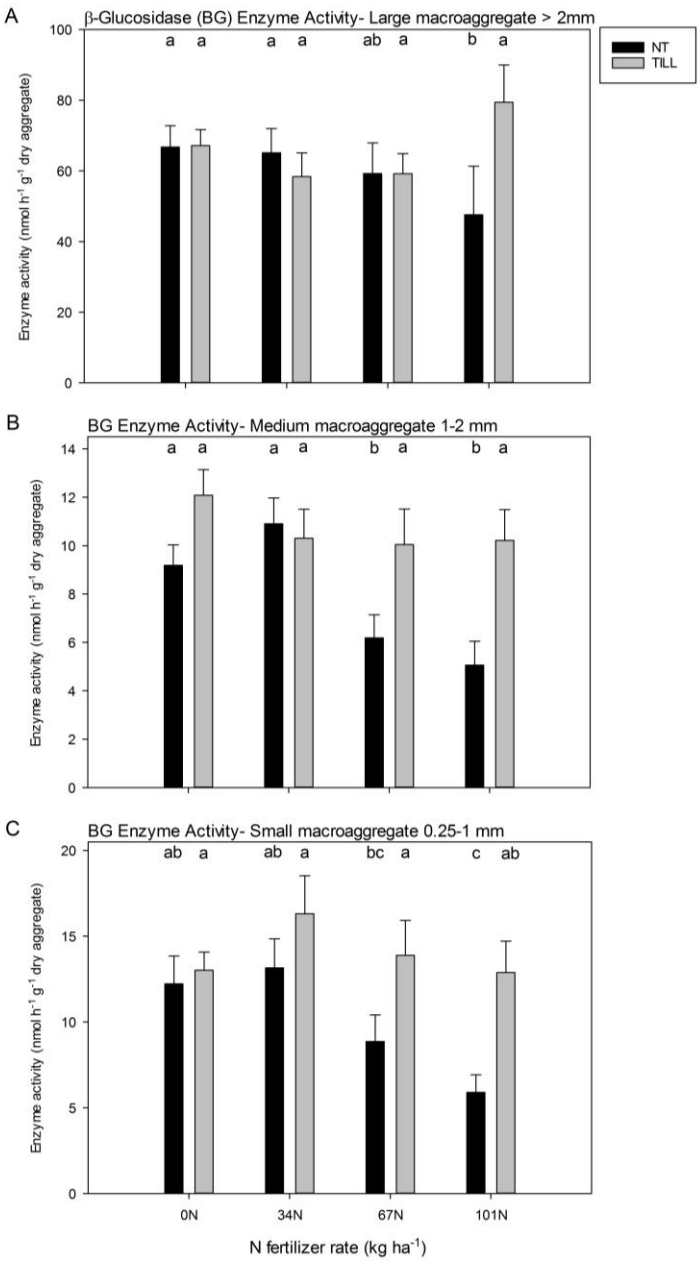


Figure 10. Potential extracellular enzyme activity of BG under N fertilizing separated by no-tillage (NT) and conventional tillage (TILL) treatments. (A, B, and C) BG potential enzyme activity is greater in all macroaggregate size fractions under TILL and high N fertilizing (101N kg ha^{-1}). Different letters denote statistically different means at $P < 0.1$ and error bars represent standard error of the mean.

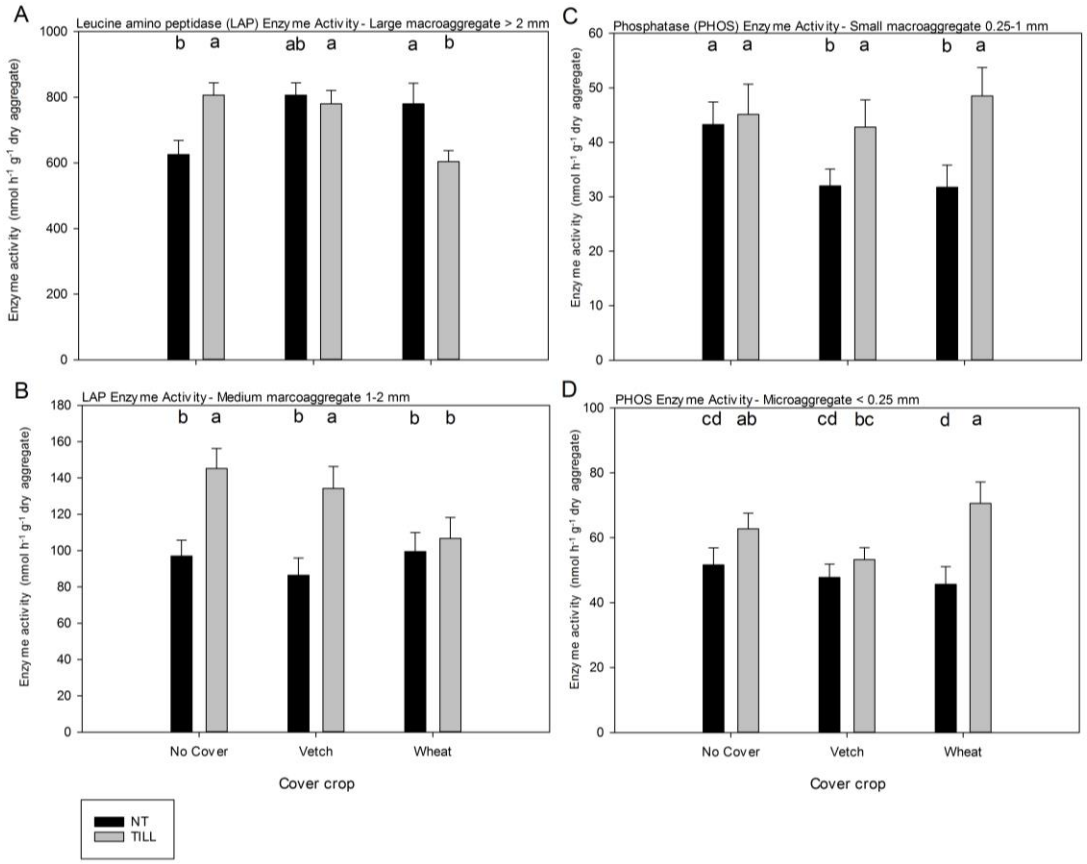


Figure 11. LAP and PHOS potential enzyme activities under cover cropping separated by no-tillage (NT) and conventional tillage (TILL) treatment. (A and B) LAP potential activity is greater in large and medium macroaggregates under no cover with TILL compared to NT. (C and D) PHOS potential enzyme activite is greater in small macroaggregates and microaggregates under wheat cover with TILL compared to NT. Different letters denote statistically different means at $P < 0.1$ and error bars represent standard error of the mean.

AG had anomalous undetectable values throughout all soil aggregate sizes (Tables A 5-8).

Broad patterns of soil nutrient acquisition within dry aggregate size classes are indicated by potential activities categorized into total C (BG+CB) and total N (LAP+NAG) enzymes. Total P nutrient acquisition is represented only by PHOS potential activity. Total C and total N enzyme activity was greatest under conventional tillage compared to no-tillage in all aggregate size fractions (Tables A 5-8). PHOS enzyme activity also follows this pattern in aggregate sizes with the exception of large macroaggregates (Table A 4-8). A second statistical analysis was conducted to compare potential enzyme activities between aggregate size fractions and tillage treatments. Total C enzyme activity ranged from 9±0.08 to 103±0.09 nmol h^{-1} g^{-1} dry aggregate, with greatest activity in large macroaggregates under conventional tillage compared to all other aggregate sizes and tillage interaction treatments (Figure 12A, Table A 5). Total N enzyme activity ranged from 94±0.12 to 227±0.05 nmol h^{-1} g^{-1} dry aggregate, and greatest activity occurred in microaggregates under conventional tillage compared to other aggregate sizes and tillage treatments (Figure 12B, Table A 8). PHOS potential activities ranged from 29±0.05 to 236±0.06 nmol h^{-1} g^{-1} dry aggregate and were greatest in large macroaggregates under either no-tillage or conventional tillage (Figure 12C, Table A 5).

Aggregate Respired Carbon

Microbial activity of intact aggregates was measured to assess whether a physical protection barrier exists for soil C. Respiration measurements were taken over

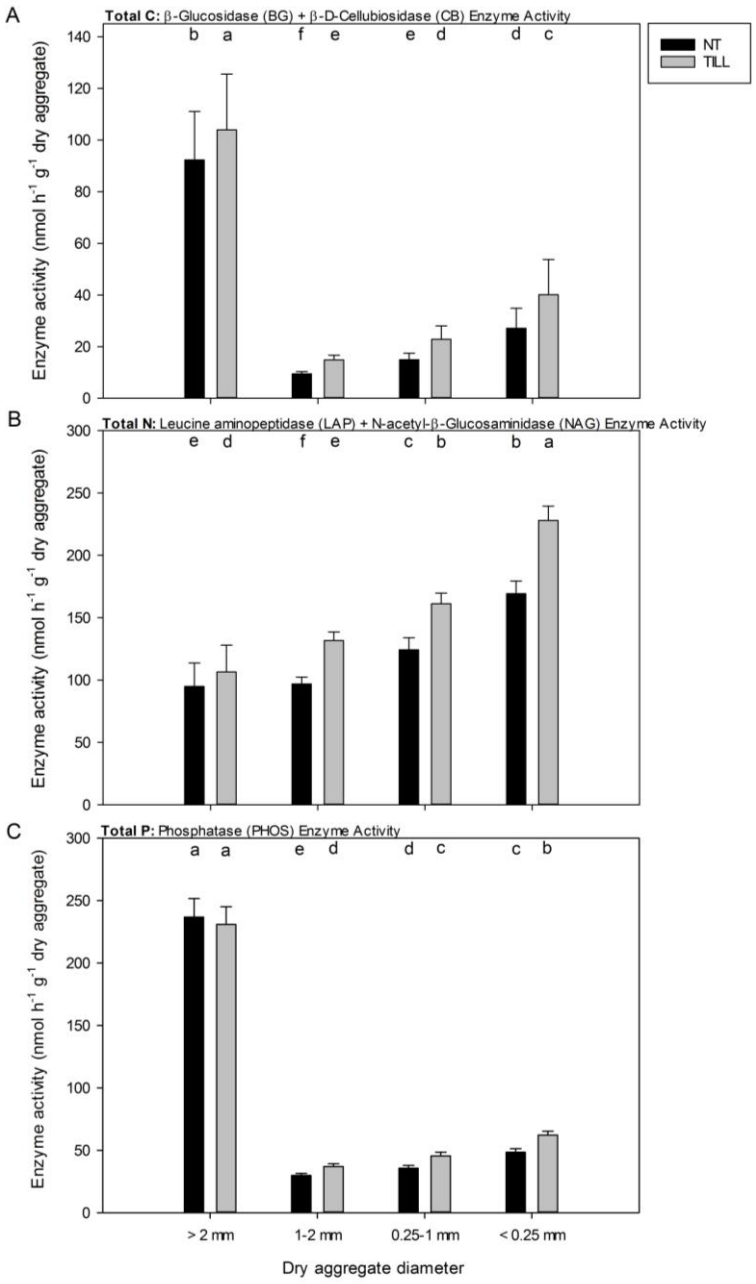


Figure 12. Total C, N, and P potential enzyme activities between aggregate fractions separated by no-tillage (NT) and conventional tillage (TILL) treatments. (A) Total C had greater activity in macroaggregates > 2mm under TILL compared to NT. (B) Total N had greater activity in microaggregates < 0.25mm under TILL compared to NT. (C) Total P activities were greater in macroaggregates < 2mm. Different letters denote statistically different means at $P < 0.1$ and error bars represent standard error of the mean.

a short-term (24 h) incubation study from each of the four aggregate sizes collected in the dry aggregate distribution. Respired C means from large macroaggregates (> 2 mm) and microaggregates (< 0.25 mm) ranged from 0.016±0.001 to 0.018±0.001 and 0.021±0.001 to 0.030±0.002 mg respired C g^{-1} dry soil, respectively, and did not differ among N fertilizer rate, cover crop, or tillage treatments (Table 4). Respired C from medium macroaggregates (1-2 mm) ranged from 0.018±0.001 to 0.026±0.002 mg g^{-1} dry soil, with greater respiration under high N fertilizer rates (101N and 67N kg ha^{-1}) compared to reduced N fertilizer (34N kg ha^{-1}) and controls (0N kg ha^{-1}) (Table 4). Differences in tillage treatment means showed 20% greater respired C under conventional tillage compared to no-tillage in the medium macroaggregates (Table 4). Respired C from small macroaggregates (0.25-1 mm) ranged from 0.024±0.001 to 0.034±0.002 mg g^{-1} dry soil, with greater respiration under high N fertilizer rates (101N kg ha^{-1}) compared to controls (Table 4). Cover crop treatment means had 22% greater respired C under vetch cover compared to wheat cover and controls (no-cover), and there was 23% greater respired C under conventional tillage compared to no-tillage in the small macroaggregates (Table 4).

When tillage treatments alone were considered, the proportion of respired C differed among each aggregate size with greatest activity in small macroaggregates (by 63%) and least activity in large macroaggregates (Figure 13). Respired soil C was 23% greater in small macroaggregates under conventional tillage compared to small macroaggregates under no-tillage, and overall had the greatest amount of respired C

Table 4. N fertilizing rate (N-rate), cover crop, and tillage effects on respired C (mg g^{-1} dry soil) from dry aggregate size classes: large macroaggregates (> 2 mm), medium macroaggregates (1-2 mm), small macroaggregates (0.25-1 mm), and microaggregates (< 0.25 mm). Numbers inside parentheses are (±1) standard error of the mean, and lettering denotes statistical significance at $P < 0.05$. Bolded ANOVA numbers show statistical significance at $P < 0.05$.

	Aggregate respired C (mg g^{-1} dry soil)			
Treatment factors	Large Macroaggregate > 2 mm	Medium Macroaggregate 1-2 mm	Small Macroaggregate 0.25-1 mm	Microaggregate < 0.25 mm
N-rate (kg ha^{-1})				
0N	0.017(0.001)a	0.018 (0.001)b	0.024 (0.001)c	0.021 (0.001)a
34N	0.016 (0.001)a	0.019 (0.001)b	0.026 (0.001)bc	0.023 (0.001)a
67N	0.018 (0.001)a	0.024 (0.001)a	0.030 (0.002)ab	0.028 (0.002)a
101N	0.019 (0.001)a	0.026 (0.002)a	0.034 (0.002)a	0.030 (0.002)a
Cover Crop				
No cover	0.017 (0.001)a	0.019 (0.001)a	0.027 (0.002)b	0.023 (0.001)a
Vetch	0.018 (0.001)a	0.023 (0.001)a	0.033 (0.001)a	0.027 (0.002)a
Wheat	0.018 (0.001)a	0.022 (0.002)a	0.027 (0.001)b	0.025 (0.001)a
Tillage				
No-tillage	0.017 (0.001)a	0.020 (0.001)b	0.026 (0.001)b	0.024 (0.001)a
Conventional tillage	0.018 (0.001)a	0.024 (0.001)a	0.032 (0.001)a	0.026 (0.001)a
Sources of Variation	ANOVA (LSD protected, $P < 0.05$)			
N-rate	0.3299	**0.0001**	**0.0052**	0.1027
Cover crop	0.5770	0.0561	**0.0011**	0.1042
Tillage	0.1084	**0.0075**	**<0.0001**	0.1012
N-rate*Cover crop	0.5697	0.6514	0.9163	0.8273
N-rate*Tillage	0.2803	0.9182	0.4094	0.9983
Cover crop*Tillage	0.5134	0.8935	0.0914	0.7369
N-rate*Cover crop*Tillage	0.1636	0.4150	0.3520	0.0873

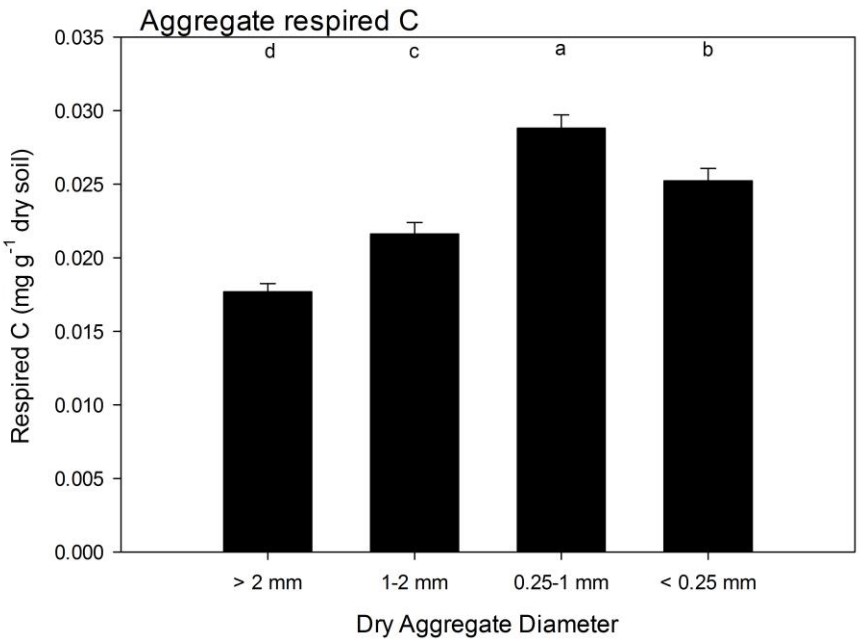


Figure 13. Proportion of mean respired C (mg g^{-1} dry soil) among dry aggregate fractions grouped by N fertilizer, cover cropping, and tillage treatments. Small macroaggregates (0.25-1 mm) had greatest respired C while large macroaggregates (> 2 mm) had the least respired C. Different letters denote statistically different means at $P < 0.05$ and error bars represent standard error of the mean.

compared to all other aggregate sizes and tillage treatments (mean equal to 0.032 ± 0.001 mg g^{-1} dry soil, respectively) (Figure 14).

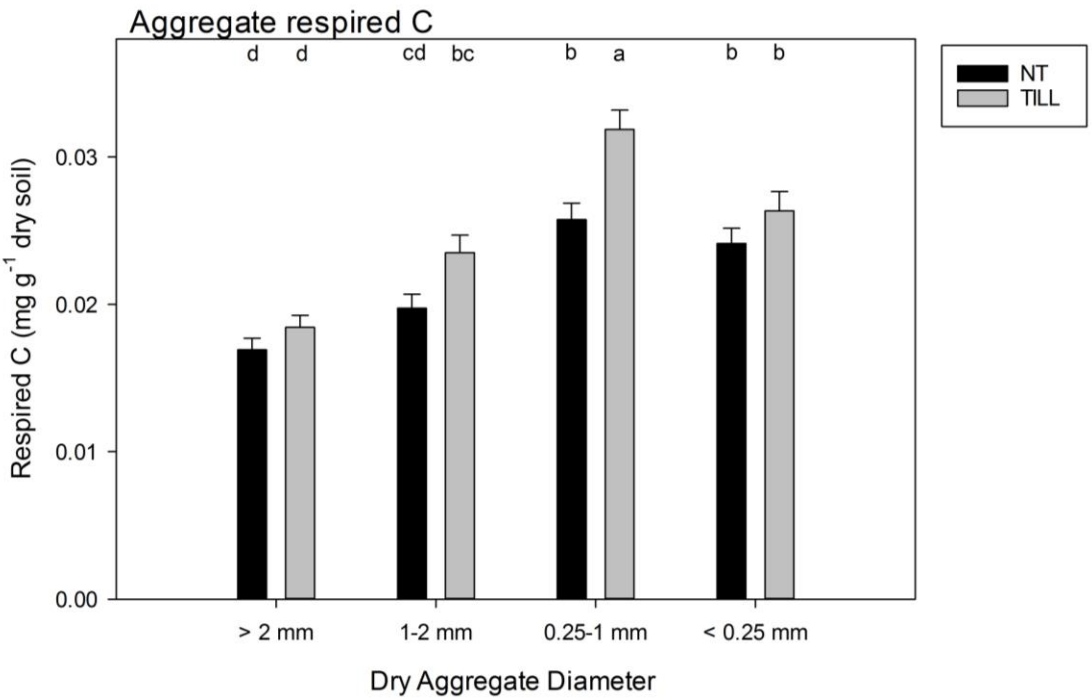


Figure 14. Proportion of respired C (mg g^{-1} dry soil) between dry aggregate fractions separated by no-tillage (NT) and conventional tillage (TILL) treatments. Small macroaggregates (0.25-1 mm) had greater respired C under TILL compared to NT, and also had greatest respired C overall. Different letters denote statistically different means at $P < 0.05$ and error bars represent standard error of the mean.

CHAPTER V DISCUSSION

Soil Organic Carbon Stability and Soil Carbon Concentration

Experimental approaches that separate bulk SOC into pools with different turnover-times in soil provide further insight to the biogeochemical processes that balance SOC compound storage and CO_2 atmospheric release. This balance is integral to long-term C storage in terrestrial ecosystems, and is useful to predict future impacts of climate change on SOC dynamics (Collins et al., 2000). My study determined soil labile and intermediate C-pools with a first order decay function and compared the effects of long-term CA practices like N fertilizer, cover cropping, and tillage. Similar to some other soil C-pooling studies, a triple pool model did not improve the total modeled respiration rate for 602 days of incubation, and thus a double pool first order model was used (Collins et al., 2000; Schädel et al., 2013). The remaining soil C stored after the 602-day incubation period was determined by subtracting cumulative mineralized C from bulk soil C data collected by Mbuthia et al. (2015).

Initial C-loss measured in the active pool [via respiration] was greater under reduced N fertilization application (34N kg ha^{-1}) combined with no-tillage compared to a reduced N rate with conventional tillage. Based on the current and past literature, I did not expect to see a greater amount of unstable [respired] C under CA practices such as reduced fertilizing and no-tillage. However, the longevity of the experimental plot management (31 years) in this study separates it from most other studies modeling C-pools, where the implementation of management systems were more recent. The initial pulse in microbial activity often observed under conventional tillage soils has likely

passed to a C equilibrium state (Blevins et al., 1983). Stimulation of SOM mineralization in the rhizosphere can occur when soils are newly tilled, and may show greater C respired than undisturbed soils (Bingeman et al., 1953; Hendrix et al., 1988). However, after extended periods of cultivation soil microbial activity can decrease as it becomes C-limited (Schimel, 1986).

The addition of high N fertilization inputs (101N kg ha^{-1}) resulted in greater active C-pool respiration under conventional tillage rather than no-tillage. Consequently, results from the active pool are distinct between applications of low and high N fertilization rates when combined with tillage, and suggest that high N-rates combined with conventional tillage results in more soil C allocated in a rapidly turning over C-pool, and reduces soil C stability overall. An *in-situ* respiration study by Hendrix et al. (1988) determined that conventional tillage incorporated fresh residue into the soil, and subsequently increased colonization of soil organisms to utilize the added substrates compared to undisturbed systems. When high N-rates were added to the tilled system in my study, inorganic substrate inputs combined with mixing induced greater microbial activity compared to no-tillage. However, high inorganic N inputs should be avoided to reduce potential leachable soil N and to efficiently utilize any applied N (Halvorson et al., 2002). Under reduced N fertilizing rates, this study and others showed greater, short-term [active] respired C with no-tillage rather than conventional tillage (Collins et al., 2000; Mpheshea, 2014). This conclusion is further supported by the analysis of extracellular enzyme activity for total N acquisition in bulk soil, which also showed greatest activity under reduced N-rates (Figure 8C). It is possible that microbial activity

is a reflection of sufficient substrate input for metabolic needs under reduced N-rates and no-tillage for short-term C-cycling.

I originally hypothesized that the slow C-pool would generate a much larger amount of respired C over a longer time period relative to the active C-pool over a short time period, thus making the system more C stable. Indeed, I observed nearly five times the amount of respired C in the slow C-pool relative to the active C-pool in both N fertilizer and tillage treatments (Figure 6). Under reduced N rates, greater respired C was observed in the slow C-pool with no-tillage compared to conventional tillage (Figure 3B). Collins et al. (2010) found similar results where greater C mineralization occurred under no-tillage treatments compared to conventional tillage in both active and slow C-pools. These results reveal that given more time, soil C-losses will stabilize under CA treatments.

Long-term tillage practices are known to influence C-cycling dynamics, and much research has concluded the absence of tillage (no-tillage) increases short-term SOC accumulation in the soil surface compared to conventional tillage practices (Al-Kaisi et al., 2005; Blevins et al., 1983; Havlin et al., 1990). Over the 602-day incubation period in this study, I observed greater soil carbon concentration under no-tillage and high N fertilization application (101N kg ha^{-1}) (Figure 7). Continuous mixing of crop residue throughout the soil surface layer used in conventional tillage can result in higher rates of OM oxidation than in no-tillage systems, where OM is lost and SOC decreases (Blevins et al., 1983). High rates of N fertilization have shown greater SOC pool concentration in the soil surface (Jagadamma et al., 2007) due to additional inputs of available

substrates and crop residue to the soil and microbial community (Hendrix et al., 1988). When the respired C-pools are compared to C concentration and bulk C previously measured at the site (Mbuthia et al., 2015), the fast-turning over active C-pool showed no differences among treatments, but greater respired C in the slow C-pool showed the same effects as C concentration and bulk C under high N fertilizer and no-tillage. These C-pools suggest the microbial community is turning over C inputs on a trajectory to greater net C gains, even after long-term (30 years) cultivation. Overall, it seems that tillage coupled with N application is more important for controlling SOC stability in these cotton plots, where cover cropping did not show any clear effects on respired C-pools.

Bulk Soil Extracellular Enzyme Activity

Respired SOC from my long-term incubation study showed distinctive patterns between N fertilizer and tillage treatments in the active and slow C-pools. There were not any significant effects under cover cropping, but because enzyme activity has a quick and sensitive response to changes in agricultural management systems (Dick et al., 1996), it was hypothesized that both organic and inorganic inputs, in addition to tillage, would emerge as driving factors for enzyme activity. Analysis of extracellular enzyme activity in this agroecosystem provided an index to compare substrate utilization by the microbial population or by stabilized enzymes in the soil matrix under the combination of long-term CA practices (31 years). A pattern of cover crop and tillage interactions effects were observed in four of the seven enzymes assayed. Different cover cropping strategies heavily influence soil C:N and was a primary control on

enzyme activity differences; plant available N is an important driver for crop production while C is an important driver for long-term soil sustainability (Salon, 2006).

C-acquiring β-glucosidase (BG), N-acquiring leucine aminopeptidase (LAP), and P-acquiring phosphatase (PHOS) enzymes showed greater activity under wheat cover with conventional tillage compared to no-tillage. The microbial community may be C-limited, where wheat cover provides rich C material (wheat cover is associated with a high C:N litter) that is rapidly processed because it is readily accessible via conventional tillage soil mixing (Salon, 2006). However, it is possible that this microbial community is N-limited and is processing high C:N litter, but driving N immobilization. This is common in systems with high a C:N because microbes have to metabolize excess C in order to access alternative sources of available N (Hartwig and Ammon, 2002; Salon, 2006; Stanford and Smith, 1972). Research by Rosecrance et al. (2000) determined N mineralization and N immobilization between vetch and rye cover crops and found that vetch decomposition resulted in net mineralization at 0.98 mg $(NO_3^- + NH_4^+)$-N d^{-1} while rye cover resulted in net N immobilization at 0.34 mg $(NO_3^- + NH_4^+)$-N d^{-1}. They also concluded that it may be possible to predict net mineralization based on C:N for different percentages of vetch and rye covers. It is generally expected that net mineralization occurs when C:N ratios are < 20, while net immobilization occurs when C:N ratios are > 20 (Jenkinson, 1981; Rosecrance et al., 2000). Additional soil analysis in the future, of net N mineralization versus immobilization, would better define with which of the two mechanisms the microbes are responding.

Conversely, cellulose degrading β-D-cellubiosidase (CB) enzymes that breakdown cellulose components resulted in greater enzyme activity under no-tillage with a vetch cover crop compared to conventional tillage with vetch cover (Figure 7E). More abundance of C-degrading enzymes under vetch, no-tillage might be an effect of C-limitation in a low C:N system, and caused the microbial community to up-regulate more complex C-acquiring enzymes (cellulose degradation from CB activity versus sugar degradation from BG activity). Greater enzymatic activity is often observed under no tillage and cover cropping systems compared to conventional tillage and no cover (Alvear et al., 2005; Bandick and Dick, 1999; Bergstrom et al., 1998; Mathew et al., 2012). However, the effects of integrative treatments are frequently overlooked, which may not account for differences in C acquiring enzymes specific to structure and complexity of available substrates. It is possible that more structurally complex substrates, like cellulose, are utilized by the microbial community for nutrient and energy sources in the absence of conventional tillage because more easily degradable substrates, like glucose, are not present without soil mixing. Mean residence times of bulk soil C are much longer under no-tillage than conventional tillage practices (Six and Jastrow, 2002). This microbial community may be slower growing and capable of breaking down more complex cellulose substrates because labile substrates included in the low C:N vetch litter are not accessible.

N fertilization was hypothesized to increase N acquisition activity with additions of inorganic N to microbes that may be N limited depending on cover cropping management and its associated C:N litter. High N-rates can lower soil C:N and increase

mineralization of substrates (Salon, 2006), and indeed, I observed greater chitin and peptidoglycan via N-acetyl-β-glucosaminidase (NAG) enzyme activity in all N fertilizing treatments. However, N acquiring LAP (protein) activity is also considered an important driver for N mineralization (Ramirez et al., 2012; Steinweg and McMahon, 2002), but did not show any clear patterns among the gradient of fertilizer treatments (Appendix Table 1). Research has shown that proteins may be a less important source of soil N in low pH systems, where the breakdown of chitin and peptidoglycan is more favored (Sinsabaugh et al., 2008). Soil pH under N fertilizer rates (101 and 67 kg N ha^{-1}) at my study site resulted in a lower, acidic pH (0.8 and 0.4 units lower, respectively) compared to zero and reduced N-rates (Mbuthia et al., 2014). Greater NAG activity under all inorganic N rates confirmed that enzymatic breakdown of chitin and peptidoglycan is favored in an acidic environment compared to protein breakdown. NAG enzyme activity contributes to N uptake, but its response depends entirely on chitin and peptidoglycan substrate availability. At present, literature has not addressed whether substrate availability, community composition, or a combination of both control NAG activity (Saiya-Cork et al., 2002). However, a study by Ramirez et al. (2012) found that soil N addition increased the relative abundan ce of *Actinobacteria*, and it is possible that the presence of peptidoglycan within the cell walls of such bacteria stimulated NAG enzyme activity in my study. Microbial community composition at this study site showed lower mycorrhiza fungi under high N-rates (Mbuthia et al., 2015), so NAG activity may only be a function of peptidoglycan located within bacterial cell walls rather than chitin associated with fungi. Ramirez et al. (2012) and others (Fierer et al., 2007; Fontaine et

al., 2003) hypothesized that fast turnover rates by microbial groups rely on more labile C sources and are able to increase in abundance under high nutrient inputs, whereas other groups with slower turnover rates would increase under lower nutrient conditions. Fast turnover of bacterial groups contrasts to the slower turnover of fungal biomass found in soil (Rousk and Bååth, 2007), and thus the possible dominant activity of peptidoglycan could be the result of a quickly turning over bacterial population that is utilizing easily decomposable inorganic N inputs from fertilizer application.

Broad nutrient acquisition efforts indicated by potential activities of total C (AG + BG + CB + XYL), N (LAP + NAG), and P (PHOS) reflected differences between N availability and N form under organic or inorganic additions to the cropping system. Results showed greater activity for total C acquisition under no cover (controls) with no-tillage compared to conventional tillage treatment (Figure 7C). This tillage effect disappeared when any cover crop was added, but it should be noted that the highest means for total C activity resulted from vetch, no-tillage plots (not significant at $P < 0.1$) (Appendix Table 1). With the addition of organic N sources via vetch cover cropping, and a lower C:N ratio, microbial activity was stimulated and is associated with better soil health (Alvear et al., 2005; Bandick and Dick, 1999; Salon, 2006). In the absence of cover cropping, it is likely that tillage disturbances are the primary control for differences in total C enzyme activity due to decreased mean residence times of whole-soil C (Six and Jastrow, 2002). Greatest total N activity occurred under reduced N rates (34 kg N ha^{-1}) (Figure 8C) and was consistent with my previous observations of greater respired C in labile and intermediate C-pools, and was also consistent with greater NAG (chitin

and peptidoglycan) activity under N additions compared to controls. It was hypothesized that greater microbial enzyme activity would occur under cover cropping and N fertilization treatments compared to controls, and the data are supportive. In terms of C-dynamics, microbes appear to be processing SOC differently between cover crop and fertilization treatments; N availability or N form also appear to be important feedback effects.

Aggregate Size Distribution

This study used dry aggregate size distribution to investigate the effects of soil physical properties and structure on soil C stability. Less soil disturbance has a clear effect on aggregate formation (Six et al., 2000), and persists when combined with cover crop management (Martens, 2000; Salon, 2006; Tisdall and Oades, 1982). Large marcoaggregates (> 2 mm) were more abundant under no-tillage combined with a cover crop (vetch or wheat) than under conventional tillage with cover. High N fertilizer (101N kg ha^{-1}) also contributed to the formation of more large marcoaggregates compared to reduced N input (34N kg ha^{-1}) and controls (0N kg ha^{-1}). Though I did not directly test the stability on these aggregates, previous research suggests that large marcoaggregate stability is also controlled by agricultural practices (Tisdall and Oades, 1982; Oades, 1992). For example, the abundance of inorganic nutrients added to the soil surface via high application rates of N fertilizing have been shown to stimulate the relative abundance of individual phyla like *Actinobacteria* and *Firmicutes* (Fontaine et al., 2003; Remirez et al., 2012), in which the excretion of microbial mucilaginous

products, such as fungal hyphae and polysaccharides, can bind soil microaggregates to form large macroaggregates (Hillel, 1998).

It is interesting to note that aggregate abundance from the smallest aggregate size (< 0.25 mm) and largest aggregate size (> 2 mm) were the only aggregates significantly influenced by cover crop and tillage interaction effects. One explanation for this is that structure is weakened by conventional tillage disturbance (Six et al., 1998). Indeed, I observed a clear relationship between no-tillage and large macroaggregate (> 2 mm) abundance. Microaggregates (< 0.25 mm) were more abundant under conventional tillage rather than no-tillage, with both wheat and no-cover treatments. These tillage differences were not observed under vetch cover, possibly due to a lower C:N litter, in which the formation of microaggregates may be less favored. Most studies associate greater microaggregate abundance with lower C:N ratios (Jastrow, 1996; Six et al., 1998). However, a study by Wick et al. (2009) found higher C:N ratios in microaggregate light fraction-C rather than macroaggregate light fraction-C. My study is supportive of this finding, and future research in C amount and composition of my aggregate sizes would further expand on these results.

The inverse tillage relationship exists for the large macroaggregates, observed under both wheat and vetch cover crops. As suggested by other studies, plant root and fungal hyphae growth are primary controls in aggregate formation, while soil microorganisms aid in stabilization (Oades, 1992; Hillel, 1998). Both cover crop species in this system, regardless of C:N input differences, add root systems which clearly effect the formation of large macroaggregates. When combined with no-tillage, cover crop

roots and microbial growth, like fungal hyphae growth, are not broken up and support macroaggregate stabilization (Six et al., 1998). All aggregate sizes were significantly affected by tillage, and literature widely accepts that intensive tillage practices inhibit large macroaggregate formation due to the degradation of soil properties that support greater stability (Kasper et al., 2009; Six et al., 2000; Tisdall and Oades, 1982).

Extracellular Enzyme Assay of Aggregate Fractions

The study of OM decomposition and nutrient acquisition by extracellular enzymes at the aggregate scale is an emerging field in soil ecology (Allison and Jastrow, 2006; Bach and Hofmockel, 2014). Aggregation is known to physically protect soil C, and OM containing labile C is typically incorporated into large aggregates with faster turnover times compared to smaller (micro) aggregates (Six and Jastrow, 2002). It was hypothesized that enzyme activities would be greater in the macroaggregates compared to microaggregates due to rapid C turnover (Allison and Jastrow, 2006).

Several enzymes show similar functional patterns among multiple aggregate size fractions and experimental treatments. BG potential activity was greater in all macroaggregate sizes under high N fertilizer rates (101 kg ha^{-1}) coupled with conventional tillage compared to high N-rates with no-tillage (Figure 9). These results expand on my long-term incubation results that observed more labile respired C rapidly mineralized under high N fertilizing rates with conventional tillage (Figure 3A). Studies have shown that intense cultivation practices, like conventional tillage, can decrease the mean residence time of whole-soil C (Six and Jastrow, 2002) and increase C-depleted microaggregates that make up macoaggregates under the aggregate hierarchy theory

(Six et al., 2000; Tisdall and Oades, 1982). A possible suggestion is that easily degradable C compounds are abundant in macroaggregates when high N rates are mixed into the soil surface, and become the labile substrate acted on by microbes for energy via BG enzymes (sugar degrading). My data supports the hypothesis that over-fertilizing while also tilling the soil results in more soil C utilized and mineralized by the microbial community in a rapidly turning over C-pool, which effectively reduces soil C stability overall.

LAP (N acquisition) potential enzyme activity showed consistent functional patterns between medium and large macroaggregate sizes. Cover crop controls (no-cover) combined with conventional tillage resulted in greater activity compared to no-cover with no-tillage (Figure 9A and B). It seems that when cover crops are absent, conventional tillage is the primary mechanism that increases plant residue mixing under the soil surface and initiates protein degrading LAP enzymes.

PHOS (P mineralization) potential activity showed similar functional patterns in small macro- and microaggregates, where greater activity occurred under wheat cover with conventional tillage compared to wheat cover with no-tillage (Figure 9C and D). These results were similar to the bulk soil PHOS enzyme assays (Figure 5B). PHOS is noted to be a bound enzyme associated with organic and inorganic colloids rather than free in solution (Burns et al., 2013). A possible explanation is that PHOS becomes externalized via conventional tillage soil mixing, whereby it would exist as a reservoir of potential activity under no-tillage. It is more accessible in smaller, broken up aggregate sizes, and the high C:N ratio wheat litter propels rich C-material processing.

Hemicellulose xylosidase (XYL) and starch α-glucosidase (AG) enzyme activities resulted in anomalous undetectable [negative] values throughout all soil aggregate sizes, but has been an occurrence also found in literature for other enzyme analyses (Keeler et al., 2009; Sinsabaugh et al., 2008). Subsequently, XYL and AG activities were not included in total C enzyme analysis within dry aggregate fractions. My general overview of individual enzyme activities highlights macroaggregate BG potential activity and long-term respiration results that suggest soil N-input coupled with tillage is more important for controlling labile C-pools, while the addition of cover crops do not show clear patterns for N-enzyme function. Smaller aggregates broken up by tillage can externalize substrates like P for enzyme breakdown that would have otherwise existed as a reservoir of potential activity.

The macro- and microaggregates within conventional tillage systems clearly differ from those in no-tillage systems due to such pronounced effects observed in extracellular total C, N, and P enzyme analyses. Activity was greatest for all C, N, and P totals under conventional tillage compared to no-tillage (Figure 10). Conventional tillage systems are a direct control on increased aggregate turnover where mixing introduces wet-dry and freeze-thaw cycles along with changes in temperature, moisture content, and aeration that can increase decomposition rates in the soil surface (Paustian et al., 1997; Beare et al., 1994). It is possible greater extracellular enzyme potentials are reflecting faster decomposition rates from conventional tillage compared to no-tillage.

The proportion of C to N enzyme activity among the aggregate sizes was contrasting in the large macroaggregate size fraction; large macroaggregates had

greater total C activity while microaggregates had greater total N activity (Figure 10A and B). PHOS activity distribution among the aggregate sizes was alike total C (Figure 10C). Differences in the proportion of total C and P enzyme activity versus total N activity could be the result of aggregate structural differences, microbial population or community differences, and soil C composition differences.

The aggregate hierarchy concept explains that macroaggregates are collections of microaggregates held together by temporary and transient organic binding agents (Tisdall and Oades, 1982). Thus, differences in aggregate proportions at the micro scale are most likely driving differences at the macro scale and structural differences between aggregate sizes in conventional versus no-tillage treatment can be eliminated as an effect on total C and N enzyme activity differences. An alternative explanation is that the accessibility of substrates to enzymes could be controlled by changes in the abundance of microbial biomass and community composition. Previous research in this cotton system by Mpheshea (2014) investigated differences in the microbial biomass over several time periods during the cotton-growing season. Lower microbial biomass was observed in conventional tillage compared to no-tillage treatment one month prior to sampling done in this study, but then there were not any treatment differences (tillage, cover crop, or N fertilizer) observed post one month sampling for this study. Composition differences in the microbial community were observed by Mbuthia et al. (2015), and showed an increased fungal:bacterial biomass ratio between conventional tillage and no-tillage treatment at two different N-rates and across all cover crops. Fungi and its mycelial growth is associated with a high C:N ratio and aids in the retardation of

macroaggregate turnover (Six et al., 2006; USDA, 1996). More fungi in conventional tillage compared to no-tillage treatments would result in a higher C:N ratio that is consistent with greater C acquiring enzyme activity in more stable macroaggregates. It is possible that N acquiring enzyme activity showed the inverse relationship due to a lower fungal:bacterial ratio in microaggregates compared to macroaggregates in conventional tillage systems (Salon, 2006), whereby N substrates would be more readily accessible and reflect greater activity potentials observed in this study. Another explanation could be that the microaggregates containing greater N potential activity were released from macroaggregates by mechanical stress during analysis, and originally was incorporated within macroaggregates via particulate OM formation encrusted with microbial mucilages and soil clay content (Beare et al., 1994). Microbial population level differences can be eliminated, but changes in community structure cannot be excluded in enzyme activity effects between aggregate size fractions.

In addition to the composition of the microbial community, the form and composition of soil C substrates (i.e. labile or recalcitrant) could be driving differences in extracellular enzyme activity of different aggregate sizes (Six et al., 2000; Six et al., 2006). The large macroaggregate fraction appears to be different than the others either because of microbial community structure, or because the C substrates in that fraction differ in chemical recalcitrance. My long-term incubation data shows greater short term, respired C in conventional tillage treatments under high N fertilizer, and data from aggregate-enzyme analyses are consistent with the long-term incubation results; soil C is rapidly cycled in conventional tillage systems compared to no-tillage systems (Six et

al., 2000; Six and Jastrow, 2002). Future research should investigate the microbial community and chemical composition of the organic C within each aggregate fraction because there is clearly some aspect of microbial function that is changing (Six et al., 2006).

Aggregate Respired Carbon

The effects of soil physical properties and structure on C stability were assessed after a 24-h incubation period measuring CO_2 respiration rates from intact, dry soil aggregate fractions to determine if a physical protection barrier exists for C, and to help identify where soil C is most vulnerable. There were similar respired C patterns among the dry aggregate size fractions and experimental treatments. Large macroaggregates (> 2 mm) and microaggregates (< 0.25 mm) are the largest and smallest aggregate sizes collected in this study, and both had no differences in respired C among N fertilizer rate, cover crop, or tillage treatments. As previously explained in the discussion of extracellular enzyme analysis within soil aggregates, differences in aggregate proportions at the micro scale are most likely driving enriched biological differences at the macro scale based on the aggregate hierarchy theory (Tisdall and Oades, 1982). Because such contrasting aggregate sizes both share no significant data, structural differences between aggregate sizes can be eliminated as an effect on large macro- and microaggregate respired C from the experimental treatments. Although, the short-term incubation period (24 h) of this study may not have been long enough to capture treatment differences between these two size fractions. We can see that soil C is less vulnerable to decomposition in large and medium macroaggregates because respired C

is significantly less compared to small macro- and microaggregates (Figure 12). This could indicate that the macroaggregates are C-depleted in comparison to the microaggregates. Future research is needed to measure total SOC concentrations within each aggregate fraction so that it can be determined if there is a protection barrier for soil C.

The medium (1-2 mm) and small (0.25-1 mm) macroaggregates both showed greater respired C under high N fertilization (101N kg ha^{-1}) and conventional tillage systems compared to reduced N-inputs and no-tillage systems (Figure 13). These results are similar to the labile C-pool from my long-term incubation (Figure 3A) and BG potential enzyme activity in all macroaggregate sizes (Figure 9), where greater microbial activity occurred under high N-rates and conventional tillage treatment. This suggests that a labile C-pool may be rapidly turning over in the medium and small macroaggregates of conventional tillage systems (Six et al., 2000), where BG substrates are more accessible via soil mixing and acted on by the microbial community (Allison and Jastrow, 2006). Additions of high N fertilizer rates allow these substrates to be readily utilized and then mineralized by the microbial community, but it is suspected that this effectively reduces soil C stability overall (Salon, 2006). As stated above, these results do not correspond with the aggregate hierarchy theory (Tisdall and Oades, 1982) where one would anticipate greater mineralization in large macroaggregates, so instead must be a function of differences in the microbial community or C composition within aggregates (Six et al., 2000; Six et al., 2006).

The proportion of respired C among aggregate sizes is contrasting in all aggregate size fractions. Amounts of respired C from greatest to least in aggregate fractions are as follows: small macroaggregates > microaggregates > medium macroaggregates > and large macroaggregates (Figure 13). It was hypothesized that greater respiration would result from microaggregates compared to large macroaggregates because macroaggregates are associated with greater OM and clay content (Jastrow, 1996), and thus would be expected to have greater protection against microbial decomposition (Balesdent et al., 2000). Another hypothesis was that respired C would be greatest in microaggregates under conventional tillage treatments compared to no-tillage treatments and other aggregate sizes due to increased aeration and temperature from disturbance (Beare et al., 1994). Tillage treatment among the aggregate size fractions showed that small macroaggregates were the only fraction with differences in tillage treatment (greater respired C in conventional tillage compared to no-tillage) (Figure 13), and overall had the greatest respired C of all aggregate sizes under conventional or no-tillage treatments. A possible explanation is that conventional tillage allowed OM from plant residue at the surface to be mixed into the soil and stimulated microbial biomass growth or activity; my results showed significant respired C under vetch cover crops in the small macroaggregate fraction (Table 4). Research by Plante and McGill (2002) proposed that aggregate turnover rates control whether incoming OM, from plant residue for example, is occluded into soil aggregates or lost via rapid mineralization. They explain occlusion can only occur when aggregates form around the incoming OM, and my results suggest that conventional tillage may increase

soil aggregate turnover rates (greater amounts of small macro- and microaggregates were observed in conventional tillage treatments) to a threshold point where OM is no longer occluded for a time period long enough before it is re-exposed in the small macroaggregates. Soil C sequestration decreases in this situation because the incoming OM lost opportunities to interact with reactive surfaces, and instead greater mineralization of the OM was observed as respired soil C.

It is important to note that my total C enzyme activity analysis and respired C analysis within the dry aggregate sizes appear to conflict: as aggregate size increased, total C enzyme activity increased while respiration rates decreased. This could be due to differences in experimental methodologies where extracellular enzyme analyses measured a potential activity after soil samples had been physically destroyed (by blending) and then flooded with a buffer solution, while microbial respiration was measured by incubating intact dry aggregates (rewetted to 15% of moisture content). The discrepancies between these two methods may be commonly found in literature concerning biological characteristics of aggregate size fractions depending on the method of fractionation used (Ashman et al., 2003). Concentrations of TOC and mineralization measured from slaking and wet sieving methods often result with greater concentration or activity in macroaggregates compared to microaggregates (Ashman et al., 2003; Puget et al., 1995; Six et al., 2000), which favor my total C enzyme activity results. Conversely, dry fractionation methods often result in greater TOC and mineralized C in microaggregates compared to macroaggregates (Seech and Beaucheamp, 1988; Ashman et al., 2003), which favor my short-term respired C results.

In conclusion, differences in enzyme activity and respired C results within aggregate sizes were most likely a factor of rewetting processes that can change relationships between aggregate sizes and their biological properties (Bach and Hofmockel, 2014).

CHAPTER VI CONCLUSIONS

The longevity of this experimental cotton system paired with a long-term incubation study provided a great opportunity to gain a mechanistic understanding of biogeochemical dynamics of SOC pools influenced by CA practices such as no-tillage, cover cropping, and N fertilizer applications. More detailed biological controls on C-cycling were determined with whole soil extracellular enzyme assays measuring enzyme activity in response to the CA treatments, and physical fractionation was used to determine activity for soil C and its vulnerability among different aggregate size fractions.

The active SOC pool showed distinct differences between applications of reduced (34N kg ha^{-1}) and high N fertilization rates (101N kg ha^{-1}) when combined with conventional tillage, and suggest that high N-rate applications and soil disturbance results in more soil C allocated in a rapidly turning over C-pool, and reduces soil C stability overall in this cotton system. The slow SOC pool revealed that given more time, soil C-losses would stabilize under reduced N-rates and no-tillage CA treatments and accounted for over four times the amount of respired C relative to the active pool. Broad nutrient acquisition efforts indicated by potential activities of whole soil total C, N, and P reflected differences between N availability and N form under organic or inorganic additions to the cropping system.

Less soil disturbance had a clear effect on the dry aggregate size fractions, and persisted when combined with cover crop management. Macro- and microaggregates within conventional tillage systems were distinct from those in no-tillage systems due to

such pronounced enzyme activity observed for all C, N, and P enzyme totals, and could indicate faster decomposition rates in conventional tillage compared to no-tillage management. Soil C was less vulnerable to decomposition in large (> 2 mm) and medium macroaggregates (1-2 mm) because respired C was significantly less compared to small macro- (0.25-1 mm) and microaggregates (< 0.25 mm). The medium and small macroaggregates both showed greater respired C under high N fertilization (101N kg ha^{-1}) combined with conventional tillage systems compared to reduced N-rates with no-tillage systems. This observation was consistent with my finding that the active C-pool and sugar (BG) degrading enzymes in all macroaggregate size fractions (>0.25 mm) showed greater microbial activity under high N-rates and conventional tillage. This suggests that an active C-pool may be rapidly turning over in the medium and small macroaggregates of conventional tillage systems, where sugar (BG) substrates are more accessible via soil mixing and acted on by the microbial community.

Additions of high N fertilizer rates incorporate labile substrates to the soil surface that are readily utilized and mineralized by the microbial community, but it is suspected that this effectively reduces soil C stability overall. Substrate occlusion can occur when aggregates form around incoming OM, and my results suggest that conventional tillage may increase soil aggregate turnover rates to a threshold point where OM is no longer occluded for a time period long enough before it is re-exposed in the small macroaggregates (greater amounts of small macro- and microaggregates were observed in conventional tillage treatments). Soil C sequestration decreases in this

situation because the incoming OM lost opportunities to interact with reactive surfaces, and instead greater mineralization of the OM was observed as respired soil C.

Agricultural management practices are integrally linked to biogeochemical processes that balance soil C inputs for storage or release as atmospheric CO_2. Respired SOC pools from this study showed a much larger portion of C in the slow pool under CA practices, where inorganic inputs coupled with tillage may be more important for controlling SOC stability in this cotton system. However, whole soil extracellular enzyme analyses showed differences between total C and N acquisition associated with N form or availability from organic cover cropping or inorganic fertilizer inputs. Dependent on future research to assess total C from aggregate fractions, we could be seeing small macro- and microaggregates turning over in a rapid C-pool where the microbial community utilizes simple glucose compounds under conventional tillage.

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APPENDIX

Table A 1. N fertilizing rate (N-rate), cover crop, and tillage effects on soil physical properties (pH and bulk density were analyzed by Mbuthia et al. (2015)). Numbers inside parentheses are (±1) standard error of the mean, and lettering denotes statistical significance at $P < 0.05$. Bolded ANOVA numbers show statistical significance at $P < 0.05$.

Soil Physical Properties			
Treatment factors	pH	Bulk Density (g cm^{-3})	Gravimetric Moisture Content (%)
N-rate (kg ha^{-1})			
0N	5.80(0.15)a	1.43(0.03)a	20.55(0.85)b
34N	5.63(0.12)a	1.43 (0.03)a	23.54(0.77)ab
67N	5.39(0.14)b	1.37(0.03)a	24.51(0.37)a
101N	5.01(0.13)c	1.40(0.03)a	24.55(0.62)a
Cover Crop			
No cover	5.44(0.06)a	1.43(0.02)a	22.85(0.77)b
Wheat	5.51(0.06)a	1.39(0.02)a	22.59(0.64)b
Vetch	5.40(0.06)a	1.40(0.02)a	24.57(0.42)a
Tillage			
No-tillage	5.40(0.05)a	1.44(0.01)a	23.23(0.55)a
Conventional tillage	5.50(0.04)a	1.37(0.01)b	23.44(0.49)a
Sources of Variation	ANOVA (LSD protected, $P < 0.05$)		
N-rate	**< 0.0001**	0.397	0.0879
Cover crop	0.390	0.392	**0.0244**
Tillage	0.091	**0.002**	0.6544
N-rate*Cover crop	0.415	0.770	0.4503
N-rate*Tillage	0.472	0.960	0.7314
Cover crop*Tillage	0.857	0.456	0.8832
N-rate*Cover crop*Tillage	0.844	0.155	0.7477

Table A 2. N fertilizing rate (N-rate), cover crop, and tillage effects on respired SOC labile and intermediate pools (mg g^{-1} dry soil). Numbers inside parentheses are (±1) standard error of the mean, and lettering denotes statistical significance at $P < 0.05$. Bolded ANOVA numbers show statistical significance at $P < 0.05$.

Respired SOC Active & Slow Pools (mg g^{-1} dry soil)		
Treatment factors	Active C-pool	Slow C-pool
N-rate (kg ha^{-1})		
0N	0.85(0.07)a	2.51(0.23)b
34N	0.83(0.07)a	2.81(0.33)b
67N	0.78(0.05)a	2.95(0.15)b
101N	0.81(0.07)a	4.12(0.25)a
Cover Crop		
No cover	0.84(0.07)a	2.94(0.29)a
Wheat	0.75(0.04)a	3.03(0.25)a
Vetch	0.87(0.06)a	3.31(0.15)a
Tillage		
No-tillage	0.83(0.05)a	3.52(0.21)a
Conventional tillage	0.81(0.04)a	2.67(0.16)b
Sources of Variation	ANOVA (LSD protected, $P < 0.05$)	
N-rate	0.9034	**0.0225**
Cover crop	0.2077	0.3686
Tillage	0.6234	**0.0004**
N-rate*Cover crop	0.0911	0.2497
N-rate*Tillage	**0.0086**	0.2025
Cover crop*Tillage	0.0648	0.3096
N-rate*Cover crop*Tillage	0.0823	0.2648

Table A 3. N fertilizing rate (N-rate), cover crop, and tillage effects on soil C concentration (g m^{-2}) where cumulative C respired after 602-days was subtracted from total C. Bulk density and total C were used by Mbuthia et al. (2015) for soil C concentration calculations. Numbers inside parentheses are (±1) standard error of the mean, and lettering denotes statistical significance at $P < 0.05$. Bolded ANOVA numbers show statistical significance at $P < 0.05$.

Soil C Concentration $Total\ soil\ C - Respired\ C\ =\ Soil\ C\ concentration$	
Treatment factors	**Soil C concentration (g m^{-2})**
N-rate (kg ha^{-1})	
0N	949.54(46.8)b
34N	1057.5(51.1)ab
67N	941.37(36.8)b
101N	1145.4(56.6)a
Cover Crop	
No Cover	999.37(42.7)a
Wheat	1011.0(43.2)a
Vetch	1056.6(45.2)a
Tillage	
No-tillage	1117.2(40.8)a
Conventional tillage	933.04(28.2)b
Sources of Variation	ANOVA (LSD protected, $P < 0.05$)
N-rate	**0.0429**
Cover crop	0.3271
Tillage	**0.0067**
N-rate*Cover crop	0.2549
N-rate*Tillage	0.1070
Cover crop*Tillage	0.4285
N-rate*Cover crop*Tillage	0.8515

Table A 4. N fertilizing rate (N-rate), cover crop, and tillage effects on whole soil extracellular enzyme activities. Extracellular enzymes assayed include: BG, CB, XYL, AG, LAP, NAG, PHOS, Total C, and Total N. Numbers inside parentheses are (±1) standard error of the mean, and lettering denotes statistical significance at $P < 0.1$. Bolded ANOVA numbers show statistical significance at $P < 0.1$.

	Whole soil extracellular enzyme activities (nmol h^{-1} g^{-1} dry aggregate)								
Treatment factors	BG	CB	XYL	AG	LAP	NAG	PHOS	Total C	Total N
N-rate (kg ha^{-1})									
0N	290.78(16.3)a	107.25(8.5)a	36.69(4.3)b	4.62(3.7)b	61.11(4.8)b	143.60(8.6)b	926.19(30.1)a	439.34(22.8)a	204.71(12.4)b
34N	363.66(16.2)a	131.44(8.6)a	53.42(5.2)a	9.78(2.6)a	108.11(8.3)a	193.42(9.7)a	966.85(45.0)a	558.31(34.8)a	289.78(15.2)a
67N	362.37(16.5)a	118.97(8.3)a	61.15(4.4)a	4.84(1.9)b	61.93(5.2)b	187.70(11.8)a	1046.5(35.9)a	546.75(27.5)a	240.07(11.3)b
101N	318.40(22.1)a	109.63(8.5)a	67.55(4.1)a	8.17(2.1)a	48.79(4.0)b	188.18(10.2)a	1017.6(46.5)a	503.75(25.5)a	236.98(7.5)b
Cover Crop									
No cover	326.70(17.7)a	110.86(6.5)a	50.19(4.2)a	5.83(2.1)a	68.97(6.2)a	178.60(9.8)a	974.27(37.4)a	493.59(29.2)a	235.04(16.7)a
Wheat	333.41(16.8)a	117.56(7.7)a	50.22(3.9)a	7.41(1.8)a	69.15(6.1)a	175.43(10.3)a	947.18(38.1)a	508.18(15.9)a	244.72(11.9)a
Vetch	341.30(14.7)a	122.05(8.1)a	63.70(4.6)a	7.31(2.8)a	71.84(6.5)a	180.65(8.0)a	1046.4(28.0)a	534.36(35.2)a	248.90(16.6)a
Tillage									
No-tillage	335.35(13.9)a	122.04(6.4)a	57.37(3.7)a	7.54(1.9)a	70.57(5.7)a	182.23(8.0)a	989.45(33.6)a	522.01(23.6)a	247.03(13.7)a
Conventional tillage	332.26(12.7)a	111.60(5.6)a	52.04(3.4)a	6.16(1.6)a	69.40(4.5)a	174.22(7.3)a	989.10(23.5)a	502.06(22.3)a	238.74(10.7)a
Sources of Variation	ANOVA (LSD protected, $P < 0.1$)								
N-rate	0.331	0.4046	**0.0004**	**0.0177**	**0.0243**	**0.0921**	0.5884	0.3709	**0.0665**
Cover crop	0.6709	0.7374	0.1217	0.1869	0.8166	0.7984	0.2049	0.5356	0.7402
Tillage	0.9929	0.2626	0.2383	0.3281	0.6946	0.3849	0.6227	0.2638	0.4060
N-rate*Cover crop	0.2134	0.3357	0.6029	**0.0281**	0.5334	0.5916	0.6689	0.1435	0.5666
N-rate*Tillage	0.3983	0.4185	0.5691	0.6101	0.1296	0.2106	0.3117	0.5295	0.1410
Cover crop*Tillage	**0.0579**	**0.0353**	0.7662	0.3199	**0.0492**	0.4221	**0.0550**	**0.0860**	0.1457
N-rate*Cover crop*Tillage	0.8688	0.7172	0.9985	0.7206	**0.0991**	0.6589	0.9614	0.9451	0.6739

Table A 5. N fertilizing rate (N-rate), cover crop, and tillage effects on extracellular enzyme activities from dry macroaggregate size > 2 mm. Extracellular enzymes assayed include: BG, CB, LAP, NAG, PHOS, Total C, and Total N. XYL and AG had analogous undetectable values and are marked with dashes (--). Numbers inside parentheses are (±1) standard error of the mean, and lettering denotes statistical significance at $P < 0.1$. Bolded ANOVA numbers show statistical significance at $P < 0.1$.

	Aggregate > 2mm Extracellular enzyme activities (nmol h dry g aggregate)								
Treatment factors	BG	CB	XYL	AG	LAP	NAG	PHOS	Total C	Total N
N-rate (kg ha^{-1})									
0N	67.0(3.7)a	10.21(1.23)a	--	--	701.2(32.6)a	18.04(2.63)a	187.6(11.7)b	80.0(5.28)a	82.51(5.25)a
34N	61.91(4.75)a	12.06(1.52)a	--	--	740.8(39.2)a	15.59(2.83)a	206.0(14.6)b	77.1(6.97)a	79.63(7.10)a
67N	59.25(5.07)a	118.8(74.3)a	--	--	696.7(43.7)a	21.54(2.78)a	220.9(10.5)b	145.0(52)a	147.5(52.3)a
101N	63.52(9.14)a	19.31(4.76)a	--	--	728.4(41.3)a	46.75(9.23)a	327.1(28.9)a	88.3(16.3)a	90.86(16.2)a
Cover Crop									
No cover	63.44(4.83)a	81.27(48.1)a	--	--	715.9(32.5)a	20.96(3.26)a	226.0(16.0)a	135.1(39.4)a	137.6(39.5)a
Wheat	63.12(5.26)a	14.79(2.64)a	--	--	694.8(38.9)a	26.59(5.13)a	228.5(18.9)a	82.58(9.55)a	85.12(9.51)a
Vetch	62.15(5.21)a	10.43(1.42)a	--	--	738.5(29.7)a	26.40(5.23)a	246.8(18.7)a	74.69(7.31)a	77.22(7.29)a
Tillage									
No-tillage	59.95(4.52)b	38.65(25.6)a	--	--	701.6(29.8)a	25.31(3.77)a	236.7(14.9)a	92.27(18.8)b	94.80(18.9)b
Conventional tillage	65.92(3.63)a	34.75(22.9)a	--	--	731.8(24.9)a	23.76(3.65)a	230.8(14.2)a	103.9(21.6)a	106.4(21.6)a
Sources of Variation	ANOVA (LSD protected, $P < 0.1$)								
N-rate	0.5931	0.7358	--	--	0.8837	0.2997	**0.0818**	0.7517	0.7919
Cover crop	0.5452	0.1758	--	--	0.5082	0.4970	0.5423	0.3746	0.3909
Tillage	**0.0681**	0.6559	--	--	0.3129	0.9178	0.5564	**0.0784**	**0.0873**
N-rate*Cover crop	0.5641	**0.0889**	--	--	0.9554	0.3034	0.6404	0.6509	0.6458
N-rate*Tillage	**0.0263**	0.7064	--	--	0.7203	0.7504	0.9534	0.1077	0.1097
Cover crop*Tillage	0.3191	0.8988	--	--	**0.0018**	0.2987	0.2168	0.3633	0.3327
N-rate*Cover crop*Tillage	0.2070	0.8844	--	--	0.6000	**0.0623**	0.1697	0.3699	0.3523

Table A 6. N fertilizing rate (N-rate), cover crop, and tillage effects on extracellular enzyme activities from dry macroaggregate size 1-2 mm. Extracellular enzymes assayed include: BG, CB, LAP, NAG, PHOS, Total C, and Total N. XYL and AG had analogous undetectable values and are marked with dashes (--). Numbers inside parentheses are (±1) standard error of the mean, and lettering denotes statistical significance at $P < 0.1$. Bolded ANOVA numbers show statistical significance at $P < 0.1$.

	Aggregate 1-2 mm Extracellular enzyme activities (nmol h^{-1} g^{-1} dry aggregate)								
Treatment factors	BG	CB	XYL	AG	LAP	NAG	PHOS	Total C	Total N
N-rate (kg ha^{-1})									
0N	10.64(0.72)a	1.62(0.21)a	--	--	116.7(8.78)ab	3.20(0.54)a	30.5(2.70)a	12.9(1.12)a	119.7(8.76)ab
34N	10.62(0.78)a	1.64(0.29)a	--	--	132.4(9.64)a	2.49(0.26)a	35.2(2.24)a	12.6(1.10)a	134.7(9.59)a
67N	8.11(0.94)b	23.9(17.7)a	--	--	104.7(10.9)bc	2.47(0.43)a	30.8(2.84)a	23.6(11.3)a	107.1(11.0)bc
101N	7.64(0.96)b	1.88(0.38)a	--	--	92.08(6.53)c	4.38(0.88)a	37.3(3.28)a	10.0(1.58)a	95.7(6.51)c
Cover Crop									
No cover	10.2(0.79)a	15.3(10.7)a	--	--	121.1(8.16)a	2.94(0.42)a	36.3(2.49)a	23.5(8.69)a	123.9(8.19)a
Wheat	8.92(0.68)a	1.59(0.23)b	--	--	103.1(7.63)a	3.39(0.56)a	30.7(2.38)a	11.0(1.02)a	106.2(7.62)a
Vetch	8.64(0.83)a	1.53(0.26)b	--	--	109.5(8.66)a	2.99(0.51)a	33.1(2.38)a	10.2(1.14)a	112.1(8.64)a
Tillage									
No-tillage	7.89(0.58)b	9.58(8.06)a	--	--	94.25(5.47)b	3.00(0.35)a	29.8(1.66)b	15.0(5.63)b	96.9(5.44)b
Conventional tillage	10.6(0.62)a	3.77(2.06)a	--	--	128.6(6.92)a	3.21(0.44)a	37.0(2.2)a	14.8(1.86)a	131.7(6.91)a
Sources of Variation	ANOVA (LSD protected, $P < 0.1$)								
N-rate	**0.0634**	0.5059	--	--	**0.0064**	0.6625	0.5377	0.4918	**0.0075**
Cover crop	0.1395	**0.0960**	--	--	0.1412	0.4736	0.2123	0.1052	0.1506
Tillage	**0.0006**	0.4560	--	--	**0.0001**	0.5464	**0.0045**	**0.0036**	**0.0001**
N-rate*Cover crop	0.7323	**0.0480**	--	--	0.1352	0.9795	0.5410	0.5072	0.1343
N-rate*Tillage	**0.0365**	0.5267	--	--	0.1515	0.4065	0.5599	**0.0901**	0.1463
Cover crop*Tillage	0.8462	0.2551	--	--	**0.0418**	0.6899	0.9334	0.9425	**0.0557**
N-rate*Cover crop*Tillage	0.3445	**0.0665**	--	--	0.3629	0.8834	0.4584	0.4775	0.3345

Table A 7. N fertilizing rate (N-rate), cover crop, and tillage effects on extracellular enzyme activities from dry macroaggregate size 0.25-1 mm. Extracellular enzymes assayed include: BG, CB, LAP, NAG, PHOS, Total C, and Total N. XYL and AG had analogous undetectable values and are marked with dashes (--). Numbers inside parentheses are (±1) standard error of the mean, and lettering denotes statistical significance at $P < 0.1$. Bolded ANOVA numbers show statistical significance at $P < 0.1$.

	Aggregate 0.25-1 mm Extracellular enzyme activities (nmol h^{-1} g^{-1} dry aggregate)								
Treatment factors	BG	CB	XYL	AG	LAP	NAG	PHOS	Total C	Total N
N-rate (kg ha^{-1})									
0N	12.6(0.95)a	1.79(0.15)a	--	--	143.9(13.4)ab	3.93(0.57)a	35.6(2.70)a	14.9(1.08)a	147.6(13.5)ab
34N	14.7(1.38)a	2.43(0.42a	--	--	174.6(14.1)a	3.71(0.48)a	47.7(4.55)a	17.9(1.96)a	178.3(14.2)a
67N	11.4(1.35)a	23.9(14.7)a	--	--	129.3(12.7)bc	4.29(0.48)a	38.9(3.99)a	29.6(10.6)a	133.6(12.9)bc
101N	9.24(1.24)a	2.28(0.53)a	--	--	107.7(9.26)c	5.50(0.94)a	39.9(3.80)a	12.3(2.06)a	112.2(9.32)c
Cover Crop									
No cover	12.7(1.2)a	16.1(10.5)a	--	--	156.4(12.2)a	3.95(0.44)a	44.1(3.35)a	26.1(8.29)a	160.3(12.4)a
Wheat	11.9(1.0)a	3.27(0.93)a	--	--	132.2(11.3)a	4.83(0.65)a	40.1(3.58)b	16.3(1.73)a	130.1(10.3)a
Vetch	11.2(1.1)a	2.29(0.28)a	--	--	126.3(10.3)a	4.16(0.51)a	37.2(3.02)b	13.8(1.50)a	136.9(11.4)a
Tillage									
No-tillage	10.0(0.84)b	5.62(3.11)b	--	--	120.6(9.57)b	3.88(0.39)b	35.6(2.27)b	14.9(2.47)b	124.3(9.63)b
Conventional tillage	14.0(0.90)a	8.26(5.74)a			156.7(8.49)a	4.76(0.49)a	45.5(2.99)a	22.8(5.22)a	161.2(8.48)a
Sources of Variation	ANOVA (LSD protected, $P < 0.1$)								
N-rate	0.1918	0.2466	--	--	**0.0315**	0.8335	0.6112	0.5021	**0.0302**
Cover crop	0.3906	0.6818	--	--	0.1175	0.8206	**0.0965**	0.3144	0.1414
Tillage	**< 0.0001**	**0.0646**	--	--	**0.0003**	**0.0261**	**0.0007**	**< 0.0001**	**0.0003**
N-rate*Cover crop	0.7280	0.3660	--	--	0.6197	0.8574	0.7213	0.8300	0.6434
N-rate*Tillage	**0.0272**	0.2330	--	--	0.8050	0.3627	0.8546	**0.0111**	0.7861
Cover crop*Tillage	0.5642	0.9730	--	--	0.6306	0.1248	**0.0738**	0.7706	0.5842
N-rate*Cover crop*Tillage	0.2423	0.5658	--	--	0.2411	**0.0989**	0.2800	**0.0367**	0.2274

Table A 8. N fertilizing rate (N-rate), cover crop, and tillage effects on extracellular enzyme activities from dry microaggregate size < 0.25 mm. Extracellular enzymes assayed include: BG, CB, LAP, NAG, PHOS, Total C, and Total N. XYL and AG had analogous undetectable values and are marked with dashes (--). Numbers inside parentheses are (±1) standard error of the mean, and lettering denotes statistical significance at $P < 0.1$. Bolded ANOVA numbers show statistical significance at $P < 0.1$.

	Aggregate < 0.25 mm Extracellular enzyme activities (nmol h^{-1} g^{-1} dry aggregate)								
Treatment Factors	BG	CB	XYL	AG	LAP	NAG	PHOS	Total C	Total N
N-rate (kg ha^{-1})									
0N	20.2(1.83)a	3.38(0.47)a	--	--	210.0(18.4)a	6.18(1.18)a	52.8(4.53)a	26.7(3.35)a	216 (18.9)ab
34N	19.3(1.69)a	3.29(0.54)a	--	--	220.7(14.9)a	5.90(1.35)a	59.6(5.26)a	23.7(2.43)a	227 (15.1)a
67N	16.8(1.49)a	62.1(37.2)a	--	--	185.1 (15.8)ab	6.15(0.80)a	55.3(4.31)a	64.9(28.7)a	191 (16.0)b
101N	13.0(1.30)a	2.35(0.51)a	--	--	151.1 (11.4)b	5.45(0.96)a	52.6(3.25)a	15.7(1.85)a	157(11.1)c
Cover Crop									
No cover	17.6(1.52)a	31.6(24.3)a	--	--	204.1 (15.2)a	5.74(1.03)a	57.0(3.67)a	44.0(20.1)a	209.6(15.1)a
Wheat	18.8(1.67)a	17.0(13.3)a	--	--	196.7 (16.1)a	6.86(1.10)a	58.1(4.79)a	36.4(11.3)a	203.6(15.9)a
Vetch	16.0(1.15)a	3.21(0.39)a	--	--	176.3 (11.0)a	5.20(0.58)a	50.4(2.77)a	20.0(1.61)a	181.3(11.1)a
Tillage									
No-tillage	14.5(1.13)b	15.2(9.79)a	--	--	164.1 (10.3)b	4.90(0.47)b	48.4(2.83)b	27.1(7.70)b	169.2(10.1)b
Conventional tillage	20.5(1.08)a	18.8(15.0)a	--	--	221.6 (11.1)a	6.99(0.96)a	62.2(3.10)a	40.0(13.6)a	227.9(11.5)a
Sources of Variation	ANOVA (LSD protected, $P < 0.1$)								
N-rate	0.1249	0.1538	--	--	**0.0415**	0.8846	0.9402	0.1876	**0.0103**
Cover crop	0.6791	0.6212	--	--	0.4611	0.8327	0.4161	0.5952	0.4880
Tillage	**< 0.0001**	0.2335	--	--	**< 0.0001**	**0.0100**	**< 0.0001**	**0.0004**	**< 0.0001**
N-rate*Cover crop	0.8440	0.5007	--	--	0.8972	0.5137	0.7985	0.7992	0.9048
N-rate*Tillage	0.3297	0.3234	--	--	0.7096	0.6386	0.4195	0.4928	0.7479
Cover crop*Tillage	0.6712	0.4559	--	--	0.4637	0.1564	**0.0380**	0.8954	0.4030
N-rate*Cover crop*Tillage	0.1262	0.1715	--	--	0.5793	0.2502	0.4639	**0.0254**	0.5608

VITA

Candace Brooke Wilson was born in Chattanooga, TN on April 2, 1990, and graduated from Notre Dame High School in 2008. Later that year, she moved to Knoxville, TN and completed her Bachelor of Science in Environmental Soil Science from the University of Tennessee in 2012. As an undergraduate, she worked in a soil chemistry laboratory for Dr. Michael E. Essington. In the fall of 2013 Candace enrolled at the University of Tennessee to pursue a Master of Science degree in Environmental and Soil Science in the Biosystems Engineering and Soil Science department while working as a graduate research assistant for Dr. Sean M. Schaeffer.