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The accumulation of new carbon input and microbial residues in soil under long-term conservation agricultural management practices

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I am submitting herewith a dissertation written by Lidong Li entitled "The accumulation of new carbon input and microbial residues in soil under long-term conservation agricultural management practices." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant, Soil and Environmental Sciences.

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Abstract

A better understanding of the mechanisms of soil organic matter (SOM) stabilization is necessary for improving soil quality, especially in agroecosystems. This doctoral dissertation research studied the effects of long-term conservation agricultural management practices on the accumulation of newly added labile carbon (C) and microbially derived SOM. To study their accumulation in soil, newly added labile C was represented by carbon-13 (^{13}C) labelled glucose and the microbially derived SOM was represented by amino sugars.

Short-term drying-rewetting cycles are common in surface soils, especially in agroecosystems, which may have different effects on different C pool. Understanding the accumulation and mineralization of newly added labile C in soil during drying-rewetting cycles is important for predicting soil organic C (SOC) storage in long-term. A 24-day incubation in microcosms was conducted with an agricultural soil under 36 years of conservation management. I added ^{13}C-labelled glucose and applied different frequencies of drying-rewetting cycles to the microcosms. At the end of the 24-day incubation, 0.08%-1% of the added glucose C was incorporated into the extractable organic C (EOC) pool, 4%-27% of the added glucose C was incorporated into the microbial biomass C (MBC) pool, and 0.7%-5% of the added glucose C was incorporated into the hydrogen peroxide (H_2O_2)-resistant C pool. The drying treatment induced higher recovery of the added glucose C in each C pool. The vetch cover crops are more favorable for the stabilization of newly added labile C under repeated drying-rewetting cycles. Structural equation model shows that chemical association and biochemical recalcitrance rather than

physical protection are major controls of labile C sequestration in soil under drying-rewetting cycles.

Understanding the physical, chemical, and microbial processes controlling the retention of microbial residues in soil is essential for predicting the accumulation of microbially derived SOM. I measured amino sugar concentration, C and nitrogen (N) concentrations microbial respiration rate, extracellular enzyme activity, and soil aggregate composition in an agricultural soil under 31-years of conservation management. Structural equation models show that physical protection plays a critical role in muramic acid stabilization, while microbial activity and substrate availability are more critical for glucosamine.

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Chapter 1 Introduction

To maintain the sustainability of agroecosystems, SOC sequestration improves and sustains agronomic productivity (Lal, 2004), and consequently food security (Lal, 2013; Lal, 2014). To sustain SOC content, conservation agricultural management practices, such as no-tillage farming and cover crops, are effective strategies (Lal, 2004). However, the degree to which these conservation agricultural management practices will affect the sequestration and long-term stability of newly added labile C in agroecosystems under water stress is largely uncertain.

Moisture pulses in surface soil are common and frequent events. They impact soil physical, chemical, and microbiological properties, including soil aggregates (Ma et al., 2015), soil swelling-shrinkage (Fernandes et al., 2015), soil microbial activities (Lado-Monserrat et al., 2014), and soil microbial community structure (Fierer et al., 2003; Barnard et al., 2015), and therefore influence the sequestration of newly added labile C. Changing the frequency of the moisture pulse events can alter the ability of soil to preserve the newly added labile C (Miller et al., 2005; Lado-Monserrat et al., 2014; Morillas et al., 2015).

Microbial residues are an important component of stabilized SOM (Kallenbach et al., 2016; Chao et al., 2017). Amino sugars can be biochemically resistant nutrient reserves. They are microbial cell wall components that can be used as biomarkers to indicate microbial community contribution to SOM turnover and sequestration (Parsons, 1981; He et al., 2011; Paul, 2014). Tillage and cover crops have been shown to impact soil

microbial activity and structure (Mbuthia et al., 2015), and therefore impact the accumulation of microbial residues.

Hence, the mineralization and accumulation of newly added labile substrates and microbially derived resistant compounds are critical processes in SOM turnover. The goal of my project is (1) to trace the fate of newly added labile C under drying-rewetting cycles and (2) to determine the controls of microbial residue (i.e. amino sugar) stabilization in agricultural soils under the effects of conservation management. My research will (1) improve the understanding of the physical, chemical, biochemical, and microbiological mechanisms controlling C sequestration under moisture pulse events in soil under conservation management, and (2) elucidate the mechanisms of the stabilization of microbially derived organic matter in soils under long-term conservation agricultural management.

1.1 SOC cycling in agroecosystems

Input of C from vegetation is a main source of SOC (Cotrufo et al., 2009), which includes aboveground and belowground inputs (Mazzilli et al., 2015). The aboveground inputs, involving leaves, stems, and floral structures, generally contribute less to SOC than belowground inputs (Mazzilli et al., 2015). Plants direct 40%-60% of photosynthetically fixed C to belowground biomass, including root biomass and root exudates (Högberg et al., 2001; Clemmensen et al., 2013; Keiluweit et al., 2015). Root biomass contributes from 2.4 times (Rasse et al., 2005) to 13 times (Kong and Six, 2010) more SOC than aboveground biomass (Redin et al., 2014). Root biomass is more biochemically resistant

compared to root exudates. Cellulose-C and lignin-C account 30.2% and 9.4% of root biomass C, respectively (Fernandez et al., 2003). Root exudates consist of small molecular compounds such as amino acids, carbohydrates, and organic acids (Nguyen, 2003; Kuzyakov and Jones, 2006). These small molecular compounds are an important source of dissolvable organic C (DOC) and a readily bioavailable supply for soil microbes (Ge et al., 2015; Keiluweit et al., 2015).

The incorporation of new C input into SOC is a complicated process. As shown in Figure 1.1, soil microbes decompose fresh plant inputs and respire CO_2 (Valentini et al., 2000). During microbial decomposition, dissolvable molecules will be released (Swift et al., 1979). The dissolvable molecules are a main source of microbial biomass because of the rapid uptake and assimilation by microbes (van Hees et al., 2005; Cotrufo et al., 2013). After microbes die, their residues accumulate in soil. Recalcitrant plant compounds also accumulate in soil due to their resistance to microbial decomposition. A portion of the SOC will be occluded by soil aggregates or adsorbed by soil mineral surfaces, and therefore are protected from microbial decomposition (Schmidt et al., 2011; Ahrens et al., 2015). As shown in Figure 1.2, biochemical recalcitrance, chemical association, and physical protection are the three main mechanisms of SOC stabilization in soil (Jastrow and Miller, 1997). The long-term preservation of SOC can be identified as two processes: stabilization processes and persistence processes (Ahrens et al., 2015). The stabilization process hypothesis claims that SOC is stabilized in soil due to physical protection and chemical association and biochemical recalcitrance (Gleixner, 2013; Ahrens et al., 2015). The persistence process hypothesis claims that SOC is retained in soil because C atoms

are continuously recycled via the synthesis of new compounds using old materials (Gleixner, 2013).

The incoming plant C consists of compounds at different turnover rates. It is difficult to describe SOC dynamics because components of SOC are of different turnover rates. Therefore, conceptual C pools with characteristic turnover rates were proposed to define turnover time and pool size of different compartments. According to the two-pool C model, the plant C input can be split into two compartments (Jenkinson, 1990), each of which represents a functionally homogeneous soil unit. Each compartment decomposes at a first order process, with one much slower than the other (Jenkinson, 1990). The passive pool includes SOC that is resistant to microbial decomposition and protected from decomposers by aggregation and mineral association (von Lützow et al., 2008; Plaza et al., 2013). The active pool includes SOC that is not occluded or associated by soil matrix. It is susceptible to microbial decomposition, and therefore is a small portion of SOC in soils (Brown et al., 2014).

The capacity of the active pool and the passive pool for C storage is different due to C saturation. Stewart et al. (2007) define soil C saturation as the limitation of soil to stabilize C represented by the collective function of the physically, chemically, biochemically protected, and the non-protected pools. Soil C content is a non-steady state equilibrium of C input and output. Thus, if something increases soil C input (cover crops, higher yield, etc.), soil C content will increase until a new equilibrium is reached. There will be a maximum equilibrium according to the definition of C saturation (Stewart et al., 2007). As with soil C pools that are without saturation, there is no limitation for soil C

content (Stewart et al., 2007). The accumulation of C in the active pool does not saturate because of no physical limitation on capacity, but the passive pool does (Stewart et al., 2007).

1.2 The impact of conservation agricultural management practices on SOC

Agricultural soils are always depleted in C because of harvesting and crop residue removal (Smith et al., 2012; Zhao et al., 2015). Applying cover crops is an efficient way to increase SOC stocks (Haque et al., 2015; Poeplau et al., 2015; Poeplau and Don, 2015). Adding crop residues can stimulate soil microbes from dormancy to activity by a flood of nutrients. Soil microbial biomass and activities rapidly respond to substrate supplies, inducing extra decomposition of SOC (Fontaine et al., 2004; Moreno-Cornejo et al., 2015), via priming effects (Dalenberg and Jager, 1989). Priming effects and extra CO_2 emission are usually coinstantaneous. The extra CO_2 can directly come from accelerated soil microbial biomass turnover, which is an apparent priming effect; the extra CO_2 can come from mineralization of SOC, which is a real priming effect (Blagodatskaya and Kuzyakov, 2008). The non-respired portion remains in soil, contributing to SOC. In addition to directly increasing SOC content, cover crops can increase SOC stability by enhancing aggregation (Chivenge et al., 2011). When crop residues are added to soils, bacteria and fungi increase their activities and therefore productivities. Soil particles are physically aggregated through fungal hyphae (Degens, 1997), extracellular polysaccharides (Chenu, 1995), and hydrophobic productivities (Capriel et al., 1990). All those microbial agents favorable for aggregation increases after addition of crop residues (Cosentino et al., 2006).

Tillage is one of the major causes of SOC depletion in agroecosystems (Six et al., 2000b). Perturbations causing faster turnover rate of macroaggregates and slower formation rate of microaggregates under conventional tillage is the dominant mechanism of SOC loss (Six et al., 1998; Six et al., 1999). Compared to conventional tillage, no-tillage systems exhibit increases in amount and stability of aggregates concomitant with increases in SOC (Paustian et al., 2000). No-tillage protects aggregate occluded SOC from microbial decomposition by not altering soil structure (Mazzoncini et al., 2016). The turnover rate of macroaggregates is reduced under no-tillage practices, and the formation of stable microaggregates is enhanced (Six et al., 2000a). As shown in Figure 1.3 (Six et al., 2000a), the fresh plant residues initiate the formation of macroaggregates and become coarse intra-aggregate particulate organic matter (iPOM). The coarse iPOM is then decomposed and fragmented into fine iPOM (Six et al., 2000a). The fine iPOM can be associated with clay and microbial products to form microaggregates (Six et al., 1999). Tillage breaks down macroaggregates, releasing coarse iPOM. The reduced physical protection caused increased decomposition.

1.3 The impact of drying-rewetting cycles on SOC

SOC turnover is mainly driven by soil microbes. Drying-rewetting cycles impact SOC turnover by affecting soil microbes. Drought affects soil microbes in two ways, one of which is resource limitation. Drying the soils can alter soil structure and micro-environment, thus the interaction pattern between soil microbes and SOC is changed. Drought decreases soil water content and increases the proportion of air filled pores (Fuchslueger et al., 2014). This decreases the mobility of both substrates and microbes,

and therefore disconnects soil microbes from substrates (Parker and Schimel, 2011). As shown in Figure 1.4, when soil is moist, bacteria, predatory protozoan, pathogen, and substrates can move through soil pores; when soil is dry, they are isolated from each other by disconnected pore water (Parker and Schimel, 2011). In the scenario of low soil water content, fungi can develop large hyphal networks to facilitate long distance transport of water and nutrients (Fuchslueger et al., 2014). Bacteria can avoid drought for a longer period of time by inhabiting smaller soil pores due to their smaller volume (Moyano et al., 2013). The other one is resource reallocation. As shown in Figure 1.5, stress forces microbes to direct resources from growth to survival, such as accumulating osmolytes to avoid dehydration (Schimel et al., 2007). Microbial cellular water potential rapidly changes with that in the soil environment when soil water content changes because of their semipermeable membranes (Schimel et al., 2007). When soil water potential decreases, soil microbes have to synthesize or import osmolytes to resist desiccation (Schimel et al., 2007). Alternatively, soil microbes can resist to desiccation by shifting to dormancy or forming spores (Fuchslueger et al., 2014).

Rewetting dry soils generally induces higher microbial activities (Wu and Brookes, 2005) and higher respiration rates than constant field moisture (Fierer and Schimel, 2002; Sponseller, 2007; Chatterjee and Jenerette, 2011), which is termed as the “Birch effect” (Birch, 1958). The effect is mainly controlled by three mechanisms: substrate exposure, hydrologic connectivity, and release of microbial biomass. (1) Substrate exposure. During droughts, shrinking collapses soil aggregates and therefore previously protected SOC are exposed (Utomo and Dexter, 1982; Appel, 1998; Denef et al., 2001; Borken and Matzner, 2009). When rewetted, soil aggregates are further disrupted by swelling (Van Gestel et

al., 1993). Crushing aggregates lead to increases in soil respiration by exposing protected SOC to microbial decomposition (Navarro-García et al., 2012). (2) Hydrologic connectivity. Droughts limit the solubility of SOC and the mobility of microbes and extracellular enzymes (Borken and Matzner, 2009). At low soil matric potentials, discontinuous water films disconnect the access of decomposers to SOC; an increase in soil water content reconnects the pathways to facilitate accessibility (Parker and Schimel, 2011). (3) Release of microbial biomass. Soil microbes can respond to severe moisture conditions by importing and synthesizing osmolytes, and therefore a high intracellular solute concentration is formed to prevent dehydration (Bonaterra et al., 2005; Sagot et al., 2010). When the soil is rewetted, soil water potential sharply increases. To avoid being burst by high osmotic pressure, microbes need to balance the water potential by rapidly releasing the osmolytes to soil (Halverson et al., 2000). Rapid rewetting induces lyses of the microbial cells that are not able to release the osmolytes out of cells in time, and therefore provokes survived microbes to decompose the released substrates (Borken and Matzner, 2009).

Albeit rewetting causes a burst of microbial activity, cumulative CO_2 emission is lower than that under optimum moisture (Shi and Marschner, 2014). Repeated drying-rewetting cycles can weaken the effects of rewetting pulses because of limited SOC stock (Mikha et al., 2005). Rewetting pulses cannot compensate for low mineralization rates during drying (Borken and Matzner, 2009). However, in soils with undepleted SOC, drying-rewetting cycles increased cumulative CO_2 emission compared with constant optimum moisture (Xiang et al., 2008). Also, the SOC mineralization is associated with the intensity and duration of drought, the amount and distribution of moisture (Borken and

Matzner, 2009; Schaeffer et al., 2017). The size of CO_2 emission pulse increased with extended drying period and enlarged amount of water (Sponseller, 2007; Shi et al., 2015) but decreased with frequency of drying-rewetting cycles (Priemé and Christensen, 2001; Fierer and Schimel, 2002; Mikha et al., 2005; Borken and Matzner, 2009). While cumulative CO_2 emission increased with length of moist period (Shi et al., 2015) but decreased with frequency of drying-rewetting cycles (Shi and Marschner, 2014). In general, a short drying period and a prolonged rewetting period will lead to greater SOC mineralization (Borken and Matzner, 2009).

Separate from increasing CO_2 emission, drying-rewetting cycles destabilize SOC through increasing the accumulation of labile C during dry period. The accumulation of labile C may contribute to microbial production of extracellular polymeric substance (EPS), continued extracellular enzyme activity, and reduced microbial uptake (Schaeffer et al., 2017). EPS is hygroscopic substance produced by microbes to maintain cellular hydration when soil dries (Or et al., 2007). So EPS may contribute to labile C accumulation under drought condition (Schaeffer et al., 2017). Though extracellular enzyme activity is low during drought, it can continue to mineralize substrates (Steinweg et al., 2012). The products of the remaining extracellular enzyme activity accumulate in soil because microbial assimilation is limited (Schaeffer et al., 2017).

1.4 Structural equation modelling

Structural equation modeling is a statistical methodology for describing linear relationships among multiple variables, which uses a confirmatory approach to analyze a

structural theory (Byrne, 2013). The structural theory represents a "causal" relationship among the multiple variables (Bentler, 1988). Structural equation modeling tests pathways of influence among those variables. It is a combination of traditional types of statistical analyses, including regression, principal components analysis, and path analysis (McCune et al., 2002). In a process, some factors have direct effects on response variable, and some others have indirect effects on response variable through the mediation of other factors. Structural equation modeling not only addresses the direct factors to the response variable but also the mediated ones and how those factors interact with each other, which goes beyond the standard multiple regression approaches (Colman and Schimel, 2013). Generally, there are three steps to build a structural model: (1) to propose an *a priori* model according to experience or background information; (2) to test if important pathways are left out, if the existing pathways are significant, and if the model fits well; (3) to revise the *a priori* model by adding missing pathways and dropping insignificant pathways.

Although structural equation modeling has not been widely used in natural sciences, especially in soil science, it is a powerful statistical technique for examining the relationship between ecosystem structure and function (Sutton-Grier et al., 2010). Understanding the relationship between ecosystem structure and function is the ultimate goal of biological study (Odum and Barrett, 1971). In underground ecosystems, ecosystem structure is the relationships between the microbes, resource supplies, and physical habitat conditions of soils; Ecosystem function is the collection of processes that cycle materials, such as C, and those that move energy through the ecosystem, such as decomposition (Sutton-Grier et al., 2010). SOC content and stability can be indicated by

many factors, involving EOC, MBC, recalcitrant C, microbial respiration, extracellular enzyme activity, and soil aggregate composition, which are affected by drying-rewetting cycles, and conservation management practices. Structural equation modeling can be used to describe each factor's direct and indirect contributions to SOC stabilization and how those factors interact among each other to stabilize SOC.

1.5 Amino sugars

New C input can go through microbial transformation and incorporate into passive SOC (Cotrufo et al., 2013). Microbial metabolites and necromass can be precursors of passive SOC (Chao et al., 2017). The incorporation of microbially derived compounds into passive SOC is important to soil C storage. Since the microbially derived compounds consist of components at different turnover rate, it is difficult to trace microbially derived SOC as a whole. However, amino sugars are widely used biomarkers of microbially originated SOM (Parsons, 1981; Amelung, 2001) due to their absence in plants (Stevenson, 1982) and resistance to decomposition (Bondietti et al., 1972).

In a polymeric form, amino sugars are major components of cell walls of bacteria and fungi (Appuhn and Joergensen, 2006; Roberts et al., 2007). Only a trace amount of amino sugars has been found in plants and lower soil animals, so soil amino sugars are assumed to originated mainly from microbes (Dai et al., 2002; Appuhn and Joergensen, 2006). Since the exudative loss of amino sugars from living microbes is extremely small, amino sugars can be assumed to enter soil as microbial necromass (Glaser and Gross, 2005). Soil contains at least two orders of magnitude more amino sugars than living microbial

biomass, so amino sugars are significantly stabilized and accumulated in soil (Glaser et al., 2004). Therefore, amino sugars are suitable biomarkers to evaluate the contribution of microbial residues to SOM (Glaser et al., 2004).

There are 11 amino sugars that have been proven to exist in soil, 4 of which has been quantified so far, namely muramic acid, glucosamine, galactosamine, and mannosamine (Zhang and Amelung, 1996). Muramic acid presents exclusively in bacteria (Parsons, 1981; Engelking et al., 2007). N-acetylmuramic acid is component of peptidoglycan in bacteria cell walls (Wilkinson, 1977; Kenne and Lindberg, 1983). Fungus cell walls are the primary source of glucosamine in soil (Kortemaa et al., 1997; Engelking et al., 2007). Chitin and chitosan are components of fungal cell walls. Chitin consists of β-1,4-N-acetylglucosamine, and chitosan consists of non-acetylated glucosamine. Galactosamine is considered mainly from bacteria. The origin of mannosamine is still unclear.

Climate and soil management practices influence the accumulation and turnover of amino sugars (Amelung et al., 1999; Liang et al., 2007). The enrichment of muramic acid is positively related to mean annual precipitation (Zhang et al., 1998). The ratio of glucosamine to muramic acid increases with increasing soil water content (Zhang et al., 1997; Amelung et al., 1998; Zhang et al., 1998; Amelung et al., 1999). Compared with conventional tillage, the amount of amino sugars, especially glucosamine, increases in conservation tillage systems because of reduced disturbance (Guggenberger et al., 1999). Adding different types of crop residues in soil induces different accumulation patterns of amino sugars because substrate quantity and quality affect the microbial syntheses and decomposition of amino sugars (Liang et al., 2007).

1.6 Research questions

Soil C can be stored in an active pool with a fast turnover rate or a passive pool with slow turnover rate. New labile C input can accumulate in the active pool through microbial transformation or in the passive pool due to physical protection, chemical association, and biochemical recalcitrance. Short-term drying-rewetting cycles and long-term conservation management practices alter microbial activities and soil properties, and therefore affect the fate and accumulation of the new labile C input in soils. Soil amino sugar content indicates the contribution of microbial residues to SOM. They are sensitive to land use. Hence, my research questions are as follows,

a) What are the effects of short-term drying-rewetting cycles and long-term conservation agricultural management practices on the fate of new labile C input in soil? (Chapter 2)
b) What are the mechanisms of the accumulation of new labile C input in soil under short-term drying-rewetting cycles and long-term conservation agricultural management practices? (Chapter 3)
c) What are the controls of amino sugar accumulation in soil under long-term conservation agricultural management practices? (Chapter 4)

1.7 Hypotheses

a) The short-term drying-rewetting cycles will cause depletion of the new labile C input and the long-term conservation agricultural management practices will offset the depletion.

Drying-rewetting cycles are known as a causation of soil C depletion by disturbing soil structure and microbial activity. Varied frequencies of drying-rewetting cycles are known to cause different sizes of CO_2 fluxes. While conservation agricultural management practices, such as no-tillage and cover crops, have been recognized to conserve soil C. No-tillage increases soil C content by maintaining soil structure and microbial activity. Cover crops increase C input and maintain soil structure and microbial activity.

b) Chemical association and biochemical recalcitrance rather than physical protection will be the stabilization mechanisms of new labile C input under short-term drying-rewetting cycles in soil under long-term conservation agricultural management practices.

The mechanisms of C stabilization in soil are categorized as physical protection, chemical association, and biochemical recalcitrance. The physical protection occurs when SOC is occluded within soil aggregates. Drying-rewetting cycles may disrupt soil aggregates and expose SOC to microbial decomposition. So physical protection of soil aggregates may not be the major control for the stabilization of new labile C input in soil under drying-rewetting cycles. Chemical association and biochemical recalcitrance are less likely reduced by drying-rewetting cycles than physical protection.

c) Chemical association, biochemical recalcitrance, and physical protection will jointly control the accumulation of amino sugars in soil under long-term conservation agricultural management practices.

Conservation agricultural management practices are known to facilitate the accumulation

of SOM. Under conservation agricultural management practices, the accumulation of amino sugars may increase, the mechanisms of which can be chemical association, physical protection, and/or biochemical recalcitrance. Those mechanisms may be of different importance for different types of amino sugars.

1.8 Approach

To test these hypotheses, I developed a series of laboratory and field experiments. To test if long-term conservation agricultural management practices will offset the depletion of new labile C input caused by drying-rewetting cycles, a 24-day mesocosm incubation was conducted using an agricultural soil from western Tennessee under 35-years of conservation management practices. Different frequencies of moisture pulses were applied on the mesocosms: 0, 1, 4, and 8 pulses. To trace the fate of new labile C input, ^{13}C-labeled glucose was added to the mesocosms at the beginning of the incubation. After 24 days, EOC, MBC, and microbial respiration were analyzed to evaluate the active C pool; H_2O_2 oxidation and aggregate size fractionation were used to examine the passive C pool.

To test if chemical association, biochemical recalcitrance, and physical protection are the mechanisms of the accumulation of new labile C input in soil under short-term drying-rewetting cycles and long-term conservation agricultural management practices, structural equation modelling was conducted to determine the relative importance of physical, chemical, and biochemical controls on the accumulation of the new labile C input in soil. The structural equation modelling allows to evaluate the relative importance

of each C pool to the accumulation of new labile C input and to determine the causal relationship between different C pools.

To test if chemical stabilization, biochemical recalcitrance, and physical protection control the accumulation of amino sugars in soil under long-term conservation agricultural management practices, amino sugar concertation, microbial respiration, and extracellular enzyme activity were analyzed in both bulk soil and soil aggregate fractions in a western Tennessee agricultural soil under 31-year of conservation management practices. Structural equation modelling was conducted to identify drivers for soil amino sugar turnover. The structural equation model allows us to determine the microbial mechanisms of soil amino sugar decomposition and accumulation under different C and N availabilities and makes it possible to use short-term microbial processes to predict long-term SOM accumulation potential.

1.9 Rationale and justification

SOM is stored either in a relatively rapidly cycling active pool or in a more slowly cycling passive pool. The passive C pool is responsible for long-term SOM storage, but its size is limited by physical saturation. The active C pool is not limited by saturation but has a shorter turnover time. Conservation agricultural management maintains soil structure and properties, which may alter the distribution of SOM between the active and passive pools. Drying-rewetting cycles are shown to destabilize SOM by pushing SOM toward the active pool, which increases CO_2 flux. Since SOM stores more organic C than the atmosphere and the terrestrial vegetation combined (Lehmann and Kleber, 2015), a

minor change in the distribution of SOM among different pools may cause quantitative fluctuation in CO_2 emission to the atmosphere. Improving the sequestration of new C input in soil is a prominent strategy to mitigate atmospheric CO_2. Understanding the effect of drying-rewetting cycles on the accumulation of new C input in soil is important for predicting soil C storage in the face of climate change. In agroecosystems, increasing SOC sustains and improvs soil quality, and therefore ensure agronomic productivity and food security. However, the effects of short-term drying-rewetting cycles on the accumulation of new C input in soils under long-term conservation agricultural management practices has receive enough attention yet. In addition, despite of the fact that the accumulation of microbially derived organic matter is critical in long-term SOM storage, it has not well studied yet. Especially, the accumulation of microbially derived organic matter under long-term conservation agricultural management practices is still unclear.

This research will examine the efficiency of conservation agricultural management practices on the sequestration newly added C and microbially derived residues by studying the physical, chemical, biochemical, and microbiological mechanisms of SOM sequestration. Microbial respiration can be a good measure of the loss of SOM through CO_2 efflux and a proxy of microbial activity (Liu, 2013). EOC is one of the most mobile fractions of SOM and readily available for soil microbes (Chantigny et al., 2014). Since MBC comprises the living component of SOM (Brookes, 2001), it is a good indicator of microbial activity. H_2O_2 oxidation of SOM can isolate the functionally passive SOM pool from the active SOM pool (von Lützow et al., 2007). Extracellular enzymes can be indicators of the potential of nutrient utilization by soil microbes (Sinsabaugh et al.,

2008). Aggregate size fractionation can indicate the relationship between soil structure and SOM stabilization (Devine et al., 2014). Amino sugars can be used as biomarkers to indicate the contribution of microbially derived organic matter to SOM (Parsons, 1981).

1.10 Novelty

Examining the efficiency of conservation agricultural management practices on the sequestration newly added C and microbially derived residues is important for maintaining SOM and agroecosystem sustainability in long-term. This will be the first time that the relative importance of physical, chemical, biological controls on SOM stabilization is identified and quantified. It could be a useful approach for understanding and modeling biogeochemical transformations of N and C in soil. My dissertation will improve the understanding of physical, chemical, biological controls on SOM stabilization under long-term conservation agricultural management practices. My findings can help to maximize SOM storage in agricultural soils and adapt agriculture in the face of climate change.

References

Ahrens, B., Braakhekke, M.C., Guggenberger, G., Schrumpf, M., Reichstein, M., 2015. Contribution of sorption, DOC transport and microbial interactions to the 14 C age of a soil organic carbon profile: Insights from a calibrated process model. Soil Biology and Biochemistry 88, 390-402.

Amelung, W., 2001. Methods using amino sugars as markers for microbial residues in soil. Assessment methods for soil carbon, 233-272.

Amelung, W., Flach, K., Zhang, X., Zech, W., Blume, H., Eger, H., Fleischhauer, E., Hebel, A., Reij, C., Steiner, K., 1998. Climatic effects on C pools of native and cultivated prairie. Advances in Geoecology 31, 217-224.

Amelung, W., Zhang, X., Zech, W., Flach, K.W., 1999. Amino sugars in native grassland soils along a climosequence in North America. Soil Science Society of America Journal 63, 86-92.

Appel, T., 1998. Non-biomass soil organic N—the substrate for N mineralization flushes following soil drying–rewetting and for organic N rendered CaCl 2-extractable upon soil drying. Soil Biology and Biochemistry 30, 1445-1456.

Appuhn, A., Joergensen, R.G., 2006. Microbial colonisation of roots as a function of plant species. Soil Biology and Biochemistry 38, 1040-1051.

Barnard, R.L., Osborne, C.A., Firestone, M.K., 2015. Changing precipitation pattern alters soil microbial community response to wet-up under a Mediterranean-type climate. The ISME journal 9, 946-957.

Bentler, P.M., 1988. Causal modeling via structural equation systems, Handbook of multivariate experimental psychology. Springer, pp. 317-335.

Birch, H., 1958. The effect of soil drying on humus decomposition and nitrogen availability. Plant and soil 10, 9-31.

Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biology and fertility of soils 45, 115-131.

Bonaterra, A., Camps, J., Montesinos, E., 2005. Osmotically induced trehalose and glycine betaine accumulation improves tolerance to desiccation, survival and efficacy of the postharvest biocontrol agent Pantoea agglomerans EPS125. FEMS Microbiology Letters 250, 1-8.

Bondietti, E., Martin, J., Haider, K., 1972. Stabilization of amino sugar units in humic-type polymers. Soil Science Society of America Journal 36, 597-602.

Borken, W., Matzner, E., 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Global change biology 15, 808-824.

Brookes, P., 2001. The soil microbial biomass: concept, measurement and applications in soil ecosystem research. Microbes and Environments 16, 131-140.

Brown, K.H., Bach, E.M., Drijber, R.A., Hofmockel, K.S., Jeske, E.S., Sawyer, J.E., Castellano, M.J., 2014. A long-term nitrogen fertilizer gradient has little effect on soil organic matter in a high-intensity maize production system. Global change biology 20, 1339-1350.

Byrne, B.M., 2013. Structural equation modeling with AMOS: Basic concepts, applications, and programming, 2nd ed. New York: Routledge.

Capriel, P., Beck, T., Borchert, H., Härter, P., 1990. Relationship between soil aliphatic fraction extracted with supercritical hexane, soil microbial biomass, and soil aggregate stability. Soil Science Society of America Journal 54, 415-420.

Chantigny, M.H., Harrison-Kirk, T., Curtin, D., Beare, M., 2014. Temperature and duration of extraction affect the biochemical composition of soil water-extractable organic matter. Soil Biology and Biochemistry 75, 161-166.

Chao, L., Joshua, P.S., Julie, D.J., 2017. The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology 2, 17105.

Chatterjee, A., Jenerette, G., 2011. Changes in soil respiration Q 10 during drying–rewetting along a semi-arid elevation gradient. Geoderma 163, 171-177.

Chenu, C., 1995. Extracellular polysaccharides: an interface between microorganisms and soil constituents. Environmental impact of soil component interactions 1, 217-233.

Chivenge, P., Vanlauwe, B., Gentile, R., Six, J., 2011. Organic resource quality influences short-term aggregate dynamics and soil organic carbon and nitrogen accumulation. Soil Biology and Biochemistry 43, 657-666.

Clemmensen, K., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Stenlid, J., Finlay, R., Wardle, D., Lindahl, B., 2013. Roots and associated fungi drive long-term carbon sequestration in boreal forest. Science 339, 1615-1618.

Colman, B.P., Schimel, J.P., 2013. Drivers of microbial respiration and net N mineralization at the continental scale. Soil Biology and Biochemistry 60, 65-76.

Cosentino, D., Chenu, C., Le Bissonnais, Y., 2006. Aggregate stability and microbial community dynamics under drying–wetting cycles in a silt loam soil. Soil Biology and Biochemistry 38, 2053-2062.

Cotrufo, M.F., Del Galdo, I., Piermatteo, D., 2009. 5 r Litter decomposition: concepts, methods and future perspectives. Soil Carbon Dynamics, 76.

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Global change biology 19, 988-995.

Dai, X., Ping, C., Hines, M., Zhang, X., Zech, W., 2002. Amino sugars in arctic soils. Communications in soil science and plant analysis 33, 789-805.

Dalenberg, J., Jager, G., 1989. Priming effect of some organic additions to 14 C-labelled soil. Soil Biology and Biochemistry 21, 443-448.

Degens, B.P., 1997. Macro-aggregation of soils by biological bonding and binding mechanisms and the factors affecting these: a review. Soil Research 35, 431-460.

Denef, K., Six, J., Bossuyt, H., Frey, S.D., Elliott, E.T., Merckx, R., Paustian, K., 2001. Influence of dry–wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Biology and Biochemistry 33, 1599-1611.

Devine, S., Markewitz, D., Hendrix, P., Coleman, D., 2014. Soil aggregates and associated organic matter under conventional tillage, no-tillage, and forest succession after three decades. PloS one 9, e84988.

Engelking, B., Flessa, H., Joergensen, R.G., 2007. Shifts in amino sugar and ergosterol contents after addition of sucrose and cellulose to soil. Soil Biology and Biochemistry 39, 2111-2118.

Fernandes, M., Denis, A., Fabre, R., Lataste, J.-F., Chrétien, M., 2015. In situ study of the shrinkage-swelling of a clay soil over several cycles of drought-rewetting. Engineering Geology 192, 63-75.

Fernandez, I., Mahieu, N., Cadisch, G., 2003. Carbon isotopic fractionation during decomposition of plant materials of different quality. Global Biogeochemical Cycles 17.

Fierer, N., Schimel, J., Holden, P., 2003. Influence of drying–rewetting frequency on soil bacterial community structure. Microbial ecology 45, 63-71.

Fierer, N., Schimel, J.P., 2002. Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. Soil Biology and Biochemistry 34, 777-787.

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

Fuchslueger, L., Bahn, M., Fritz, K., Hasibeder, R., Richter, A., 2014. Experimental drought reduces the transfer of recently fixed plant carbon to soil microbes and alters the bacterial community composition in a mountain meadow. New Phytologist 201, 916-927.

Ge, T., Liu, C., Yuan, H., Zhao, Z., Wu, X., Zhu, Z., Brookes, P., Wu, J., 2015. Tracking the photosynthesized carbon input into soil organic carbon pools in a rice soil fertilized with nitrogen. Plant and soil 392, 17-25.

Glaser, B., Gross, S., 2005. Compound-specific δ13C analysis of individual amino sugars–a tool to quantify timing and amount of soil microbial residue stabilization. Rapid Communications in Mass Spectrometry 19, 1409-1416.

Glaser, B., Turrión, M.a.-B., Alef, K., 2004. Amino sugars and muramic acid—biomarkers for soil microbial community structure analysis. Soil Biology and Biochemistry 36, 399-407.

Gleixner, G., 2013. Soil organic matter dynamics: a biological perspective derived from the use of compound-specific isotopes studies. Ecological research 28, 683-695.

Guggenberger, G., Frey, S.D., Six, J., Paustian, K., Elliott, E.T., 1999. Bacterial and fungal cell-wall residues in conventional and no-tillage agroecosystems. Soil Science Society of America Journal 63, 1188-1198.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F., Ekblad, A., Högberg, M.N., Nyberg, G., Ottosson-Löfvenius, M., Read, D.J., 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411, 789-792.

Halverson, L.J., Jones, T.M., Firestone, M.K., 2000. Release of intracellular solutes by four soil bacteria exposed to dilution stress. Soil Science Society of America Journal 64, 1630-1637.

Haque, M.M., Kim, S.Y., Kim, G.W., Kim, P.J., 2015. Optimization of removal and recycling ratio of cover crop biomass using carbon balance to sustain soil organic carbon stocks in a mono-rice paddy system. Agriculture, ecosystems & environment 207, 119-125.

He, H., Li, X., Zhang, W., Zhang, X., 2011. Differentiating the dynamics of native and newly immobilized amino sugars in soil frequently amended with inorganic nitrogen and glucose. European Journal of Soil Science 62, 144-151.

Jastrow, J., Miller, R., 1997. Soil aggregate stabilization and carbon sequestration: feedbacks through organomineral associations. Soil processes and the carbon cycle, 207-223.

Jenkinson, D., 1990. The turnover of organic carbon and nitrogen in soil. Phil. Trans. R. Soc. Lond. B 329, 361-368.

Kallenbach, C.M., Frey, S.D., Grandy, A.S., 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nature communications 7, 13630.

Keiluweit, M., Bougoure, J.J., Nico, P.S., Pett-Ridge, J., Weber, P.K., Kleber, M., 2015. Mineral protection of soil carbon counteracted by root exudates. Nature Climate Change.

Kenne, L., Lindberg, B., 1983. Bacterial polysaccharides. The polysaccharides 2, 287-363.

Kong, A.Y., Six, J., 2010. Tracing root vs. residue carbon into soils from conventional and alternative cropping systems. Soil Science Society of America Journal 74, 1201-1210.

Kortemaa, H., Pennanen, T., Smolander, A., Haahtela, K., 1997. Distribution of antagonistic Streptomyces griseoviridis in rhizosphere and nonrhizosphere sand. Journal of Phytopathology 145, 137-143.

Kuzyakov, Y., Jones, D., 2006. Glucose uptake by maize roots and its transformation in the rhizosphere. Soil Biology and Biochemistry 38, 851-860.

Lado-Monserrat, L., Lull, C., Bautista, I., Lidón, A., Herrera, R., 2014. Soil moisture increment as a controlling variable of the "Birch effect". Interactions with the pre-wetting soil moisture and litter addition. Plant and soil 379, 21-34.

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

Lal, R., 2013. Food security in a changing climate. Ecohydrology & Hydrobiology 13, 8-21.

Lal, R., 2014. Abating climate change and feeding the world through soil carbon sequestration, Soil as World Heritage. Springer, pp. 443-457.

Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. Nature 528, 60.

Liang, C., Zhang, X., Balser, T.C., 2007. Net microbial amino sugar accumulation process in soil as influenced by different plant material inputs. Biology and fertility of soils 44, 1-7.

Liu, H., 2013. Thermal response of soil microbial respiration is positively associated with labile carbon content and soil microbial activity. Geoderma 193, 275-281.

Ma, R., Cai, C., Li, Z., Wang, J., Xiao, T., Peng, G., Yang, W., 2015. Evaluation of soil aggregate microstructure and stability under wetting and drying cycles in two Ultisols using synchrotron-based X-ray micro-computed tomography. Soil and Tillage Research 149, 1-11.

Mazzilli, S.R., Kemanian, A.R., Ernst, O.R., Jackson, R.B., Piñeiro, G., 2015. Greater humification of belowground than aboveground biomass carbon into particulate soil organic matter in no-till corn and soybean crops. Soil Biology and Biochemistry 85, 22-30.

Mazzoncini, M., Antichi, D., Di Bene, C., Risaliti, R., Petri, M., Bonari, E., 2016. Soil carbon and nitrogen changes after 28 years of no-tillage management under Mediterranean conditions. European Journal of Agronomy 77, 156-165.

Mbuthia, L.W., Acosta-Martínez, V., DeBruyn, J., Schaeffer, S., Tyler, D., Odoi, E., Mpheshea, M., Walker, F., Eash, N., 2015. Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: Implications for soil quality. Soil Biology and Biochemistry 89, 24-34.

McCune, B., Grace, J.B., Urban, D.L., 2002. Analysis of ecological communities. MjM software design Gleneden Beach, OR.

Mikha, M.M., Rice, C.W., Milliken, G.A., 2005. Carbon and nitrogen mineralization as affected by drying and wetting cycles. Soil Biology and Biochemistry 37, 339-347.

Miller, A.E., Schimel, J.P., Meixner, T., Sickman, J.O., Melack, J.M., 2005. Episodic rewetting enhances carbon and nitrogen release from chaparral soils. Soil Biology and Biochemistry 37, 2195-2204.

Moreno-Cornejo, J., Zornoza, R., Doane, T.A., Faz, Á., Horwath, W.R., 2015. Influence of cropping system management and crop residue addition on soil carbon turnover through the microbial biomass. Biology and fertility of soils 51, 839-845.

Morillas, L., Durán, J., Rodríguez, A., Roales, J., Gallardo, A., Lovett, G.M., Groffman, P.M., 2015. Nitrogen supply modulates the effect of changes in drying-rewetting

frequency on soil C and N cycling and greenhouse gas exchange. Global change biology.

Moyano, F.E., Manzoni, S., Chenu, C., 2013. Responses of soil heterotrophic respiration to moisture availability: An exploration of processes and models. Soil Biology and Biochemistry 59, 72-85.

Navarro-García, F., Casermeiro, M.Á., Schimel, J.P., 2012. When structure means conservation: Effect of aggregate structure in controlling microbial responses to rewetting events. Soil Biology and Biochemistry 44, 1-8.

Nguyen, C., 2003. Rhizodeposition of organic C by plants: mechanisms and controls. Agronomie-Sciences des Productions Vegetales et de l'Environnement 23, 375-396.

Odum, E.P., Barrett, G.W., 1971. Fundamentals of ecology. Saunders Philadelphia.

Or, D., Phutane, S., Dechesne, A., 2007. Extracellular polymeric substances affecting pore-scale hydrologic conditions for bacterial activity in unsaturated soils. Vadose Zone Journal 6, 298-305.

Parker, S.S., Schimel, J.P., 2011. Soil nitrogen availability and transformations differ between the summer and the growing season in a California grassland. Applied Soil Ecology 48, 185-192.

Parsons, J., 1981. Chemistry and distribution of amino sugars in soils and soil organisms. Soil biochemistry.

Paul, E.A., 2014. Soil microbiology, ecology and biochemistry. Academic press.

Paustian, K., Six, J., Elliott, E., Hunt, H., 2000. Management options for reducing CO2 emissions from agricultural soils. Biogeochemistry 48, 147-163.

Plaza, C., Courtier-Murias, D., Fernández, J.M., Polo, A., Simpson, A.J., 2013. Physical, chemical, and biochemical mechanisms of soil organic matter stabilization under conservation tillage systems: a central role for microbes and microbial by-products in C sequestration. Soil Biology and Biochemistry 57, 124-134.

Poeplau, C., Aronsson, H., Myrbeck, Å., Kätterer, T., 2015. Effect of perennial ryegrass cover crop on soil organic carbon stocks in southern Sweden. Geoderma Regional 4, 126-133.

Poeplau, C., Don, A., 2015. Carbon sequestration in agricultural soils via cultivation of cover crops–A meta-analysis. Agriculture, ecosystems & environment 200, 33-41.

Priemé, A., Christensen, S., 2001. Natural perturbations, drying–wetting and freezing–thawing cycles, and the emission of nitrous oxide, carbon dioxide and methane from farmed organic soils. Soil Biology and Biochemistry 33, 2083-2091.

Rasse, D.P., Rumpel, C., Dignac, M.-F., 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant and soil 269, 341-356.

Redin, M., Guénon, R., Recous, S., Schmatz, R., de Freitas, L.L., Aita, C., Giacomini, S.J., 2014. Carbon mineralization in soil of roots from twenty crop species, as affected by their chemical composition and botanical family. Plant and soil 378, 205-214.

Roberts, P., Bol, R., Jones, D.L., 2007. Free amino sugar reactions in soil in relation to soil carbon and nitrogen cycling. Soil Biology and Biochemistry 39, 3081-3092.

Sagot, B., Gaysinski, M., Mehiri, M., Guigonis, J.-M., Le Rudulier, D., Alloing, G., 2010. Osmotically induced synthesis of the dipeptide N-acetylglutaminylglutamine

amide is mediated by a new pathway conserved among bacteria. Proceedings of the National Academy of Sciences 107, 12652-12657.

Schaeffer, S.M., Homyak, P.M., Boot, C.M., Roux-Michollet, D., Schimel, J.P., 2017. Soil carbon and nitrogen dynamics throughout the summer drought in a California annual grassland. Soil Biology and Biochemistry 115, 54-62.

Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88, 1386-1394.

Schmidt, M.W., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49-56.

Shi, A., Marschner, P., 2014. Drying and rewetting frequency influences cumulative respiration and its distribution over time in two soils with contrasting management. Soil Biology and Biochemistry 72, 172-179.

Shi, A., Yan, N., Marschner, P., 2015. Cumulative respiration in two drying and rewetting cycles depends on the number and distribution of moist days. Geoderma 243, 168-174.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., 2008. Stoichiometry of soil enzyme activity at global scale. Ecology letters 11, 1252-1264.

Six, J., Elliott, E., Paustian, K., 1999. Aggregate and soil organic matter dynamics under conventional and no-tillage systems. Soil Science Society of America Journal 63, 1350-1358.

Six, J., Elliott, E., Paustian, K., 2000a. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology and Biochemistry 32, 2099-2103.

Six, J., Elliott, E., Paustian, K., Doran, J., 1998. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Science Society of America Journal 62, 1367-1377.

Six, J., Paustian, K., Elliott, E., Combrink, C., 2000b. Soil structure and organic matter I. Distribution of aggregate-size classes and aggregate-associated carbon. Soil Science Society of America Journal 64, 681-689.

Smith, W., Grant, B., Campbell, C., McConkey, B., Desjardins, R., Kröbel, R., Malhi, S., 2012. Crop residue removal effects on soil carbon: measured and inter-model comparisons. Agriculture, ecosystems & environment 161, 27-38.

Sponseller, R.A., 2007. Precipitation pulses and soil CO2 flux in a Sonoran Desert ecosystem. Global change biology 13, 426-436.

Steinweg, J.M., Dukes, J.S., Wallenstein, M.D., 2012. Modeling the effects of temperature and moisture on soil enzyme activity: linking laboratory assays to continuous field data. Soil Biology and Biochemistry 55, 85-92.

Stevenson, F.J., 1982. Nitrogen in agricultural soils. American Society of Agronomy.

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

Sutton-Grier, A.E., Kenney, M.A., Richardson, C.J., 2010. Examining the relationship between ecosystem structure and function using structural equation modelling: A

case study examining denitrification potential in restored wetland soils. Ecological Modelling 221, 761-768.

Swift, M.J., Heal, O.W., Anderson, J.M., 1979. Decomposition in terrestrial ecosystems. Univ of California Press.

Utomo, W., Dexter, A., 1982. Changes in soil aggregate water stability induced by wetting and drying cycles in non-saturated soil. Journal of Soil Science 33, 623-637.

Valentini, R., Matteucci, G., Dolman, A., Schulze, E.-D., Rebmann, C., Moors, E., Granier, A., Gross, P., Jensen, N., Pilegaard, K., 2000. Respiration as the main determinant of carbon balance in European forests. Nature 404, 861-865.

Van Gestel, M., Merckx, R., Vlassak, K., 1993. Microbial biomass responses to soil drying and rewetting: the fate of fast-and slow-growing microorganisms in soils from different climates. Soil Biology and Biochemistry 25, 109-123.

van Hees, P.A., Jones, D.L., Finlay, R., Godbold, D.L., Lundström, U.S., 2005. The carbon we do not see—the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. Soil Biology and Biochemistry 37, 1-13.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007. SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. Soil Biology and Biochemistry 39, 2183-2207.

von Lützow, M., Kögel-Knabner, I., Ludwig, B., Matzner, E., Flessa, H., Ekschmitt, K., Guggenberger, G., Marschner, B., Kalbitz, K., 2008. Stabilization mechanisms of

organic matter in four temperate soils: development and application of a conceptual model. Journal of Plant Nutrition and Soil Science 171, 111-124.

Wilkinson, S.G., 1977. Composition and structure of bacterial lipopolysaccharides, Surface carbohydrates of the prokaryotic cell. Academic Press New York, pp. 97-175.

Wu, J., Brookes, P., 2005. The proportional mineralisation of microbial biomass and organic matter caused by air-drying and rewetting of a grassland soil. Soil Biology and Biochemistry 37, 507-515.

Xiang, S.-R., Doyle, A., Holden, P.A., Schimel, J.P., 2008. Drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface California grassland soils. Soil Biology and Biochemistry 40, 2281-2289.

Zhang, X., Amelung, W., 1996. Gas chromatographic determination of muramic acid, glucosamine, mannosamine, and galactosamine in soils. Soil Biology and Biochemistry 28, 1201-1206.

Zhang, X., Amelung, W., Yuan, Y., Zech, W., 1997. Amino sugars in soils of the North American cultivated prairie. Zeitschrift für Pflanzenernährung und Bodenkunde 160, 533-538.

Zhang, X., Amelung, W., Yuan, Y., Zech, W., 1998. AMINO SUGAR SIGNATURE OF PARTICLE-SIZE FRACTIONS IN SOILS OF THE NATIVE PRAIRIE AS AFFECTED BY CLIMATE. Soil Science 163, 220-229.

Zhao, G., Bryan, B.A., King, D., Luo, Z., Wang, E., Yu, Q., 2015. Sustainable limits to crop residue harvest for bioenergy: maintaining soil carbon in Australia's agricultural lands. GCB Bioenergy 7, 479-487.

Appendix 1

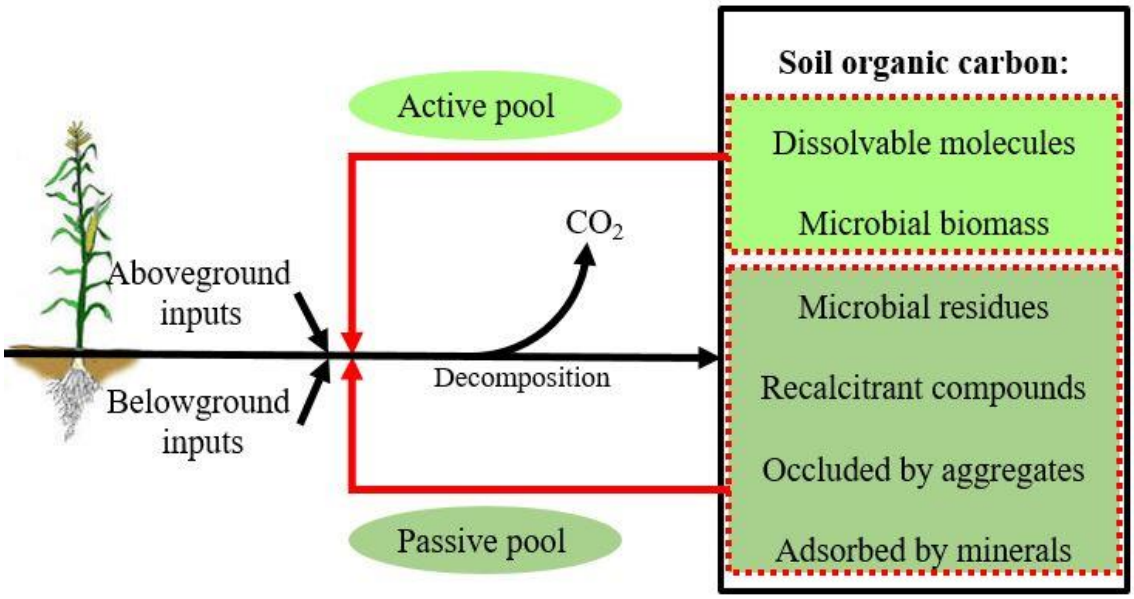

Figure 1.1 The incorporation of new C input into SOM.

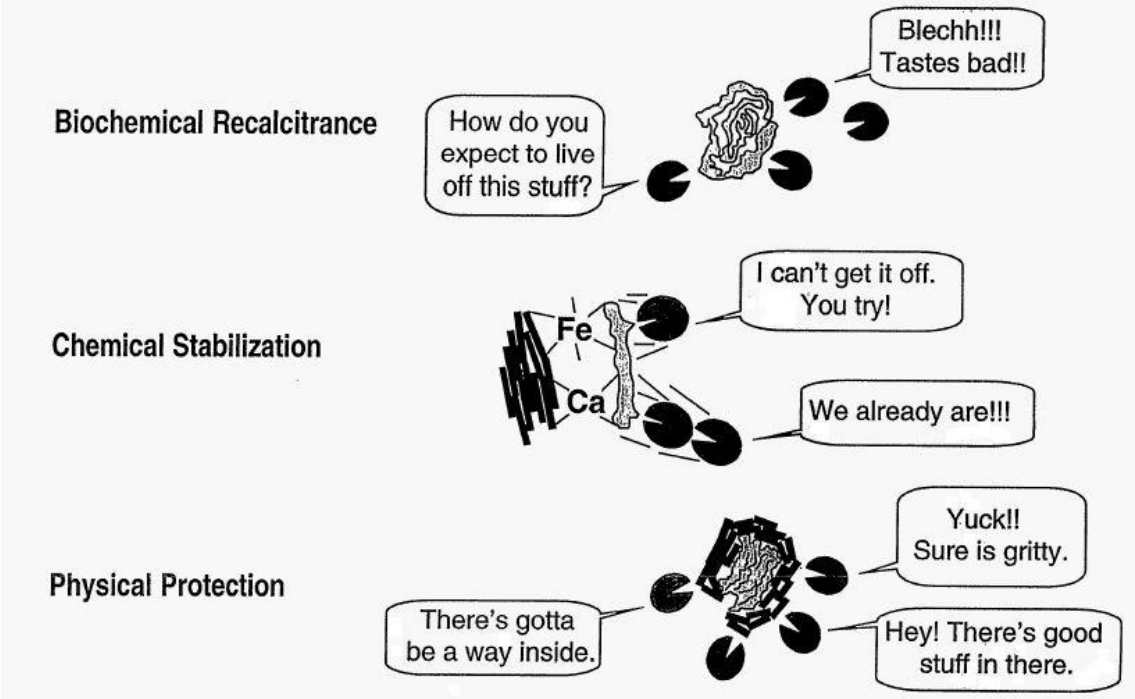

Figure 1.2 Mechanisms of SOM stabilization (Jastrow and Miller, 1997).

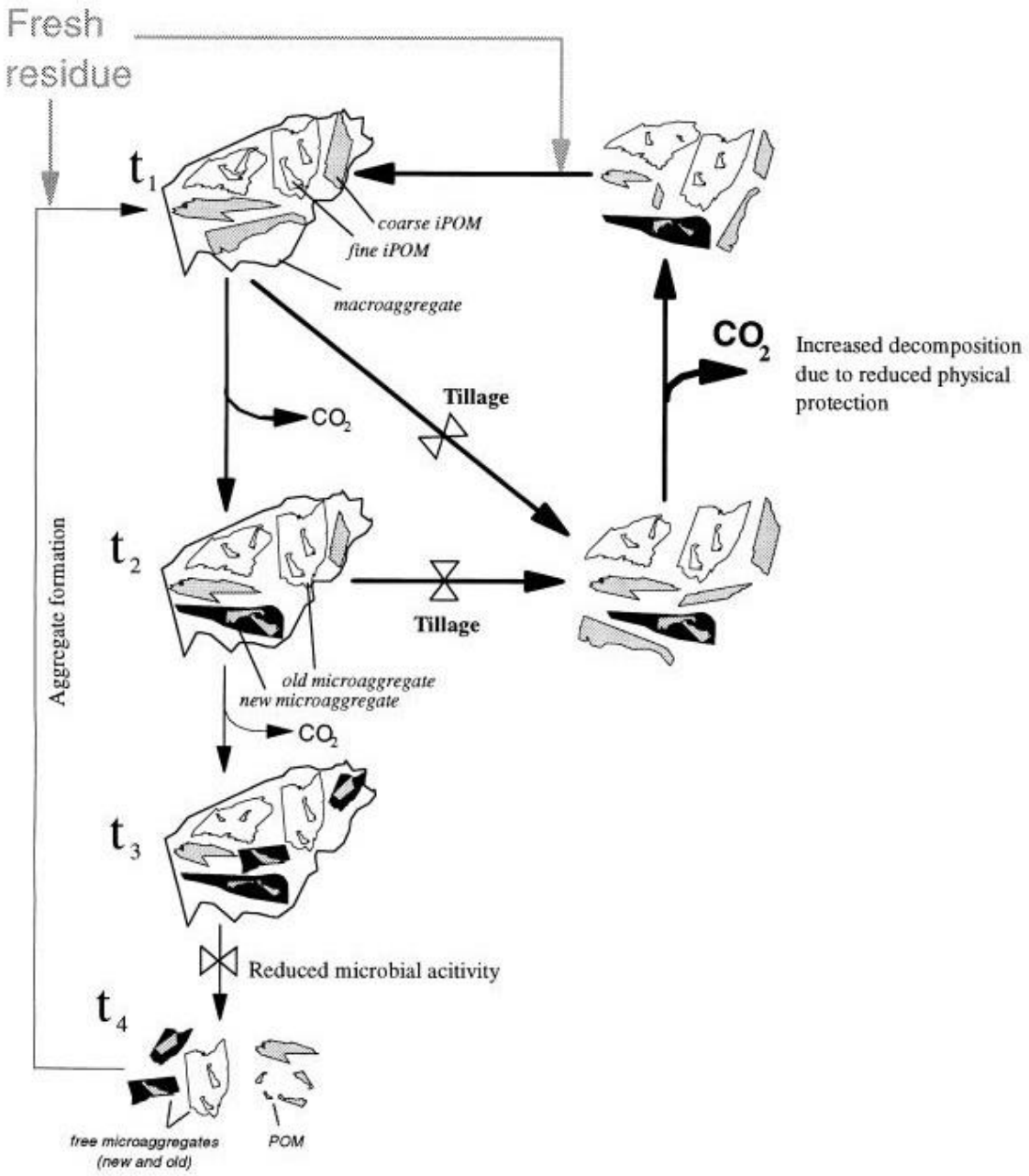

Figure 1.3 Macro- and microaggregate turnover as affected by tillage (Six et al., 2000).

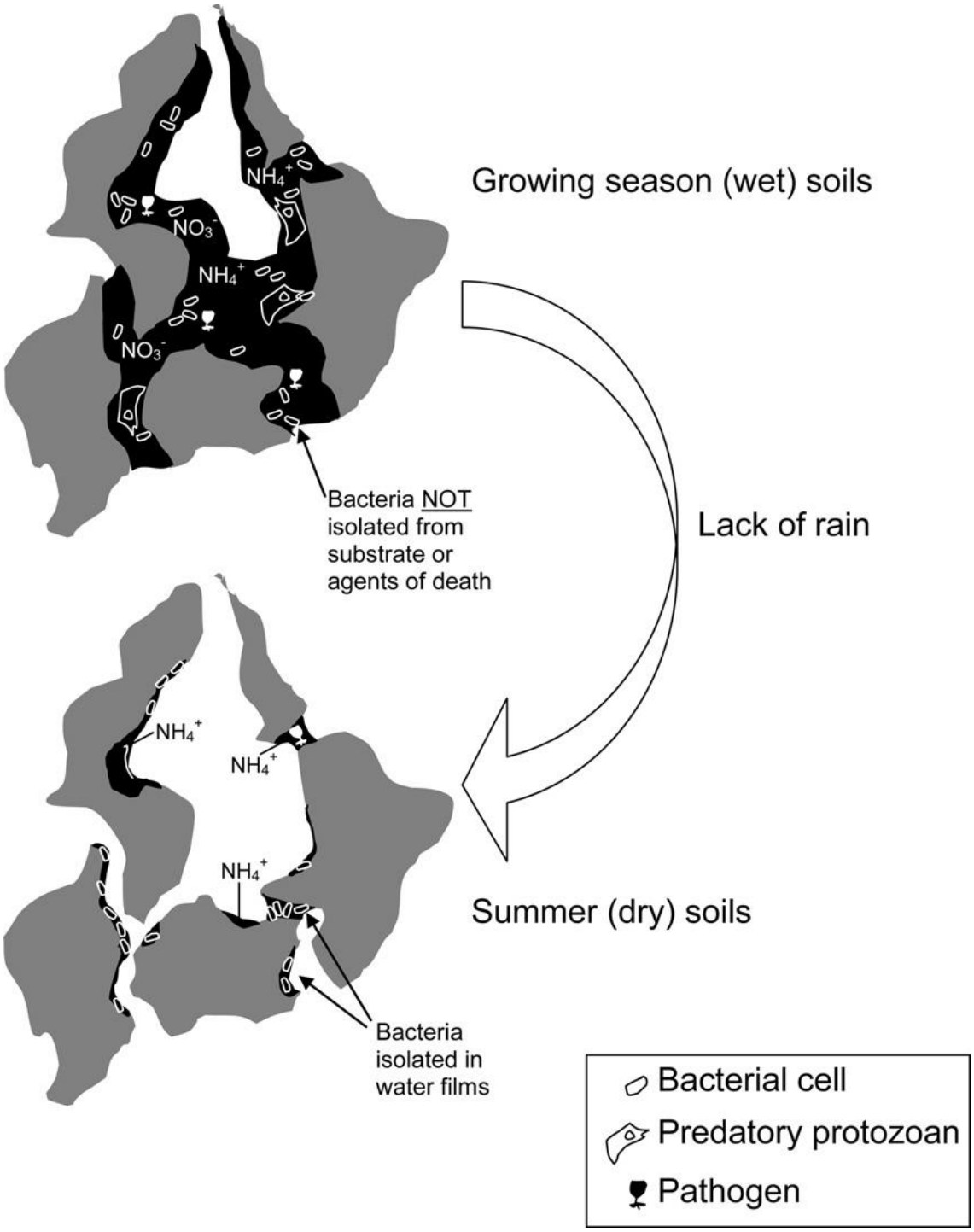

Figure 1.4 The effect of hydrological connectivity on substrate availability for soil microbes (Parker and Schimel, 2011).

Grey area represents soil colloid; black area represents water filled pore space.

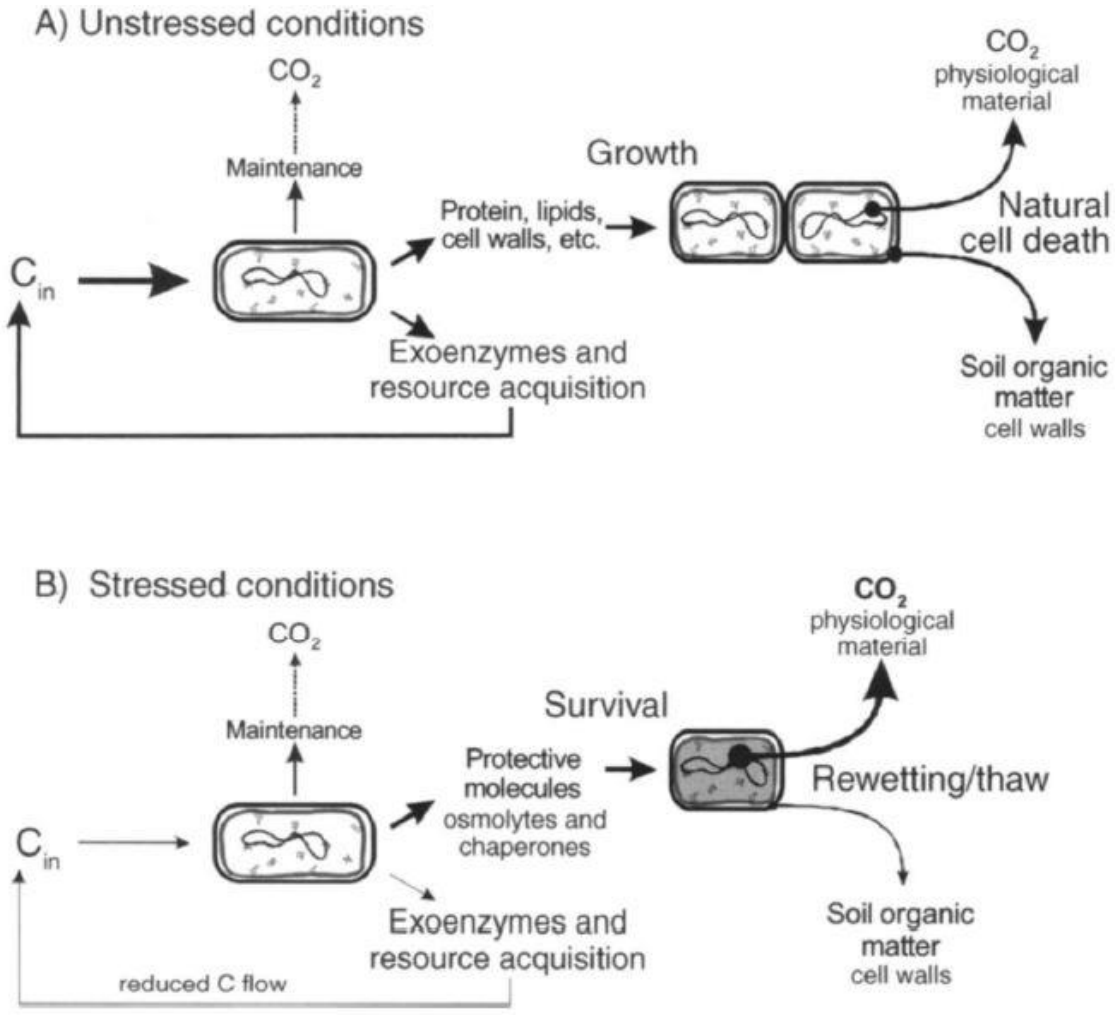

Figure 1.5 The change of resource allocation in soil microbes caused by stress (Schimel et al., 2007).

Chapter 2 The impacts of drying-rewetting cycles and conservation agricultural management practices on the fate of added glucose-C in soil

A version of this chapter will be submitted for publication to Soil Biology and Biochemistry by Lidong Li and Sean M. Schaeffer.

Abstract

Short-term drying-rewetting cycles are common in surface soils, especially in agroecosystems, which may have a significant effect on long-term carbon (C) storage. To test the effect of short-term drying-rewetting cycles on the accumulation of new C input in soil under long-term conservation agricultural management practices, a 24-day microcosm incubation was conducted with an agricultural soil under 36 years of conservation agricultural management practices, involving no-tillage, N-fixing vetch cover crops, and wheat cover crops. I added ^{13}C-labelled glucose to the microcosms at the beginning of the incubation. During the 24-day incubation, I applied different frequencies of drying-rewetting cycles: 0, 1, 4, and 8 moisture pulses. In each moisture pulse, soil water content was brought up to 40% gravimetric water content and then air dried. The fate of the ^{13}C-labelled substrate in active and passive C pools was traced. My results indicate that repeated drying-rewetting cycles decrease soil microbial C use efficiency (CUE). At the end of the 24-day incubation, regardless of treatments, 0.08%-1% of the added glucose-C was incorporated in the extractable organic C (EOC) pool, 4%-27% of the added glucose-C was incorporated in the microbial biomass C (MBC) pool, and 0.7%-5% of the added glucose-C was incorporated in the hydrogen peroxide (H_2O_2)-resistant C pool. The longest drought treatment (24 days, i.e., 0 moisture pulses) induced higher recovery of the added glucose-C in each C pool, which could be caused by the increased accumulation during drying period and the rapid consumption upon rewetting.

Under different conservation agricultural management practices, my results suggest that soil microbes preferentially utilize the added glucose-C instead of the endogenous soil organic C (SOC) when soil C resource is relatively scarce regardless of soil moisture conditions. N-fixing vetch cover crops are more favorable for the stabilization of the added glucose-C under repeated drying-rewetting cycles. Taken together, these results demonstrate that short-term drying-rewetting cycles changed microbial process and therefore changed the accumulation pattern of the added glucose-C. This suggests that climate change may induce an altered biogeochemical cycle of soil C in agroecosystems.

2.1 Introduction

One prediction of climate change is an intensified hydrological cycle, namely extended soil drought coupled with extreme precipitation (Xiang et al., 2008). This would likely intensify the effect of drying-rewetting cycles on agriculture. It is importance to understand the effects of drying-rewetting cycles on the accumulation of new C input in soil in order to adapt agriculture in the face of climate change. Drying-rewetting cycles are known to impact SOC turnover through increasing SOC decomposition (Birch, 1958). SOC consists of various functional pools, involving EOC, MBC, and H_2O_2-resistant C. Since those pools have different sizes and turnover rates, drying-rewetting cycles may have different impacts on those pools.

Soil EOC is a form of SOC that is extracted by agitating soil with aqueous solutions (Chantigny, 2003). EOC consists of SOC ranging from low molecular weight compounds to colloidal substances (von Lützow et al., 2007), involving carbohydrates, phenols,

amino acids, and organic acids (Chantigny, 2003). Despite the fact that EOC only accounts for 0.05–0.4% of SOC in agricultural soil (Haynes, 2005), it plays a critical role in soil C cycle process due to its high turnover rate and solubility (Chantigny et al., 2014). EOC is found to increase upon crop residue amendment and reduced tillage (Chantigny, 2003).

MBC is one of the most labile C pool in soil (Jenkinson, 1981), which comprises the living component of SOM (Brookes, 2001). The turnover time of MBC is estimated to be 0.9-5 years (Brookes, 2001; von Lützow et al., 2007). Due to the short turnover time, MBC is considered to be an active C pool (Wardle, 1992; von Lützow et al., 2007). Although MBC only accounts for 0.3-4% of SOC in agricultural topsoil (von Lützow et al., 2007), soil microbes regulates all SOC transformations (Smith and Paul, 1990). In agricultural soils, MBC is also a sensitive indicator of changes in soil property that are caused by management practices and environmental stresses (Moore et al., 2000). The changes in MBC caused by soil management practices can be detected much earlier than that in soil total C (Powlson et al., 1987; Brookes, 2001). For instance, detectable changes in MBC can be indicative of alterations in soil fertility (Brookes, 2001).

H_2O_2 oxidation removes SOC that is accessible to exoenzymes, i.e., active C pool, therefore H_2O_2-resistant C is considered a major component of functionally passive C pool (von Lützow et al., 2007). The H_2O_2-resistant SOM is found to be 500-3000 years older than SOM in bulk soil (Eusterhues et al., 2005), suggesting that H_2O_2-resistant C has a slower turnover rate. Since H_2O_2 oxidation is similar with biological mineralization, it may be a useful tool for isolating the passive SOC (Plante et al., 2004). H_2O_2 oxidation

is found to be more efficient in removing SOC in disaggregated soils due to its reduced dispersion effect on microaggregates (von Lützow et al., 2007). Since aliphatic plant materials are also found to be resistant to H_2O_2 oxidation (von Lützow et al., 2007), H_2O_2 oxidation cannot separate organominerally protected SOC from biochemically recalcitrant SOC.

The fate of the added glucose-C in those C pools is critical for its stabilization in soil. Our objectives of this study were to (1) examine the effects of repeated drying-rewetting cycles on soil microbial utilization of the added glucose-C, (2) determine the effects of soil drought on the transformation and accumulation of the added glucose-C, and (3) evaluate the effects of conservation agricultural management practices on the transformation patterns of the added glucose-C in different soil C pools. My hypotheses were: (1) Repeated drying-rewetting cycles decrease soil microbial CUE. By definition, microbial CUE is the ratio of organic C allocated to growth and organic C assimilated by microbes (Spohn et al., 2016). Drying-rewetting is known to increase soil CO_2 emission (Miller et al., 2005), which will decrease the organic C allocated to microbial growth and therefore decrease microbial CUE. (2) Soil drought causes accumulation of the added glucose-C. The decomposition of C substrates in soil is mainly driven by microbes. Soil drought decreases microbial decomposition (Borken and Matzner, 2009), which will decrease the loss of C through microbial decomposition. (3) C abundant condition is more favorable for the stabilization of the added glucose-C than C scarce condition. Newly introduced C is incorporated into soil through biochemical alteration of microbes. C abundant condition is more favorable for microbial activity and growth, which will facilitate the incorporation of new C input into soil. To test those hypotheses, I measured

the concentrations of the added glucose-C in EOC pool, MBC pool, H_2O_2-resistant C pool, macroaggregate fraction, microaggregate fraction, and bulk soil from a microcosm experiment with drying-rewetting cycle and conservation management treatments.

2.2 Materials and methods

2.2.1 Site descriptions and experiment design

Soil was collected from the West Tennessee Research and Education Center located in Jackson, TN (35°37'23.1"N 88°50'47.4"W). The climate data has been compiled since 1981. The mean annual temperature is 15.6°C at the experimental location (Ritchey et al., 2015). The mean annual precipitation is 1375 mm (Mbuthia et al., 2015). The soil is derived from red marine deposit overlaid by loess deposits, classified as Lexington silt loam (fine-silty, mixed, thermic, Ultic Hapludalf), and well-drained with a 0 to 2 percent slope (Ritchey et al., 2015). The fields are managed by long-term conservation agricultural management practices under continuous cotton (*Gossypium hirsutum*) planting, which were established in 1981. More detailed information about soil physicochemical properties and site descriptions can be found in the article by Mbuthia et al. (2015).

Our experiment is a factorial design with 2 factors: conservation management and drying-rewetting cycle. Conservation management has 6 levels: (1) no-cover crop and no-tillage (NCNT), (2) no-cover crop and conventional tillage (NCCT), (3) vetch cover and no-tillage (VCNT), (4) vetch cover and conventional tillage (VCCT), (5) wheat cover and no-tillage (WCNT), and (6) wheat cover and conventional tillage (WCCT). Drying-

rewetting cycle has 4 levels: (1) rewetting to 40% gravimetric water content (GWC) everyday, (2) rewetting to 40% GWC every 3 days, (3) rewetting to 40% GWC every 6 days, and (4) rewetting to 40% GWC and then drying.

Soil was collected in April 2017 using stainless steel cores (7.6 cm diameter and 7.6 cm height) to a depth of 7.5 cm. Sampling spots were randomly picked and located 10-15 cm from the root zone. Composite samples were made by homogenizing soils from each plot. Sampling gears were disinfected with 70% ethanol to avoid contamination between treatments. The composite samples were sealed in plastic bags (Whirl-pak, Nasco) and stored in a cooler under dry ice during being transported to laboratory.

In laboratory, the soil was homogenized by passing through a 2 mm sieve. ^{13}C labeled glucose (99 atom% ^{13}C, Cambridge Isotope Laboratories, Inc.) was added at 100 μg ^{13}C g^{-1} dry soil as solution into 50 g soil on oven dried basis in a 500 mL mason jar. All soil samples were wetted to 40% GWC and then placed in an incubator under 25 ℃ for 24 days, during which soil water content was adjusted by adding sterilized water according to soil weight. Soil was destructively sampled at the last day of incubation for future analyses.

2.2.2 Soil microbial respiration

Soil microbial respiration rate was measured using a CO_2 infrared gas analyzer (IRGA, LiCOR-820, LiCor Inc., Lincoln NE) from batch mesocosms (500 mL Ball Mason Jar) fitted with butyl rubber septa (Kimble Stoppers for Headspace Vials). Each soil sample is placed in a mason jar. A septum port is constructed at the top of each aluminum lid in

order to draw gas samples (0.5 mL) with a syringe. Respiration measurements are conducted after gravimetric water content is adjusted every day. After each CO_2 measurement, the mason jars are fanned with ambient air to obtain a 'zero' starting point for next measurement and to avoid anaerobic conditions. Cumulative soil respiration was calculated by multiplying respiration rate by time and then being normalized by total soil C.

2.2.3 H_2O_2 oxidation

The passive C pool was separated by a H_2O_2 oxidation method(Helfrich et al., 2007; Jagadamma et al., 2010). Soil (1 g) was wetted with 10 mL distilled water for 10 min. 90 mL of 10% H_2O_2 was added to soil. The oxidation was conducted at 50 ℃ in a water bath. After 3 days when the frothing completely stops, the suspension was centrifuged at 2500×g for 15 min. After the supernatant being decanted, the soil was rinsed with 40 mL of deionized water 3 times and then freeze dried on a lyophilizer (Labconco). The freeze-dried materials were analyzed for ^{13}C and total C contents on a Combustion Module-Cavity Ringdown Spectrometer (Picarro, Inc).

2.2.4 EOC, MBC, and CUE

EOC and MBC were measured by a slurry fumigation extraction method (Fierer and Schimel, 2002). Briefly, 10 g of soil was weighed into a 100 mL extraction jar. Two jars of soil ware weighed for each sample, one for fumigated and one for unfumigated. 40 mL of 0.03M K_2SO_4 was transferred into each jar and 0.5 mL of chloroform was added into the fumigated jars. Samples ware agitated on a shaker at 150 rpm for 4 h and then settled

for 30 minutes. 30 mL of supernatant was transferred into a 50 mL conical centrifuge tube. The fumigated samples ware aerated for 30 minutes to remove the chloroform by an air spurge. All samples are centrifuged at 226 × g for 20 minutes on a centrifugal machine (Thermo Scientific Sorvall STR16 Centrifuge) and then filtered through glass fiber prefilters (Merck Millipore Ltd.) on a vacuum filter hold (Hoefer Inc.). The filtrate was freeze dried on a lyophilizer (Labconco). The freeze-dried materials were analyzed for ^{13}C and total C contents on a Combustion Module-Cavity Ringdown Spectrometer (Picarro, Inc). MBC was calculated according to $MBC = EOC_{fumigated} - EOC_{unfumigated}$. When MBC was calibrated by fumigation efficiency coefficient, the total recovery of 13C exceeded 100%. Therefore, the fumigation efficiency coefficient was not applied. CUE was calculated according to CUE = MBC / (MBC + Cumulative CO_2 emission).

2.2.5 Soil aggregate fractionation

Air-dried soil (10 g) was placed on a 0.25 mm sieve. The sieve was shaken both horizontally and vertically using a sieve shaker (CSC Scientific) at 50 Hz for 3 minutes. The soil was fractionated into 2 fractions: macroaggregate fraction (> 0.25 mm) and microaggregate fraction (< 0.25 mm). Soil was ground and analyzed for ^{13}C and total C contents on a Combustion Module-Cavity Ringdown Spectrometer (Picarro, Inc.).

2.2.6 Statistical analyses

A two-way ANOVA was conducted with SAS 9.4 (Glimmix procedure, SAS Institute Inc., Cary, NC) and least square means were compared by Fisher's LSD at 5% significance level. The model was used for ANOVA:

$Y = \mu + CM + DRC + CM*DRC + Rep*(CM*DRC)$

μ = mean

CM = conservation management treatment

DRC = drying-rewetting cycle treatment

Rep = replication

The model was used for examining the main effects of conservation management treatment and drying-rewetting cycle treatment and the interaction between the two.

2.3 Results

2.3.1 Soil gravimetric water content

Soil gravimetric water content decreased to different extents with different frequencies of moisture pulses and different conservation agricultural management practices (Figure 2.1). After 3 days of drought (8 pulses throughout the experiment), soil gravimetric water content decreased to 0.14 g water g^{-1} dry soil. After 6 days of drought (4 pulses throughout the experiment), soil gravimetric water content decreased to 0.002 g water g^{-1} dry soil. After 24 days of drought (1 pulse throughout the experiment), soil gravimetric water content decreased to 0.03 g water g^{-1} dry soil. Soil gravimetric water content decreased to a lower level in the NCCT treatment than that in the NCNT treatment regardless of moisture pulse treatments. Under the 8-pulse treatment, soil gravimetric

water content decreased to a lower level in the VCNT and WCNT treatments than that in the VCCT and WCCT treatments, respectively, during the drought period.

2.3.2 Soil microbial respiration

Different frequencies of drying-rewetting cycles induced different sizes of CO_2 emission pulses (Figure 2.2). Rewetting after the 6-day drought induced larger CO_2 emission pulses than rewetting after the 3-day drought. The soil microbial respiration rate under the different moisture treatments was generally ordered as 4 pulses > 8 pulses > 0 pulse > 1 pulse, ranging from 0.00-3.48 μg C-CO_2 g^{-1} dry soil h^{-1}. The soil microbial respiration rate under the different cover crop treatments was generally ordered as vetch cover crops > no cover crop > wheat cover crops.

Cumulative soil microbial respiration under the different moisture treatments was ordered as 4 pulses > 8 pulses > 0 pulse > 1 pulse, ranging from 9.41-92.89 mg C-CO_2 g^{-1} total C (Figure 2.3). Cumulative soil microbial respiration under the 4 moisture pulse treatment was 2.9-5.2 times of that under the 1 moisture pulse treatment. Cumulative soil microbial respiration under the different cover crop treatments was ordered as no cover crop > vetch cover crops > wheat cover crops. Cumulative soil microbial respiration under the no cover crop treatment was 1.5-2.8 times of that under the wheat cover crop treatment. Under the no cover crop treatment, cumulative soil microbial respiration in the no tillage treatment was significantly smaller than that in the conventional tillage treatment ($P < 0.0001$). Under the vetch cover crop treatment, cumulative soil microbial respiration in the no tillage treatment was significantly larger than that in the conventional tillage treatment ($P < 0.0001$). Under the wheat cover crop treatment, cumulative soil microbial

respiration in the no tillage treatment was significantly smaller than that in the conventional tillage treatment ($P < 0.0001$).

2.3.3 Recovered ^{13}C and total C concentrations in bulk soil

In bulk soil, the concentration of recovered ^{13}C in the WCCT treatment under the 1 moisture pulse treatment (50.45 μg g^{-1} dry bulk soil) was the highest (Figure 2.4). The lowest concentration of recovered ^{13}C was found in the treatment WCNT with the 8 moisture pulse treatment (18.49 μg g^{-1} dry bulk soil). The concentration of recovered ^{13}C in the NCCT treatment was significantly lower than that in the WCCT treatment ($P = 0.0041$). The concentration of recovered ^{13}C in the 1 moisture pulse treatment was significantly higher than that in the other moisture pulse treatments ($P < 0.0001$). The concentrations of recovered ^{13}C in the 0 and 8 moisture pulse treatments were not significantly different from each other ($P = 0.9244$). Under the 0 pulse moisture treatment, the NCNT treatment had the highest concentration of recovered ^{13}C among the conservation management treatments. Under the 1 moisture pulse treatment, the lowest concentration of recovered ^{13}C was found in the NCCT treatment.

The concentration of total C in bulk soil ranged from 8.10 mg g^{-1} dry bulk soil to 14.75 mg g^{-1} dry bulk soil (Figure 2.4). The concentration of total C in the different conservation management treatments was generally ordered as WCNT > VCNT > NCNT > VCCT > WCCT > NCCT. The concentration of total C in the NCCT treatment was significantly lower than that in all the other conservation management treatments ($P < 0.0001$). The concentrations of total C in the NCNT and the WCCT treatments were not significantly different from each other ($P = 0.4655$). The concentrations of total C in the

VCCT and the WCCT treatments were not significantly different from each other (P = 0.1135). The concentration of total C in the 1 moisture pulse treatment was significantly higher than that in the 4 moisture pulse treatment ($P < 0.0001$) but not significantly different from that in the 0 moisture pulse treatment (P = 0.1834). The concentrations of total C in the 4 and 8 moisture pulse treatment were not significantly different from each other (P = 0.7114).

2.3.4 Recovered ^{13}C and total C in different soil C pools

The recovery of glucose derived ^{13}C in different soil C pools was distinctly different from each other (Figure 2.5). The concentration of recovered H_2O_2-resistant ^{13}C ranged from 0.68 μg g^{-1} dry bulk soil to 5.21 μg g^{-1} dry bulk soil, the concentration of recovered $EO^{13}C$ ranged from 0.11 μg g^{-1} dry bulk soil to 1.35 μg g^{-1} dry bulk soil, and the concentration of recovered $MB^{13}C$ ranged from 7.45 μg g^{-1} dry bulk soil to 36.48 μg g^{-1} dry bulk soil. The concentration of recovered ^{13}C under the 1 moisture pulse treatment was significantly higher than that in the other moisture pulse treatments regardless of C pools and conservation management treatments ($P < 0.0001$). In H_2O_2-resistant C pool, the concentration of recovered ^{13}C in the VCNT treatment was significantly higher than that in the WCNT treatment ($P < 0.0001$), and the concentration of recovered ^{13}C in the VCCT treatment was significantly higher than that in the WCCT treatment (P = 0.0004). In EOC and MBC pool, the conservation management treatment did not cause any significant differences in the concentration of recovered ^{13}C ($P > 0.0500$). The $EO^{13}C$ concentration under the different moisture pulse treatments was ordered as 1 pulse > 4 pulses > 0 pulses > 8 pulses regardless of the conservation management treatments, and

the MB^{13}C concentration was generally ordered as 1 pulse > 0 pulse > 8 pulses > 4 pulses.

Total C concentration had a different distribution pattern among the soil C pools from the recovered ^{13}C (Figure 2.5). The H_2O_2-resistant C concentration ranged from 0.52 mg g^{-1} dry bulk soil to 1.85 mg g^{-1} dry bulk soil, EOC concentration ranged from 0.03 mg g^{-1} dry bulk soil to 0.10 mg g^{-1} dry bulk soil, and MBC concentration ranged from 0.09 mg g^{-1} dry bulk soil to 0.18 mg g^{-1} dry bulk soil. The moisture pulse treatment did not have any significant effects on H_2O_2-resistant C concentration (P = 0.3922). The concentration of H_2O_2-resistant C in the VCNT treatment was significantly higher than that in the other conservation management treatments (P < 0.0001). EOC concentration under different moisture pulse treatment was ordered as 1 pulse > 4 pulses > 0 pulse > 8 pulses. The VCNT and WCNT treatments induced higher EOC concentration than the other conservation management treatments. MBC concentrations under the moisture pulse treatments were not significantly different from each other (P > 0.1000). The VCNT treatment induced significantly higher MBC concentration than the NCCT treatment (P = 0.0164).

2.3.5 Soil microbial CUE

The 1 moisture pulse treatment induced significantly higher soil microbial CUE under all conservation management treatments (P < 0.0001, Figure 2.6). CUE in the 4 and 8 moisture pulse treatments was significantly lower than that in constant control (P < 0.0001 and P = 0.0008, respectively). CUE in the 4 and 8 moisture pulse treatments was not significantly different from each other (P = 0.4891). The highest CUE (0.59) was

observed under the 1 moisture pulse in the WCCT treatment. The lowest CUE (0.11) was found under the 4 moisture pulse in the NCNT treatment.

2.3.6 Recovery of macroaggregates and microaggregates after soil aggregate size fractionation

The recovery of macroaggregates after soil aggregate size fractionation under the 0 and 1 moisture pulse treatments was significantly higher than that in the 4 and 8 moisture pulse treatments (Figure 2.7, $P < 0.0001$). The recovery of macroaggregates in the 4 and 8 moisture pulse treatments was not significantly different from each other ($P = 0.4245$). The lowest recovery of 76% was found in the NCCT treatment with the 4 moisture pulse treatment and the highest recovery of 97% was found in NCCT treatment with the 0 moisture pulse treatment. Macroaggregate recovery in the VCNT treatment was significantly lower than that in the VCCT treatment regardless of moisture pulse treatments ($P = 0.0002$).

The recovery of microaggregates ranged from 2% to 22% (Figure 2.7). Microaggregate recovery under the 0 and 1 moisture pulse treatments was significantly lower than that in the 4 and 8 moisture pulse treatments regardless of conservation management treatments ($P < 0.0001$). The VCNT treatment caused higher microaggregate recovery than the VCCT treatment regardless of moisture pulse treatments ($P = 0.0052$).

2.3.7 Recovered ^{13}C and total C in soil aggregate fractions

In macroaggregate fraction, the concentration of recovered ^{13}C ranged from 35.03 μg g^{-1} dry fraction soil to 82.37 μg g^{-1} dry fraction soil (Figure 2.8). Under the no cover crop

treatment, the no tillage treatment caused lower concentration of recovered ^{13}C than the conventional tillage treatment, while under the wheat cover crop treatment, the no tillage treatment caused higher concentration of recovered ^{13}C than the conventional tillage treatment. The concentration of recovered ^{13}C under the 1 moisture pulse treatment was significantly higher than that in the 0, 4, and 8 moisture pulse treatments ($P = 0.0172$, $P = 0.0002$, and $P = 0.0022$, respectively). In microaggregate fraction, the concentration of recovered ^{13}C ranged from 32.59 μg g^{-1} dry fraction soil to 73.87 μg g^{-1} dry fraction soil. The highest ^{13}C recovery was found in the WCNT treatment under the 1 moisture pulse treatment. The lowest ^{13}C recovery was found in the NCNT treatment under the 4 moisture pulse treatment.

In macroaggregate fraction, the highest and the lowest concentration of total C were 17.05 mg g^{-1} dry fraction soil and 10.17 mg g^{-1} dry fraction soil (Figure 2.8). The moisture pulse treatment did not cause any significant differences on the concentration of total C ($P > 0.0800$). The concentration of total C in the NCCT treatment was significantly lower than that in the NCNT treatment ($P = 0.0009$), the VCNT treatment ($P < 0.0001$), the VCCT treatment ($P < 0.0001$), the WCNT treatment ($P < 0.0001$), and the WCCT treatment ($P = 0.0305$). In microaggregate fraction, the concentration of total C ranged from 9.19 mg g^{-1} dry fraction soil to 17.37 mg g^{-1} dry fraction soil. The concentration of total C under the 0 moisture pulse treatments was significantly higher than that under the 4 and 8 moisture pulse treatments ($P < 0.0001$). The concentration of total C under the 1 moisture pulse treatments was significantly higher than that under the 4 and 8 moisture pulse treatments ($P = 0.0010$ and $P = 0.0002$, respectively). The VCNT

treatment induced the highest concentration of total C than the other conservation management treatments ($P < 0.0001$).

2.4 Discussion

2.4.1 Effects of repeated drying-rewetting cycles on soil total C loss and soil microbial CUE

Cumulative CO_2 emission was significantly greater in soils that went through repeated drying-rewetting cycles than that in soils that were kept at a constant soil moisture content (Figure 2.3), but MBC was significantly lower in soils that went through repeated drying-rewetting cycles than that in soils that were kept constant soil moisture content (Figure 2.5). It indicates that soil microbial CUE is lower in soils that went through repeated drying-rewetting cycles than that in soils that were kept constant soil moisture content (Figure 2.6). Repeated drying-rewetting cycles decrease CUE in several ways. (1) Low soil water content impedes substrate availability. Since water-filled capillaries become disconnected (Moldrup et al., 2001) and water-filled pores become smaller (Or et al., 2007) during the drying periods, the diffusivity of substrates and the mobility of microbes and enzymes are reduced by low soil water content (Manzoni et al., 2012a). Substrate availability become the most limiting factor lowering microbial growth under dry conditions, which can lower CUE. (2) Low soil water content alters the balance between microbial growth and maintenance. Facing moisture stress, soil microbes have to switch their allocation of resources from growth to maintenance in order to survive (Schimel et al., 2007). Soil microbes can cope with drought by producing mucilage and accumulating osmolytes (Schimel et al., 2007; Borken and Matzner, 2009; Manzoni et

al., 2012a). This will alter the investment of C from microbial growth to survival strategies, which can decrease CUE. Soil microbes can produce a layer of polysaccharide-rich mucilage to resist desiccation (Borken and Matzner, 2009). The other response to drought is to accumulate osmolytes to avoid dehydrating and dying under osmotic stress (Tiemann and Billings, 2011). Soil microbes usually use simple organic compounds that are highly soluble and have few physiological effects as osmolytes, such as amino compounds like glutamine, glycine betaine, and proline for bacteria and polyols like mannitol, erythritol, and glycerol for fungi (Csonka, 1989; Witteveen and Visser, 1995; Schimel et al., 2007). The osmolyte C take up about 7%-20% of total C in bacteria and the polyols take up over 10% of cell mass in fungi (Koujima et al., 1978; Tibbett et al., 2002; Schimel et al., 2007). Synthesizing osmolytes is vary energy- and resource-consuming for soil microbes. Soil microbes need to consume about 5% of total annual net primary production for synthesizing the osmolytes in order to survive one drought period (Schimel et al., 2007). (3) Soil microbes release osmolytes upon rewetting. Facing rapidly decreased soil water potential, soil microbes have to dispose the previously accumulated osmolytes either by respiring, polymerizing, or transporting in order to avoid being ruptured (Kieft, 1987; Wood et al., 2001; Schimel et al., 2007). Rewetting can induce a release of up to 50% of the microbial biomass (Kieft, 1987; Schimel et al., 2007). Rewetting decreases CUE through increasing the excretion of microbial cellular materials (Manzoni et al., 2012b). (4) Soil microbes can avoid desiccation becoming dormant. If the moisture stress were too severe, soil microbes will be forced into dormancy (Suzina et al., 2004; Schimel et al., 2007). Becoming dormant is the strategy of soil microbes to avoid desiccation at the expense of lowering C uptake (Manzoni et al., 2014). Dormancy

also can lead to delayed recovery of microbial activities following rewetting, therefore it can lower the use efficiency of the C made available by the rewetting (Placella et al., 2012; Manzoni et al., 2014). (5) Water saturated condition changes metabolic end products. Under anaerobic conditions, instead of CO_2 only, acetate and CH_4 are also metabolic products, which are not completely oxidized (Šantrůčková et al., 2004; Burgin et al., 2011). This can lower CUE.

2.4.2 Effects of drying-rewetting cycles on microbial utilization of the added glucose-C in different soil C pools

At the end of the 24-day incubation, 0.08%-1% of the added glucose-C was incorporated in the EOC pool, 4%-27% of the added glucose-C was incorporated in the MBC pool, and 0.7%-5% of the added glucose-C was incorporated in the H_2O_2-resistant C pool. Most of the added glucose-C was recovered in the MBC pool, especially in the drying treatment. The added glucose-C can be rapidly utilized by microbes once added to soil because glucose can be assimilated by microbial cells without being fully metabolized (Witter and Dahlin, 1995). The concentration of $MB^{13}C$ was higher in the dry soils than that in the rewetted soils (Figure 2.5) possibly due to osmoregulation. Osmoregulation is the mechanism that soil microbes accumulate osmolytes to maintain intracellular water potential in response to dry conditions (Schimel et al., 2007). Manzoni et al. (2014) summarized the 3 stages of microbial metabolism when soil is drying: (1) stable microbial metabolism stage under favorable soil moisture conditions, (2) osmolyte accumulating stage when soil is becoming dry, and (3) dormant stage when the osmolyte concentration in microbial cells reaches the maximum. Therefore, the added glucose-C was accumulated in the dry soil in the form of MBC. By contrast, in the rewetted soils,

the $MB^{13}C$ accumulated during the drying periods was released to the environment upon rewetting, which was rapidly mineralized by soil microbes.

The concentration of $EO^{13}C$ was higher in the dried soils than that in the rewetted soils (Figure 2.5), which could be caused by the increased accumulation of $EO^{13}C$ during the drying period and the rapid consumption of $EO^{13}C$ upon rewetting. This may happen for several reasons. (1) Reduced microbial utilization during drying. All soils started with a gravimetric water content of 40% and a ^{13}C-labeled glucose addition. The addition of labile substrates induced rapid microbial utilization of the readily available EOC. In the drying treatment, along with the decrease of soil water content, the microbial consumption of EOC became limited. Hence, the $EO^{13}C$ was remained in the soil while the soil was dried. Under field condition instead of in the microcosm incubation, the physical loss of the $EO^{13}C$ through leaching will also be reduced, which leads to $EO^{13}C$ accumulation. (2) Activated microbial utilization upon rewetting. In the rewetted soils, the rewetting events can accelerate microbial activity and may transport the increase the $EO^{13}C$ to microbial cells. Therefor the microbial utilization of $EO^{13}C$ increases, which will lower the $EO^{13}C$ content in the rewetted soils. (3) Extracellular enzyme activity during drying periods. Although microbial activity was low during the drying periods, extracellular enzymes were still active. This is in agreement with previous findings that extracellular enzymes may be less active when soil is dry (Steinweg et al., 2012), but they may still degrade organic matter under dry conditions (Zeglin et al., 2013; Manzoni et al., 2014). Extracellular enzymes could possibly hydrolyze organic C and readily biodegradable $EO^{13}C$ accumulated during the drying periods. The readily biodegradable $EO^{13}C$ can be immediately used by soil microbes upon rewetting. (4) Disruption of soil

aggregates by drying-rewetting cycles. The drying-rewetting cycles caused disruption of soil aggregates (Figure 2.7), which lead to release of previously protected $EO^{13}C$ within soil aggregates. The released $EO^{13}C$ can be rapidly used by soil microbes upon rewetting.

2.4.3 Effects of conservation agricultural management practices on utilization and stabilization of new labile C input

Under the dry conditions, $MB^{13}C$ accounted for 29% of MBC in the no-cover-crop-conventional-tillage treatment, while $MB^{13}C$ only accounted for 13% of MBC in the vetch-cover-crop-no-tillage treatment. Under optimum moisture conditions, $MB^{13}C$ accounted for 17% of MBC in the no-cover-crop-conventional-tillage treatment, while $MB^{13}C$ only accounted for 11% of MBC in the vetch-cover-crop-no-tillage treatment. The total C concentration in the no-cover-crop-conventional-tillage treatment and the vetch-cover-crop-no-tillage treatment is 9.04 mg g^{-1} dry bulk soil and 14.75 mg g^{-1} dry bulk soil, respectively. This suggests that soil microbes preferentially utilize the added glucose-C instead of the endogenous SOC when soil C resource is relatively scarce regardless of soil moisture conditions. Under dry conditions, $EO^{13}C$ accounted for 2.21% of EOC in the no-cover-crop-conventional-tillage treatment, while $EO^{13}C$ only accounted for 1.53% of EOC in the vetch-cover-crop-no-tillage treatment. Under well-watered conditions, $EO^{13}C$ accounted for 0.38% of EOC in the no-cover-crop-conventional-tillage treatment, while $EO^{13}C$ only accounted for 0.29% of EOC in the vetch-cover-crop-no-tillage treatment. This indicates that more of the added glucose-C was accumulated as labile C under C scarce condition than under C abundant condition. However, under dry conditions, H_2O_2-resistant ^{13}C accounted for 0.24% of H_2O_2-resistant C in the no-cover-crop-conventional-tillage treatment, while H_2O_2-resistant ^{13}C accounted for 0.21% of

H_2O_2-resistant C in the vetch-cover-crop-no-tillage treatment. Under well-watered conditions, H_2O_2-resistant ^{13}C accounted for 0.16% of H_2O_2-resistant C in the no-cover-crop-conventional-tillage treatment, while H_2O_2-resistant ^{13}C accounted for 0.13% of H_2O_2-resistant C in the vetch-cover-crop-no-tillage treatment. It suggests that more of the added glucose-C was accumulated as recalcitrant C under C scarce condition than under C abundant condition. Those transformation patterns of the added glucose-C indicate that C scarce condition has more capacity for the stabilization of the added glucose-C than C abundant condition.

The concentration of MBC in the vetch-cover-crop-no-tillage treatment is significantly higher than that in the no-cover-crop-conventional-tillage treatment (Figure 2.5). This indicates that long-term vetch cover crops and no tillage practices increased soil MBC content. This is in agreement with previous findings that 18 years of straw addition to field increased MBC by 40%-50% (Powlson et al., 1987). The concentration of EOC and H_2O_2-resistant C in the vetch-cover-crop-no-tillage treatment is also significantly higher than that in the no-cover-crop-conventional-tillage treatment (Figure 2.5). This suggests that long-term cover crops and no-tillage practices increased both the active C pool and the passive C pool. It can be explained by reduced C loss through CO_2 emission. The amount of C loss in per unit of total C was significantly lower in the vetch-cover-crop-no-tillage treatment compared in the no-cover-crop-conventional-tillage treatment (Figure 2.3). This suggests that cover crops and no-tillage practices both decreased the amount of C loss in per unit of total C. In conventional tillage systems, both vetch cover crops and wheat cover crops decreased the amount of C loss in per unit of total C, with wheat cover crops being more effective than vetch cover crops. This could possibly be

explained by the difference in cover crop quality. Vetch cover crops have a more suitable C/N ratio for microbial activities. The high C/N ratio of wheat cover crops may limit microbial respiration, and therefore reduced C loss. In no-tillage systems, only wheat cover crops decreased the amount of C loss in per unit of total C. This could be explained by the difference in soil structure between no-tillage systems and conventional tillage systems. The more abundant soil aggregates in no-tillage systems can provide stronger physical protection for SOC than conventional tillage systems, which can reduce C loss thought microbial respiration. This may diminish the effect of vetch cover crops compared to wheat cover crops.

2.5 Conclusions

My results indicate that repeated drying-rewetting cycles decrease soil microbial CUE. This may be because (1) low soil water content impedes substrate availability, (2) low soil water content alters the balance between microbial growth and maintenance, (3) soil microbes release osmolytes upon rewetting, (4) soil microbes can avoid desiccation becoming dormant, and (5) water saturated condition changes metabolic end products. At the end of the 24-day incubation, 0.08%-1% of the added glucose-C was incorporated in the EOC pool, 4%-27% of the added glucose-C was incorporated in the MBC pool, and 0.7%-5% of the added glucose-C was incorporated in the H_2O_2-resistant C pool. The drying treatment induced higher incorporation of the added glucose-C in the EOC pool, MBC pool, and H_2O_2-resistant C pool, which could be caused by the increased accumulation during dry period and the rapid consumption upon rewetting. Under different conservation agricultural management practices, my results suggest that soil

microbes preferentially utilize the added glucose-C instead of the endogenous SOC when soil C resource is relatively scarce regardless of soil moisture conditions. More of the added glucose-C was mineralized under C scarce condition than under C abundant condition. The difference in the accumulation of the added glucose-C under different conservation agricultural management practices indicates that C scarce condition has more capacity for the stabilization of the added glucose-C than C abundant condition..

References

Birch, H., 1958. The effect of soil drying on humus decomposition and nitrogen availability. Plant and soil 10, 9-31.

Borken, W., Matzner, E., 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Global change biology 15, 808-824.

Brookes, P., 2001. The soil microbial biomass: concept, measurement and applications in soil ecosystem research. Microbes and Environments 16, 131-140.

Burgin, A.J., Yang, W.H., Hamilton, S.K., Silver, W.L., 2011. Beyond carbon and nitrogen: how the microbial energy economy couples elemental cycles in diverse ecosystems. Frontiers in Ecology and the Environment 9, 44-52.

Chantigny, M.H., 2003. Dissolved and water-extractable organic matter in soils: a review on the influence of land use and management practices. Geoderma 113, 357-380.

Chantigny, M.H., Harrison-Kirk, T., Curtin, D., Beare, M., 2014. Temperature and duration of extraction affect the biochemical composition of soil water-extractable organic matter. Soil Biology and Biochemistry 75, 161-166.

Csonka, L.N., 1989. Physiological and genetic responses of bacteria to osmotic stress. Microbiological reviews 53, 121-147.

Eusterhues, K., Rumpel, C., Kögel-Knabner, I., 2005. Stabilization of soil organic matter isolated via oxidative degradation. Organic Geochemistry 36, 1567-1575.

Fierer, N., Schimel, J.P., 2002. Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. Soil Biology and Biochemistry 34, 777-787.

Haynes, R., 2005. Labile organic matter fractions as central components of the quality of agricultural soils: an overview. Advances in Agronomy 85, 221-268.

Helfrich, M., Flessa, H., Mikutta, R., Dreves, A., Ludwig, B., 2007. Comparison of chemical fractionation methods for isolating stable soil organic carbon pools. European Journal of Soil Science 58, 1316-1329.

Jagadamma, S., Lal, R., Ussiri, D.A., Trumbore, S.E., Mestelan, S., 2010. Evaluation of structural chemistry and isotopic signatures of refractory soil organic carbon fraction isolated by wet oxidation methods. Biogeochemistry 98, 29-44.

Jastrow, J., Miller, R., 1997. Soil aggregate stabilization and carbon sequestration: feedbacks through organomineral associations. Soil processes and the carbon cycle, 207-223.

Jenkinson, D., 1981. Microbial biomass in soil-measurement and turnover. Soil biochemistry 6, 415-471.

Kieft, T.L., 1987. Microbial biomass response to a rapid increase in water potential when dry soil is wetted. Soil Biology and Biochemistry 19, 119-126.

Koujima, I., Hayashi, H., Tomochika, K., Okabe, A., Kanemasa, Y., 1978. Adaptational change in proline and water content of Staphylococcus aureus after alteration of environmental salt concentration. Applied and Environmental Microbiology 35, 467-470.

Manzoni, S., Schaeffer, S., Katul, G., Porporato, A., Schimel, J., 2014. A theoretical analysis of microbial eco-physiological and diffusion limitations to carbon cycling in drying soils. Soil Biology and Biochemistry 73, 69-83.

Manzoni, S., Schimel, J.P., Porporato, A., 2012a. Responses of soil microbial communities to water stress: results from a meta-analysis. Ecology 93, 930-938.

Manzoni, S., Taylor, P., Richter, A., Porporato, A., Ågren, G.I., 2012b. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytologist 196, 79-91.

Mbuthia, L.W., Acosta-Martinez, V., DeBruyn, J., Schaeffer, S., Tyler, D., Odoi, E., Mpheshea, M., Walker, F., Eash, N., 2015. Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: Implications for soil quality. Soil Biology and Biochemistry 89, 24-34.

Miller, A.E., Schimel, J.P., Meixner, T., Sickman, J.O., Melack, J.M., 2005. Episodic rewetting enhances carbon and nitrogen release from chaparral soils. Soil Biology and Biochemistry 37, 2195-2204.

Moldrup, P., Olesen, T., Komatsu, T., Schjønning, P., Rolston, D., 2001. Tortuosity, diffusivity, and permeability in the soil liquid and gaseous phases. Soil Science Society of America Journal 65, 613-623.

Moore, J., Klose, S., Tabatabai, M., 2000. Soil microbial biomass carbon and nitrogen as affected by cropping systems. Biology and fertility of soils 31, 200-210.

Or, D., Smets, B.F., Wraith, J., Dechesne, A., Friedman, S., 2007. Physical constraints affecting bacterial habitats and activity in unsaturated porous media–a review. Advances in Water Resources 30, 1505-1527.

Parker, S.S., Schimel, J.P., 2011. Soil nitrogen availability and transformations differ between the summer and the growing season in a California grassland. Applied Soil Ecology 48, 185-192.

Placella, S.A., Brodie, E.L., Firestone, M.K., 2012. Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. Proceedings of the National Academy of Sciences 109, 10931-10936.

Plante, A.F., Chenu, C., Balabane, M., Mariotti, A., Righi, D., 2004. Peroxide oxidation of clay-associated organic matter in a cultivation chronosequence. European Journal of Soil Science 55, 471-478.

Powlson, D., Prookes, P., Christensen, B.T., 1987. Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. Soil Biology and Biochemistry 19, 159-164.

Ritchey, E.L., Tyler, D.D., Essington, M.E., Mullen, M.D., Saxton, A.M., 2015. Nitrogen rate, cover crop, and tillage practice alter soil chemical properties. Agronomy Journal 107, 1259-1268.

Šantrůčková, H., Picek, T., Tykva, R., Šimek, M., Pavlů, B., 2004. Short-term partitioning of 14C-[U]-glucose in the soil microbial pool under varied aeration status. Biology and fertility of soils 40, 386-392.

Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88, 1386-1394.

Six, J., Elliott, E., Paustian, K., 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology and Biochemistry 32, 2099-2103.

Smith, J., Paul, E., 1990. The significance of soil microbial biomass estimations. Soil biochemistry. Volume 6., 357-396.

Spohn, M., Klaus, K., Wanek, W., Richter, A., 2016. Microbial carbon use efficiency and biomass turnover times depending on soil depth–Implications for carbon cycling. Soil Biology and Biochemistry 96, 74-81.

Steinweg, J.M., Dukes, J.S., Wallenstein, M.D., 2012. Modeling the effects of temperature and moisture on soil enzyme activity: linking laboratory assays to continuous field data. Soil Biology and Biochemistry 55, 85-92.

Suzina, N., Mulyukin, A., Kozlova, A., Shorokhova, A., Dmitriev, V., Barinova, E., Mokhova, O., Duda, V., 2004. Ultrastructure of resting cells of some non-spore-forming bacteria. Microbiology 73, 435-447.

Tibbett, M., Sanders, F., Cairney, J., 2002. Low-temperature-induced changes in trehalose, mannitol and arabitol associated with enhanced tolerance to freezing in ectomycorrhizal basidiomycetes (Hebeloma spp.). Mycorrhiza 12, 249-255.

Tiemann, L.K., Billings, S.A., 2011. Changes in variability of soil moisture alter microbial community C and N resource use. Soil Biology and Biochemistry 43, 1837-1847.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007. SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. Soil Biology and Biochemistry 39, 2183-2207.

Wardle, D., 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biological reviews 67, 321-358.

Witter, E., Dahlin, S., 1995. Microbial utilization of [U 14C]-labelled straw and [U 13C]-labelled glucose in soils of contrasting pH and metal status. Soil Biology and Biochemistry 27, 1507-1516.

Witteveen, C.F., Visser, J., 1995. Polyol pools in Aspergillus niger. FEMS Microbiology Letters 134, 57-62.

Wood, J.M., Bremer, E., Csonka, L.N., Kraemer, R., Poolman, B., van der Heide, T., Smith, L.T., 2001. Osmosensing and osmoregulatory compatible solute accumulation by bacteria. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 130, 437-460.

Xiang, S.-R., Doyle, A., Holden, P.A., Schimel, J.P., 2008. Drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface California grassland soils. Soil Biology and Biochemistry 40, 2281-2289.

Zeglin, L., Bottomley, P.J., Jumpponen, A., Rice, C., Arango, M., Lindsley, A., McGowan, A., Mfombep, P., Myrold, D., 2013. Altered precipitation regime affects the function and composition of soil microbial communities on multiple time scales. Ecology 94, 2334-2345.

Appendix 2

Appendix 2A Soil gravimetric water content

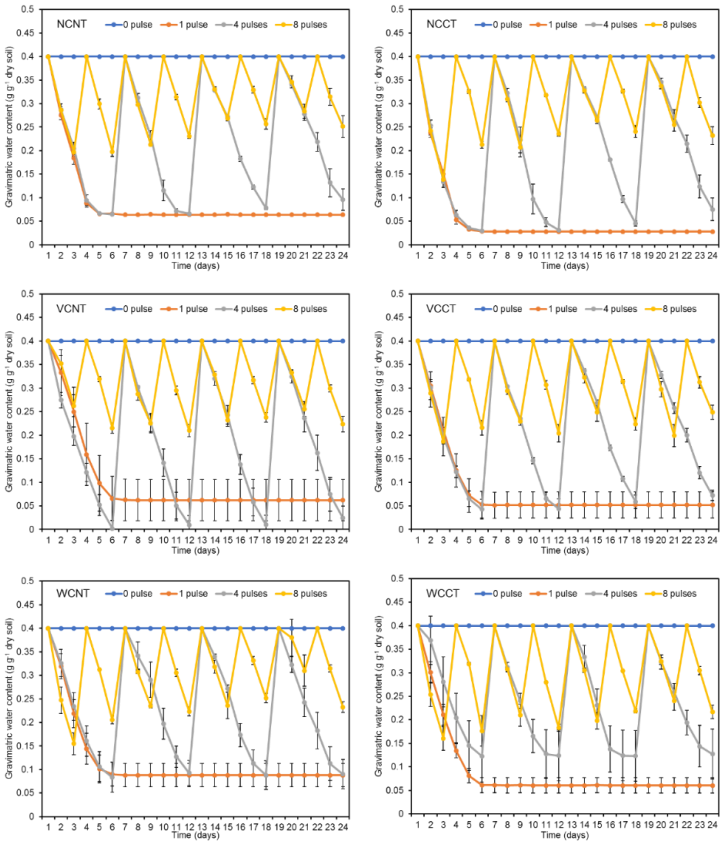

Figure 2.1 Mean soil gravimetric water content during the incubation under drying-rewetting cycles.

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. 0 pulse: 0 day of drought; 1 pulse: 24 days of drought; 4 pulses: 6 days of drought; 8 pulses: 3 days of drought. Bars indicate standard error. Error bars are at 95% confident interval.

Appendix 2B Soil microbial respiration

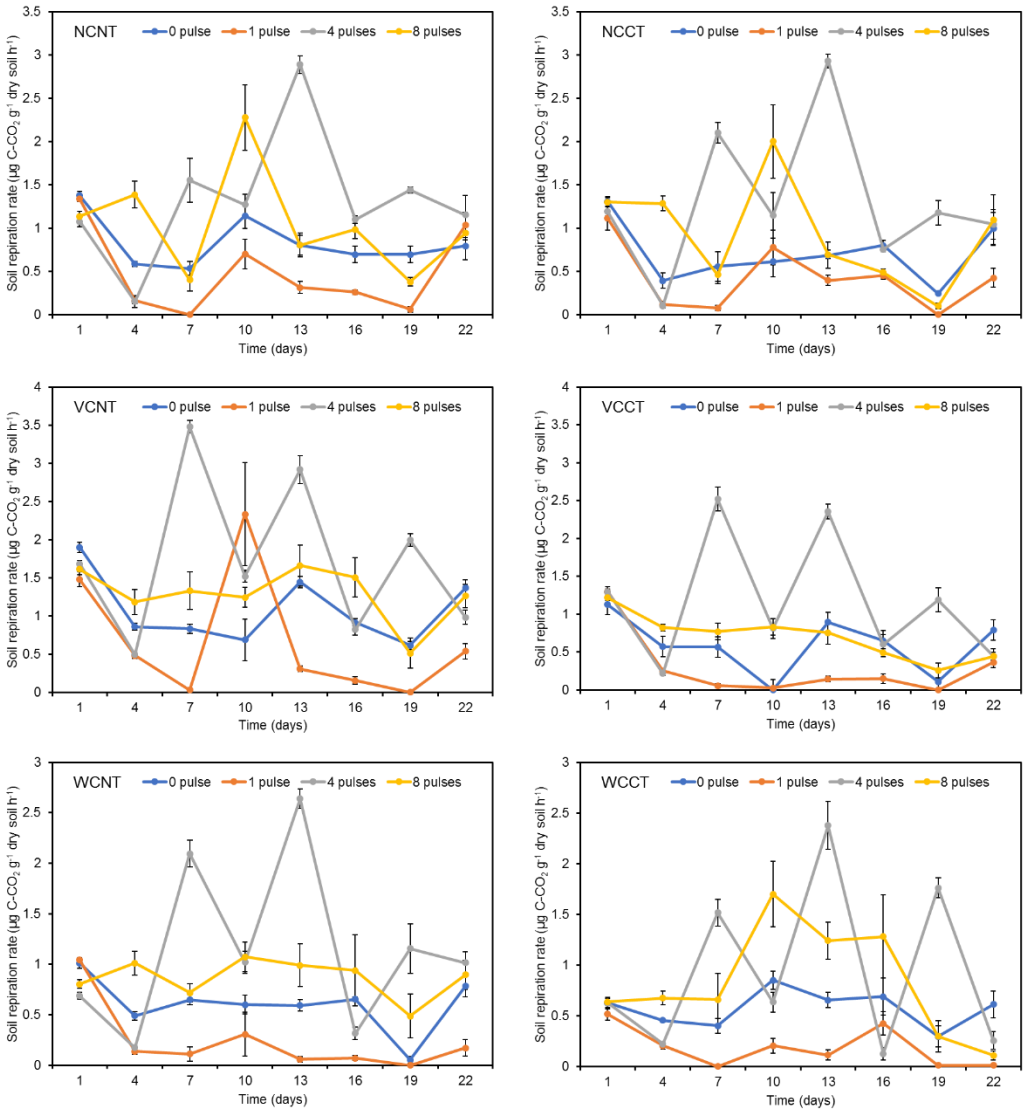

Figure 2.2 Mean soil microbial respiration rate during the incubation under drying-rewetting cycles.

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Bars indicate standard error. Error bars are at 95% confident interval.

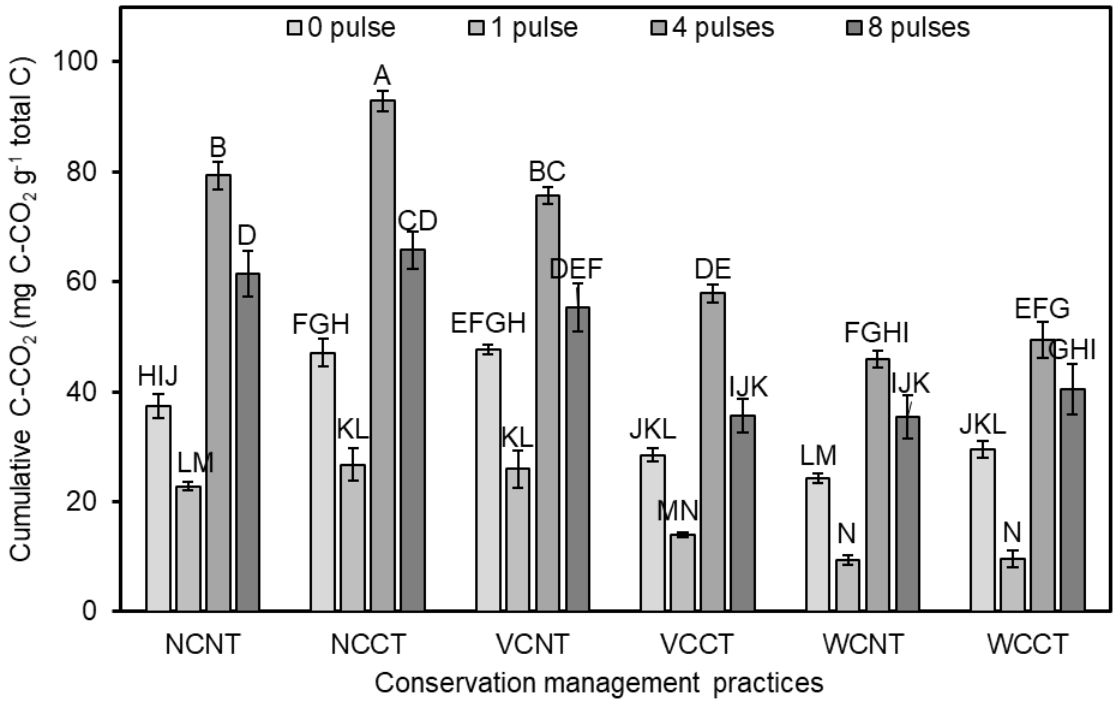

Figure 2.3 Cumulative soil respiration during the incubation.

The cumulative soil respiration was normalized by total soil C concentration. NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Bars indicate standard error. Error bars are at 95% confident interval. Shared letters denote no significant difference at 5% level between all treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.1 Main effect of conservation management on cumulative soil microbial respiration.

Conservation management	Mean	Standard Error of Mean	Letter Group
NCCT	58.0938	1.8414	A
NCNT	50.2957	1.8414	B
VCCT	34.0154	1.8414	C
VCNT	51.2845	1.8414	B
WCCT	32.2823	1.8414	C
WCNT	28.8742	1.8414	C

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.2 Main effect of drying-rewetting cycles on cumulative soil microbial respiration.

Drying-rewetting cycles	Mean	Standard Error of Mean	Letter Group
0 pulse	35.7003	1.5035	C
1 pulse	18.0973	1.5035	D
4 pulses	67.0231	1.5035	A
8 pulses	49.0765	1.5035	B

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.3 Interaction effect of conservation management and drying-rewetting cycles on cumulative soil microbial respiration.

Conservation management	Drying-rewetting cycles	Mean	Standard Error of Mean	Letter Group
NCCT	0 pulse	47.0690	3.6828	FGH
NCCT	1 pulse	26.5396	3.6828	KL
NCCT	8 pulses	65.6551	3.6828	CD
NCCT	4 pulses	93.1118	3.6828	A
NCNT	0 pulse	37.3252	3.6828	HIJ
NCNT	1 pulse	23.0130	3.6828	LM
NCNT	8 pulses	61.4373	3.6828	D
NCNT	4 pulses	79.4073	3.6828	B
VCCT	0 pulse	28.4906	3.6828	JKL
VCCT	1 pulse	13.9811	3.6828	MN
VCCT	8 pulses	35.7192	3.6828	IJK
VCCT	4 pulses	57.8707	3.6828	DE
VCNT	0 pulse	47.6960	3.6828	EFGH
VCNT	1 pulse	26.1121	3.6828	KL
VCNT	8 pulses	55.5288	3.6828	DEF
VCNT	4 pulses	75.8011	3.6828	BC
WCCT	0 pulse	29.4481	3.6828	JKL
WCCT	1 pulse	9.5309	3.6828	N
WCCT	8 pulses	40.2970	3.6828	GHI
WCCT	4 pulses	49.8533	3.6828	EFG
WCNT	0 pulse	24.1730	3.6828	LM
WCNT	1 pulse	9.4073	3.6828	N
WCNT	8 pulses	35.8219	3.6828	IJK
WCNT	4 pulses	46.0946	3.6828	FGHI

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Appendix 2C Concentrations of ^{13}C and total C in bulk soil

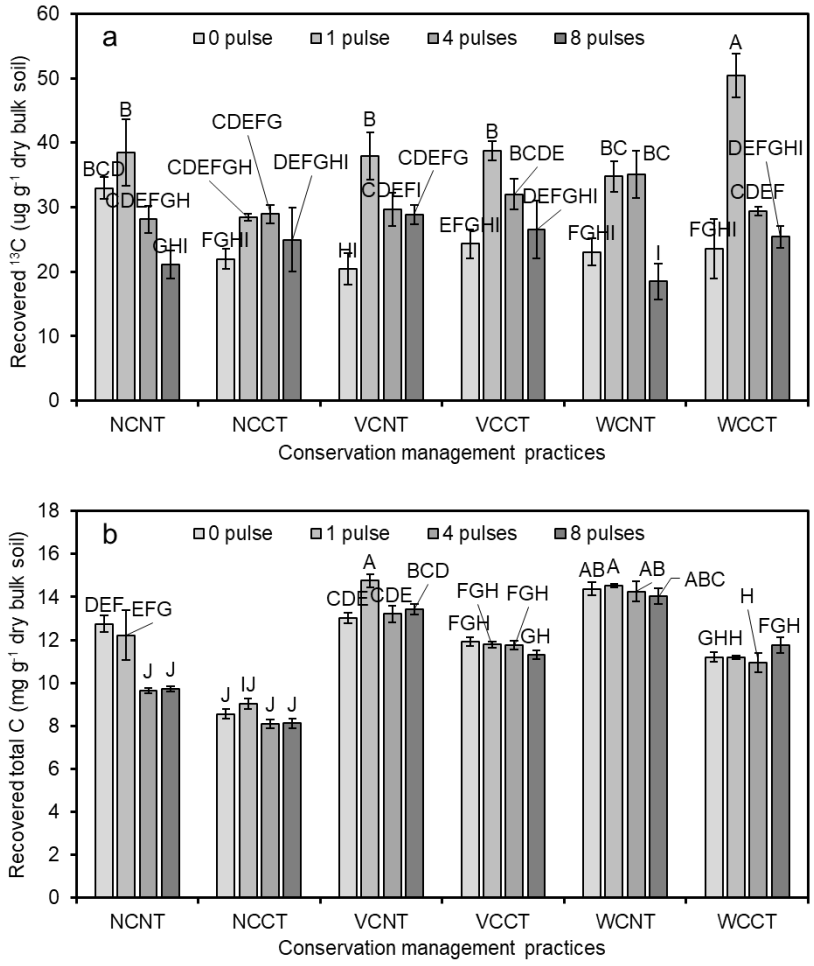

Figure 2.4 Recovered ^{13}C (upper) and total C (lower) in bulk soil at the end of the incubation.

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage; a: ^{13}C; b: total C. Bars indicate standard error. Error bars are at 95% confident interval. Shared letters denote no significant difference at 5% level between all treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.4 Main effect of conservation management on recovered ^{13}C in bulk soil.

Conservation management	Mean	Standard Error of Mean	Letter Group
NCCT	26.0903	1.4337	C
NCNT	30.1687	1.4337	AB
VCCT	30.4012	1.4337	AB
VCNT	29.2239	1.4337	ABC
WCCT	32.2108	1.4337	A
WCNT	27.8741	1.4337	BC

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.5 Main effect of drying-rewetting cycles on recovered ^{13}C in bulk soil.

Drying-rewetting cycles	Mean	Standard Error of Mean	Letter Group
0 pulse	24.3890	1.1706	C
1 pulse	38.1381	1.1706	A
4 pulses	30.5587	1.1706	B
8 pulses	24.2268	1.1706	C

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.6 Interaction effect of conservation management and drying-rewetting cycles on recovered ^{13}C in bulk soil.

Conservation management	Drying-rewetting cycles	Mean	Standard Error of Mean	Letter Group
NCCT	0 pulse	21.9993	2.8673	FGHI
NCCT	1 pulse	28.4437	2.8673	CDEFGH
NCCT	8 pulses	24.9725	2.8673	DEFGHI
NCCT	4 pulses	28.9456	2.8673	CDEFG
NCNT	0 pulse	32.9701	2.8673	BCD
NCNT	1 pulse	38.4937	2.8673	B
NCNT	8 pulses	21.0950	2.8673	GHI
NCNT	4 pulses	28.1161	2.8673	CDEFGH
VCCT	0 pulse	24.3164	2.8673	EFGHI
VCCT	1 pulse	38.7394	2.8673	B
VCCT	8 pulses	26.5178	2.8673	DEFGHI
VCCT	4 pulses	32.0310	2.8673	BCDE
VCNT	0 pulse	20.3923	2.8673	HI
VCNT	1 pulse	37.9377	2.8673	B
VCNT	8 pulses	28.8645	2.8673	CDEFG
VCNT	4 pulses	29.7010	2.8673	CDEF
WCCT	0 pulse	23.5712	2.8673	FGHI
WCCT	1 pulse	50.4469	2.8673	A
WCCT	8 pulses	25.4195	2.8673	DEFGHI
WCCT	4 pulses	29.4055	2.8673	CDEF
WCNT	0 pulse	23.0849	2.8673	FGHI
WCNT	1 pulse	34.7668	2.8673	BC
WCNT	8 pulses	18.4914	2.8673	I
WCNT	4 pulses	35.1534	2.8673	BC

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.7 Main effect of conservation management on recovered total C in bulk soil.

Conservation management	Mean	Standard Error of Mean	Letter Group
NCCT	8.4533	0.1800	E
NCNT	11.0893	0.1800	D
VCCT	11.6871	0.1800	C
VCNT	13.6013	0.1800	B
WCCT	11.2766	0.1800	CD
WCNT	14.2994	0.1800	A

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.8 Main effect of drying-rewetting cycles on recovered total C in bulk soil.

Dring-rewetting cycles	Mean	Standard Error of Mean	Letter Group
0 pulse	11.9711	0.1470	A
1 pulse	12.2517	0.1470	A
4 pulses	11.3189	0.1470	B
8 pulses	11.3963	0.1470	B

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.9 Interaction effect of conservation management and drying-rewetting cycles on recovered total C in bulk soil.

Conservation management	Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
NCCT	0 pulse	8.5529	0.3601	J
NCCT	1 pulse	9.0368	0.3601	IJ
NCCT	8 pulses	8.1252	0.3601	J
NCCT	4 pulses	8.0984	0.3601	J
NCNT	0 pulse	12.7508	0.3601	DEF
NCNT	1 pulse	12.2229	0.3601	EFG
NCNT	8 pulses	9.7363	0.3601	I
NCNT	4 pulses	9.6470	0.3601	I
VCCT	0 pulse	11.9109	0.3601	FGH
VCCT	1 pulse	11.7853	0.3601	FGH
VCCT	8 pulses	11.3045	0.3601	GH
VCCT	4 pulses	11.7475	0.3601	FGH
VCNT	0 pulse	13.0286	0.3601	CDE
VCNT	1 pulse	14.7541	0.3601	A
VCNT	8 pulses	13.4152	0.3601	BCD
VCNT	4 pulses	13.2075	0.3601	CDE
WCCT	0 pulse	11.2028	0.3601	GH
WCCT	1 pulse	11.1870	0.3601	H
WCCT	8 pulses	11.7625	0.3601	FGH
WCCT	4 pulses	10.9540	0.3601	H
WCNT	0 pulse	14.3804	0.3601	AB
WCNT	1 pulse	14.5240	0.3601	A
WCNT	8 pulses	14.0340	0.3601	ABC
WCNT	4 pulses	14.2591	0.3601	AB

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Appendix 2D Concentrations of ^{13}C and total C in H_2O_2-resistant C pool, EOC, and MBC

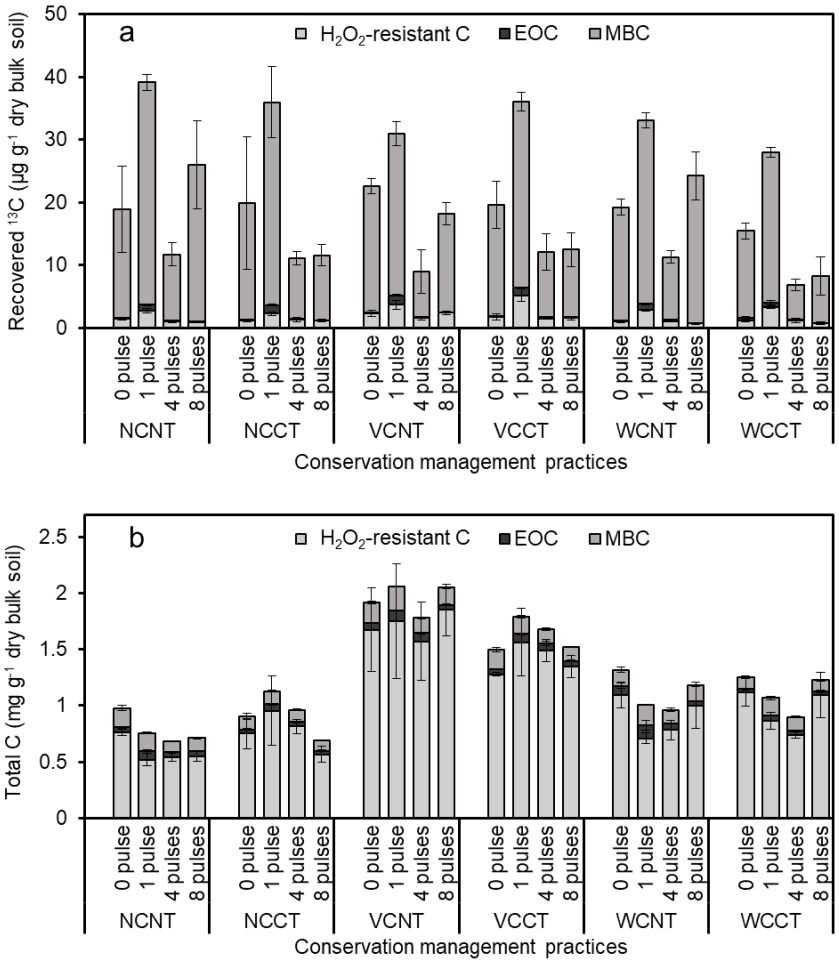

Figure 2.5 Distribution of recovered ^{13}C (upper) and total C (lower) in soil C pools at the end of the incubation.

EOC: extractable organic C; MBC: microbial biomass C. NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage; a: ^{13}C; b: total C. Bars indicate standard error. Error bars are at 95% confident interval.

Table 2.10 Main effect of conservation management on extractable organic ^{13}C.

Conservation management	Mean	Standard Error of Mean	Letter Group
NCCT	0.4489	0.05542	A
NCNT	0.3603	0.05542	A
VCCT	0.4345	0.05542	A
VCNT	0.5088	0.05542	A
WCCT	0.3677	0.05542	A
WCNT	0.3764	0.05542	A

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.11 Main effect of drying rewetting cycles on extractable organic ^{13}C.

Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
0 pulse	0.1963	0.04525	B
1 pulse	1.1334	0.04525	A
4 pulses	0.2130	0.04525	B
8 pulses	0.1217	0.04525	B

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.12 Interaction effect of conservation management and drying-rewetting cycles on extractable organic ^{13}C.

Conservation management	Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
NCCT	0 pulse	0.1484	0.1108	D
NCCT	1 pulse	1.3507	0.1108	A
NCCT	8 pulses	0.1103	0.1108	D
NCCT	4 pulses	0.1861	0.1108	D
NCNT	0 pulse	0.1473	0.1108	D
NCNT	1 pulse	0.9627	0.1108	BC
NCNT	8 pulses	0.1197	0.1108	D
NCNT	4 pulses	0.2114	0.1108	D
VCCT	0 pulse	0.1761	0.1108	D
VCCT	1 pulse	1.2148	0.1108	AB
VCCT	8 pulses	0.1457	0.1108	D
VCCT	4 pulses	0.2014	0.1108	D
VCNT	0 pulse	0.1709	0.1108	D
VCNT	1 pulse	1.5079	0.1108	A
VCNT	8 pulses	0.1296	0.1108	D
VCNT	4 pulses	0.2266	0.1108	D
WCCT	0 pulse	0.3446	0.1108	D
WCCT	1 pulse	0.7748	0.1108	C
WCCT	8 pulses	0.1139	0.1108	D
WCCT	4 pulses	0.2376	0.1108	D
WCNT	0 pulse	0.1904	0.1108	D
WCNT	1 pulse	0.9895	0.1108	BC
WCNT	8 pulses	0.1108	0.1108	D
WCNT	4 pulses	0.2151	0.1108	D

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.13 Main effect of conservation management on extractable organic C.

Conservation management	Mean	Standard Error of Mean	Letter Group
NCCT	1.7815	0.2497	BC
NCNT	2.1506	0.2497	BC
VCCT	2.4037	0.2497	AB
VCNT	2.9123	0.2497	A
WCCT	1.4825	0.2497	C
WCNT	2.9402	0.2497	A

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.14 Main effect of drying rewetting cycles on extractable organic C.

Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
0 pulse	1.5705	0.2038	C
1 pulse	3.5163	0.2038	A
4 pulses	2.4786	0.2038	B
8 pulses	1.5483	0.2038	C

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.15 Interaction effect of conservation management and drying-rewetting cycles on extractable organic C.

Conservation management	Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
NCCT	0 pulse	1.2042	0.4993	HI
NCCT	1 pulse	2.8478	0.4993	CDEF
NCCT	8 pulses	1.2048	0.4993	HI
NCCT	4 pulses	1.8691	0.4993	EFGHI
NCNT	0 pulse	1.4155	0.4993	GHI
NCNT	1 pulse	3.3250	0.4993	BCD
NCNT	8 pulses	1.6665	0.4993	EFGHI
NCNT	4 pulses	2.1953	0.4993	CDEFGHI
VCCT	0 pulse	1.4794	0.4993	FGHI
VCCT	1 pulse	3.3066	0.4993	BCD
VCCT	8 pulses	1.9194	0.4993	DEFGHI
VCCT	4 pulses	2.9093	0.4993	CDE
VCNT	0 pulse	1.8269	0.4993	EFGHI
VCNT	1 pulse	4.4066	0.4993	AB
VCNT	8 pulses	1.8229	0.4993	EFGHI
VCNT	4 pulses	3.5929	0.4993	BC
WCCT	0 pulse	0.8866	0.4993	I
WCCT	1 pulse	2.1585	0.4993	DEFGHI
WCCT	8 pulses	1.2610	0.4993	HI
WCCT	4 pulses	1.6238	0.4993	EFGHI
WCNT	0 pulse	2.6104	0.4993	CDEFGH
WCNT	1 pulse	5.0534	0.4993	A
WCNT	8 pulses	1.4154	0.4993	GHI
WCNT	4 pulses	2.6815	0.4993	CDEFG

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.16 Main effect of conservation management on microbial biomass ^{13}C.

Conservation management	Mean	Standard Error of Mean	Letter Group
NCCT	0.4489	0.05437	A
NCNT	0.3603	0.05437	A
VCCT	0.4345	0.05437	A
VCNT	0.5088	0.05437	A
WCCT	0.3940	0.05437	A
WCNT	0.3764	0.05437	A

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.17 Main effect of drying-rewetting cycles on microbial biomass ^{13}C.

Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
0 pulse	0.2138	0.04439	B
1 pulse	1.1334	0.04439	A
4 pulses	0.2130	0.04439	B
8 pulses	0.1217	0.04439	B

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.18 Interaction effect of conservation management and drying-rewetting cycles on microbial biomass ^{13}C.

Conservation management	Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
NCCT	0 pulse	0.1484	0.1087	DE
NCCT	1 pulse	1.3507	0.1087	A
NCCT	8 pulses	0.1103	0.1087	E
NCCT	4 pulses	0.1861	0.1087	DE
NCNT	0 pulse	0.1473	0.1087	DE
NCNT	1 pulse	0.9627	0.1087	BC
NCNT	8 pulses	0.1197	0.1087	E
NCNT	4 pulses	0.2114	0.1087	DE
VCCT	0 pulse	0.1761	0.1087	DE
VCCT	1 pulse	1.2148	0.1087	AB
VCCT	8 pulses	0.1457	0.1087	DE
VCCT	4 pulses	0.2014	0.1087	DE
VCNT	0 pulse	0.1709	0.1087	DE
VCNT	1 pulse	1.5079	0.1087	A
VCNT	8 pulses	0.1296	0.1087	E
VCNT	4 pulses	0.2266	0.1087	DE
WCCT	0 pulse	0.4495	0.1087	D
WCCT	1 pulse	0.7748	0.1087	C
WCCT	8 pulses	0.1139	0.1087	E
WCCT	4 pulses	0.2376	0.1087	DE
WCNT	0 pulse	0.1904	0.1087	DE
WCNT	1 pulse	0.9895	0.1087	BC
WCNT	8 pulses	0.1108	0.1087	E
WCNT	4 pulses	0.2151	0.1087	DE

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.19 Main effect of conservation management on microbial biomass C.

Conservation management	Mean	Standard Error of Mean	Letter Group
NCCT	106.80	6.4489	D
NCNT	135.20	6.4489	BC
VCCT	144.84	6.4489	B
VCNT	170.79	6.4489	A
WCCT	122.43	6.4489	CD
WCNT	146.43	6.4489	B

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.20 Main effect of drying-rewetting cycles on microbial biomass C.

Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
0 pulse	149.35	5.2655	A
1 pulse	163.70	5.2655	A
4 pulses	116.11	5.2655	B
8 pulses	121.84	5.2655	B

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.21 Interaction effect of conservation management and drying-rewetting cycles on microbial biomass C.

Conservation management	Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
NCCT	0 pulse	112.75	12.8977	GHI
NCCT	1 pulse	114.37	12.8977	GHI
NCCT	8 pulses	93.6560	12.8977	I
NCCT	4 pulses	106.41	12.8977	GHI
NCNT	0 pulse	172.95	12.8977	BC
NCNT	1 pulse	161.80	12.8977	BCD
NCNT	8 pulses	114.91	12.8977	GHI
NCNT	4 pulses	91.1621	12.8977	I
VCCT	0 pulse	174.00	12.8977	BC
VCCT	1 pulse	154.03	12.8977	BCDEF
VCCT	8 pulses	125.75	12.8977	DEFGHI
VCCT	4 pulses	125.58	12.8977	DEFGHI
VCNT	0 pulse	184.51	12.8977	AB
VCNT	1 pulse	211.04	12.8977	A
VCNT	8 pulses	153.95	12.8977	BCDEF
VCNT	4 pulses	133.66	12.8977	DEFGH
WCCT	0 pulse	108.90	12.8977	GHI
WCCT	1 pulse	156.27	12.8977	BCDE
WCCT	8 pulses	103.35	12.8977	HI
WCCT	4 pulses	121.20	12.8977	EFGHI
WCNT	0 pulse	142.98	12.8977	CDEFG
WCNT	1 pulse	184.69	12.8977	AB
WCNT	8 pulses	139.43	12.8977	CDEFGH
WCNT	4 pulses	118.63	12.8977	FGHI

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.22 Main effect of conservation management on hydrogen peroxide resistant ^{13}C.

Conservation management	Mean	Standard Error of Mean	Letter Group
NCCT	0.7705	0.09878	CD
NCNT	0.5929	0.09878	D
VCCT	1.4204	0.09878	B
VCNT	1.7118	0.09878	A
WCCT	0.9527	0.09878	C
WCNT	0.8967	0.09878	C

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.23 Main effect of drying-rewetting cycles on hydrogen peroxide resistant ^{13}C.

Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
0 pulse	1.1124	0.08066	A
1 pulse	1.0598	0.08066	A
4 pulses	0.9897	0.08066	A
8 pulses	1.0682	0.08066	A

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.24 Interaction effect of conservation management and drying-rewetting cycles on hydrogen peroxide resistant ^{13}C.

conservation	Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
NCCT	0 pulse	0.7500	0.1976	GHIJ
NCCT	1 pulse	0.9525	0.1976	EFGHIJ
NCCT	8 pulses	0.5664	0.1976	HIJ
NCCT	4 pulses	0.8130	0.1976	FGHIJ
NCNT	0 pulse	0.7601	0.1976	GHIJ
NCNT	1 pulse	0.5190	0.1976	J
NCNT	8 pulses	0.5514	0.1976	IJ
NCNT	4 pulses	0.5410	0.1976	IJ
VCCT	0 pulse	1.2798	0.1976	BCDEFG
VCCT	1 pulse	1.5643	0.1976	ABCD
VCCT	8 pulses	1.3470	0.1976	ABCDEF
VCCT	4 pulses	1.4904	0.1976	ABCDE
VCNT	0 pulse	1.6748	0.1976	ABC
VCNT	1 pulse	1.7511	0.1976	AB
VCNT	8 pulses	1.8504	0.1976	A
VCNT	4 pulses	1.5711	0.1976	ABCD
WCCT	0 pulse	1.1176	0.1976	CDEFGH
WCCT	1 pulse	0.8620	0.1976	FGHIJ
WCCT	8 pulses	1.0920	0.1976	DEFGHI
WCCT	4 pulses	0.7393	0.1976	GHIJ
WCNT	0 pulse	1.0918	0.1976	DEFGHI
WCNT	1 pulse	0.7099	0.1976	HIJ
WCNT	8 pulses	1.0020	0.1976	EFGHIJ
WCNT	4 pulses	0.7832	0.1976	GHIJ

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.25 Main effect of conservation management on hydrogen peroxide resistant C.

Conservation management	Mean	Standard Error of Mean	Letter Group
NCCT	0.7714	0.1227	B
NCNT	0.7257	0.1227	B
VCCT	1.4020	0.1227	A
VCNT	1.6435	0.1227	A
WCCT	0.9061	0.1227	B
WCNT	0.8586	0.1227	B

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.26 Main effect of drying-rewetting cycles on hydrogen peroxide resistant C.

Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
0 pulse	1.1091	0.1002	A
1 pulse	1.0677	0.1002	A
4 pulses	0.9928	0.1002	A
8 pulses	1.0353	0.1002	A

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.27 Interaction effect of conservation management and drying-rewetting cycles on hydrogen peroxide resistant C.

Conservation management	Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
NCCT	0 pulse	0.6850	0.2454	DE
NCCT	1 pulse	1.0216	0.2454	BCDE
NCCT	8 pulses	0.5343	0.2454	E
NCCT	4 pulses	0.8448	0.2454	CDE
NCNT	0 pulse	1.3144	0.2454	ABCD
NCNT	1 pulse	0.5212	0.2454	E
NCNT	8 pulses	0.5434	0.2454	E
NCNT	4 pulses	0.5239	0.2454	E
VCCT	0 pulse	1.2485	0.2454	ABCD
VCCT	1 pulse	1.5697	0.2454	AB
VCCT	8 pulses	1.2902	0.2454	ABCD
VCCT	4 pulses	1.4996	0.2454	ABC
VCNT	0 pulse	1.4840	0.2454	ABC
VCNT	1 pulse	1.7276	0.2454	A
VCNT	8 pulses	1.7220	0.2454	A
VCNT	4 pulses	1.6403	0.2454	AB
WCCT	0 pulse	0.9728	0.2454	BCDE
WCCT	1 pulse	0.8607	0.2454	CDE
WCCT	8 pulses	1.1070	0.2454	ABCDE
WCCT	4 pulses	0.6841	0.2454	DE
WCNT	0 pulse	0.9502	0.2454	BCDE
WCNT	1 pulse	0.7052	0.2454	DE
WCNT	8 pulses	1.0150	0.2454	BCDE
WCNT	4 pulses	0.7640	0.2454	DE

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Appendix 2E Microbial C use efficiency

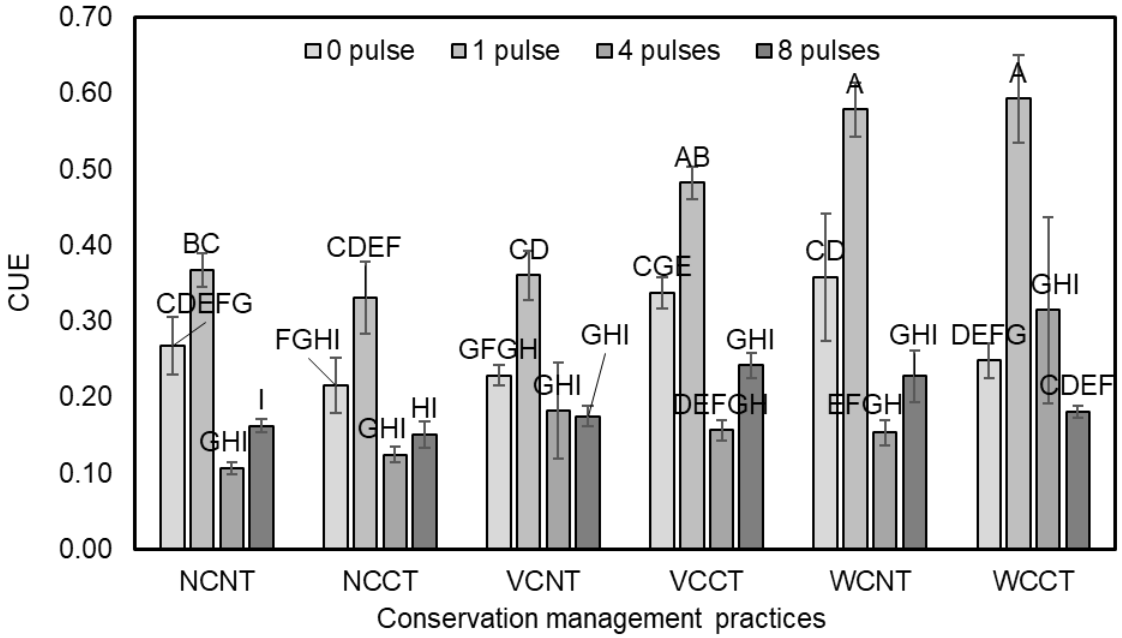

Figure 2.6 Soil microbial CUE during the 24-day incubation.

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Bars indicate standard error. Error bars are at 95% confident interval. Shared letters denote no significant difference at 5% level between all treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.28 Main effect of conservation management on microbial C use efficiency.

Conservation management	Mean	Standard Error of Mean	Letter Group
NCCT	0.2050	0.02086	B
NCNT	0.2259	0.02086	B
VCCT	0.3041	0.02086	A
VCNT	0.2362	0.02086	B
WCCT	0.3340	0.02086	A
WCNT	0.3291	0.02086	A

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.29 Main effect of drying-rewetting cycles on microbial C use efficiency.

Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
0 pulse	0.2754	0.01703	B
1 pulse	0.4519	0.01703	A
4 pulses	0.1727	0.01703	C
8 pulses	0.1895	0.01703	C

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.30 Interaction effect of conservation management and drying-rewetting cycles on microbial C use efficiency.

Conservation management	Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
NCCT	0 pulse	0.2147	0.04172	FGHI
NCCT	1 pulse	0.3305	0.04172	CDEF
NCCT	8 pulses	0.1507	0.04172	GHI
NCCT	4 pulses	0.1242	0.04172	HI
NCNT	0 pulse	0.2670	0.04172	CDEFG
NCNT	1 pulse	0.3678	0.04172	BC
NCNT	8 pulses	0.1623	0.04172	GHI
NCNT	4 pulses	0.1067	0.04172	I
VCCT	0 pulse	0.3369	0.04172	CDE
VCCT	1 pulse	0.4819	0.04172	AB
VCCT	8 pulses	0.2416	0.04172	DEFGH
VCCT	4 pulses	0.1562	0.04172	GHI
VCNT	0 pulse	0.2287	0.04172	EFGH
VCNT	1 pulse	0.3602	0.04172	CD
VCNT	8 pulses	0.1744	0.04172	GHI
VCNT	4 pulses	0.1817	0.04172	GHI
WCCT	0 pulse	0.2480	0.04172	DEFG
WCCT	1 pulse	0.5927	0.04172	A
WCCT	8 pulses	0.1805	0.04172	GHI
WCCT	4 pulses	0.3146	0.04172	CDEF
WCNT	0 pulse	0.3574	0.04172	CD
WCNT	1 pulse	0.5784	0.04172	A
WCNT	8 pulses	0.2276	0.04172	EFGH
WCNT	4 pulses	0.1530	0.04172	GHI

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Appendix 2F Recovery of macroaggregates and microaggregates after fractionation

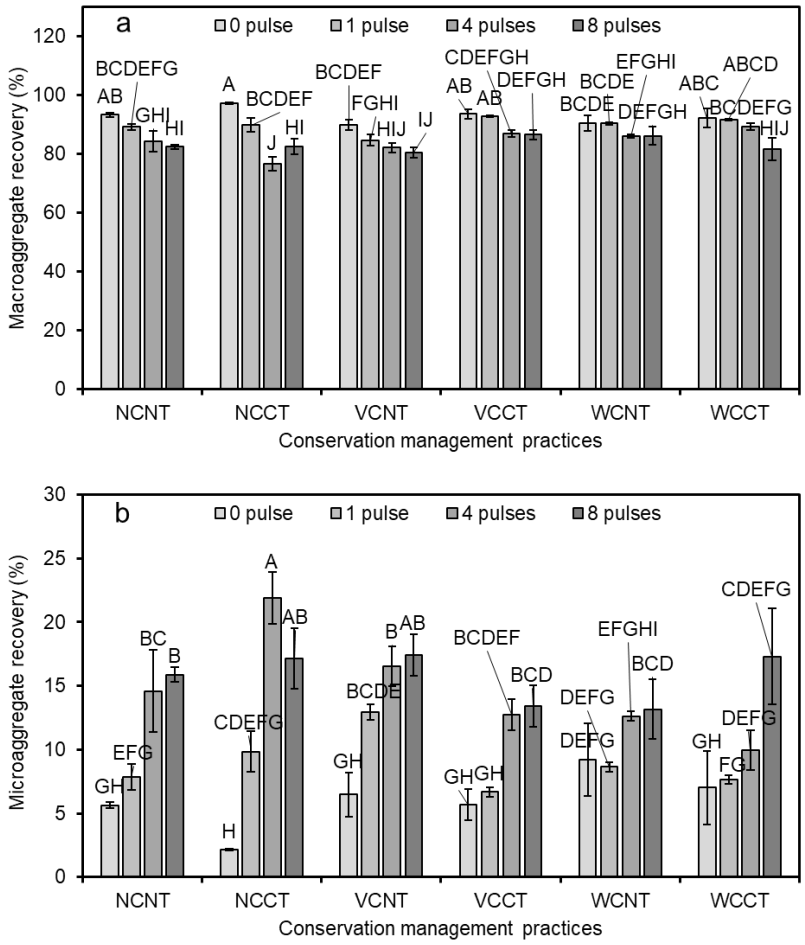

Figure 2.7 Recovery of macroaggregates (upper) and microaggregates (lower) after soil aggregate size fractionation.

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage; a: macroaggregates; b: microaggregates. Bars indicate standard error. Error bars are at 95% confident interval. Shared letters denote no significant difference at 5% level between all treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.31 Main effect of conservation management on macroaggregate recovery.

Conservation management	Mean	Standard Error of Mean	Letter Group
NCCT	0.8657	0.009896	BC
NCNT	0.8729	0.009896	AB
VCCT	0.8994	0.009896	A
VCNT	0.8430	0.009896	C
WCCT	0.8874	0.009896	AB
WCNT	0.8825	0.009896	AB

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.32 Main effect of drying-rewetting cycles on macroaggregate recovery.

Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
0 pulse	0.9276	0.008080	A
1 pulse	0.8975	0.008080	B
4 pulses	0.8423	0.008080	C
8 pulses	0.8331	0.008080	C

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.33 Interaction effect of conservation management and drying-rewetting cycles on macroaggregate recovery.

Conservation management	Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
NCCT	0 pulse	0.9717	0.01979	A
NCCT	1 pulse	0.8984	0.01979	BCDEF
NCCT	8 pulses	0.8255	0.01979	HI
NCCT	4 pulses	0.7674	0.01979	J
NCNT	0 pulse	0.9332	0.01979	AB
NCNT	1 pulse	0.8924	0.01979	BCDEFG
NCNT	8 pulses	0.8242	0.01979	HI
NCNT	4 pulses	0.8421	0.01979	GHI
VCCT	0 pulse	0.9358	0.01979	AB
VCCT	1 pulse	0.9267	0.01979	AB
VCCT	8 pulses	0.8659	0.01979	DEFGH
VCCT	4 pulses	0.8692	0.01979	CDEFGH
VCNT	0 pulse	0.8988	0.01979	BCDEF
VCNT	1 pulse	0.8471	0.01979	FGHI
VCNT	8 pulses	0.8047	0.01979	IJ
VCNT	4 pulses	0.8214	0.01979	HIJ
WCCT	0 pulse	0.9222	0.01979	ABC
WCCT	1 pulse	0.9169	0.01979	ABCD
WCCT	8 pulses	0.8167	0.01979	HIJ
WCCT	4 pulses	0.8936	0.01979	BCDEFG
WCNT	0 pulse	0.9042	0.01979	BCDE
WCNT	1 pulse	0.9036	0.01979	BCDE
WCNT	8 pulses	0.8619	0.01979	DEFGH
WCNT	4 pulses	0.8604	0.01979	EFGHI

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.34 Main effect of conservation management on microaggregate recovery.

Conservation management	Mean	Standard Error of Mean	Letter Group
NCCT	0.1274	0.008987	AB
NCNT	0.1099	0.008987	ABC
VCCT	0.09626	0.008987	C
VCNT	0.1335	0.008987	A
WCCT	0.1049	0.008987	BC
WCNT	0.1091	0.008987	ABC

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.35 Main effect of drying-rewetting cycles on microaggregate recovery.

Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
0 pulse	0.06026	0.007338	C
1 pulse	0.08933	0.007338	B
4 pulses	0.1472	0.007338	A
8 pulses	0.1572	0.007338	A

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.36 Interaction effect of conservation management and drying-rewetting cycles on microaggregate recovery.

Conservation management	Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
NCCT	0 pulse	0.02139	0.01797	H
NCCT	1 pulse	0.09831	0.01797	CDEFG
NCCT	8 pulses	0.1712	0.01797	AB
NCCT	4 pulses	0.2189	0.01797	A
NCNT	0 pulse	0.05644	0.01797	GH
NCNT	1 pulse	0.07851	0.01797	EFG
NCNT	8 pulses	0.1588	0.01797	B
NCNT	4 pulses	0.1458	0.01797	BC
VCCT	0 pulse	0.05671	0.01797	GH
VCCT	1 pulse	0.06670	0.01797	GH
VCCT	8 pulses	0.1341	0.01797	BCD
VCCT	4 pulses	0.1275	0.01797	BCDEF
VCNT	0 pulse	0.06478	0.01797	GH
VCNT	1 pulse	0.1296	0.01797	BCDE
VCNT	8 pulses	0.1744	0.01797	AB
VCNT	4 pulses	0.1652	0.01797	B
WCCT	0 pulse	0.07025	0.01797	GH
WCCT	1 pulse	0.07644	0.01797	FG
WCCT	8 pulses	0.1731	0.01797	AB
WCCT	4 pulses	0.09974	0.01797	CDEFG
WCNT	0 pulse	0.09201	0.01797	DEFG
WCNT	1 pulse	0.08640	0.01797	DEFG
WCNT	8 pulses	0.1315	0.01797	BCD
WCNT	4 pulses	0.1263	0.01797	BCDEF

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Appendix 2G Concentrations of ^{13}C and total C in macroaggregate and microaggregate fractions

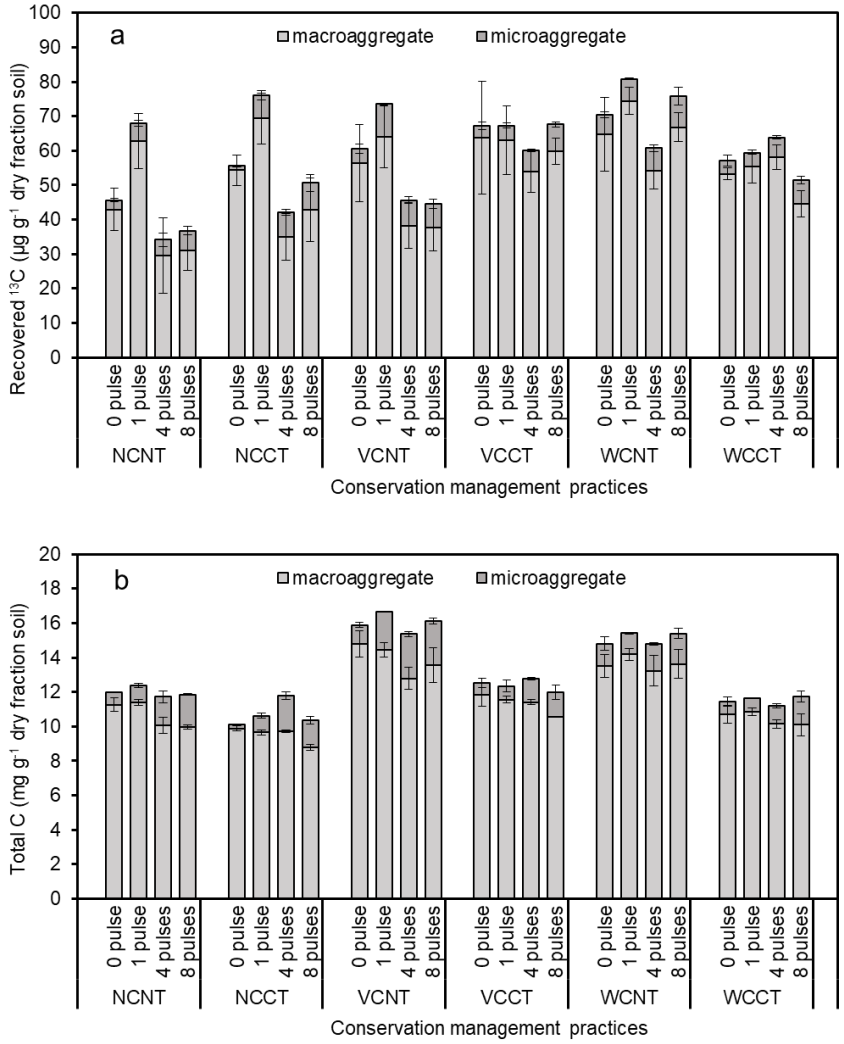

Figure 2.8 Recovered ^{13}C (upper) and total C (lower) in soil aggregate fractions at the end of the incubation.

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage; a: ^{13}C; b: total C. Bars indicate standard error. Error bars are at 95% confident interval.

Table 2.37 Main effect of conservation management on recovered ^{13}C in macroaggregates.

Conservation management	Mean	Standard Error of Mean	Letter Group
NCCT	57.7379	4.2462	BC
NCNT	47.2643	4.2462	C
VCCT	66.7732	4.2462	AB
VCNT	57.7378	4.2462	BC
WCCT	59.4428	4.2462	B
WCNT	73.6373	4.2462	A

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.38 Main effect of drying-rewetting cycles on recovered ^{13}C in macroaggregates.

Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
0 pulse	60.2240	3.4670	B
1 pulse	72.3236	3.4670	A
4 pulses	52.7381	3.4670	B
8 pulses	56.4432	3.4670	B

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.39 Interaction effect of conservation management and drying-rewetting cycles on recovered ^{13}C in macroaggregates.

conservation	Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
NCCT	0 pulse	55.8635	8.4924	BCDEFG
NCCT	1 pulse	77.3731	8.4924	AB
NCCT	8 pulses	52.6127	8.4924	CDEFG
NCCT	4 pulses	45.1022	8.4924	EFG
NCNT	0 pulse	45.9978	8.4924	EFG
NCNT	1 pulse	70.2892	8.4924	ABCD
NCNT	8 pulses	37.7414	8.4924	FG
NCNT	4 pulses	35.0287	8.4924	G
VCCT	0 pulse	68.1561	8.4924	ABCDE
VCCT	1 pulse	68.0172	8.4924	ABCDE
VCCT	8 pulses	69.0211	8.4924	ABCDE
VCCT	4 pulses	61.8984	8.4924	ABCDE
VCNT	0 pulse	62.2916	8.4924	ABCDE
VCNT	1 pulse	75.5327	8.4924	ABC
VCNT	8 pulses	46.8117	8.4924	DEFG
VCNT	4 pulses	46.3150	8.4924	DEFG
WCCT	0 pulse	57.9362	8.4924	BCDEFG
WCCT	1 pulse	60.3596	8.4924	ABCDEF
WCCT	8 pulses	54.4453	8.4924	BCDEFG
WCCT	4 pulses	65.0303	8.4924	ABCDE
WCNT	0 pulse	71.0986	8.4924	ABC
WCNT	1 pulse	82.3700	8.4924	A
WCNT	8 pulses	78.0270	8.4924	AB
WCNT	4 pulses	63.0538	8.4924	ABCDE

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.40 Main effect of conservation management on total C in macroaggregates.

Conservation management	Mean	Standard Error of Mean	Letter Group
NCCT	11.0753	0.2302	E
NCNT	12.2333	0.2302	CD
VCCT	12.6140	0.2302	C
VCNT	16.4783	0.2302	A
WCCT	11.8011	0.2302	D
WCNT	15.4418	0.2302	B

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.41 Main effect of drying-rewetting cycles on total C in macroaggregates.

Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
0 pulse	12.9748	0.1879	A
1 pulse	13.4377	0.1879	A
4 pulses	13.3499	0.1879	A
8 pulses	13.3335	0.1879	A

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.42 Interaction effect of conservation management and drying-rewetting cycles on total C in macroaggregates.

Conservation management	Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
NCCT	0 pulse	10.1748	0.4603	I
NCCT	1 pulse	10.7607	0.4603	HI
NCCT	8 pulses	10.6815	0.4603	HI
NCCT	4 pulses	12.6843	0.4603	EFG
NCNT	0 pulse	12.0722	0.4603	EFG
NCNT	1 pulse	12.7963	0.4603	EF
NCNT	8 pulses	12.0931	0.4603	EFG
NCNT	4 pulses	11.9715	0.4603	EFGH
VCCT	0 pulse	12.6445	0.4603	EFG
VCCT	1 pulse	12.4762	0.4603	EFG
VCCT	8 pulses	12.2040	0.4603	EFG
VCCT	4 pulses	13.1314	0.4603	E
VCNT	0 pulse	16.4411	0.4603	ABC
VCNT	1 pulse	17.0484	0.4603	A
VCNT	8 pulses	16.8484	0.4603	AB
VCNT	4 pulses	15.5754	0.4603	BCD
WCCT	0 pulse	11.5980	0.4603	FGH
WCCT	1 pulse	11.8414	0.4603	EFGH
WCCT	8 pulses	12.3838	0.4603	EFG
WCCT	4 pulses	11.3810	0.4603	GHI
WCNT	0 pulse	14.9185	0.4603	D
WCNT	1 pulse	15.7031	0.4603	BCD
WCNT	8 pulses	15.7900	0.4603	ABCD
WCNT	4 pulses	15.3557	0.4603	CD

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.43 Main effect of conservation management on recovered ^{13}C in microaggregates.

Conservation management	Mean	Standard Error of Mean	Letter Group
NCCT	51.3078	3.7967	BC
NCNT	45.9364	3.7967	C
VCCT	58.5817	3.7967	AB
VCNT	55.4219	3.7967	ABC
WCCT	51.3329	3.7967	BC
WCNT	64.3382	3.7967	A

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.44 Main effect of drying-rewetting cycles on recovered ^{13}C in microaggregates.

Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
0 pulse	59.1366	3.1000	A
1 pulse	66.2675	3.1000	A
4 pulses	47.4776	3.1000	B
8 pulses	45.0642	3.1000	B

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.45 Interaction effect of conservation management and drying-rewetting cycles on recovered ^{13}C in microaggregates.

Conservation management	Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
NCCT	0 pulse	59.7573	7.5935	ABCDEF
NCCT	1 pulse	68.4563	7.5935	AB
NCCT	8 pulses	43.4401	7.5935	DEFGH
NCCT	4 pulses	33.5776	7.5935	GH
NCNT	0 pulse	47.5428	7.5935	BCDEFGH
NCNT	1 pulse	67.4052	7.5935	ABC
NCNT	8 pulses	36.2122	7.5935	GH
NCNT	4 pulses	32.5852	7.5935	H
VCCT	0 pulse	62.4935	7.5935	ABCDE
VCCT	1 pulse	63.6927	7.5935	ABCD
VCCT	8 pulses	59.1612	7.5935	ABCDEF
VCCT	4 pulses	48.9796	7.5935	BCDEFGH
VCNT	0 pulse	63.1881	7.5935	ABCD
VCNT	1 pulse	73.3596	7.5935	A
VCNT	8 pulses	39.1615	7.5935	FGH
VCNT	4 pulses	45.9784	7.5935	CDEFGH
WCCT	0 pulse	54.4345	7.5935	ABCDEFG
WCCT	1 pulse	50.8255	7.5935	BCDEFGH
WCCT	8 pulses	41.4238	7.5935	EFGH
WCCT	4 pulses	58.6477	7.5935	ABCDEF
WCNT	0 pulse	67.4035	7.5935	ABC
WCNT	1 pulse	73.8658	7.5935	A
WCNT	8 pulses	65.4665	7.5935	ABC
WCNT	4 pulses	50.6169	7.5935	BCDEFGH

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.46 Main effect of conservation management on total C in microaggregates.

Conservation management	Mean	Standard Error of Mean	Letter Group
NCCT	9.8671	0.2355	E
NCNT	12.1414	0.2355	C
VCCT	11.2739	0.2355	D
VCNT	16.1023	0.2355	A
WCCT	10.3298	0.2355	E
WCNT	13.7021	0.2355	B

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.47 Main effect of drying-rewetting cycles on total C in microaggregates.

Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
0 pulse	13.1086	0.1922	A
1 pulse	12.6250	0.1922	A
4 pulses	11.6714	0.1922	B
8 pulses	11.5394	0.1922	B

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 2.48 Interaction effect of conservation management and drying-rewetting cycles on total C in microaggregates.

conservation	Drying rewetting cycles	Mean	Standard Error of Mean	Letter Group
NCCT	0 pulse	10.9978	0.4709	GHI
NCCT	1 pulse	9.7618	0.4709	IJK
NCCT	8 pulses	9.1871	0.4709	K
NCCT	4 pulses	9.5216	0.4709	JK
NCNT	0 pulse	12.7261	0.4709	DE
NCNT	1 pulse	12.4620	0.4709	DEF
NCNT	8 pulses	11.9510	0.4709	EFG
NCNT	4 pulses	11.4264	0.4709	EFGH
VCCT	0 pulse	11.9247	0.4709	EFG
VCCT	1 pulse	11.8624	0.4709	EFG
VCCT	8 pulses	10.6520	0.4709	GHIJ
VCCT	4 pulses	10.6565	0.4709	GHIJ
VCNT	0 pulse	17.3747	0.4709	A
VCNT	1 pulse	17.1341	0.4709	A
VCNT	8 pulses	14.3877	0.4709	BC
VCNT	4 pulses	15.5127	0.4709	B
WCCT	0 pulse	11.1591	0.4709	FGH
WCCT	1 pulse	10.1871	0.4709	HIJK
WCCT	8 pulses	9.5660	0.4709	JK
WCCT	4 pulses	10.4068	0.4709	HIJK
WCNT	0 pulse	14.4690	0.4709	BC
WCNT	1 pulse	14.3425	0.4709	BC
WCNT	8 pulses	13.4927	0.4709	CD
WCNT	4 pulses	12.5041	0.4709	DE

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Chapter 3 Stabilization mechanisms of new labile C input as affected by drying-rewetting cycles in soils under conservation agricultural management practices

A version of this chapter will be submitted for publication to Soil Biology and Biochemistry by Lidong Li and Sean M. Schaeffer.

Abstract

Understanding the stabilization mechanisms of new C input in soil under drying-rewetting cycles is essential for predicting the terrestrial C pool facing climate change. To evaluate the relative importance of the stabilization mechanisms, a 24-day incubation in microcosms was conducted with an agricultural soil under 36 years of conservation management. We added ^{13}C-labelled glucose and applied different frequencies of drying-rewetting cycles to the microcosms. The concentrations of the added glucose-C in different soil C pools were measured. Structural equation modelling was conducted to determine the relative importance of physical, chemical, and biochemical controls of stabilization of the added glucose-C in soil. The structural equation model shows that H_2O_2-resistant C pool is the major control of the stabilization of the added glucose-C in soil under drying-rewetting cycles. It indicates that chemical association and biochemical recalcitrance rather than physical protection are major stabilization mechanisms of the added glucose-C in soil under drying-rewetting cycles. The model also demonstrates that conservation agricultural management can only offset the loss of the added glucose-C in soil caused by drying-rewetting cycles to a limited extent. Understanding the stabilization mechanisms of new C input in soils under drying-rewetting cycles can help develope strategy and policy for agriculture in the face of climate change.

3.1 Introduction

Soil stores more organic C than the atmosphere and global vegetation combined (Lehmann and Kleber, 2015). Soil C sequestration impacts global climate change and food security (Lal, 2004). There is consensus that the mechanisms of C sequestration in soil are categorized as (1) biochemical recalcitrance, (2) physical protection, and (3) chemical stabilization (Lal et al., 1997; Six et al., 2002; von Lützow et al., 2008). Biochemical recalcitrance derives from the complex chemical composition of compounds in soil that make them more, or less resistant to microbial decomposition (Six et al., 2002). The complex chemical composition may be an inherent molecular property of plant materials, or be obtained through biochemical alteration during microbial decomposition (Six et al., 2002). Biochemical recalcitrance is identified as primary recalcitrance and secondary recalcitrance, where primary recalcitrance is that of plant materials and secondary recalcitrance involves that of microbial products, humic polymers, and charred materials (Lützow et al., 2006). Physically occluded soil organic matter (SOM) is spatially protected from decomposition because of reduced accessibility of soil microbes and their enzymes to their substrates and/or reduced aerobic decomposition caused by limited diffusion of oxygen (Lützow et al., 2006). Labile substrates are often physically protected by being occluded within soil aggregates (Navarro-García et al., 2012). Chemical stabilization occurs when SOM is chemically or physicochemically bound to soil minerals (Lal et al., 1997). Mechanisms of chemical stabilization include interaction with mineral surfaces (e.g., ligand exchange, polyvalent cation bridges), intercalation within phyllosilicates, and hydrophobic interactions (e.g., van der Waals forces, H-bonding) (Lal et al., 1997; Lützow et al., 2006).

While these mechanisms, in and of themselves, are important for predicting terrestrial C pool distribution, they also interact with chronic environmental factors such as climate change. Climate change severely influences soil moisture regime, changing drying-rewetting cycles and resulting in extended drought periods or intensified precipitation events (Qafoku, 2015). Wetting the dry soil induces higher mineralization and respiration rates than remaining constant field moisture (Lado-Monserrat et al., 2014; Evans et al., 2016) (e.g. Birch Effect) (Birch, 1958). Drying-rewetting dynamics are mainly caused by 3 processes: substrate exposure, increased hydrologic connectivity, and release of microbial biomass. Drying-rewetting cycles causes soil aggregate disruption due to soil shrinking during drying and soil swelling upon abrupt wetting (Shi and Marschner, 2014), thus previously protected SOM is exposed to microbial mineralization (Navarro-García et al., 2012). Soil drought limits the solubility of SOM and the mobility of microbes and extracellular enzymes (Borken and Matzner, 2009). At low soil matric potentials, discontinuous water films impede the access of decomposers to substrates; increases in soil water content upon rewetting then reconnect the decomposers and substrates (Parker and Schimel, 2011). Soil microbes can respond to severe moisture conditions through physiological responses such as by importing and synthesizing osmolytes, so a high intracellular solute concentration is formed to prevent dehydration (Bonaterra et al., 2005; Sagot et al., 2010). When soil is rewetted, soil water potential sharply increases and, to avoid being ruptured by high osmotic pressure, microbes then balance the water potential in and out of their cells either by respiring, polymerizing, or transporting the osmolytes (Halverson et al., 2000; Wood et al., 2001; Schimel et al., 2007). Rapid rewetting may induce rupture of the microbial cells that are not able to

rapidly release the osmolytes out of cells, and therefore provokes survived microbes to decompose the released substrates (Borken and Matzner, 2009).

Soil C sequestration is important for improving and sustaining agronomic productivity (Lal, 2004). To sustain soil C content, conservation agricultural management practices, such as cover crops and no-tillage farming, are effective strategies (Lal, 2004). However, rarely are the effects of cover crops and no-tillage on C sequestration evaluated together. The mean increase in C sequestration was estimated to be 0.32 Mg C ha^{-1} yr^{-1} within the first ~50 years of cover crop application (Poeplau and Don, 2015). Separate from increasing biomass input (Chivenge et al., 2011), applying plant residues to the field can increase SOC content and stability by enhancing aggregation (Cotrufo et al., 2013) because of stimulation of microbial productivity (Cosentino et al., 2006). Soil microbes produce extracellular polysaccharides that can improve interparticle cohesion (Chenu, 1995) and fungal hyphae can mechanically enmesh soil particles (Degens, 1997). No-tillage management is also proved to increase SOC storage (Mazzoncini et al., 2016) and stability (Plaza et al., 2013) compared to conventional tillage. No-tillage can facilitate C sequestration by slowing macroaggregate turnover and boosting microaggregate formation (Six et al., 2000). The average increase in SOC storage was estimated to be 0.57 ± 0.14 Mg C ha^{-1} yr^{-1} within the first 40-60 years of no-tillage farming (West and Post, 2002; Mazzoncini et al., 2016).

Although the impact of conservation agricultural management practices or drying-rewetting cycles on soil C has been intensively studied, the controlling mechanism of the sequestration of labile C input in soil under the impact is still unclear. My objectives of

this study were to (1) reveal the effects of moisture pulses on the stabilization of added glucose-C in soil, (2) determine the effects of conservation management on the stabilization of added glucose-C in soil, and (3) evaluate the relative importance of physical, chemical, and biochemical controls on sequestration of added glucose C. My hypotheses were that (1) moisture pulses make added glucose-C vulnerable to loss from soil, (2) conservation management can offset the loss of added glucose-C caused by moisture pulses, and (3) chemical association and biochemical recalcitrance are the key controls of the sequestration of added glucose-C under moisture pulses. To test these hypotheses, we traced the fate of ^{13}C-labelled glucose in bulk soil, H_2O_2-oxidized soil, macroaggregate fraction, microaggregate fraction, MBC, and EOC from a microcosm experiment with drying-rewetting cycle and conservation management treatments. I then applied structural equation modelling to these data to identify the key mechanisms for the stabilization of the added glucose-C.

3.2 Materials and methods

Total C and ^{13}C concetrations in different soil C pools were measured in **Chapter 2**. Meterials and methods please see **2.2 Materials and methods**. I then applied those data to structural equation modelling.

Structural equation modeling was used to test how experimental treatments affect soil C pools and how soil C pools interact with each other. Structural equation modeling is a statistical methodology for describing linear relationships among multiple variables, which uses a confirmatory approach to analyze a structural theory (Byrne, 2013). The

structural theory represents relationships among multiple variables (Byrne, 2013). Briefly, there are 3 steps to build a structural equation model: (1) to propose an *a priori* model according to experience or background information; (2) to test if important pathways are left out, if the existing pathways are significant, and if the model fits well; (3) to revise the *a priori* model by adding missing pathways and dropping insignificant pathways. I used AMOS 25.0 (IBM Corporation, Meadville, PA) to conduct the structural equation modeling. All variables were log transformed for normality before modelling.

The path coefficients in the model are tested with maximum likelihood estimation. The significance test of the paths is conducted on unstandardized path coefficients. The unstandardized coefficients are expressed in original units of the variables. It is difficult to compare unstandardized coefficients since the original units are different among different pathways. To make coefficients comparable, standardized coefficients based on standard deviations of the variables are usually used in scientific reports (Grace and Bollen, 2005). Further detailed information about how to interpret the results from structural equation modelling is available in the commentary by Grace and Bollen (2005) and the article by Colman and Schimel (2013).

I used CMIN/DF, *P*, CFI, RMSEA, and PCLOSE to evaluate model fit. CMIN/DF is the minimum discrepancy divided by its degrees of freedom and evaluated by the likelihood ratio test, representing the discrepancy between the model and the data(Byrne, 2013). *P* is the probability value of the likelihood ratio test; values more than 0.05 indicate no significant discrepancy between the model and the data. CFI is the comparative fit index; values close to 1 indicates a very good model fit (Byrne, 2013). RMSEA is the root mean

square error of aprixiamation; values less than 0.05 indicate good fit (Byrne, 2013). PCLOSE is test for closeness of fit; values more than 0.50 indicate good fit. Squared multiple correlation (R^2) represents the percentage of the variance being explained in a variable by the model, ranging from 0.00-1.00.

3.3 Results

3.3.1 Structural equation modeling for total C concentration in bulk soil

The *a priori* model was created according to background knowledge. As shown in Figure 3.1, boxes indicate variables. Single headed arrows indicate causal relationships. Double headed arrows indication correlations. I assumued that moisture pulse frequancy, vetch cover crops, wheat cover crops, reduced tillage, MBC, EOC, H_2O_2-resistant C, cumulative CO_2, macroaggregate C, and microaggregate C have direct effects on bulk soil C. Moisture pulse frequancy has effects on MBC, EOC, and cumulative CO_2. Vetch cover crops and wheat cover crops have effects on MBC and are correlated with H_2O_2-resistant C. Reduced tillage has effects on macroaggregate C and microaggregate C. The model fit indices of the *a priori* model were CMIN/DF = 6.481, P = 0.000, CFI = 0.589, RMSEA = 0.278, PCLOSE = 0.000, which indicated a mdoerate model fit. To improve the model fit, I droped the insignificant ($P > 0.05$) pathways: vetch cover crops and wheat cover crops to MBC; moisture pulse frequancy, MBC, EOC, H_2O_2-resistant C, and macroaggregate C to bulk soil C; the corelations of H_2O_2-resistant C with vetch cover crops and wheat cover crops. Only one of the pathways was droped at a time. After droping one pathway, model fit was checked to see if there was improvement. After all the insignificant pathways were droped, the model fit indices of the *a priori* model were

CMIN/DF = 6.844, P = 0.000, CFI = 0.428, RMSEA = 0.287, PCLOSE = 0.000, which indicated no improvement of model fit. To further improve the model fit, according to modification indices, missing pathways were added: cumulative CO_2 to EOC, MBC to cumulative CO_2, macroaggregate C to microaggregate C, moisture pulse frequancy to microaggregate C, vetch cover crops to EOC and macroaggregate C, reduced tillage to EOC, wheat cover crops to macroaggregate C, and H_2O_2-resistant C to macroaggregate C. Only one of the pathways was added at a time. After adding one pathway, model fit was checked to see if there was improvement. After all the missing pathways were added, the model fit indices were CMIN/DF = 0.726, P = 0.875, CFI = 1.000, RMSEA = 0.000, PCLOSE = 0.960, which indicated a very good model fit. The final model was shown in Figure 3.2

As shown in Figure 3.2 and Table 3.1, moisture pulse frequency had a negative effect on bulk soil C concentration (-0.13), indicating that 1.00 unit of increase in moisture pulse frequency will cause 0.13 unit of decrease in bulk soil C concentration. The H_2O_2-resistant C concentration did not have any significant effects on bulk soil C concentration. Microaggregate C had a direct effect on bulk soil C (-0.12), and macroaggregate C had a minor indirect effect on bulk soil C (-0.06) through microaggregates. Vetch cover crop, wheat cover crop, and no-tillage had positive effects on bulk soil C content, with effect sizes of 0.68, 0.70, and 0.59, respectively.

3.3.2 Structural equation modeling for added glucose-C concentration in bulk soil

To creat the *a priori* model, I assumued that moisture pulse frequancy, vetch cover crops, wheat cover crops, reduced tillage, $MB^{13}C$, $EO^{13}C$, H_2O_2-resistant ^{13}C, macroaggregate

^{13}C, and microaggregate ^{13}C have direct effects on bulk soil ^{13}C. Moisture pulse frequancy have effects on MB^{13}C, EO^{13}C, H_2O_2-resistant ^{13}C, macroaggregate ^{13}C, and microaggregate ^{13}C. Vetch cover crops and wheat cover crops have effects on MB^{13}C. Reduced tillage has effects on macroaggregate ^{13}C and microaggregate ^{13}C. The model fit indices of the *a priori* model were CMIN/DF = 9.725, P = 0.000, CFI = 0.331, RMSEA = 0.351, PCLOSE = 0.000, which indicated a poor model fit. To improve the model fit, I droped the insignificant ($P > 0.05$) pathways: reduced tillage macroaggregate ^{13}C, moisture pulse frequancy to macroaggregate ^{13}C, wheat cover crops to MB^{13}C, and vethc cover crops to MB^{13}C and bulk soil ^{13}C. Only one of the pathways was droped at a time. After droping one pathway, model fit was checked to see if there was improvement. After all the insignificant pathways were droped, the model fit indices of the *a priori* model were CMIN/DF = 8.244, P = 0.000, CFI = 0.321, RMSEA = 0.319, PCLOSE = 0.000, which indicated a minor improvement of model fit. To further improve the model fit, according to modification indices, missing pathways were added: vetch cover crops to H_2O_2-resistant ^{13}C and macroaggregate ^{13}C, wheat cover crops to microaggregate ^{13}C, MB^{13}C to EO^{13}C, macroaggregate ^{13}C to microaggregate ^{13}C, and EO^{13}C to macroaggregate ^{13}C and H_2O_2-resistant ^{13}C. Only one of the pathways was added at a time. After adding one pathway, model fit was checked to see if there was improvement. After all the missing pathways were added, the model fit indices were CMIN/DF = 0.861, $P = 0.668$, CFI = 1.000, RMSEA = 0.000, PCLOSE = 0.839, which indicated a very good model fit. The final model was shown in Figure 3.4.

As shown in Figure 3.4 and Table 3.4, vetch and wheat cover crops both had a positive effect on bulk soil ^{13}C concentration (0.17 and 0.16, respectively). No-tillage did not have

any significant effects on bulk soil ^{13}C concentration ($P > 0.05$). Moisture pulse frequency had a negative effect on bulk soil ^{13}C concentration (-0.29). Neither macroaggregates nor microaggregates had any positive effects on bulk soil ^{13}C concentration. Macroaggregates had a major positive effect on microaggregate ^{13}C concentration (0.94). H_2O_2-resistant ^{13}C concentration had a direct positive effect on bulk soil ^{13}C concentration (0.60). MB^{13}C concentration had an indirect positive effect (0.20) on H_2O_2-resistant ^{13}C concentration through EOC ^{13}C concentration.

3.4 Discussions

3.4.1 Effects of moisture pulses

Moisture pulse frequency had a negative effect for both total C and ^{13}C concentrations in soil (Table 3.2 and Table 3.1), which can be explained by that extended drought increased EOC and EO^{13}C (Figure 2.5). This is consistent with the findings of the grassland studies in California (Parker and Schimel, 2011). The amount of EOC depends on the balance between gross immobilization and gross mineralization. Although the mineralization rate is high when soil is moist (Mikha et al., 2005), massive immobilization was observed as well (Figure 2.5). This can lead to net immobilization of C during moist days. When soil is dry, the immobilization could be low as indicated by low respiration (Figure 2.3), but the mineralization could continue (Parker and Schimel, 2011). This can result in net mineralization of C during drought, explaining the accumulation of EOC and EO^{13}C.

3.4.2 Physical protection within soil aggregates

My structural equation model shows that no-tillage did not have any effects on the stabilization of the added glucose-C in bulk soil, cover crops facilitated the stabilization of the added glucose-C, while moisture pulses caused the depletion of the added glucose-C (Figure 3.4). By comparing the standardized total effect sizes of no-tillage, cover crops, and moisture pulses on the concentrations of the added glucose-C in bulk soil (Table 3.4), conservation agricultural management practices were not able to completely offset the loss of the added glucose-C caused by drying-rewetting cycles. Conventionally, no-tillage and cover crops are considered to sustain SOC through enhancing aggregation (Garcia-Franco et al., 2015). However, my structural equation model shows that soil aggregates were not able to provide physical protection for the added glucose-C under the 24-day drying-rewetting cycles (Figure 3.4). Macroaggregate C pool greatly facilitated the accumulation of the added glucose-C in microaggregate C pool (Figure 3.4). It indicates that the incorporation of the added glucose-C to microaggregate C pool is mediated by macroaggregates. This is in agreement with previous findings that there is a redistribution of C from macroaggregates to microaggregates with time (Angers et al., 1997). Physical protection of C within microaggregates only can occur after the protection within macroaggregates (von Lützow et al., 2007). While the drying-rewetting cycles severely disrupted macroaggregates (Figure 2.7), the added glucose-C was not able to complete the translocation from macroaggregates to microaggregates. This may be the reason that physical protection is not responsible for accumulation of the added glucose-C under drying-rewetting cycles.

3.4.3 Chemical association and biochemical alteration of the added glucose-C

My structural equation model also shows that H_2O_2-resistant C pool is the major control of the stabilization of the added glucose-C in soil under drying-rewetting cycles (Figure 3.4). It implies that chemical association and biochemical recalcitrance are the major mechanisms of the stabilization of the added glucose-C in soil under drying-rewetting cycles. SOM chemically resistant to H_2O_2 oxidation mainly consists of two fractions. One fraction is inaccessible SOM (von Lützow et al., 2007) protected from microbes and extracellular enzymes through organomineral interactions (Helfrich et al., 2007). Alkyl chains may bind to hydrophobic parts of soil surfaces through van der Waals forces (Deng and Dixon, 2002; Eusterhues et al., 2005). The hydrophobicity of soil surfaces is a consequence of the increase in organic substances with the intensity of drought (Borken and Matzner, 2009). While alcoholic, carboxylic, and amino functional groups can bind to polar sites on soil surfaces through ionic, hydrogen, and coordination bonds (Deng and Dixon, 2002; Eusterhues et al., 2005), which are more resistant to drying-rewetting disruption compared to aggregate occlusion. The other fraction is highly aliphatic materials, e.g., fatty acids, waxes (Eusterhues et al., 2005), which could be derived from microbes or plants. The added glucose-C was possibly transformed to recalcitrant C through biochemical alteration during microbial decomposition. This can be proved by that MBC pool facilitated the accumulation of the added glucose-C in H_2O_2-resistant C pool (Table 3.2). Most of the added glucose-C was recovered in the MBC pool at the 24th day of substrate addition, while most of the total C was recovered in the H_2O_2-resistant C pool (Table 3.3). This indicates that the MBC is a transitional pool for the added glucose-

C. The added glucose-C may pass through the MBC pool and then be stabilized in the H_2O_2-resistant C pool.

3.5 Conclusions

In summary, my structural equation model demonstrates that chemical association and biochemical recalcitrance rather than physical protection are the major mechanisms stabilizing of the added glucose-C in soil under the short-term moisture pulse events. Although the long-term agricultural conservation management practices can completely counteract the loss of soil total C caused by the short-term moisture pulse events, they are only able to offset the loss of the added glucose-C to a limited extent. The vetch cover crops and wheat cover crops are effective on offset the C loss caused by the short-term moisture pulse event. No-tillage did not have any significant effects because the soil structure is disrupted rather than in field condition.

References

Angers, D., Recous, S., Aita, C., 1997. Fate of carbon and nitrogen in water-stable aggregates during decomposition of 13C15N-labelled wheat straw in situ. European Journal of Soil Science 48, 295-300.

Birch, H., 1958. The effect of soil drying on humus decomposition and nitrogen availability. Plant and soil 10, 9-31.

Bonaterra, A., Camps, J., Montesinos, E., 2005. Osmotically induced trehalose and glycine betaine accumulation improves tolerance to desiccation, survival and efficacy of the postharvest biocontrol agent Pantoea agglomerans EPS125. FEMS Microbiology Letters 250, 1-8.

Borken, W., Matzner, E., 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Global Change Biology 15, 808-824.

Byrne, B.M., 2013. Structural equation modeling with AMOS: Basic concepts, applications, and programming, 2nd ed. New York: Routledge.

Chenu, C., 1995. Extracellular polysaccharides: an interface between microorganisms and soil constituents. Environmental impact of soil component interactions 1, 217-233.

Chivenge, P., Vanlauwe, B., Gentile, R., Six, J., 2011. Organic resource quality influences short-term aggregate dynamics and soil organic carbon and nitrogen accumulation. Soil Biology and Biochemistry 43, 657-666.

Colman, B.P., Schimel, J.P., 2013. Drivers of microbial respiration and net N mineralization at the continental scale. Soil Biology and Biochemistry 60, 65-76.

Cosentino, D., Chenu, C., Le Bissonnais, Y., 2006. Aggregate stability and microbial community dynamics under drying–wetting cycles in a silt loam soil. Soil Biology and Biochemistry 38, 2053-2062.

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Global change biology 19, 988-995.

Degens, B., 1997. Macro-aggregation of soils by biological bonding and binding mechanisms and the factors affecting these: a review. Soil Research 35, 431-460.

Deng, Y., Dixon, J.B., 2002. Soil organic matter and organic-mineral interactions. Soil mineralogy with environmental applications, 69-107.

Eusterhues, K., Rumpel, C., Kögel-Knabner, I., 2005. Stabilization of soil organic matter isolated via oxidative degradation. Organic Geochemistry 36, 1567-1575.

Evans, S., Dieckmann, U., Franklin, O., Kaiser, C., 2016. Synergistic effects of diffusion and microbial physiology reproduce the Birch effect in a micro-scale model. Soil Biology and Biochemistry 93, 28-37.

Garcia-Franco, N., Albaladejo, J., Almagro, M., Martínez-Mena, M., 2015. Beneficial effects of reduced tillage and green manure on soil aggregation and stabilization of organic carbon in a Mediterranean agroecosystem. Soil and Tillage Research 153, 66-75.

Grace, J.B., Bollen, K.A., 2005. Interpreting the results from multiple regression and structural equation models.

Halverson, L.J., Jones, T.M., Firestone, M.K., 2000. Release of intracellular solutes by four soil bacteria exposed to dilution stress. Soil Science Society of America Journal 64, 1630-1637.

Helfrich, M., Flessa, H., Mikutta, R., Dreves, A., Ludwig, B., 2007. Comparison of chemical fractionation methods for isolating stable soil organic carbon pools. European Journal of Soil Science 58, 1316-1329.

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

Lado-Monserrat, L., Lull, C., Bautista, I., Lidón, A., Herrera, R., 2014. Soil moisture increment as a controlling variable of the "Birch effect". Interactions with the pre-wetting soil moisture and litter addition. Plant and soil 379, 21-34.

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

Lal, R., Kimble, J.M., Follett, R.F., Stewart, B.A., 1997. Soil processes and the carbon cycle. CRC Press.

Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. Nature 528, 60.

Mazzoncini, M., Antichi, D., Di Bene, C., Risaliti, R., Petri, M., Bonari, E., 2016. Soil carbon and nitrogen changes after 28 years of no-tillage management under Mediterranean conditions. European Journal of Agronomy 77, 156-165.

Mikha, M.M., Rice, C.W., Milliken, G.A., 2005. Carbon and nitrogen mineralization as affected by drying and wetting cycles. Soil Biology and Biochemistry 37, 339-347.

Navarro-García, F., Casermeiro, M.Á., Schimel, J.P., 2012. When structure means conservation: Effect of aggregate structure in controlling microbial responses to rewetting events. Soil Biology and Biochemistry 44, 1-8.

Parker, S.S., Schimel, J.P., 2011. Soil nitrogen availability and transformations differ between the summer and the growing season in a California grassland. Applied Soil Ecology 48, 185-192.

Plaza, C., Courtier-Murias, D., Fernández, J.M., Polo, A., Simpson, A.J., 2013. Physical, chemical, and biochemical mechanisms of soil organic matter stabilization under conservation tillage systems: a central role for microbes and microbial by-products in C sequestration. Soil Biology and Biochemistry 57, 124-134.

Poeplau, C., Don, A., 2015. Carbon sequestration in agricultural soils via cultivation of cover crops–A meta-analysis. Agriculture, ecosystems & environment 200, 33-41.

Qafoku, N.P., 2015. Chapter Two-Climate-Change Effects on Soils: Accelerated Weathering, Soil Carbon, and Elemental Cycling. Advances in Agronomy 131, 111-172.

Sagot, B., Gaysinski, M., Mehiri, M., Guigonis, J.-M., Le Rudulier, D., Alloing, G., 2010. Osmotically induced synthesis of the dipeptide N-acetylglutaminylglutamine amide is mediated by a new pathway conserved among bacteria. Proceedings of the National Academy of Sciences 107, 12652-12657.

Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88, 1386-1394.

Shi, A., Marschner, P., 2014. Drying and rewetting frequency influences cumulative respiration and its distribution over time in two soils with contrasting management. Soil Biology and Biochemistry 72, 172-179.

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

Six, J., Elliott, E., Paustian, K., 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology and Biochemistry 32, 2099-2103.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007. SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. Soil Biology and Biochemistry 39, 2183-2207.

von Lützow, M., Kögel-Knabner, I., Ludwig, B., Matzner, E., Flessa, H., Ekschmitt, K., Guggenberger, G., Marschner, B., Kalbitz, K., 2008. Stabilization mechanisms of organic matter in four temperate soils: development and application of a conceptual model. Journal of Plant Nutrition and Soil Science 171, 111-124.

West, T.O., Post, W.M., 2002. Soil organic carbon sequestration rates by tillage and crop rotation. Soil Science Society of America Journal 66, 1930-1946.

Wood, J.M., Bremer, E., Csonka, L.N., Kraemer, R., Poolman, B., van der Heide, T., Smith, L.T., 2001. Osmosensing and osmoregulatory compatible solute accumulation by bacteria. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 130, 437-460.

Appendix 3

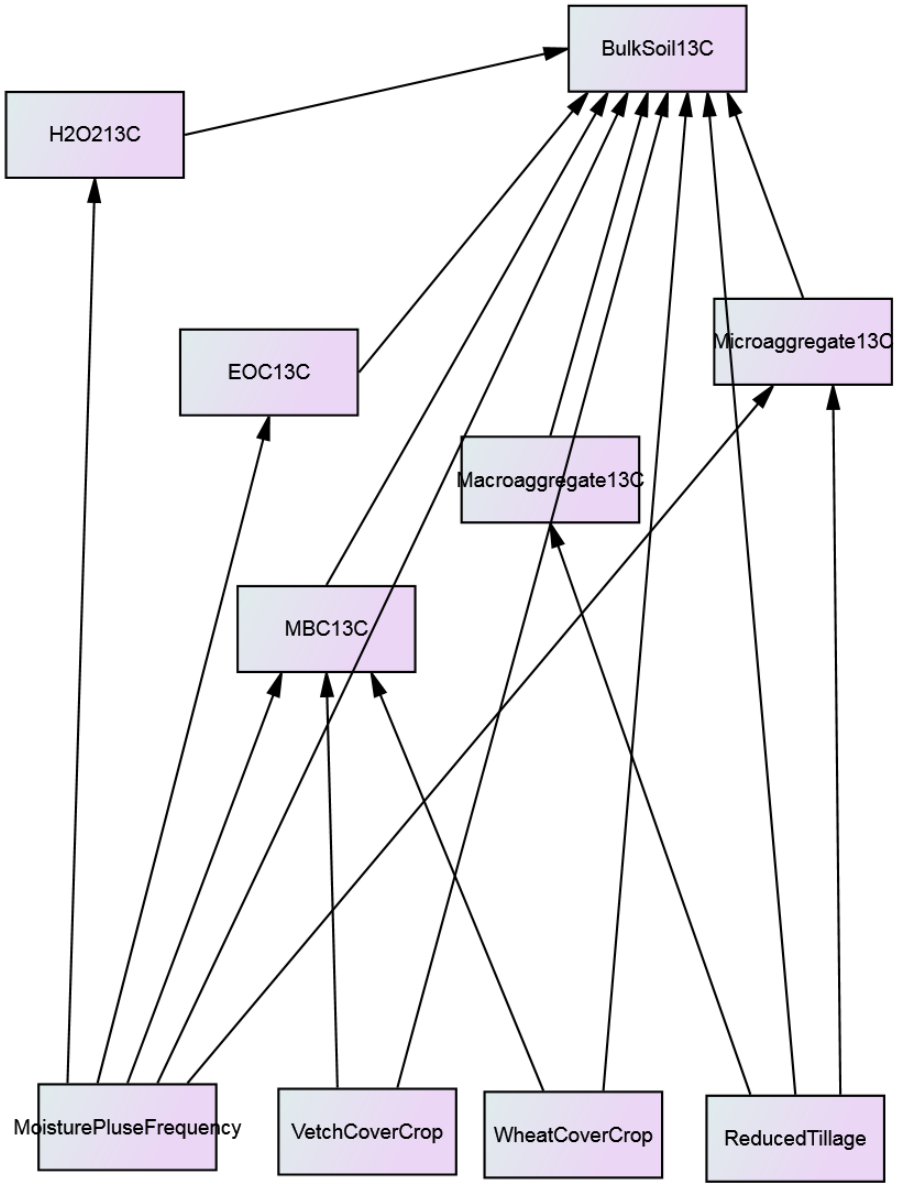

Figure 3.1 The *a priori* model for pools of total C in soil at the end of the incubation.

EOC: extractable organic carbon; MBC: microbial biomass carbon; H_2O_2: hydrogen peroxide; TC: total carbon. Boxes indicate variables. Single headed arrows indicate causal relationships. Double headed arrows indication correlations.

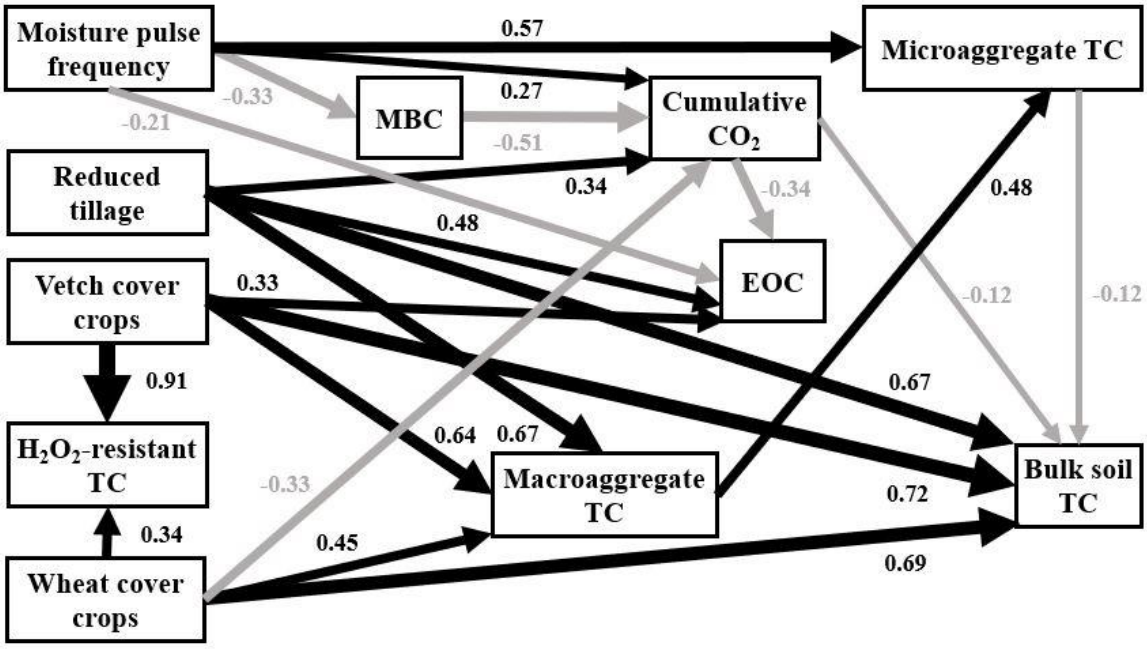

Figure 3.2 Structural equation model for pools of total C in soil at the end of the incubation.

EOC: extractable organic carbon; MBC: microbial biomass carbon; TC: total carbon. Boxes indicate variables. A arrow represents a causal relationship ($P < 0.05$). Arrow direction indicates the direction of causation. Arrow width indicates effect size. A black arrow denotes positive relationship, and gray arrow negative relationship. Numbers beside arrows are standardized path coefficients, i.e., effect size. CMIN/DF = 0.726, P = 0.875, CFI = 1.000, RMSEA = 0.000, PCLOSE = 0.960, R^2 = 0.85. Variables were log transformed for normality.

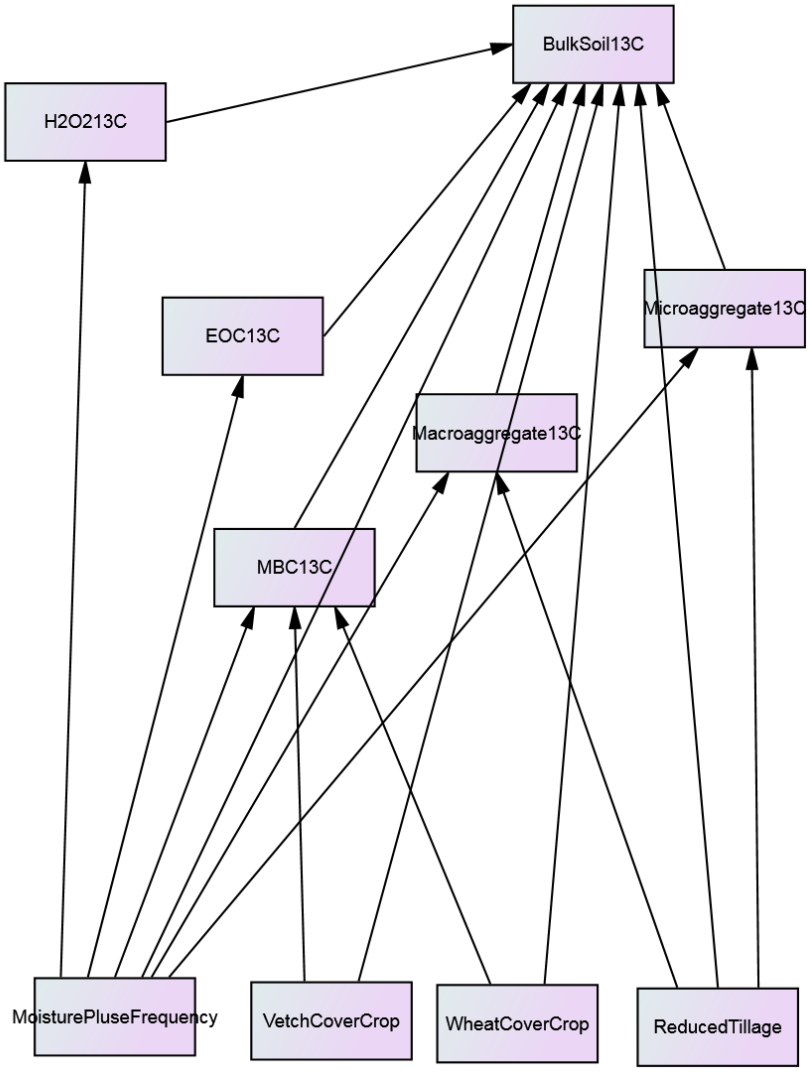

Figure 3.3 The *a priori* model for pools of newly added labile C in soil at the end of the incubation.

EOC: extractable organic carbon; MBC: microbial biomass carbon; H_2O_2: hydrogen peroxide. Boxes indicate variables. Single headed arrows indicate causal relationships. Double headed arrows indication correlations.

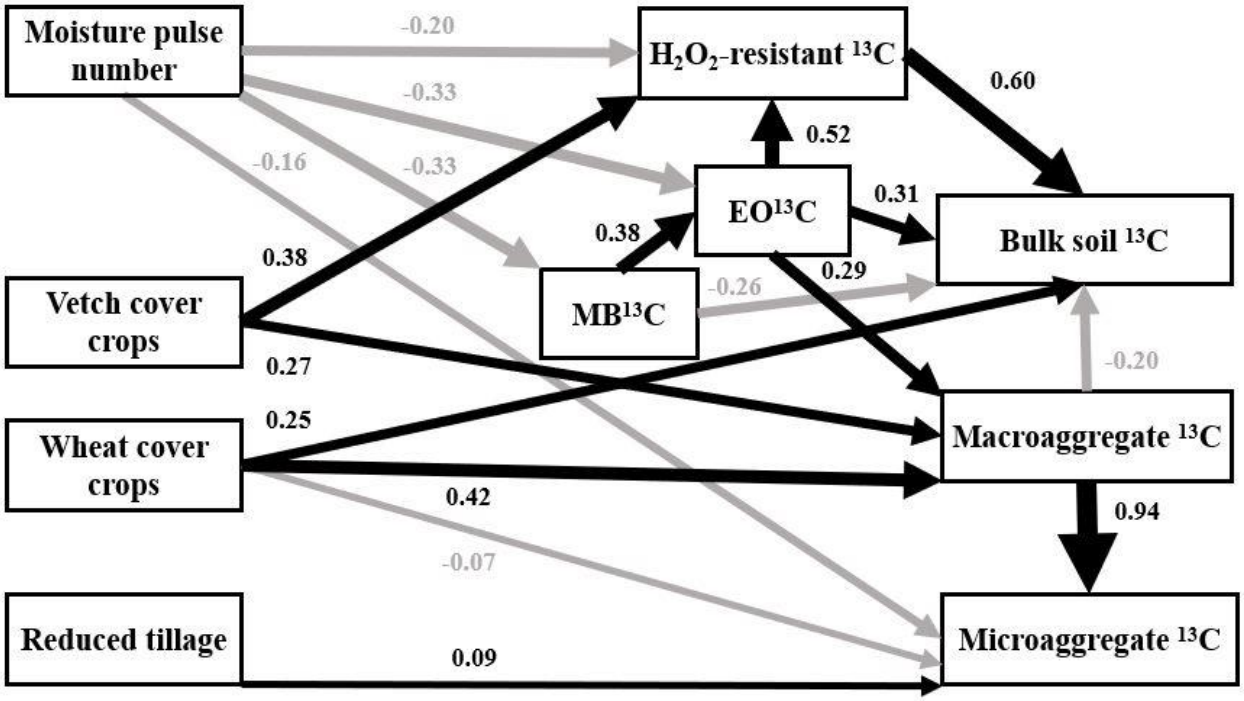

Figure 3.4 Structural equation model for pools of newly added labile C in soil at the end of the incubation.

$EO^{13}C$: ^{13}C labeled extractable organic carbon; $MB^{13}C$: ^{13}C labeled microbial biomass carbon. Boxes indicate variables. A arrow represents a causal relationship ($P < 0.05$). Arrow direction indicates the direction of causation. Arrow width indicates effect size. A black arrow denotes positive relationship, and gray arrow negative relationship. Numbers beside arrows are standardized path coefficients, i.e., effect size. CMIN/DF = 0.861, P = 0.668, CFI = 1.000, RMSEA = 0.000, PCLOSE = 0.839, R^2 = 0.52. Variables were log transformed for normality.

Table 3.1 Standardized total effects of vetch cover crop, wheat cover crop, tillage, and drying-rewetting frequency on different soil C pools.

Factors	Bulk soil TC	Macroaggregate TC	Microaggregate TC	EOC	MBC	Cumulative CO_2
Vetch cover crop→	0.68	0.64	0.31	0.33	0.00	0.00
Wheat cover crop →	0.70	0.45	0.22	0.11	0.00	-0.33
No-tillage →	0.59	0.68	0.33	0.36	0.00	0.34
Moisture pulse number →	-0.13	0.00	0.57	-0.36	-0.33	0.44
H_2O_2-resistant TC→	0.01	-0.16	-0.08	0.00	0.00	0.00
MBC→	0.06	0.00	0.00	0.17	0.00	-0.51
Macroaggregate TC →	-0.06	0.00	0.49	0.00	0.00	0.00
Microaggregate TC →	-0.12	0.00	0.00	0.00	0.00	0.00
Cumulative CO_2 →	-0.12	0.00	0.00	-0.34	0.00	0.00

EOC: extractable organic carbon; MBC: microbial biomass carbon; TC: total carbon. Arrows indicate directions of causal relationships. All effects are significant ($P < 0.05$).

Table 3.2 Standardized total effects of vetch cover crop, wheat cover crop, tillage, and drying-rewetting frequency on the added glucose C in different soil C pools.

Factors	Bulk soil ^{13}C	H_2O_2-resistant ^{13}C	Macroaggregate ^{13}C	Microaggregate ^{13}C	EOC ^{13}C	MBC ^{13}C
Vetch cover crop→	0.17	0.38	0.27	0.25	0.00	0.00
Wheat cover crop →	0.16	0.00	0.42	0.33	0.00	0.00
No-tillage →	0.00	0.00	0.00	0.09	0.00	0.00
Moisture pulse number →	-0.29	-0.43	-0.13	-0.28	-0.46	-0.33
H_2O_2-resistant ^{13}C→	0.60	0.00	0.00	0.00	0.00	0.00
Macroaggregate ^{13}C →	-0.20	0.00	0.00	0.94	0.00	0.00
$EO^{13}C$ →	0.57	0.32	0.29	0.43	0.00	0.00
$MB^{13}C$ →	-0.05	0.20	0.11	0.10	0.38	0.00

$EO^{13}C$: ^{13}C labeled extractable organic carbon; $MB^{13}C$: ^{13}C labeled microbial biomass carbon. Arrows indicate directions of causal relationships. All effects are significant ($P < 0.05$).

Table 3.3 Distribution of total C in different soil C pools used in the structural equation model.

Conservation management	Moisture pulses	Bulk soil C (ug C g^{-1} dry bulk soil)	Macroaggregate C (ug C g^{-1} dry fraction soil)	Microaggregate C (ug C g^{-1} dry fraction soil)	H_2O_2-resistant C (ug C g^{-1} dry bulk soil)	EOC (ug C g^{-1} dry bulk soil)	MBC (ug C g^{-1} dry bulk soil)	Cumulative C-CO_2 (ug g^{-1} dry bulk soil)
NCNT	0	12.66	11.41	0.77	0.70	39.82	39.82	472.00
NCNT	0	13.47	12.52	0.67	0.78	46.83	46.83	533.58
NCNT	0	12.11	12.28	0.72	0.80	49.55	49.55	425.27
NCNT	1	13.10	12.94	0.77	0.51	67.76	67.76	285.83
NCNT	1	13.66	12.92	1.13	0.62	102.00	102.00	298.60
NCNT	1	9.91	12.53	1.02	0.43	53.10	53.10	251.33
NCNT	8	9.87	12.17	1.92	0.47	33.57	33.57	531.02
NCNT	8	9.85	12.37	1.97	0.55	39.08	39.08	713.42
NCNT	8	9.49	11.74	1.80	0.64	58.65	58.65	551.09
NCNT	4	9.66	11.47	1.86	0.52	49.55	49.55	728.05
NCNT	4	9.43	12.07	0.98	0.61	49.01	49.01	820.56
NCNT	4	9.84	12.37	2.11	0.50	50.27	50.27	747.15
NCCT	0	8.22	9.98	0.27	0.53	26.15	26.15	416.18
NCCT	0	9.00	10.49	0.22	0.73	43.30	43.30	447.44
NCCT	0	8.44	10.05	0.22	0.99	46.68	46.68	344.83
NCCT	1	8.55	11.03	1.24	1.57	59.76	59.76	160.38
NCCT	1	9.22	10.71	0.85	0.68	60.66	60.66	282.51
NCCT	1	9.34	10.55	0.78	0.61	63.24	63.24	282.22
NCCT	8	8.46	9.87	1.31	0.60	33.76	33.76	607.67
NCCT	8	7.69	11.37	1.99	0.67	18.89	18.89	490.24
NCCT	8	8.23	10.80	1.41	0.43	39.46	39.46	504.90
NCCT	4	8.18	13.47	2.33	0.86	37.75	37.75	756.90
NCCT	4	8.41	11.82	1.62	0.69	41.33	41.33	720.41
NCCT	4	7.71	12.77	2.32	0.89	43.78	43.78	779.35
VCNT	0	12.61	17.33	1.19	1.51	55.23	55.23	643.71
VCNT	0	13.48	16.55	1.48	2.38	53.47	53.47	607.95
VCNT	0	12.99	15.44	0.62	1.13	68.31	68.31	610.04
VCNT	1	15.06	17.19	2.18	1.17	100.74	100.74	298.04
VCNT	1	15.03	17.57	1.93	2.76	90.52	90.52	378.85
VCNT	1	14.18	16.39	2.55	1.32	104.22	104.22	472.60
VCNT	8	13.27	15.86	3.12	1.98	24.50	24.50	676.65
VCNT	8	13.08	18.69	2.04	2.17	57.86	57.86	918.37
VCNT	8	13.90	15.99	2.41	1.41	56.98	56.98	630.54
VCNT	4	12.66	14.74	3.15	1.27	63.92	63.92	965.09
VCNT	4	12.97	17.08	2.34	2.27	73.58	73.58	1020.83
VCNT	4	13.99	14.91	2.22	1.18	91.05	91.05	1014.02
VCCT	0	12.24	13.20	0.43	1.31	56.76	56.76	333.40
VCCT	0	11.94	13.16	0.69	1.27	30.62	30.62	329.50
VCCT	0	11.56	11.58	0.90	1.26	44.92	44.92	353.95
VCCT	1	11.63	12.24	0.75	1.35	55.43	55.43	155.33
VCCT	1	12.07	12.39	0.76	1.18	85.78	85.78	164.55
VCCT	1	11.66	12.80	0.85	2.16	78.61	78.61	174.32
VCCT	8	11.01	12.71	1.74	1.19	68.49	68.49	372.12
VCCT	8	11.21	12.06	1.39	1.53	38.71	38.71	507.21
VCCT	8	11.69	11.85	1.15	1.33	41.92	41.92	328.81
VCCT	4	11.67	12.98	1.21	1.52	45.71	45.71	715.23

Table 3.3 continued

Conservation management	Moisture pulses	Bulk soil C (ug C g^{-1} dry bulk soil)	Macroaggregate C (ug C g^{-1} dry fraction soil)	Microaggregate C (ug C g^{-1} dry fraction soil)	H_2O_2-resistant C (ug C g^{-1} dry bulk soil)	EOC (ug C g^{-1} dry bulk soil)	MBC (ug C g^{-1} dry bulk soil)	Cumulative C-CO_2 (ug g^{-1} dry bulk soil)
VCCT	4	12.13	13.13	1.66	1.64	47.42	47.42	676.88
VCCT	4	11.44	13.28	1.21	1.31	100.56	100.56	646.73
WCNT	0	13.94	14.28	2.07	1.29	76.29	76.29	323.64
WCNT	0	14.98	14.93	0.88	1.10	127.69	127.69	349.66
WCNT	0	14.22	15.55	1.00	0.89	47.58	47.58	369.17
WCNT	1	14.62	14.92	1.16	0.63	65.19	65.19	172.57
WCNT	1	14.36	16.37	1.32	0.78	193.84	193.84	129.29
WCNT	1	14.58	15.82	1.23	0.73	85.10	85.10	108.21
WCNT	8	14.66	16.83	1.40	0.61	40.56	40.56	339.45
WCNT	8	13.43	15.19	2.38	1.31	26.63	26.63	624.94
WCNT	8	14.01	15.35	1.53	1.09	40.85	40.85	529.34
WCNT	4	14.16	15.18	1.72	0.88	67.57	67.57	633.45
WCNT	4	13.49	13.85	1.57	0.86	57.70	57.70	691.15
WCNT	4	15.13	17.03	1.44	0.61	58.60	58.60	640.13
WCCT	0	11.62	11.29	0.40	0.91	28.15	58.60	324.87
WCCT	0	11.15	12.20	0.57	1.12	23.39	23.39	354.03
WCCT	0	10.84	11.30	1.30	1.32	32.92	32.92	310.36
WCCT	1	11.04	11.88	0.81	0.72	48.91	48.91	81.06
WCCT	1	11.21	12.24	0.71	0.88	53.12	53.12	106.15
WCCT	1	11.31	11.40	0.81	0.99	42.25	42.25	133.26
WCCT	8	11.04	12.27	1.01	1.42	51.87	51.87	398.29
WCCT	8	12.02	13.58	1.99	0.73	20.63	20.63	595.84
WCCT	8	12.23	11.30	1.89	1.12	21.76	21.76	430.87
WCCT	4	10.26	11.67	1.04	0.69	33.54	33.54	575.74
WCCT	4	11.80	10.64	0.81	0.78	52.13	52.13	454.22
WCCT	4	10.80	11.83	1.23	0.75	32.75	32.75	593.58

All variables were log transformed for normality before conducting structural equation modelling. NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage.

Table 3.4 Distribution of added glucose C in different soil C pools used in the structural equation model.

Conservation management	Moisture pulses	Bulk soil ^{13}C (ug ^{13}C g^{-1} dry bulk soil)	Macroaggregate ^{13}C (ug ^{13}C g^{-1} dry fraction soil)	Microaggregate ^{13}C (ug ^{13}C g^{-1} dry fraction soil)	H_2O_2-resistant ^{13}C (ug ^{13}C g^{-1} dry bulk soil)	EO^{13}C (ug ^{13}C g^{-1} dry bulk soil)	MB^{13}C (ug ^{13}C g^{-1} dry bulk soil)
NCNT	0	35.66	50.72	55.41	1.03	0.16	28.78
NCNT	0	33.51	54.24	52.71	1.49	0.14	26.54
NCNT	0	29.74	33.04	34.51	1.66	0.15	81.77
NCNT	1	34.22	75.97	75.57	2.42	1.06	100.18
NCNT	1	48.70	81.87	78.37	3.45	0.71	90.71
NCNT	1	32.56	53.03	48.28	2.24	1.12	89.55
NCNT	8	16.99	52.01	47.42	0.71	0.13	56.53
NCNT	8	24.50	28.83	29.00	0.83	0.13	101.39
NCNT	8	21.80	32.38	32.21	1.03	0.10	39.40
NCNT	4	25.32	9.48	9.15	0.69	0.16	21.65
NCNT	4	26.76	44.93	43.46	1.25	0.23	37.66
NCNT	4	32.27	50.68	45.15	0.99	0.24	23.98
NCCT	0	22.58	64.34	75.97	1.46	0.10	18.98
NCCT	0	18.96	52.55	51.55	0.75	0.20	104.61
NCCT	0	24.46	50.70	51.75	1.35	0.15	23.20
NCCT	1	27.55	86.29	72.94	2.39	1.06	111.79
NCCT	1	29.55	60.11	55.77	2.70	1.32	83.03
NCCT	1	28.24	85.72	76.66	1.78	1.67	60.21
NCCT	8	33.20	29.80	27.92	1.51	0.10	19.25
NCCT	8	16.19	73.93	58.09	1.08	0.09	27.34
NCCT	8	25.52	54.10	44.32	0.77	0.14	34.82
NCCT	4	31.80	32.31	21.73	1.94	0.17	29.06
NCCT	4	27.51	57.85	42.80	0.96	0.18	19.73
NCCT	4	27.52	45.14	36.20	0.95	0.21	27.28
VCNT	0	18.65	79.61	76.86	1.38	0.19	58.51
VCNT	0	17.31	65.97	62.75	2.33	0.17	53.13
VCNT	0	25.22	41.29	49.95	3.06	0.16	47.34
VCNT	1	31.36	72.96	64.93	2.77	1.77	73.98
VCNT	1	38.42	93.50	84.66	5.07	1.30	57.72
VCNT	1	44.03	60.14	70.48	3.17	1.45	71.59
VCNT	8	29.72	48.41	46.29	3.00	0.07	45.56
VCNT	8	25.88	59.49	43.71	2.20	0.14	32.19
VCNT	8	31.00	32.54	27.49	1.95	0.18	46.06
VCNT	4	29.28	38.19	41.35	1.62	0.23	34.19
VCNT	4	34.31	40.01	33.34	1.91	0.25	2.35
VCNT	4	25.51	60.74	63.24	1.10	0.20	20.51
VCCT	0	28.47	53.14	48.13	2.08	0.21	59.35
VCCT	0	20.83	103.40	92.57	0.82	0.10	27.38
VCCT	0	23.64	47.93	46.79	2.38	0.22	52.77
VCCT	1	35.82	67.75	68.42	3.30	1.26	71.80
VCCT	1	39.59	49.42	42.68	6.31	1.39	85.49
VCCT	1	40.80	86.88	79.97	6.01	1.00	77.17
VCCT	8	32.21	63.44	51.46	1.43	0.16	41.58
VCCT	8	17.65	76.08	66.84	1.10	0.11	17.28
VCCT	8	29.69	67.53	59.18	2.10	0.16	26.37
VCCT	4	28.34	61.14	49.92	1.20	0.17	39.95

Table 3.4 continued

Conservation management	Moisture pulses	Bulk soil ^{13}C (ug ^{13}C g^{-1} dry bulk soil)	Macroaggregate ^{13}C (ug ^{13}C g^{-1} dry fraction soil)	Microaggregate ^{13}C (ug ^{13}C g^{-1} dry fraction soil)	H_2O_2-resistant ^{13}C (ug ^{13}C g^{-1} dry bulk soil)	EO^{13}C (ug ^{13}C g^{-1} dry bulk soil)	MB^{13}C (ug ^{13}C g^{-1} dry bulk soil)
VCCT	4	31.23	51.29	41.86	1.81	0.19	13.31
VCCT	4	36.52	73.26	55.16	1.54	0.24	28.69
WCNT	0	26.49	51.83	49.43	1.38	0.26	50.09
WCNT	0	23.42	74.74	73.81	0.64	0.14	52.03
WCNT	0	19.34	86.73	78.98	0.92	0.18	40.69
WCNT	1	39.41	86.96	83.46	3.09	0.90	82.27
WCNT	1	31.58	73.99	65.00	2.56	0.84	77.74
WCNT	1	33.31	86.16	73.14	2.79	1.23	71.51
WCNT	8	15.39	74.98	58.82	0.90	0.10	54.31
WCNT	8	16.04	91.97	79.05	0.43	0.08	81.74
WCNT	8	24.04	67.13	58.53	0.71	0.16	49.24
WCNT	4	32.83	73.22	62.62	1.33	0.28	28.64
WCNT	4	30.28	51.84	44.39	0.98	0.20	21.09
WCNT	4	42.35	64.10	44.84	0.91	0.17	29.23
WCCT	0	15.83	57.48	57.10	0.78	0.13	37.19
WCCT	0	22.92	52.91	50.40	1.09	0.12	42.43
WCCT	0	31.96	63.42	55.80	1.63	0.78	30.29
WCCT	1	56.14	51.92	43.60	3.32	0.99	59.81
WCCT	1	44.27	69.06	56.77	2.86	1.27	62.91
WCCT	1	50.93	60.10	52.10	3.54	0.07	66.58
WCCT	8	24.68	58.09	47.72	0.72	0.15	33.71
WCCT	8	22.85	55.08	37.09	1.03	0.10	19.58
WCCT	8	28.74	50.16	39.46	0.31	0.09	5.99
WCCT	4	29.62	71.52	69.41	0.69	0.31	16.84
WCCT	4	28.10	63.47	59.54	1.75	0.40	17.18
WCCT	4	30.49	60.11	46.99	0.87	0.01	9.63

All variables were log transformed for normality before conducting structural equation modelling. NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage.

Chapter 4 The controls of accumulation of microbially derived residues in soil under long-term conservation management practices

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Abstract

Understanding the processes controlling retention of amino sugars in soil is essential for quantifying the accumulation of microbially derived organic matter in soil. Many previous studies have examined the effect of cover crops and no-tillage on soil amino sugar content, but few have done so in soil aggregates, and fewer have been combined with microbial activities or extracellular enzyme activities to determine physical and biological controls on amino sugar retention. My project examined amino sugar content, microbial respiration, and extracellular enzyme activities in soil aggregates in a western Tennessee agricultural soil under 31years of conservation management practices. I used structural equation modelling to identify drivers for soil amino sugar turnover. The structural equation model allows to determine the microbial mechanisms of soil amino sugar decomposition and accumulation under different C and N availabilities and makes it possible to use short-term microbial processes to predict long-term SOM accumulation potential. My results show that a N-fixing vetch cover crop with no-tillage treatment facilitated amino sugar accumulation in large macroaggregates, while a wheat cover crop with conventional tillage treatment facilitated amino sugar accumulation in microaggregates. My structural equation model shows that N availability had a negative effect on β-N-acetylglucosaminidase (NAG) activity but a positive effect on amino sugar content; leucine aminopeptidase (LAP) had a negative effect on NAG but a positive

effect on amino sugars. The structural equation model demonstrates that when N is scarce in soil, amino sugars can be used as an alternative N source for microbes after amino acids.

4.1 Introduction

Amino sugars are used as biomarkers to trace microbial residues in soil (Glaser et al., 2004) because they are major constituents of microbial cell walls (Roberts et al., 2007). Since living plants and living microbial biomass contain negligible amounts of amino sugars compared with SOM (Glaser et al., 2004; Roberts et al., 2007), amino sugars in soil are assumed to result from microbial necromass accumulation, making up a relatively resistant reserve compared with phospholipid fatty acids, nucleic acids, proteins, etc. (Bremner and Shaw, 1954; Glaser et al., 2004; Engelking et al., 2007). Thus, amino sugars are suitable for characterizing microbial residues to the medium- to long-term (Glaser et al., 2004). Decomposition of amino sugar polymers, as with other polymers in soil, depends on extracellular enzymes (Beier and Bertilsson, 2014a). β-glucosidase (BG), α-glucosidase (AG), β-D-cellobiosidase (CB), and β-xylosidase (XYL) are hydrolytic enzymes that decompose polysaccharides (Deng and Tabatabai, 1994; Jian et al., 2016), which are associated with microbial carbon (C) acquisition. β-N-acetylglucosaminidase (NAG) and leucine aminopeptidase (LAP) are associated with microbial N acquisition (Tabatabai and Bremner, 1972; Jian et al., 2016). NAG breaks down chitin and other β-1,4-linked glucosamine polymers (Beier and Bertilsson, 2014a). LAP hydrolyzes polypeptides from the N terminus (Sinsabaugh et al., 2008). Since the production of extracellular enzymes is regulated by microbes, extracellular enzyme

activities reflect substrate composition and consumption in relation to microbial biomass and nutrient demand (Sinsabaugh et al., 2008; Bowles et al., 2014).

Soil aggregates provide physical protection for SOM including amino sugars, which is central to soil C and N accumulation and stabilization (O'Brien and Jastrow, 2013; Devine et al., 2014). The physical structure of aggregates can limit the accessibility of decomposers and enzymes to SOM through the occlusion of SOM within aggregates (Pulleman and Marinissen, 2004; Plaza et al., 2013). Aggregates exist in various sizes: macroaggregates (> 0.25 mm) and microaggregates (< 0.25 mm) are the two major groups, which can be further divided by size (Tisdall and Oades, 1982). Different sized aggregates differ in functions in relation to their stability, SOM stock, and SOM retention time (Bronick and Lal, 2005). When aggregate size increases, the stability tends to decrease (Tisdall and Oades, 1982; Dexter, 1988), and SOM stock increases (Devine et al., 2014). SOM retention in macroaggregates is considered as short-term storage, while that in microaggregates as long-term sequestration (Sainju et al., 2009; Gelaw et al., 2015).

Soil aggregate dynamics play a critical role in stabilization of SOM in agroecosystems (Plaza et al., 2013) because agricultural soils are generally depleted in SOM because of harvesting and crop residue removal. Soil particles can be physically aggregated by fungal hyphae (Degens, 1997), bacterial extracellular polysaccharides (Chenu, 1995), and hydrophobic compounds (Capriel et al., 1990). All these microbial products, favorable for aggregation, increase after addition of plant residues (Cosentino et al., 2006). On the other hand, tillage is a major cause of SOM depletion in agroecosystems (Six et al.,

2000b). This perturbation causes faster turnover rate of macroaggregates and slower formation rate of microaggregates within macroaggregates and is a dominant mechanism of SOM loss in agroecosystems (Six et al., 1998; Six et al., 1999). Compared to conventional tillage, no-tillage systems exhibit increases in amount and stability of aggregates concomitant with increases in SOM (Paustian et al., 2000). Planting of cover crops is another conservation management practice that can increase SOM content (Haque et al., 2015; Poeplau et al., 2015; Poeplau and Don, 2015). In addition to directly increasing SOM content, cover crops increase SOM stability by enhancing aggregation (Chivenge et al., 2011).

Although the impacts of cover crops and no-tillage on soil aggregates have been intensively studied (Elliott, 1986; Six et al., 2002; Austin et al., 2017), our understanding of how cover crops and tillage affect amino sugar accumulation in soil aggregates is still limited. Net amino sugar accumulation in soil implies either intensified microbial proliferation, reduced consumption, or both. Accumulation in soil aggregates also implies enhanced aggregate formation.

Amino sugar content in soil is highly dependent on microbial accessibility to substrates (Liang et al., 2007) and increases rapidly in response to plant residue amendment (Liang et al., 2007; Ding et al., 2011). Net amino sugar accumulation is also affected by soil N availability, provided either as inorganic fertilizer (He et al., 2011) or as high N-content residues by N-fixing plants (Liang et al., 2007). As with plant residue amendment, no-tillage facilitates amino sugar accumulation (Guggenberger et al., 1999; Zhang et al., 2014). Even though amino sugar-N only accounts for 4.5-7.4% of total soil N (Schulten

and Schnitzer, 1997), it may be an important source of N for soil microbes in certain scenarios because microbes can preferentially decompose their own cell residues (Schlegel and Zaborosch, 1993; Liang et al., 2007). Because amino sugar content is positively correlated with content of SOM (Liang et al., 2007) and degree of humification (Lowe, 1973), they may be used as a tool for N pool assessment. Also, amino sugars may be a good measure of the effect of soil microbes on aggregation since they are involved in aggregate formation and stabilization (Chantigny et al., 1997; Guggenberger et al., 1999).

To determine the long-term effect of agricultural management on amino sugar accumulation in soil aggregates, I conducted my study in a western Tennessee agricultural soil under 31-years treatment of cover crops and no-tillage practices. My objectives for this study were to: (1) examine the combined effects of cover crops and no tillage on amino sugar accumulation in soil; (2) reveal the microbiological and physical controls on amino sugar accumulation in soil aggregates; (3) evaluate the role of extracellular enzymes and microbial activity in soil amino sugar accumulation. My hypotheses are: (1) a N-fixing vetch cover crop with no-tillage treatment enhances C, N, and amino sugar accumulation in bulk soil; (2) using a cover crop with no-tillage treatment facilitates amino sugar accumulation in micro- and macroaggregates; (3) N availability has a positive effect on amino sugar accumulation through the mediation of NAG production/activity; and (4) C availability has a positive effect on soil amino sugar accumulation through the mediation of BG and LAP production/activity. To test these hypotheses, I examined amino sugar accumulation, extracellular enzyme activity, and microbial respiration in aggregate size fractions of soils from long-term experimental

plots with tillage and cover crop treatments. I then applied these data to a structural equation model to identify the key drivers of amino sugar accumulation.

4.2 Materials and methods

4.2.1 Site descriptions, experiment design, and soil sampling

Soil was collected from the West Tennessee Research and Education Center located in Jackson, TN (35°37'23.1"N 88°50'47.4"W) in July of 2013. The soil is derived from red marine deposit overlaid by loess deposits, classified as Lexington silt loam (fine-silty, mixed, thermic, Ultic Hapludalf), and well-drained with a 0 to 2 percent slope, with a mean pH of 5.5. The fields are managed by long-term conservation management practices under continuous cotton (*Gossypium hirsutum*) planting, which are established since 1981. In the field, there is a randomized complete blocked design with split-split plot, with 4 levels of inorganic N fertilizer treatment on whole plot (0 kg N ha^{-1}, 34 kg N ha^{-1}, 67 kg N ha^{-1}, and 101 kg N ha^{-1}), 4 levels of cover crop treatment on split plot (no-cover, hairy vetch, winter wheat, and clover), 2 levels of tillage treatment on split-split plot (tillage and no-tillage)65. The blocks are on field location. Experiment units are 12 m by 8 m in size with 8 rows of cotton.

In my study, I selected 3 levels of the cover crop treatment (no-cover, hairy vetch, and winter wheat) to compare the effect of a N-fixing cover crop with that of a C-enriched one, 1 level of the inorganic N fertilizer treatment (0 kg N ha-1) to make the cover crops the exclusive N source, and 2 levels of the tillage treatment (tillage and no-tillage). The treatments are: (1) no-cover crop and no-tillage (NCNT), (2) no-cover crop and

conventional tillage (NCCT), (3) vetch cover and no-tillage (VCNT), (4) vetch cover and conventional tillage (VCCT), (5) wheat cover and no-tillage (WCNT), and (6) wheat cover and conventional tillage (WCCT).

For aggregate fractionation analyses, intact soil cores were collected during cotton flowering stage in July 2014 using metal cores (7.62 cm diameter and 7.62 cm height) to a depth of 7.5 cm. The metal cores were sterilized with 70% ethanol between each sampling. Two soil cores per plot were sampled randomly. Soil cores were gently removed from the metal cores and sealed in heavy-paper lined sample bags (Fisher 6.4 × 24 × 8.6 cm) to keep intact during the transportation to laboratory. The samples were then air-dried in laboratory for aggregate fractionation analyses.

4.2.2 Soil aggregate size fractionation

Air-dried soil (200 g) was placed on a on a stack of sieves, including 2, 1, and 0.25 mm mesh openings. The sieve was shaken both horizontally and vertically using a sieve shaker (CSC Scientific) at 50 Hz for 3 minutes. The soil was fractionated into 4 fractions: large macroaggregate fraction (> 2 mm), medium macroaggregate fraction (1-2 mm), small macroaggregate fraction (0.25-1 mm), and microaggregate fraction (< 0.25 mm).

4.2.3 Soil microbial respiration rate of aggregate fractions

Soil microbial respiration rate was measured on 5 g of aggregate samples by a CO_2 infrared gas analyzer (IRGA, LiCOR-820, LiCor Inc., Lincoln NE) from batch microcosms (60 mL Wheaton vial), fitted with butyl rubber septa (Kimble Stoppers for

Headspace Vials) in order to draw gas samples (0.5 mL) with a syringe. Respiration measurements are conducted after gravimetric water content was adjusted to 15%.

4.2.4 Extracellular enzyme activity analyses

Activities of β-glucosidase (BG), α-glucosidase (AG), β-D-cellobiosidase (CB), β-xylosidase (XYL), β-N-acetylglucosaminidase (NAG), and leucine aminopeptidase (LAP) were measured by a fluorometric method (Steinweg and McMahon, 2012). A fluorescent dye is released during an enzyme-catalyzed reaction, and enzyme reaction is measured by a difference in the fluorescence between substrates and products (Steinweg and McMahon, 2012). A buffer solution of sodium acetate was used to match the mean soil pH of 5.5. The synthetic substrates used in this method were fluorescently labeled with one of two fluorescent indicators: 4-methylumbelliferone (MUB) or 7-amino-4-methylcoumarin (MUC, Sigma-Aldrich Co.). Air-dried soil (2.75 g) and buffer solution (91 mL) were mixed into a soil slurry using a blender. Soil slurry (800 µL) was pipetted into deep 96-well plates (2 mL Deep 96-Well PlateOne Polypropylene Plate) for enzyme activity measurement and standard curves. 5 µM, 10 µM, 25 µM, 50µM, 100 µM, and 1000 µM of MUB and MUC were prepared to create standard curves. Standard curves of MUB and MUC were prepared for each sample separately due to background auto fluorescence. Enzymes assayed and their corresponding functions and substrates are shown in Table 4.1. Substrate (200 µL of 200 µM) was pipetted into wells for enzyme activity measurement. After being incubated at 25℃ for 3 h, samples were centrifuged at 226 × g for 3 minutes (Thermo Scientific Sorvall STR16 Centrifuge) and 250 µL of supernatant was transferred into flat-bottomed 96-well plates (Corning 96-Well

Polypropylene Assay Plates). Fluorescence was measured on a Synergy HT microplate reader (BioTek, Winooski, VT) at two wavelengths: excitation 365 nm, emission 450 nm.

4.2.5 Amino sugar analyses

Three types of amino sugars were quantified in our work: muramic acid, glucosamine, and galactosamine, which were summed to calculate total amino sugars. Before amino sugar analyses the content of total N and C was measured by an elemental analyzer (vario MACRO cube, Elementar, Germany). Amino sugars were analyzed by the method developed by Zhang and Amelung (1996). Briefly, 3 g of air-dried soil was sieved (< 0.25 mm). After being hydrolyzed with 6 M HCl for 8 h, the soil slurry was filtered, rinsed, adjusted to pH 6.6-6.8, and centrifuged. The liquid supernatant was freeze-dried overnight. Dried methanol was used to wash amino sugars out from the inorganic salts. Amino sugars were dried under a gentle stream of dry N_2. Derivatization reagents were added and kept at 75-80°C for 35 minutes. After being cooled to room temperature, acetic anhydride was added and kept at 75-80°C for 25 minutes. Excessive anhydride was reacted with 1 M HCl and removed with water. The derivatives were extracted from the dried methanol with dichloromethane. After removing dichloromethane using N_2 stream, the dried amino sugar derivatives were dissolved in mixed hexane and ethyl acetate solvent (v:v = 1:1) for analyses. Myo-inositol was added as an internal standard before hydrolyses, and Methyl-glucamine as the recovery standard before derivatization. The amino sugar derivatives were separated on a DB-5MS column (30 m × 0.25 mm × 0.25 mm) with temperature program set by Zhang and Amelung (1996).

4.2.6 Statistical analyses.

ANOVA was conducted with SAS 9.4 (Glimmix procedure, SAS Institute Inc., Cary, NC) and least square means were compared by Fisher's LSD at both 5% significance level. Mixed model was used for determining the main effects of cover crops and tillage and the interaction effect of cover crops and tillage:

$$Y = \mu + CC + B + B*CC + T + T*CC + B*(T*CC)$$

μ = mean

B = block

CC = cover crop treatment

T = tillage treatment

Structural equation modeling was conducted by AMOS 25.0 (IBM Corporation, Meadville, PA). The path weights in the model were tested with maximum likelihood estimation. All variables were log transformed for normality. We used CMIN/DF, P, CFI, RMSEA, and PCLOSE to evaluate model fit. CMIN/DF is the minimum discrepancy divided by its degrees of freedom and evaluated by the likelihood ratio test, representing the discrepancy between the model and the data (Byrne, 2013). P is the probability value of the likelihood ratio test; values more than 0.05 indicate no significant discrepancy between the model and the data. CFI is the comparative fit index; values close to 1 indicates a very good model fit (Byrne, 2013). RMSEA is the root mean square error of

aprixiamation; values less than 0.05 indicate good fit (Byrne, 2013). PCLOSE is test for closeness of fit; values more than 0.50 indicate good fit. Squared multiple correlation (R^2) represents the percentage of the variance being explained in a variable by the model, ranging from 0.00-1.00. The data used for condeucting the strucrural equation modelling is shown in Table 4.108 and Table 4.110.

4.3 Results

4.3.1 Soil total C, total N, and C/N ratio in bulk soil

The main effects of cover crops and no tillage farming on concentrations of soil total C were significantly different from each other ($P < 0.0001$, Figure 4.1). Concentration of soil total C in the VCNT treatment was significantly higher than that in the NCCT and WCCT treatments ($P = 0.0199$ and $P = 0.0315$, respectively), while concentrations of soil total C in the other conservation management treatments were not significantly different from each other ($P > 0.0500$). The largest amount of total C (15.64 g kg^{-1}) appeared in the VCNT treatment. Total C in other treatments varied between 9.37 g kg^{-1} and 12.01 g kg^{-1}. Soil total N followed a similar pattern as total C (Figure 4.1). Total N in the VCNT treatment (1.52 g kg^{-1}) was significantly higher than that the NCCT, WCCT, and WCNT treatments ($P = 0.0127$, $P = 0.0098$, and $P = 0.0420$, respectively). Total N in other treatments varied between 0.92 g kg^{-1} and 1.19 g kg^{-1} but was not significantly different from each other ($P > 0.0500$). The C/N ratio varied between 9.87 and 10.81. The C/N ratio in the WCCT treatment was significantly higher than that in the NCCT and NCNT treatments ($P = 0.0457$ and $P = 0.0372$, respectively), while the C/N ratio in the other treatments was not significantly different from each other ($P > 0.0500$).

4.3.2 Amino sugar contents in bulk soil

Total amino sugar content in bulk soil (Figure 4.2) showed an identical pattern to that of total C and N in response to the treatments. Total amino sugar concentrations in the no-tillage and the conventional tillage treatments did not show any significant differences ($P = 0.0877$), and those in the wheat cover crop treatment were significantly lower than those in the vetch cover crop and the no-cover crop treatments ($P = 0.0069$ and $P = 0.0254$, respectively). Total amino sugar concentrations in the vetch cover crop and the no-cover crop treatments were not significantly different from each other ($P = 0.4937$). The lowest concentration (935.23 mg kg^{-1}) was found in the WCCT treatment. The highest concentration (1538.59 mg kg^{-1}) was found in the NCNT treatment. Total amino sugar concentration in the NCNT treatment was significantly higher than that in the NCCT, WCNT, and WCCT treatments ($P = 0.0170$, $P = 0.0122$, and $P = 0.0050$, respectively), but was not significantly different from that in the VCNT and VCCT treatments ($P = 0.3864$ and $P = 0.4006$, respectively). The similar patterns were observed for glucosamine, muramic acid, and galactosamine separately as for the total amino sugar concentration (Figure 4.2).

4.3.3 Soil total C, total N, and C/N ratio in aggregate fractions

Soil total C contents were the greatest in large macroaggregate fraction, which varied between 5.08 mg kg^{-1} and 7.68 mg kg^{-1}, compared to the other aggregate fractions, which varied between 0.81 mg kg^{-1} and 2.39 mg kg^{-1} (Figure 4.3). In large macroaggregate fraction, soil total C concentration in the VCNT treatment was higher than that in the other treatments but was not significant ($P > 0.1000$). In medium macroaggregate and

small macroaggregate fractions, VCCT caused the highest soil total C content. In microaggregate fraction, the highest soil total C content was found in WCCT. Soil total N, which varied between 0.08 mg kg^{-1} and 0.81 mg kg^{-1} (Figure 4.4), had a similar pattern as soil total C. Soil C/N ratio varied between 9.09 and 11.70 (Figure 4.5).

4.3.4 Soil total amino sugar contents in aggregate fractions

The distribution of amino sugars amongst aggregate hierarchies (Figure 4.6) followed that of aggregate proportion. The amount of amino sugars in large macroaggregate fraction was 2-18 times larger than that in other fractions. Generally, the amounts of amino sugars in aggregate fractions were ordered as large macroaggregates > microaggregates > small macroaggregates > medium macroaggregates. The smallest amount of amino sugars in large macroaggregates and the largest amount in microaggregates were both found in the WCCT treatment, while the largest amount in large macroaggregates was observed in the VCNT treatment. In each aggregate fraction, the amount of amino sugars varied differently in response to the treatments. In the large macroaggregate fraction, VCNT and WCCT treatments induced the highest and lowest amount of amino sugars (978.65 mg kg^{-1} and 359.82 mg kg^{-1}, respectively), while the amounts of amino sugars in the other treatments did not show any significant differences from each other ($P > 0.0500$). In the medium macroaggregate fraction, the largest amount (110.05 mg kg^{-1}) appeared in the VCCT treatment. In the small macroaggregate fraction, the amount of amino sugars in the NCNT treatment (156.39 mg kg^{-1}) was about 2 times as large as that in the NCCT treatment. In the microaggregate fraction, the amounts of amino sugars in WCCT and WCNT treatments (274.30 mg kg^{-1} and 115.00 mg kg^{-1}) were

the largest and the smallest, respectively, but the amounts did not show significant differences between NCNT and NCCT treatments or between VCNT and VCCT treatments (P = 0.6346 and P = 0.7192, respectively). Again, the same pattern was observed for glucosamine, muramic acid, and galactosamine separately as for the total amino sugar content (Figure 4.7 through Figure 4.9).

4.3.5 Structural equation model of total amino sugar concentration in bulk soil.

As shown in Figure 4.11, microbial respiration, microaggregate content, tillage, and wheat cover crop have direct effects on total amino sugar concentration in bulk soil. Microbial respiration has a direct effect in the size of 0.51, indicating that 1.00 unit of increase in microbial respiration will cause 0.51 unit of increase in total amino sugar concentration. Likewise, microaggregate content has a direct effect in the size of 0.44 on total amino sugar concentration. Tillage has both direct negative and indirect positive effects on total amino sugar concentration, which sums up to a total effect size of -0.26 (Table 4.107), suggesting that 1.00 unit of increase in tillage will cause 0.26 unit of decrease in total amino sugar concentration. Similarly, wheat cover crop has a total effect size of -0.48. Large macroaggregate content and microbial respiration respectively have the largest negative and positive effect size on total amino sugar concentration (-0.49 and 0.51, Table 4.107).

4.3.6 Structural equation model of individual amino sugar concentration in bulk soil

Glucosamine concentration in bulk soil is only associated with microbial respiration and wheat cover crop (Figure 4.13). Microbial respiration and wheat cover crop have an

effect on glucosamine concentration in the size of 0.50 and -0.48, respectively. As for muramic acid, its concentration in bulk soil is only directly affected by microaggregate content, with an effect size of -0.53 (Figure 4.15). Although small macroaggregate contents also has a direct effect in the size of 0.42 on muramic acid concentration, it is not significant ($P = 0.07$). Small macroaggregates do have a significant indirect effect on muramic acid concentration through large macroaggregates, medium macroaggregates, and microaggregates ($P < 0.05$), with a total effect size of -0.23 (Table 4.107). Tillage and wheat cover crop also have indirect effects on muramic acid through large macroaggregates, medium macroaggregates, and microaggregates, with a total effect size of -0.33 and -0.20, respectively (Table 4.107). Galactosamine has the same pattern as total amino sugars in bulk soil (Figure 4.17).

4.3.7 Structural equation model of total amino sugar concentration in soil aggregate fractions

As shown in Figure 4.19, microbial respiration rate, BG activity, and NAG activity had direct effects on the concentration of total amino sugars in soil aggregate fractions, with path coeffitients of 0.33, 0.31, and -0.41, respectively. LAP activity, N concentration, and C concentration had indirect effects on total amino sugar in soil aggregate fractions. LAP activity had a positive effect on total amino sugars through NAG activity, BG activity, C concentration, N concentration, and microbial respiration rate (standardized total effect = 0.26, Table 4.109), while it had a negative effect on NAG activity (stadardized total effect = -0.26, Table 4.109). N concentration had a positive effect on total amino sugars through NAG activity and microbial respiration rate (stadardized total effect = 0.28, Table 4.109), while it had a negtive effect on NAG activity (path coeffitient = -0.27,

Figure 4.19). C concentration had a nagative effect on on BG activity (path coeffitient = -0.24, Figure 4.19).

4.4 Discussion

4.4.1 Effects of cover crops and no-tillage on accumulations of C, N, and amino sugars in bulk soil

The utilization of cover crops has been proved to increase soil C storage by increasing C input (Poeplau and Don, 2015), while long-term no-tillage increases soil C content through enhanced physical protection of aggregates (Devine et al., 2014). We evaluated the combined effects of cover crops and no-tillage on soil C and N accumulation, specifically that in microbial residues. When no-tillage is combined with different cover crops, a N-fixing vetch cover crop induced higher accumulation of soil C and N than a wheat cover crop (Figure 4.1), indicating the quality of the cover crop litter influences total C and N incorporation in soil. Since the mineralization and immobilization of cover crop residues are driven by soil microorganisms (Wagger et al., 1998), litter quality, such as C/N ratio and lignin concentration (Baldock et al., 2004), of the cover crops may control the processes. Vetch has a low C/N ratio of 10:1 to 15:1 (Spargo et al., 2016), while wheat has a much higher C/N ratio of ~80:1 (Tardy et al., 2015). Lignin C accounts for ~14% in vetch, while ~22% in wheat (Baldock et al., 2004; Baumann et al., 2009). Differences in the initial composition of cover crops may explain the different C and N content between the vetch-cover-crop-with-no-tillage treatment and the wheat-cover-crop-with-no-tillage treatment, but no significant difference was found between the vetch-cover-crop-with-conventional-tillage treatment and the wheat-cover-crop-with-

conventional-tillage treatment. This suggests that the advantages of vetch cover crops over wheat cover crops might be realized only when combined with no-tillage.

The amount of amino sugars retained in soil depends on the balance of consumption and production of microbial residues (Zhang et al., 2014). Together, soil aggregate structure and substrate availability are two factors that can control this balance. We found that no-tillage induced greater amino sugar accumulation in bulk soil compared with conventional tillage regardless of cover crops (Figure 4.2), indicating no-tillage greatly facilitates amino sugar retention compared with conventional tillage. Under no-tillage, the turnover rate of macroaggregates is reduced, and the formation of stable microaggregates is enhanced (Six et al., 2000a). Once occluded in soil aggregates, SOM, including amino sugars, can be spatially protected from microbial decomposition because of reduced access of soil microbes and their enzymes to substrates (Lützow et al., 2006). As to amino sugar production, it may be increased by intensified microbial activity and/or growth (Liang et al., 2007). Amino sugar content in vetch cover crop treatments was higher than that in no-cover crop and wheat cover treatments regardless of tillage treatment (Figure 4.2). This suggests that cover crops have a greater positive effect on soil amino sugar accumulation despite tillage disturbances, unlike bulk soil C and N. However, the greatest amino sugar content was observed in vetch-cover-crop-with-no-tillage treatment, indicating that a combination of management practices provides the most favorable nutrient condition and physical environment for amino sugar accumulation in soil. Vetch cover crops could possibly increase soil microbial activity and/or growth through sufficient C and N availability and suitable C/N ratio, and therefore result in increased amino sugar production.

Compared to vetch, the high C/N ratio of wheat cover crops create relatively poor nutrient conditions in the soil, thus soil microbes may activate corresponding N-acquisition mechanisms. Amino sugar concentration in the wheat-cover-crop-with-no-tillage treatment was 26% lower than those in the vetch-cover-crop-with-no-tillage treatment, and that in the wheat-cover-crop-with-conventional-tillage treatment was 32% lower than that in the vetch-cover-crop-with-conventional-tillage treatment. In my study, cover crop treatments represent extremes in N availability; since no inorganic N fertilizer was applied to the field plots we studied, the vetch cover crops are the exclusive supply of N. When N availability is relatively low, soil microbes may decompose recalcitrant SOM, including amino sugars, to meet their N requirement (Chen et al., 2014). Thus, wheat cover crops might cause greater amino sugar consumption by inducing microbial N mining.

4.4.2 Effects of cover crops and no-tillage on amino sugar accumulation among soil aggregate hierarchies

The balance of the consumption and production of amino sugars varied greatly among soil aggregate hierarchies, especially in the large macroaggregate fraction. The most significant amino sugar accumulation in the large macroaggregate fraction was found in the vetch-cover-crop-with-no-tillage treatment (Figure 4.6), possibly due to increased production of amino sugars. The amounts of large macroaggregates showed no significant difference under the treatments, so the effect of aggregate amount can be ruled out (except the wheat-cover-crop-with-conventional-tillage treatment, Figure 4.6).

We also observed, in some cases, depletion of amino sugars in the large aggregate fraction, possibly due to increased consumption stimulated by aggregate disruption. The lowest amino sugar content in the large macroaggregate fraction appeared in the wheat-cover-crop-with-conventional-tillage treatment (Figure 4.6), concomitant with the smallest amount of large macroaggregates (Table 4.108). This could be the result of reduced large macroaggregate amount caused by tillage disturbance. As the large macroaggregates were disrupted, the amino sugars previously protected within the large macroaggregates were exposed, allowing microbes access to N from large macroaggregates. Concomitantly, amino sugar content in the microaggregate fraction of the wheat-cover-crop-with-conventional-tillage treatment was significantly higher than that in the other treatments (Figure 4.6), as was the amount of microaggregates (Table 4.108), indicating that microaggregates contribute to amino sugar accumulation. Conventional tillage causes accelerated turnover of large macroaggregates and subsequent release of microaggregates (Six et al., 2000a), which may be responsible for the significantly increased amount of microaggregates. Likewise, in the medium macroaggregate fraction and the small macroaggregate fraction, the vetch-cover-crop-with-conventional-tillage treatment and the no-cover-with-no-tillage treatment respectively induced higher amino sugar contents than other treatments (Figure 4.6). This also can be explained by increased aggregate content in each fraction (Table 4.108).

4.4.3 Mechanisms of amino sugar stabilization in soil as revealed by structural equation modelling

In bulk soil, our structural equation model indicates that soil structure, microbial activity, and conservation agricultural management practices jointly control total amino sugar

content, explaining 65% of changes in total amino sugar content (Figure 4.11). Amino sugar content in soil depends on the balance between production and decomposition of microbial residues (Zhang et al., 2014). Conventionally, it is well known that once occluded in soil aggregates, SOM, including amino sugars, can be spatially protected from microbial decomposition (Lützow et al., 2006). However, our models show that physical protection of soil aggregates does not have any significant effects on glucosamine content ($P > 0.05$, Figure 4.13), although it has a major control over muramic acid content ($P < 0.05$, Figure 4.15). This can be explained by the difference in biochemical recalcitrance between glucosamine and muramic acid. Glucosamine is more resistant to microbial decomposition than muramic acid and tends to accumulate in soil (He et al., 2011), which can be proved by our results that glucosamine content was 15-27 times larger than muramic acid (Table 4.108). Muramic acid being biochemically labile makes physical protection of soil aggregates more critical for its accumulation in soil, while the accumulation of glucosamine is controlled by microbial activity and substrate availability rather than soil structure (Figure 4.13).

In soil aggregate fractions, our structural equation model reveals the microbial and enzymatic controls over the production and decomposition of total amino sugars. The model shows that soil microbial respiration, β-1,4-glucosidase (BG) activity, and β-N-acetylglucosaminidase (NAG) activity jointly control total amino sugar content (Figure 4.19). Microbial respiration rate can be a proxy of microbial activity and/or microbial biomass (Wang et al., 2003; Liu, 2013). Similarly, BG can be used a proxy as it hydrolyzes cellobiose into glucose (Sinsabaugh et al., 2008), providing energy for microbial syntheses of amino sugars. Increases in microbial respiration and BG activity

cause increases in the production of amino sugars. NAG is responsible for the hydrolysis of chitin into N-acetyl-glucosamine (Ghuysen, 1968; Beier and Bertilsson, 2014b) and, when active, could possibly drive amino sugar decomposition as an N source. On the other hand, leucine aminopeptidase (LAP) is associated with protein decomposition (Sinsabaugh et al., 2008), potentially representing a different N source. Although LAP did not have a direct effect on total amino sugar content, it has an indirect positive effect on amino sugars through suppressing NAG (Figure 4.19), indicating that soil microbes prefer amino acids before amino sugars as N source. Additionally, we measured activities of LAP that were 22-60 times larger than those of NAG (Table 4.110), which also supports our hypothesis that soil microbes more actively consume polypeptides than amino saccharides. Like LAP, soil N availability also had an indirect positive effect on amino sugars through suppressing NAG (Figure 4.19). When N is scarce, NAG activity would be greater, which can be explained by the microbial N mining theory. The microbial N mining model assumes soil microbes use labile C as energy source to decompose recalcitrant SOM to meet N acquisition (Moorhead and Sinsabaugh, 2006). Indeed, in each soil aggregate fraction, NAG activity in the wheat-cover-crop treatments was significantly higher than that in the vetch-cover-crop treatments (Table 4.110). Vetch has a low C/N ratio of 10:1 to 15:1 (Spargo et al., 2016), while wheat has a much higher C/N ratio of ~80:1 (Tardy et al., 2015). In the wheat-cover-crop treatments, with excessive C but scarce N, microbes may need to synthesize and excrete more NAG to breakdown polymers to monomers before N can be assimilated. Since enzyme production is nutrient and energy intensive (Koch, 1985; Allison and Vitousek, 2005), in the vetch-cover-crop treatments, with sufficient N, NAG exhibited lower activity (Table 4.110).

Our results are in agreement with previous findings that the addition of C source can induced soil microbial N mining (Chen et al., 2014). The model also shows that soil N content has a negative effect on NAG activity and C content has a negative effect on BG activity (Figure 4.19). This is consistent with previous studies that an available nutrient usually suppresses the activity of the corresponding nutrient-releasing enzyme (Sinsabaugh and Moorhead, 1994; Allison and Vitousek, 2005; Sinsabaugh et al., 2005).

4.5 Conclusions

In summary, our work shows an overview of how physical protection, biochemical recalcitrance, microbial activity, and substrate availability jointly control amino sugar accumulation in bulk soil and aggregate fractions. Our structural equation model shows that physical protection of soil aggregates has a major control over the accumulation of microbial residues with less biochemical resistance, while microbial activity and substrate availability are more critical for the accumulation of more recalcitrant microbial residues. We also found that amino sugars were used by soil microbes as an N source when soil N was scarce but amino acids were utilized prior to amino sugars. This demonstrates how soil amino sugars can constitute a transitional pool in the soil N cycle, and act as a readily available N pool when soil N is scarce but as a recalcitrant N pool when soil N is abundant. Recent research (Chao et al., 2017) highlights the need to explore the cumulative legacy of microbial assimilation and substrate preference as means to understand the contribution of microbially derived organic matter to passive SOM. Proposed conceptual models of passive SOM formation that show plant derived organic matter can contribute to passive SOM in the form of microbial necromass (Chao

et al., 2017). Indeed, direct evidence has proved that soil microbes produce recalcitrant SOM (Kallenbach et al., 2016). Long-term experimental results from this agroecosystem show that extracellular enzyme activity and microbial respiration rate are suitable indicators of amino sugar decomposition and production in soil. Since soil microbial processes control the transformation from plant derived organic matter to microbial necromass (Cotrufo et al., 2013; Chao et al., 2017), it may be possible to use short-term microbial processes to predict long-term SOM accumulation. In the future, amino sugar analyses should be combined with isotope labeling technique to determine how much labeled C and N atoms have been incorporated into microbial residues.

References

Allison, S.D., Vitousek, P.M., 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biology and Biochemistry 37, 937-944.

Austin, E.E., Wickings, K., McDaniel, M.D., Robertson, G.P., Grandy, A.S., 2017. Cover crop root contributions to soil carbon in a no-till corn bioenergy cropping system. GCB Bioenergy.

Baldock, J., Masiello, C., Gelinas, Y., Hedges, J., 2004. Cycling and composition of organic matter in terrestrial and marine ecosystems. Marine Chemistry 92, 39-64.

Baumann, K., Marschner, P., Smernik, R.J., Baldock, J.A., 2009. Residue chemistry and microbial community structure during decomposition of eucalypt, wheat and vetch residues. Soil Biology and Biochemistry 41, 1966-1975.

Beier, S., Bertilsson, S., 2014a. Bacterial chitin degradation—mechanisms and ecophysiological strategies. The Microbial Regulation of Global Biogeochemical Cycles, 9.

Beier, S., Bertilsson, S., 2014b. Bacterial chitin degradation—mechanisms and ecophysiological strategies. The Microbial Regulation of Global Biogeochemical Cycles 9.

Bell, C.W., Fricks, B.E., Rocca, J.D., Steinweg, J.M., McMahon, S.K., Wallenstein, M.D., 2013. High-throughput fluorometric measurement of potential soil extracellular enzyme activities. Journal of visualized experiments: JoVE 81, 50961.

Bowles, T.M., Acosta-Martínez, V., Calderón, F., Jackson, L.E., 2014. Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic

agroecosystems across an intensively-managed agricultural landscape. Soil Biology and Biochemistry 68, 252-262.

Bremner, J., Shaw, K., 1954. Studies on the estimation and decomposition of amino sugars in soil. The Journal of Agricultural Science 44, 152-159.

Bronick, C.J., Lal, R., 2005. Soil structure and management: a review. Geoderma 124, 3-22.

Byrne, B.M., 2013. Structural equation modeling with AMOS: Basic concepts, applications, and programming, 2nd ed. New York: Routledge.

Capriel, P., Beck, T., Borchert, H., Härter, P., 1990. Relationship between soil aliphatic fraction extracted with supercritical hexane, soil microbial biomass, and soil aggregate stability. Soil Science Society of America Journal 54, 415-420.

Chantigny, M.H., Angers, D.A., Prévost, D., Vézina, L.-P., Chalifour, F.-P., 1997. Soil aggregation and fungal and bacterial biomass under annual and perennial cropping systems. Soil Science Society of America Journal 61, 262-267.

Chao, L., Joshua, P.S., Julie, D.J., 2017. The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology 2, 17105.

Chen, R., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., Lin, X., Blagodatskaya, E., Kuzyakov, Y., 2014. Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories. Global change biology 20, 2356-2367.

Chenu, C., 1995. Extracellular polysaccharides: an interface between microorganisms and soil constituents. Environmental impact of soil component interactions 1, 217-233.

Chivenge, P., Vanlauwe, B., Gentile, R., Six, J., 2011. Organic resource quality influences short-term aggregate dynamics and soil organic carbon and nitrogen accumulation. Soil Biology and Biochemistry 43, 657-666.

Cosentino, D., Chenu, C., Le Bissonnais, Y., 2006. Aggregate stability and microbial community dynamics under drying–wetting cycles in a silt loam soil. Soil Biology and Biochemistry 38, 2053-2062.

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Global change biology 19, 988-995.

Degens, B.P., 1997. Macro-aggregation of soils by biological bonding and binding mechanisms and the factors affecting these: a review. Soil Research 35, 431-460.

Deng, S.P., Tabatabai, M.A., 1994. Cellulase activity of soils. Soil Biology and Biochemistry 26, 1347-1354.

Devine, S., Markewitz, D., Hendrix, P., Coleman, D., 2014. Soil aggregates and associated organic matter under conventional tillage, no-tillage, and forest succession after three decades. PloS one 9, e84988.

Dexter, A., 1988. Advances in characterization of soil structure. Soil and Tillage Research 11, 199-238.

Ding, X., He, H., Zhang, B., Zhang, X., 2011. Plant-N incorporation into microbial amino sugars as affected by inorganic N addition: a microcosm study of 15 N-labeled maize residue decomposition. Soil Biology and Biochemistry 43, 1968-1974.

Elliott, E., 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Science Society of America Journal 50, 627-633.

Engelking, B., Flessa, H., Joergensen, R.G., 2007. Shifts in amino sugar and ergosterol contents after addition of sucrose and cellulose to soil. Soil Biology and Biochemistry 39, 2111-2118.

Gelaw, A.M., Singh, B., Lal, R., 2015. Organic carbon and nitrogen associated with soil aggregates and particle sizes under different land uses in Tigray, Northern Ethiopia. Land Degradation & Development 26, 690-700.

Ghuysen, J.-M., 1968. Use of bacteriolytic enzymes in determination of wall structure and their role in cell metabolism. Bacteriological reviews 32, 425.

Glaser, B., Turrión, M.a.-B., Alef, K., 2004. Amino sugars and muramic acid—biomarkers for soil microbial community structure analysis. Soil Biology and Biochemistry 36, 399-407.

Guggenberger, G., Frey, S.D., Six, J., Paustian, K., Elliott, E.T., 1999. Bacterial and fungal cell-wall residues in conventional and no-tillage agroecosystems. Soil Science Society of America Journal 63, 1188-1198.

Haque, M.M., Kim, S.Y., Kim, G.W., Kim, P.J., 2015. Optimization of removal and recycling ratio of cover crop biomass using carbon balance to sustain soil organic carbon stocks in a mono-rice paddy system. Agriculture, ecosystems & environment 207, 119-125.

He, H., Zhang, W., Zhang, X., Xie, H., Zhuang, J., 2011. Temporal responses of soil microorganisms to substrate addition as indicated by amino sugar differentiation. Soil Biology and Biochemistry 43, 1155-1161.

Jian, S., Li, J., Chen, J., Wang, G., Mayes, M.A., Dzantor, K.E., Hui, D., Luo, Y., 2016. Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: A meta-analysis. Soil Biology and Biochemistry 101, 32-43.

Kallenbach, C.M., Frey, S.D., Grandy, A.S., 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nature communications 7, 13630.

Koch, A.L., 1985. The macroeconomics of bacterial growth. Special Publications of the Society for General Microbiology[SPEC. PUBL. SOC. GEN. MICROBIOL.]. 1985.

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

Liang, C., Zhang, X., Rubert, K.F., Balser, T.C., 2007. Effect of plant materials on microbial transformation of amino sugars in three soil microcosms. Biology and fertility of soils 43, 631-639.

Liu, H., 2013. Thermal response of soil microbial respiration is positively associated with labile carbon content and soil microbial activity. Geoderma 193, 275-281.

Lowe, L., 1973. Amino acid distribution in forest humus layers in British Columbia. Soil Science Society of America Journal 37, 569-572.

Moorhead, D.L., Sinsabaugh, R.L., 2006. A theoretical model of litter decay and microbial interaction. Ecological Monographs 76, 151-174.

O'Brien, S.L., Jastrow, J.D., 2013. Physical and chemical protection in hierarchical soil aggregates regulates soil carbon and nitrogen recovery in restored perennial grasslands. Soil Biology and Biochemistry 61, 1-13.

Paustian, K., Six, J., Elliott, E., Hunt, H., 2000. Management options for reducing CO2 emissions from agricultural soils. Biogeochemistry 48, 147-163.

Plaza, C., Courtier-Murias, D., Fernández, J.M., Polo, A., Simpson, A.J., 2013. Physical, chemical, and biochemical mechanisms of soil organic matter stabilization under conservation tillage systems: a central role for microbes and microbial by-products in C sequestration. Soil Biology and Biochemistry 57, 124-134.

Poeplau, C., Aronsson, H., Myrbeck, Å., Kätterer, T., 2015. Effect of perennial ryegrass cover crop on soil organic carbon stocks in southern Sweden. Geoderma Regional 4, 126-133.

Poeplau, C., Don, A., 2015. Carbon sequestration in agricultural soils via cultivation of cover crops–A meta-analysis. Agriculture, ecosystems & environment 200, 33-41.

Pulleman, M., Marinissen, J., 2004. Physical protection of mineralizable C in aggregates from long-term pasture and arable soil. Geoderma 120, 273-282.

Roberts, P., Bol, R., Jones, D.L., 2007. Free amino sugar reactions in soil in relation to soil carbon and nitrogen cycling. Soil Biology and Biochemistry 39, 3081-3092.

Sainju, U.M., Caesar-TonThat, T., Jabro, J.D., 2009. Carbon and nitrogen fractions in dryland soil aggregates affected by long-term tillage and cropping sequence. Soil Science Society of America Journal 73, 1488-1495.

Schlegel, H.G., Zaborosch, C., 1993. General microbiology. Cambridge university press.

Schulten, H.-R., Schnitzer, M., 1997. The chemistry of soil organic nitrogen: a review. Biology and fertility of soils 26, 1-15.

Sinsabaugh, R., Moorhead, D., 1994. Resource allocation to extracellular enzyme production: a model for nitrogen and phosphorus control of litter decomposition. Soil Biology and Biochemistry 26, 1305-1311.

Sinsabaugh, R.L., Gallo, M.E., Lauber, C., Waldrop, M.P., Zak, D.R., 2005. Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. Biogeochemistry 75, 201-215.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., 2008. Stoichiometry of soil enzyme activity at global scale. Ecology letters 11, 1252-1264.

Six, J., Elliott, E., Paustian, K., 1999. Aggregate and soil organic matter dynamics under conventional and no-tillage systems. Soil Science Society of America Journal 63, 1350-1358.

Six, J., Elliott, E., Paustian, K., 2000a. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology and Biochemistry 32, 2099-2103.

Six, J., Elliott, E., Paustian, K., Doran, J., 1998. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Science Society of America Journal 62, 1367-1377.

Six, J., Feller, C., Denef, K., Ogle, S., de Moraes Sa, J.C., Albrecht, A., 2002. Soil organic matter, biota and aggregation in temperate and tropical soils-Effects of no-tillage. Agronomie 22, 755-775.

Six, J., Paustian, K., Elliott, E., Combrink, C., 2000b. Soil structure and organic matter I. Distribution of aggregate-size classes and aggregate-associated carbon. Soil Science Society of America Journal 64, 681-689.

Spargo, J., Cavigelli, M., Mirsky, S., Meisinger, J., Ackroyd, V., 2016. Organic Supplemental Nitrogen Sources for Field Corn Production after a Hairy Vetch Cover Crop. Agronomy Journal 108, 1992-2002.

Steinweg, M., McMahon, S., 2012. Microplate Enzyme Assay Using Fluorescence.

Tabatabai, M., Bremner, J., 1972. Assay of urease activity in soils. Soil Biology and Biochemistry 4, 479-487.

Tardy, V., Chabbi, A., Charrier, X., De Berranger, C., Reignier, T., Dequiedt, S., Faivre-Primot, C., Terrat, S., Ranjard, L., Maron, P.-A., 2015. Land use history shifts in situ fungal and bacterial successions following wheat straw input into the soil. PloS one 10, e0130672.

Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. Journal of Soil Science 33, 141-163.

Wagger, M., Cabrera, M., Ranells, N., 1998. Nitrogen and carbon cycling in relation to cover crop residue quality. Journal of Soil and Water Conservation 53, 214-218.

Wang, W., Dalal, R., Moody, P., Smith, C., 2003. Relationships of soil respiration to microbial biomass, substrate availability and clay content. Soil Biology and Biochemistry 35, 273-284.

Wilson, C.B., 2015. Long-term Impacts of Conservation Management Practices on Soil Carbon Storage, Stability, and Utilization under Cotton Production in West Tennessee. Master's Thesis, University of Tennessee, 32-33.

Zhang, B., Drury, C.F., Yang, X., Reynolds, W.D., Zhang, X., 2014. Effects of long-term and recently imposed tillage on the concentration and composition of amino sugars in a clay loam soil in Ontario, Canada. Soil and Tillage Research 135, 9-17.

Zhang, X., Amelung, W., 1996. Gas chromatographic determination of muramic acid, glucosamine, mannosamine, and galactosamine in soils. Soil Biology and Biochemistry 28, 1201-1206.

Appendix 4

Appendix 4A Enzymes assayed and their corresponding functions and substrates

Table 4.1 Enzymes assayed and their corresponding functions and substrates (Bell et al., 2013).

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

Appendix 4B Soil total C, total N, and C/N ratio in bulk soil

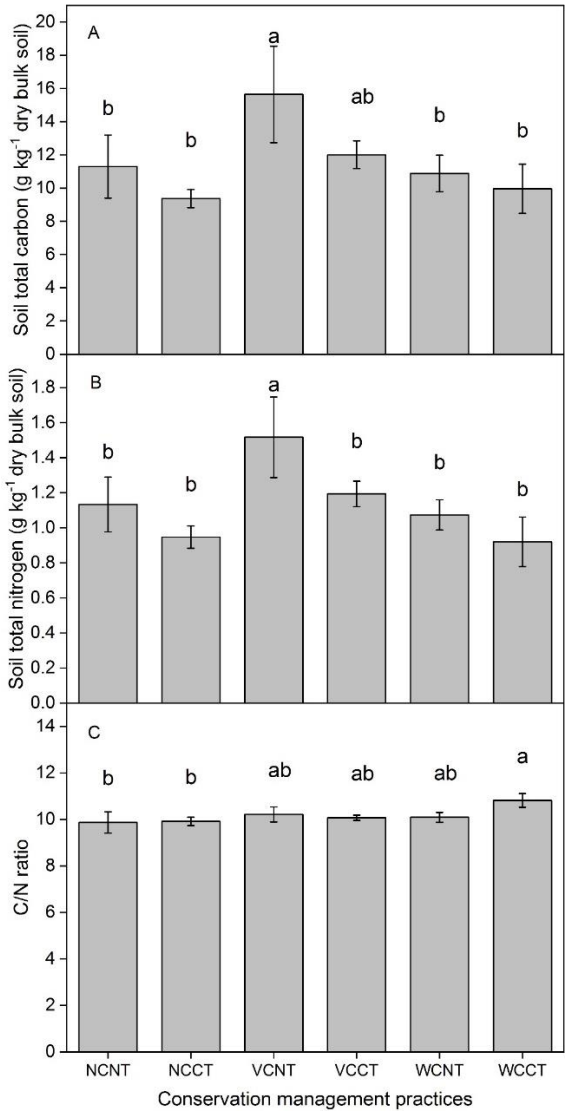

Figure 4.1 Soil total C, soil total N, and soil C/N ratio in bulk soil.

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Bars indicate standard error. Error bars are at 95% confident interval. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.2 Main effect of cover crops on total C in bulk soil.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	10.3350	1.4403	A
Vetch cover crops	13.8250	1.4403	A
Wheat cover crops	10.4183	1.4403	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.3 Main effect of tillage on total C in bulk soil.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	10.4456	1.2413	A
No-tillage	12.6067	1.2413	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.4 Interaction effect of cover crops and tillage on total C in bulk soil.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	9.3733	1.6526	B
No-cover crop	No-tillage	11.2967	1.6526	B
Vetch cover crops	Conventional tillage	12.0067	1.6526	AB
Vetch cover crops	No-tillage	15.6433	1.6526	A
Wheat cover crops	Conventional tillage	9.9567	1.6526	B
Wheat cover crops	No-tillage	10.8800	1.6526	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.5 Main effect of cover crops on total N in bulk soil.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	1.0400	0.1231	AB
Vetch cover crops	1.3550	0.1231	A
Wheat cover crops	0.9967	0.1231	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.6 Main effect of tillage on total N in bulk soil.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	1.0200	0.1062	B
No-tillage	1.2411	0.1062	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3)

Table 4.7 Interaction effect of cover crops and tillage on total N in bulk soil.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	0.9467	0.1377	B
No-cover crop	No-tillage	1.1333	0.1377	B
Vetch cover crops	Conventional tillage	1.1933	0.1377	B
Vetch cover crops	No-tillage	1.5167	0.1377	A
Wheat cover crops	Conventional tillage	0.9200	0.1377	B
Wheat cover crops	No-tillage	1.0733	0.1377	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3)

Table 4.8 Main effect of cover crops on total C/N ratio in bulk soil.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	9.8881	0.2137	A
Vetch cover crops	10.1406	0.2137	A
Wheat cover crops	10.4509	0.2137	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3)

Table 4.9 Main effect of tillage on total C/N ratio in bulk soil.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	10.2646	0.1708	A
No-tillage	10.0552	0.1708	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3)

Table 4.10 Interaction effect of cover crops and tillage on C/N ratio in bulk soil.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	9.9111	0.2864	B
No-cover crop	No-tillage	9.8651	0.2864	B
Vetch cover crops	Conventional tillage	10.0688	0.2864	AB
Vetch cover crops	No-tillage	10.2124	0.2864	AB
Wheat cover crops	Conventional tillage	10.8139	0.2864	A
Wheat cover crops	No-tillage	10.0880	0.2864	AB

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3)

Appendix 4C Concentrations of total amino sugars, glucosamine, muramic acid, and galactosamine in bulk soil

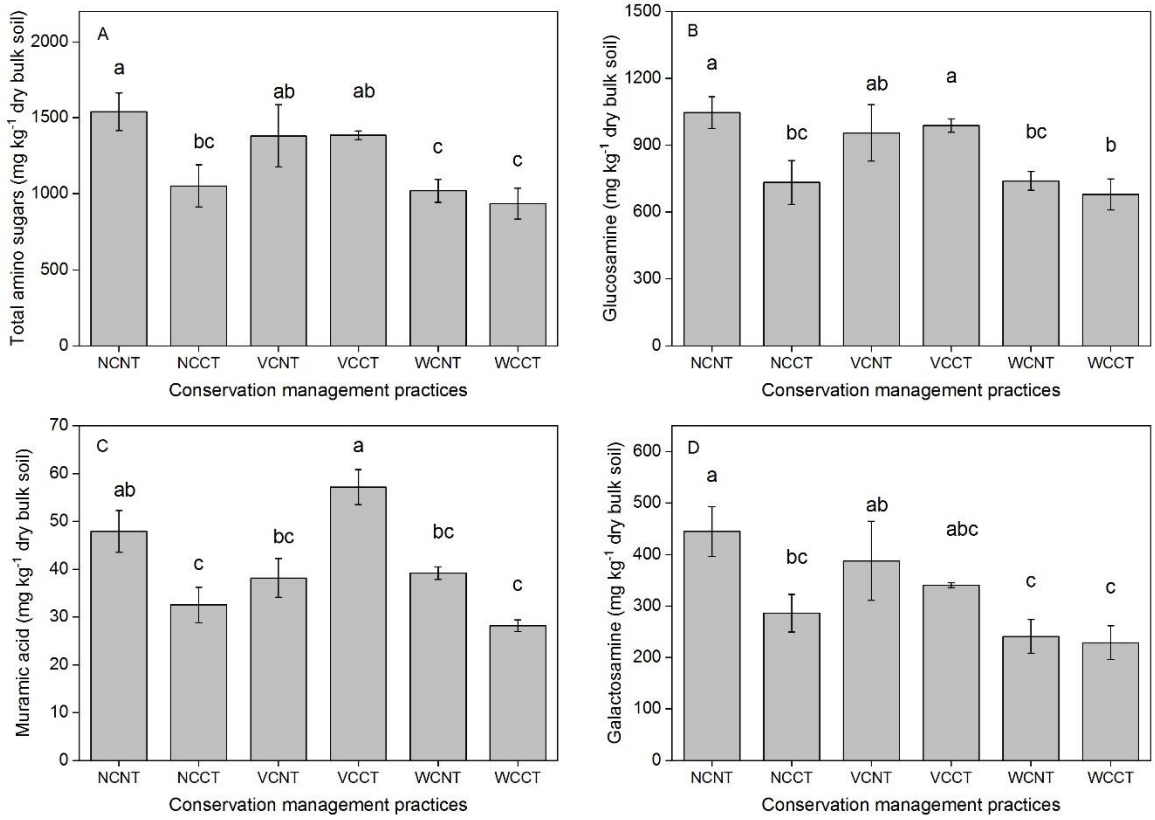

Figure 4.2 Amino sugar concentrations in bulk soil.

NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Bars indicate standard error. Error bars are at 95% confident interval. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.11 Main effect of cover crops on total amino sugar in bulk soil.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	1294.88	95.5982	A
Vetch cover crops	1382.76	95.5982	A
Wheat cover crops	977.37	95.5982	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3)

Table 4.12 Main effect of tillage on total amino sugars in bulk soil.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	1123.86	83.7474	A
No-tillage	1312.81	83.7474	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3)

Table 4.13 Interaction effect of cover crops and tillage on total amino sugars in bulk soil.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	1051.17	124.56	BC
No-cover crop	No-tillage	1538.59	124.56	A
Vetch cover crops	Conventional tillage	1385.18	124.56	AB
Vetch cover crops	No-tillage	1380.34	124.56	AB
Wheat cover crops	Conventional tillage	935.23	124.56	C
Wheat cover crops	No-tillage	1019.51	124.56	C

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3)

Table 4.14 Main effect of cover crops on glucosamine in bulk soil.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	889.20	60.7625	A
Vetch cover crops	971.13	60.7625	A
Wheat cover crops	708.92	60.7625	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3)

Table 4.15 Main effect of tillage on glucosamine in bulk soil.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	799.53	52.9337	A
No-tillage	913.31	52.9337	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3)

Table 4.16 Interaction effect of cover crops and tillage on glucosamine in bulk soil.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	732.54	79.7645	BC
No-cover crop	No-tillage	1045.86	79.7645	A
Vetch cover crops	Conventional tillage	987.67	79.7645	A
Vetch cover crops	No-tillage	954.59	79.7645	AB
Wheat cover crops	Conventional tillage	678.36	79.7645	C
Wheat cover crops	No-tillage	739.47	79.7645	BC

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3)

Table 4.17 Main effect of cover crops on muramic acid in bulk soil.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	30.5592	40.2258	2.3686
Vetch cover crops	37.6613	47.6613	2.3686
Wheat cover crops	28.3545	33.6879	2.3686

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3)

Table 4.18 Main effect of tillage on muramic acid in bulk soil.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	39.2860	1.9166	A
No-tillage	41.7641	1.9166	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3)

Table 4.19 Interaction effect of cover crops and tillage on muramic acid in bulk soil.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	32.5237	3.3196	C
No-cover crop	No-tillage	47.9280	3.3196	AB
Vetch cover crops	Conventional tillage	57.1557	3.3196	A
Vetch cover crops	No-tillage	38.1669	3.3196	BC
Wheat cover crops	Conventional tillage	28.1785	3.3196	C
Wheat cover crops	No-tillage	39.1972	3.3196	BC

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3)

Table 4.20 Main effect of cover crops on galactosamine in bulk soil.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	365.45	34.8727	A
Vetch cover crops	363.97	34.8727	A
Wheat cover crops	234.77	34.8727	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3)

Table 4.21 Main effect of tillage on galactosamine in bulk soil.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	285.05	31.1099	B
No-tillage	357.74	31.1099	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3)

Table 4.22 Interaction effect of cover crops and tillage on galactosamine in bulk soil.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	286.10	44.2826	BC
No-cover crop	No-tillage	444.80	44.2826	A
Vetch cover crops	Conventional tillage	340.35	44.2826	ABC
Vetch cover crops	No-tillage	387.58	44.2826	AB
Wheat cover crops	Conventional tillage	228.69	44.2826	C
Wheat cover crops	No-tillage	240.84	44.2826	C

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3)

Appendix 4D Concentrations of total C in aggregate fractions

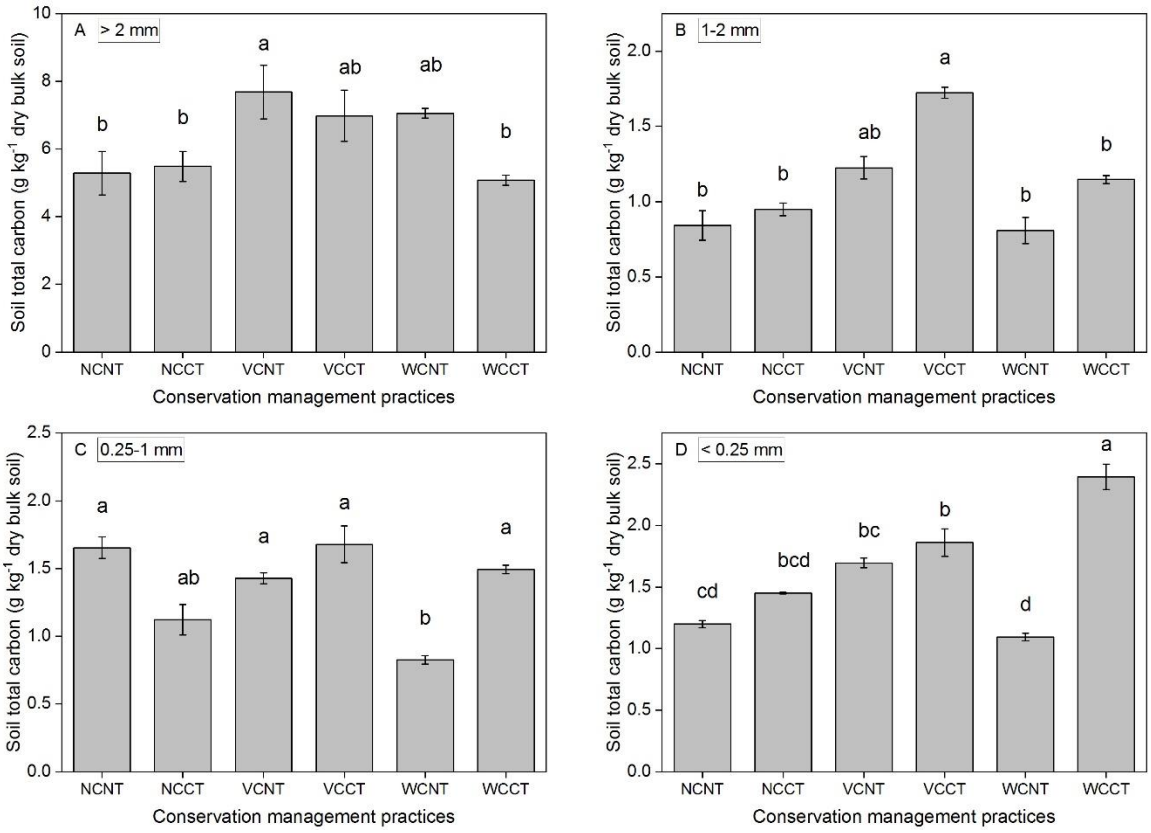

Figure 4.3 Soil total C concentrations in aggregate fractions.

A (> 2 mm): large macroaggregates; B (1-2 mm): medium macroaggregates; C (0.25-1 mm): small macroaggregates; D (< 0.25 mm): microaggregates; NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Bars indicate standard error. Error bars are at 95% confident interval. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.23 Main effect of cover crops on total C in large macroaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	5.3821	1.0682	B
Vetch cover crops	7.3270	1.0682	A
Wheat cover crops	5.8271	1.0682	AB

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.24 Main effect of tillage on total C in large macroaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	5.8450	1.0317	A
No-tillage	6.5123	1.0317	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.25 Interaction effect of cover crops and tillage on total C in large macroaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	5.4827	1.1707	B
No-cover crop	No-tillage	5.2815	1.1707	B
Vetch cover crops	Conventional tillage	6.9745	1.1707	AB
Vetch cover crops	No-tillage	7.6794	1.1707	A
Wheat cover crops	Conventional tillage	5.0779	1.1707	B
Wheat cover crops	No-tillage	6.5762	1.1707	AB

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.26 Main effect of cover crops on total C in medium macroaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	0.8950	0.1169	B
Vetch cover crops	1.4733	0.1169	A
Wheat cover crops	0.9770	0.1169	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.27 Main effect of tillage on total C in medium macroaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	1.2724	0.09543	A
No-tillage	0.9578	0.09543	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.28 Interaction effect of cover crops and tillage on total C in medium macroaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	0.9486	0.1653	B
No-cover crop	No-tillage	0.8415	0.1653	B
Vetch cover crops	Conventional tillage	1.7225	0.1653	A
Vetch cover crops	No-tillage	1.2241	0.1653	AB
Wheat cover crops	Conventional tillage	1.1462	0.1653	B
Wheat cover crops	No-tillage	0.8078	0.1653	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.29 Main effect of cover crops on total C in small macroaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	1.3878	0.2237	A
Vetch cover crops	1.5528	0.2237	A
Wheat cover crops	1.1593	0.2237	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.30 Main effect of tillage on total C in small macroaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	1.4313	0.1944	A
No-tillage	1.3019	0.1944	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.31 Interaction effect of cover crops and tillage on total C in small macroaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	1.1225	0.2946	AB
No-cover crop	No-tillage	1.6531	0.2946	A
Vetch cover crops	Conventional tillage	1.6785	0.2946	A
Vetch cover crops	No-tillage	1.4270	0.2946	A
Wheat cover crops	Conventional tillage	1.4929	0.2946	A
Wheat cover crops	No-tillage	0.8256	0.2946	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.32 Main effect of cover crops on total C in microaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	1.3251	0.1728	B
Vetch cover crops	1.7783	0.1728	A
Wheat cover crops	1.7440	0.1728	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.33 Main effect of tillage on total C in microaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	1.9019	0.1585	A
No-tillage	1.3297	0.1585	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.34 Interaction effect of cover crops and tillage on total C in microaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	1.4507	0.2097	BCD
No-cover crop	No-tillage	1.1994	0.2097	CD
Vetch cover crops	Conventional tillage	1.8611	0.2097	B
Vetch cover crops	No-tillage	1.6955	0.2097	BC
Wheat cover crops	Conventional tillage	2.3938	0.2097	A
Wheat cover crops	No-tillage	1.0941	0.2097	D

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Appendix 4E Concentrations of total N in aggregate fractions

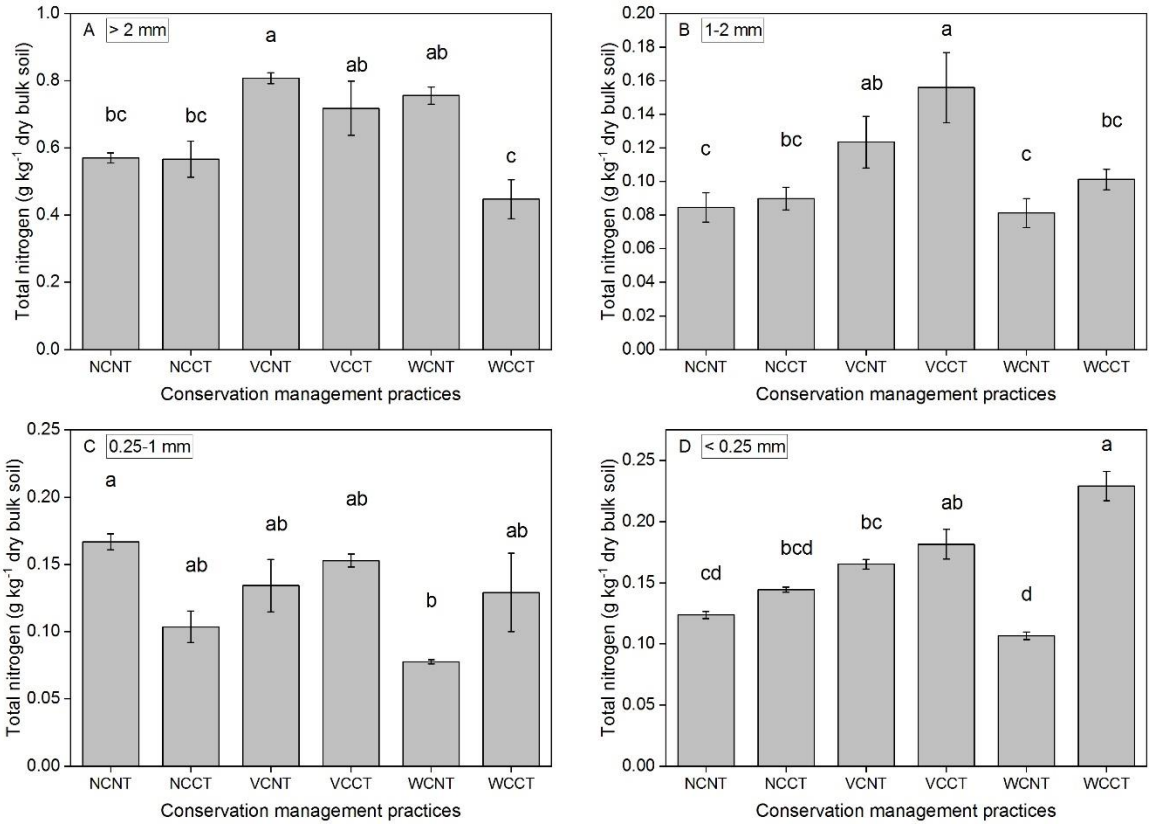

Figure 4.4 Soil total N concentrations in aggregate fractions.

A (> 2 mm): large macroaggregates; B (1-2 mm): medium macroaggregates; C (0.25-1 mm): small macroaggregates; D (< 0.25 mm): microaggregates; NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Bars indicate standard error. Error bars are at 95% confident interval. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.35 Main effect of cover crops on total N in large macroaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	0.5682	0.09896	A
Vetch cover crops	0.7623	0.09896	A
Wheat cover crops	0.5708	0.09896	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.36 Main effect of tillage on total N in large macroaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	0.5770	0.09238	B
No-tillage	0.6905	0.09238	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.37 Interaction effect of cover crops and tillage on total N in large macroaggregate fraction.

Cover	Standard Error	Mean	Standard Error of Mean	Letter Group
No-cover crop	0.1066	0.5662	0.1066	BC
No-cover crop	0.1066	0.5701	0.1066	BC
Vetch cover crops	0.1066	0.7177	0.1066	AB
Vetch cover crops	0.1066	0.8069	0.1066	A
Wheat cover crops	0.1066	0.4472	0.1066	C
Wheat cover crops	0.1066	0.6944	0.1066	AB

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.38 Main effect of cover crops on total N in medium macroaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	0.08708	0.008683	B
Vetch cover crops	0.1396	0.008683	A
Wheat cover crops	0.09120	0.008683	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.39 Main effect of tillage on total N in medium macroaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	0.1156	0.007090	A
No-tillage	0.09634	0.007090	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.40 Interaction effect of cover crops and tillage on total N in medium macroaggregate fraction.

Cover	Standard Error	Mean	Standard Error of Mean	Letter Group
No-cover crop	0.01228	0.08975	0.01228	BC
No-cover crop	0.01228	0.08441	0.01228	C
Vetch cover crops	0.01228	0.1559	0.01228	A
Vetch cover crops	0.01228	0.1234	0.01228	AB
Wheat cover crops	0.01228	0.1012	0.01228	BC
Wheat cover crops	0.01228	0.08122	0.01228	C

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.41 Main effect of cover crops on total N in small macroaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	0.1352	0.02129	A
Vetch cover crops	0.1435	0.02129	A
Wheat cover crops	0.1034	0.02129	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.42 Main effect of tillage on total N in small macroaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	0.1285	0.01827	A
No-tillage	0.1262	0.01827	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.43 Interaction effect of cover crops and tillage on total N in small macroaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	0.1035	0.02850	AB
No-cover crop	No-tillage	0.1668	0.02850	A
Vetch cover crops	Conventional tillage	0.1528	0.02850	AB
Vetch cover crops	No-tillage	0.1342	0.02850	AB
Wheat cover crops	Conventional tillage	0.1292	0.02850	AB
Wheat cover crops	No-tillage	0.07755	0.02850	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.44 Main effect of cover crops on total N in microaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	0.1341	0.01775	B
Vetch cover crops	0.1734	0.01775	A
Wheat cover crops	0.1679	0.01775	AB

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.45 Main effect of tillage on total N in microaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	0.1850	0.01645	A
No-tillage	0.1318	0.01645	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.46 Interaction effect of cover crops and tillage on total N in microaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	0.1444	0.02118	BCD
No-cover crop	No-tillage	0.1237	0.02118	CD
Vetch cover crops	Conventional tillage	0.1815	0.02118	AB
Vetch cover crops	No-tillage	0.1652	0.02118	BC
Wheat cover crops	Conventional tillage	0.2292	0.02118	A
Wheat cover crops	No-tillage	0.1066	0.02118	D

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Appendix 4F Soil C/N ration in aggregate fractions

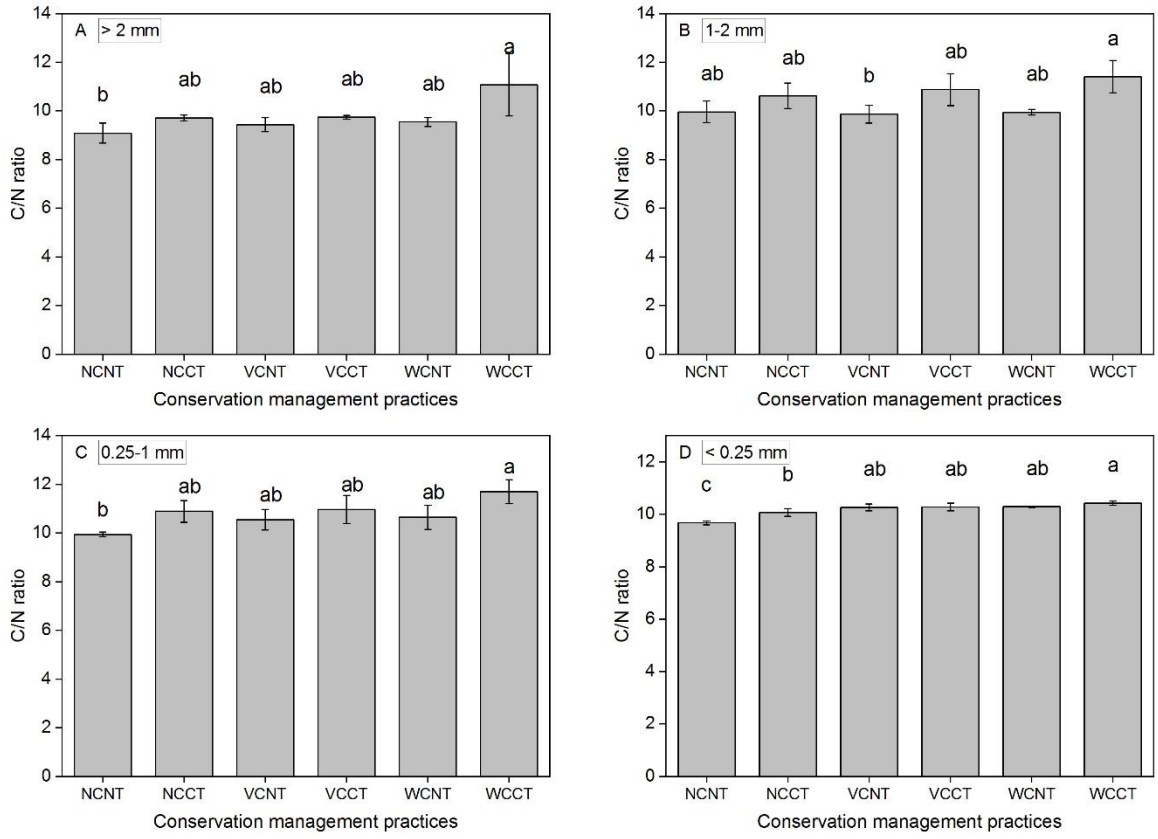

Figure 4.5 Soil C/N ratio in aggregate fractions.

A (> 2 mm): large macroaggregates; B (1-2 mm): medium macroaggregates; C (0.25-1 mm): small macroaggregates; D (< 0.25 mm): microaggregates; NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Bars indicate standard error. Error bars are at 95% confident interval. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.47 Main effect of cover crops on C/N ratio in large macroaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	9.3987	0.4126	A
Vetch cover crops	9.5854	0.4126	A
Wheat cover crops	10.3073	0.4126	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.48 Main effect of tillage on C/N ratio in large macroaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	10.1729	0.3458	A
No-tillage	9.3547	0.3458	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.49 Interaction effect of cover crops and tillage on C/N ratio in large macroaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	9.7117	0.5676	AB
No-cover crop	No-tillage	9.0856	0.5676	B
Vetch cover crops	Conventional tillage	9.7370	0.5676	AB
Vetch cover crops	No-tillage	9.4338	0.5676	AB
Wheat cover crops	Conventional tillage	11.0698	0.5676	A
Wheat cover crops	No-tillage	9.5449	0.5676	AB

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.50 Main effect of cover crops on C/N ratio in medium macroaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	10.2905	0.3518	A
Vetch cover crops	10.3727	0.3518	A
Wheat cover crops	10.6747	0.3518	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.51 Main effect of tillage on C/N ratio in medium macroaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	10.9690	0.2872	A
No-tillage	9.9230	0.2872	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.52 Interaction effect of cover crops and tillage on C/N ratio in medium macroaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	10.6220	0.4975	AB
No-cover crop	No-tillage	9.9590	0.4975	AB
Vetch cover crops	Conventional tillage	10.8751	0.4975	AB
Vetch cover crops	No-tillage	9.8703	0.4975	B
Wheat cover crops	Conventional tillage	11.4099	0.4975	A
Wheat cover crops	No-tillage	9.9395	0.4975	AB

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.53 Main effect of cover crops on C/N ratio in small macroaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	10.4147	0.3325	A
Vetch cover crops	10.7527	0.3325	A
Wheat cover crops	11.1748	0.3325	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.54 Main effect of tillage on C/N ratio in small macroaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	11.1856	0.2594	A
No-tillage	10.3759	0.2594	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.55 Interaction effect of cover crops and tillage on C/N ratio in small macroaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	10.8896	0.4492	AB
No-cover crop	No-tillage	9.9398	0.4492	B
Vetch cover crops	Conventional tillage	10.9628	0.4492	AB
Vetch cover crops	No-tillage	10.5427	0.4492	AB
Wheat cover crops	Conventional tillage	11.7043	0.4492	A
Wheat cover crops	No-tillage	10.6453	0.4492	AB

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.56 Main effect of cover crops on C/N ratio in microaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	9.8677	0.07817	B
Vetch cover crops	10.2689	0.07817	A
Wheat cover crops	10.3549	0.07817	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.57 Main effect of tillage on C/N ratio in microaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	10.2524	0.06382	A
No-tillage	10.0753	0.06382	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.58 Interaction effect of cover crops and tillage on C/N ratio in microaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	10.0627	0.1105	B
No-cover crop	No-tillage	9.6727	0.1105	C
Vetch cover crops	Conventional tillage	10.2733	0.1105	AB
Vetch cover crops	No-tillage	10.2645	0.1105	AB
Wheat cover crops	Conventional tillage	10.4211	0.1105	A
Wheat cover crops	No-tillage	10.2887	0.1105	AB

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Appendix 4G Concentrations of total amino sugars in aggregate fractions

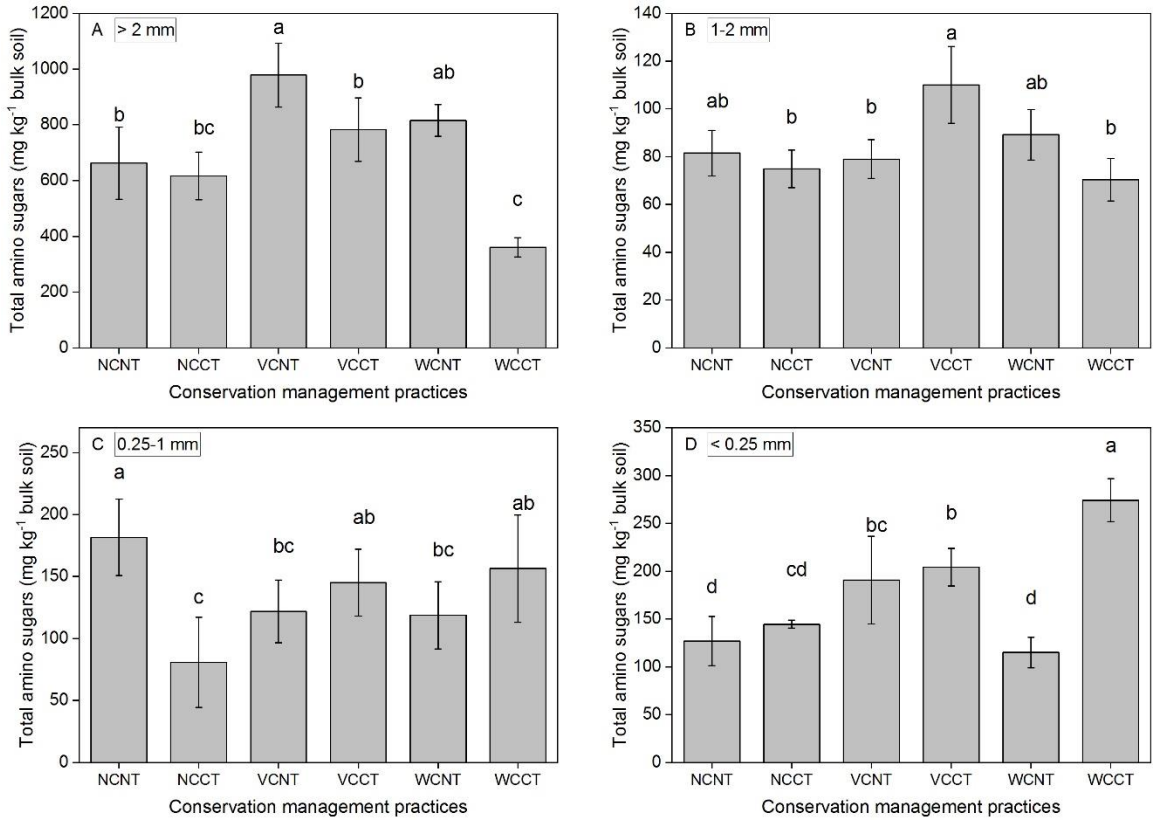

Figure 4.6 Soil total amino sugar concentrations in aggregate fractions.

A (> 2 mm): large macroaggregates; B (1-2 mm): medium macroaggregates; C (0.25-1 mm): small macroaggregates; D (< 0.25 mm): microaggregates; NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Bars indicate standard error. Error bars are at 95% confident interval. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.59 Main effect of cover crops on total amino sugars in large macroaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	639.51	88.8719	AB
Vetch cover crops	880.34	88.8719	A
Wheat cover crops	587.67	88.8719	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.60 Main effect of tillage on total amino sugars in large macroaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	586.21	71.2266	B
No-tillage	818.81	71.2266	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.61 Interaction effect of cover crops and tillage on total amino sugars in large macroaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	616.75	95.3242	BC
No-cover crop	No-tillage	662.28	95.3242	B
Vetch cover crops	Conventional tillage	782.04	95.3242	B
Vetch cover crops	No-tillage	978.65	95.3242	A
Wheat cover crops	Conventional tillage	359.83	95.3242	C
Wheat cover crops	No-tillage	815.51	95.3242	AB

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.62 Main effect of cover crops on total amino sugars in medium macroaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	78.2039	8.8381	A
Vetch cover crops	94.5218	8.8381	A
Wheat cover crops	79.7333	8.8381	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.63 Main effect of tillage on total amino sugars in medium macroaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	85.0901	6.0870	A
No-tillage	83.2159	6.0870	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.64 Interaction effect of cover crops and tillage on total amino sugars in medium macroaggregate fraction.

Cover	Standard Error	Mean	Standard Error of Mean	Letter Group
No-cover crop	10.5430	74.8861	10.5430	B
No-cover crop	10.5430	81.5218	10.5430	AB
Vetch cover crops	10.5430	110.05	10.5430	A
Vetch cover crops	10.5430	78.9938	10.5430	B
Wheat cover crops	10.5430	70.3344	10.5430	B
Wheat cover crops	10.5430	89.1322	10.5430	AB

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.65 Main effect of cover crops on total amino sugars in small macroaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	131.20	35.0390	A
Vetch cover crops	133.57	35.0390	A
Wheat cover crops	137.56	35.0390	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.66 Main effect of tillage on total amino sugars in small macroaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	127.46	31.3264	A
No-tillage	140.76	31.3264	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.67 Interaction effect of cover crops and tillage on total amino sugars in small macroaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	80.7437	44.3496	C
No-cover crop	No-tillage	181.66	44.3496	A
Vetch cover crops	Conventional tillage	145.23	44.3496	AB
Vetch cover crops	No-tillage	121.91	44.3496	BC
Wheat cover crops	Conventional tillage	156.39	44.3496	AB
Wheat cover crops	No-tillage	118.72	44.3496	BC

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.68 Main effect of cover crops on total amino sugars in microaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	135.76	21.9052	B
Vetch cover crops	197.56	21.9052	A
Wheat cover crops	194.65	21.9052	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.69 Main effect of tillage on total amino sugars in microaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	207.71	20.5247	A
No-tillage	144.28	20.5247	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.70 Interaction effect of cover crops and tillage on total amino sugars in microaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	144.59	25.6038	CD
No-cover crop	No-tillage	126.93	25.6038	D
Vetch cover crops	Conventional tillage	204.23	25.6038	B
Vetch cover crops	No-tillage	190.90	25.6038	BC
Wheat cover crops	Conventional tillage	274.30	25.6038	A
Wheat cover crops	No-tillage	115.00	25.6038	D

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Appendix 4H Concentrations of glucosamine in aggregate fractions

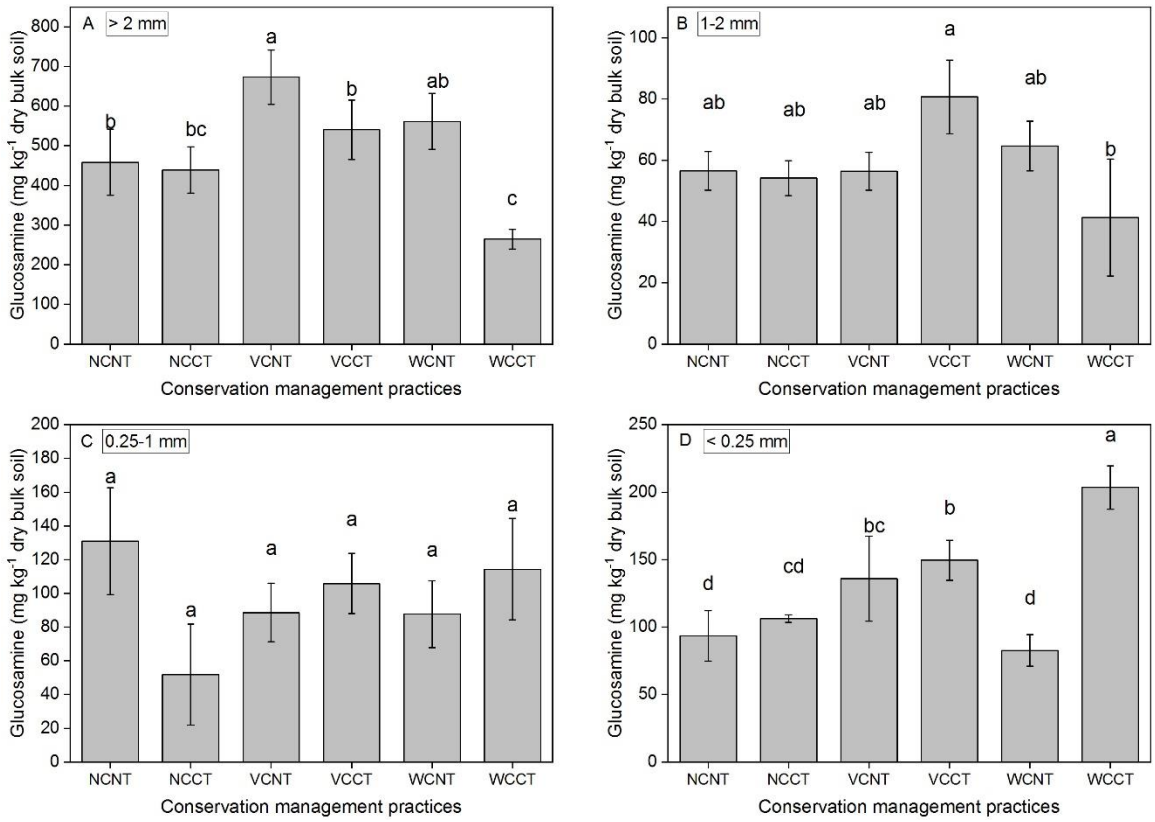

Figure 4.7 Soil glucosamine concentrations in aggregate fractions.

A (> 2 mm): large macroaggregates; B (1-2 mm): medium macroaggregates; C (0.25-1 mm): small macroaggregates; D (< 0.25 mm): microaggregates; NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Bars indicate standard error. Error bars are at 95% confident interval. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.71 Main effect of cover crops on glucosamine in large macroaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	448.38	57.0799	AB
Vetch cover crops	606.59	57.0799	A
Wheat cover crops	416.28	57.0799	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.72 Main effect of tillage on glucosamine in large macroaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	414.48	45.1030	B
No-tillage	566.35	45.1030	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.73 Interaction effect of cover crops and tillage on glucosamine in large macroaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	438.56	61.7147	BC
No-cover crop	No-tillage	458.21	61.7147	B
Vetch cover crops	Conventional tillage	540.31	61.7147	B
Vetch cover crops	No-tillage	672.88	61.7147	A
Wheat cover crops	Conventional tillage	264.57	61.7147	C
Wheat cover crops	No-tillage	567.98	61.7147	AB

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.74 Main effect of cover crops on glucosamine in medium macroaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	55.3438	8.3865	A
Vetch cover crops	68.4963	8.3865	A
Wheat cover crops	52.9540	8.3865	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.75 Main effect of tillage on glucosamine in medium macroaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	58.6993	6.1645	A
No-tillage	59.1634	6.1645	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.76 Interaction effect of cover crops and tillage on glucosamine in medium macroaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	54.1538	10.6772	AB
No-cover crop	No-tillage	56.5338	10.6772	AB
Vetch cover crops	Conventional tillage	80.6186	10.6772	A
Vetch cover crops	No-tillage	56.3740	10.6772	AB
Wheat cover crops	Conventional tillage	41.3255	10.6772	B
Wheat cover crops	No-tillage	64.5825	10.6772	AB

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.77 Main effect of cover crops on glucosamine in small macroaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	91.4496	25.0988	A
Vetch cover crops	97.1837	25.0988	A
Wheat cover crops	100.99	25.0988	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.78 Main effect of tillage on glucosamine in small macroaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	90.6651	22.4464	A
No-tillage	102.41	22.4464	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.79 Interaction effect of cover crops and tillage on glucosamine in small macroaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	51.9222	31.7535	A
No-cover crop	No-tillage	130.98	31.7535	A
Vetch cover crops	Conventional tillage	105.78	31.7535	A
Vetch cover crops	No-tillage	88.5884	31.7535	A
Wheat cover crops	Conventional tillage	114.29	31.7535	A
Wheat cover crops	No-tillage	87.6794	31.7535	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.80 Main effect of cover crops on glucosamine in microaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	99.8770	15.6369	B
Vetch cover crops	142.77	15.6369	A
Wheat cover crops	143.10	15.6369	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.81 Main effect of tillage on glucosamine in microaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	153.10	14.6866	A
No-tillage	104.06	14.6866	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.82 Interaction effect of cover crops and tillage on glucosamine in microaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	106.26	18.1105	CD
No-cover crop	No-tillage	93.4927	18.1105	D
Vetch cover crops	Conventional tillage	149.58	18.1105	B
Vetch cover crops	No-tillage	135.97	18.1105	BC
Wheat cover crops	Conventional tillage	203.47	18.1105	A
Wheat cover crops	No-tillage	82.7288	18.1105	D

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Appendix 4I Concentrations of muramic acid in aggregate fractions

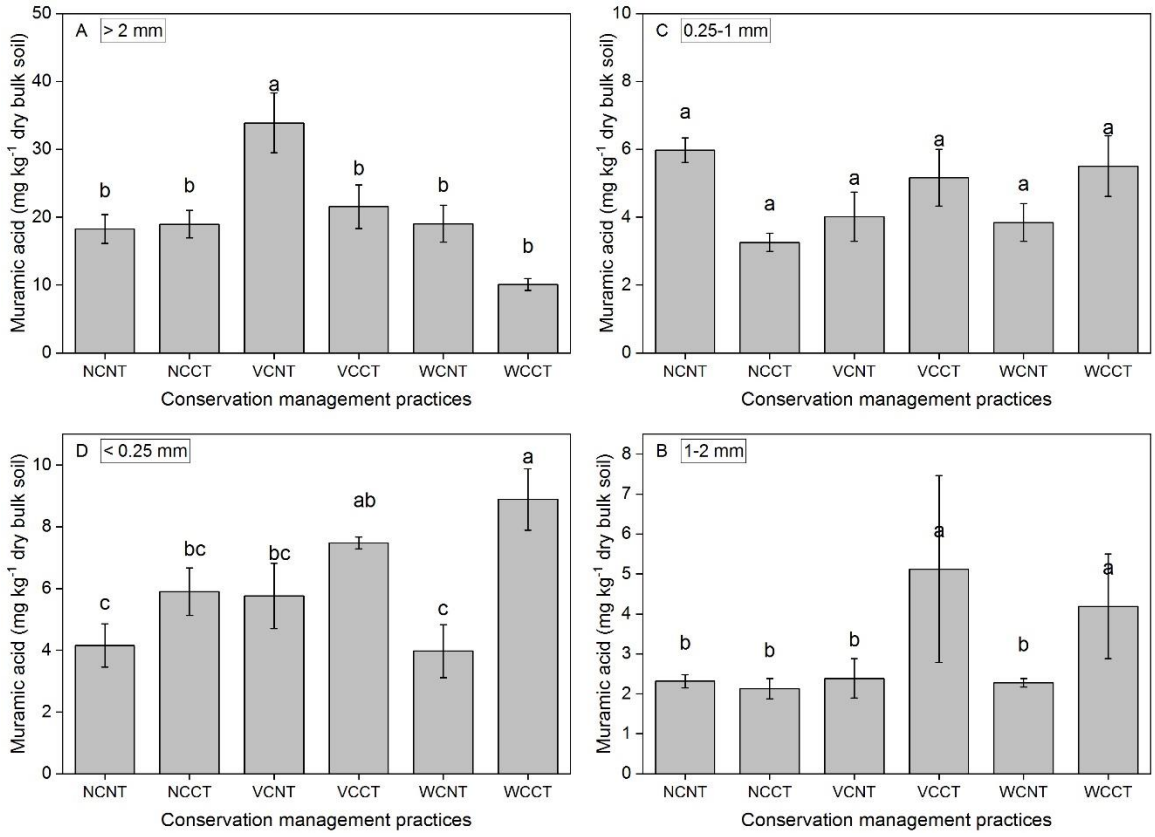

Figure 4.8 Soil muramic acid concentrations in aggregate fractions.

A (> 2 mm): large macroaggregates; B (1-2 mm): medium macroaggregates; C (0.25-1 mm): small macroaggregates; D (< 0.25 mm): microaggregates; NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Bars indicate standard error. Error bars are at 95% confident interval. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.83 Main effect of cover crops on muramic acid in large macroaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	18.6101	3.6489	A
Vetch cover crops	27.7196	3.6489	A
Wheat cover crops	14.5311	3.6489	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.84 Main effect of tillage on muramic acid in large macroaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	16.8634	2.5309	B
No-tillage	23.7105	2.5309	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.85 Interaction effect of cover crops and tillage on muramic acid in large macroaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	18.9654	4.1654	B
No-cover crop	No-tillage	18.2549	4.1654	B
Vetch cover crops	Conventional tillage	21.5603	4.1654	B
Vetch cover crops	No-tillage	33.8790	4.1654	A
Wheat cover crops	Conventional tillage	10.0644	4.1654	B
Wheat cover crops	No-tillage	18.9977	4.1654	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.86 Main effect of cover crops on muramic acid in medium macroaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	2.2204	0.9064	A
Vetch cover crops	3.7563	0.9064	A
Wheat cover crops	3.2309	0.9064	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.87 Main effect of tillage on muramic acid in medium macroaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	3.8128	0.7464	A
No-tillage	2.3255	0.7464	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.88 Interaction effect of cover crops and tillage on muramic acid in medium macroaggregate fraction.

Cover	Standard Error	Mean	Standard Error of Mean	Letter Group
No-cover crop	1.1216	2.1257	1.1216	A
No-cover crop	1.1216	2.3151	1.1216	A
Vetch cover crops	1.1216	5.1245	1.1216	A
Vetch cover crops	1.1216	2.3880	1.1216	A
Wheat cover crops	1.1216	4.1884	1.1216	A
Wheat cover crops	1.1216	2.2735	1.1216	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.89 Main effect of cover crops on muramic acid in small macroaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	4.6145	1.0797	A
Vetch cover crops	4.5877	1.0797	A
Wheat cover crops	4.6745	1.0797	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.90 Main effect of tillage on muramic acid in small macroaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	4.6405	0.9326	A
No-tillage	4.6107	0.9326	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.91 Interaction effect of cover crops and tillage on muramic acid in small macroaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	3.2566	1.4332	A
No-cover crop	No-tillage	5.9724	1.4332	A
Vetch cover crops	Conventional tillage	5.1604	1.4332	A
Vetch cover crops	No-tillage	4.0150	1.4332	A
Wheat cover crops	Conventional tillage	5.5044	1.4332	A
Wheat cover crops	No-tillage	3.8447	1.4332	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.92 Main effect of cover crops on muramic acid in microaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	5.0279	0.5752	A
Vetch cover crops	6.6183	0.5752	A
Wheat cover crops	6.4291	0.5752	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.93 Main effect of tillage on muramic acid in microaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	7.4216	0.4702	A
No-tillage	4.6286	0.4702	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.94 Interaction effect of cover crops and tillage on muramic acid in microaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	5.9000	0.8125	BC
No-cover crop	No-tillage	4.1558	0.8125	C
Vetch cover crops	Conventional tillage	7.4771	0.8125	AB
Vetch cover crops	No-tillage	5.7595	0.8125	BC
Wheat cover crops	Conventional tillage	8.8877	0.8125	A
Wheat cover crops	No-tillage	3.9704	0.8125	C

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Appendix 4J Concentrations of galactosamine in aggregate fractions

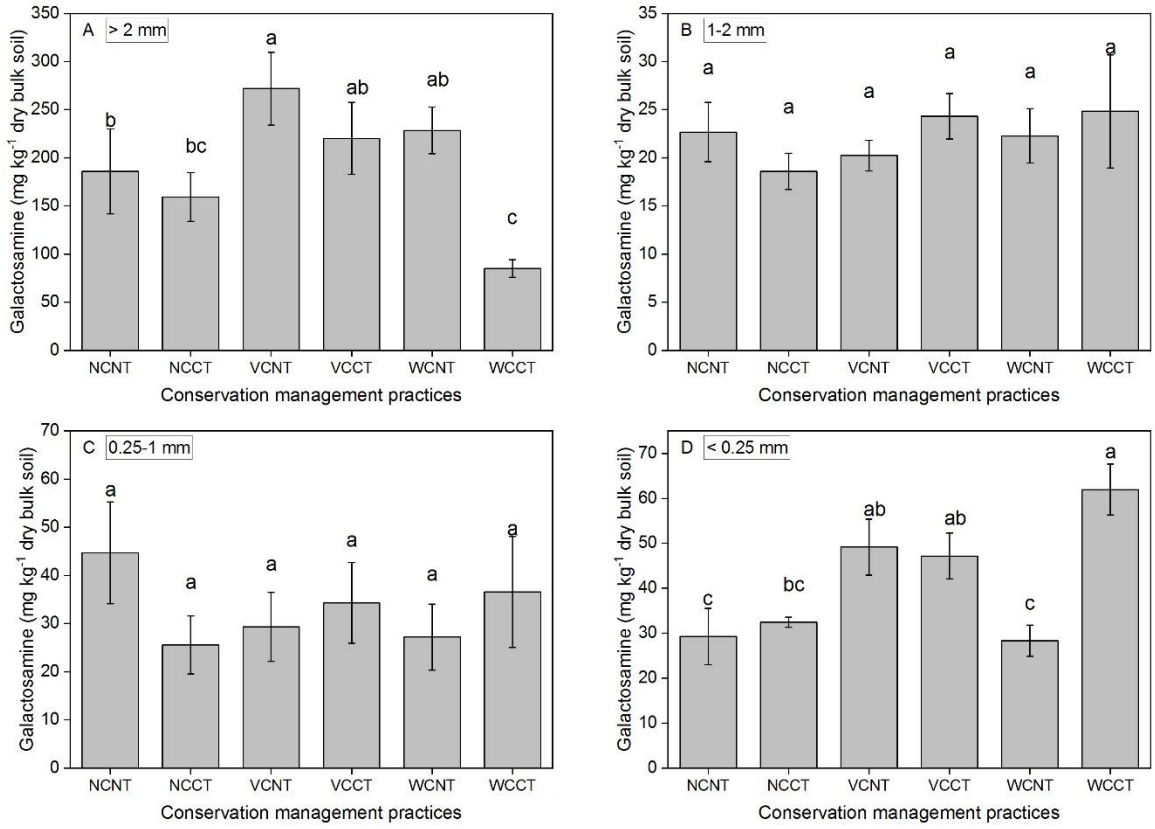

Figure 4.9 Soil galactosamine concentrations in aggregate fractions.

A (> 2 mm): large macroaggregates; B (1-2 mm): medium macroaggregates; C (0.25-1 mm): small macroaggregates; D (< 0.25 mm): microaggregates; NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage. Bars indicate standard error. Error bars are at 95% confident interval. Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.95 Main effect of cover crops on galactosamine in large macroaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	172.52	28.9350	AB
Vetch cover crops	246.03	28.9350	A
Wheat cover crops	156.86	28.9350	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.96 Main effect of tillage on galactosamine in large macroaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	154.86	23.9447	B
No-tillage	228.75	23.9447	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.97 Interaction effect of cover crops and tillage on galactosamine in large macroaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	159.23	30.8272	BC
No-cover crop	No-tillage	185.82	30.8272	B
Vetch cover crops	Conventional tillage	220.17	30.8272	AB
Vetch cover crops	No-tillage	271.89	30.8272	A
Wheat cover crops	Conventional tillage	85.1872	30.8272	C
Wheat cover crops	No-tillage	228.54	30.8272	AB

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.98 Main effect of cover crops on galactosamine in medium macroaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	20.6398	3.8641	A
Vetch cover crops	22.2692	3.8642	A
Wheat cover crops	23.5484	3.4877	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.99 Main effect of tillage on galactosamine in medium macroaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	22.5780	2.6463	A
No-tillage	21.7270	2.8016	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.100 Interaction effect of cover crops and tillage on galactosamine in medium macroaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	18.6066	4.6482	A
No-cover crop	No-tillage	22.6729	4.7538	A
Vetch cover crops	Conventional tillage	24.3067	4.6046	A
Vetch cover crops	No-tillage	20.2317	4.7582	A
Wheat cover crops	Conventional tillage	24.8206	4.2760	A
Wheat cover crops	No-tillage	22.2763	4.3662	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.101 Main effect of cover crops on galactosamine in small macroaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	35.1352	9.2352	A
Vetch cover crops	31.8020	9.0152	A
Wheat cover crops	31.8956	8.5001	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.102 Main effect of tillage on galactosamine in small macroaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	32.1513	7.9497	A
No-tillage	33.7373	7.9248	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.103 Interaction effect of cover crops and tillage on galactosamine in small macroaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	25.5649	11.7410	A
No-cover crop	No-tillage	44.7056	11.4756	A
Vetch cover crops	Conventional tillage	34.2944	11.5417	A
Vetch cover crops	No-tillage	29.3096	11.5406	A
Wheat cover crops	Conventional tillage	36.5946	10.5571	A
Wheat cover crops	No-tillage	27.1966	10.9058	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.104 Main effect of cover crops on galactosamine in microaggregate fraction.

Cover	Mean	Standard Error of Mean	Letter Group
No-cover crop	30.8563	5.8801	B
Vetch cover crops	48.1702	5.8801	A
Wheat cover crops	45.1283	5.8801	A

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.105 Main effect of tillage on galactosamine in microaggregate fraction.

Tillage	Mean	Standard Error of Mean	Letter Group
Conventional tillage	47.1844	5.4891	A
No-tillage	35.5855	5.4891	B

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Table 4.106 Interaction effect of cover crops and tillage on galactosamine in microaggregate fraction.

Cover	Tillage	Mean	Standard Error of Mean	Letter Group
No-cover crop	Conventional tillage	32.4279	6.9219	BC
No-cover crop	No-tillage	29.2847	6.9219	C
Vetch cover crops	Conventional tillage	47.1732	6.9219	AB
Vetch cover crops	No-tillage	49.1672	6.9219	A
Wheat cover crops	Conventional tillage	61.9521	6.9219	A
Wheat cover crops	No-tillage	28.3044	6.9219	C

Shared letters denote no significant difference at 5% level between treatments (ANOVA, Fisher's LSD, n = 3).

Appendix 4K Strctural equation models for total amino sugars, glucosamine, muramic acid, and galactosamine

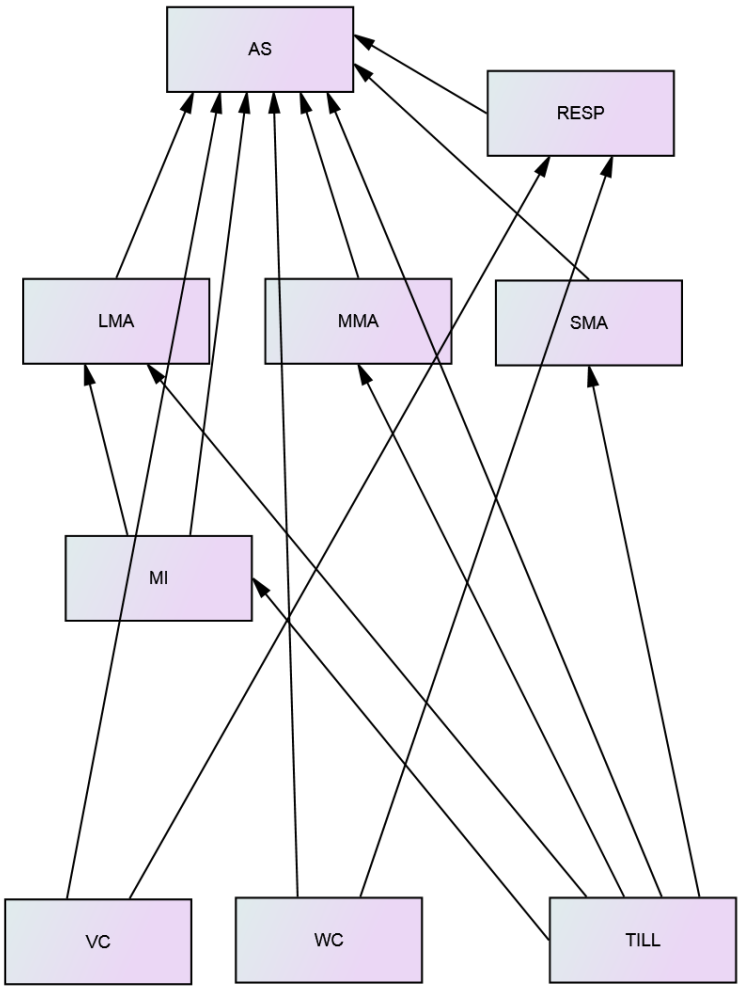

Figure 4.10 The *a priori* model for controls of total amino sugar accumulation in bulk soil.

AS: total amino sugars; RESP: soil microbial respiration; LMA: large macroaggregate content; MMA: medium macroaggregate content; SMA: small aggregate content; MI: microaggregate content; VC: vetch cover crops; WC: wheat cover crops; TILL: tillage. Boxes represent variables. Single headed arrows represent causal relationships.

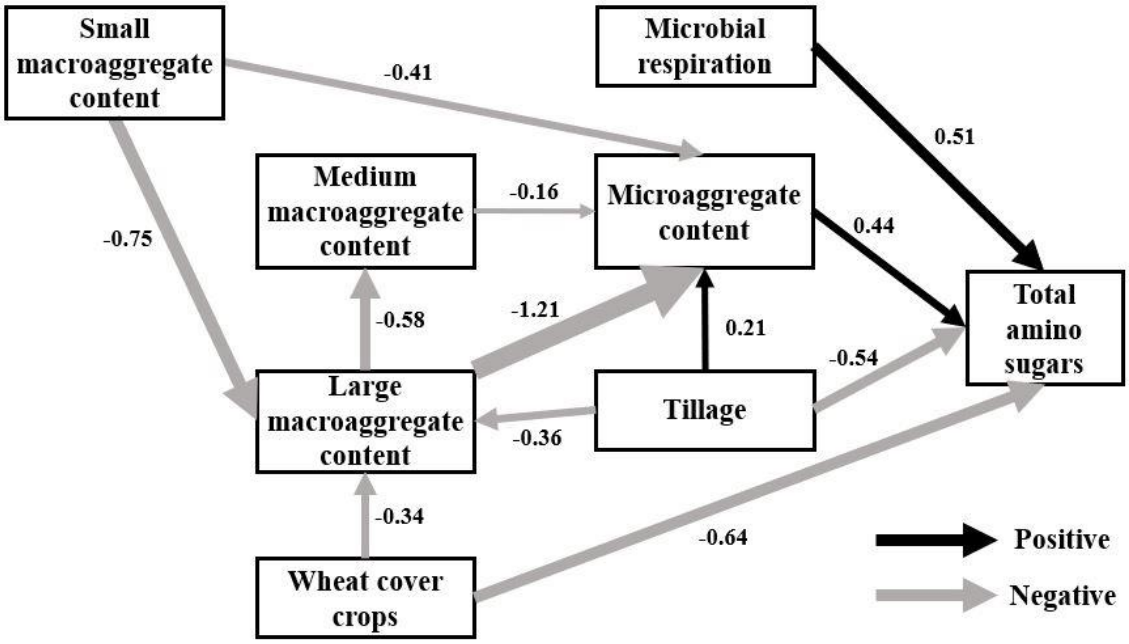

Figure 4.11 Structural equation model for controls of total amino sugar accumulation in bulk soil.

Boxes indicate variables. A arrow represents a causal relationship ($P < 0.05$). Arrow direction indicates the direction of causation. Arrow width indicates effect size. A black arrow denotes positive relationship, and gray arrow negative relationship. Numbers beside arrows are standardized path coefficients. CMIN/DF = 0.747, $P = 0.747$, CFI = 1.000, RMSEA = 0.000, PCLOSE = 0.780, $R^2 = 0.65$. All variables were log transformed for normality.

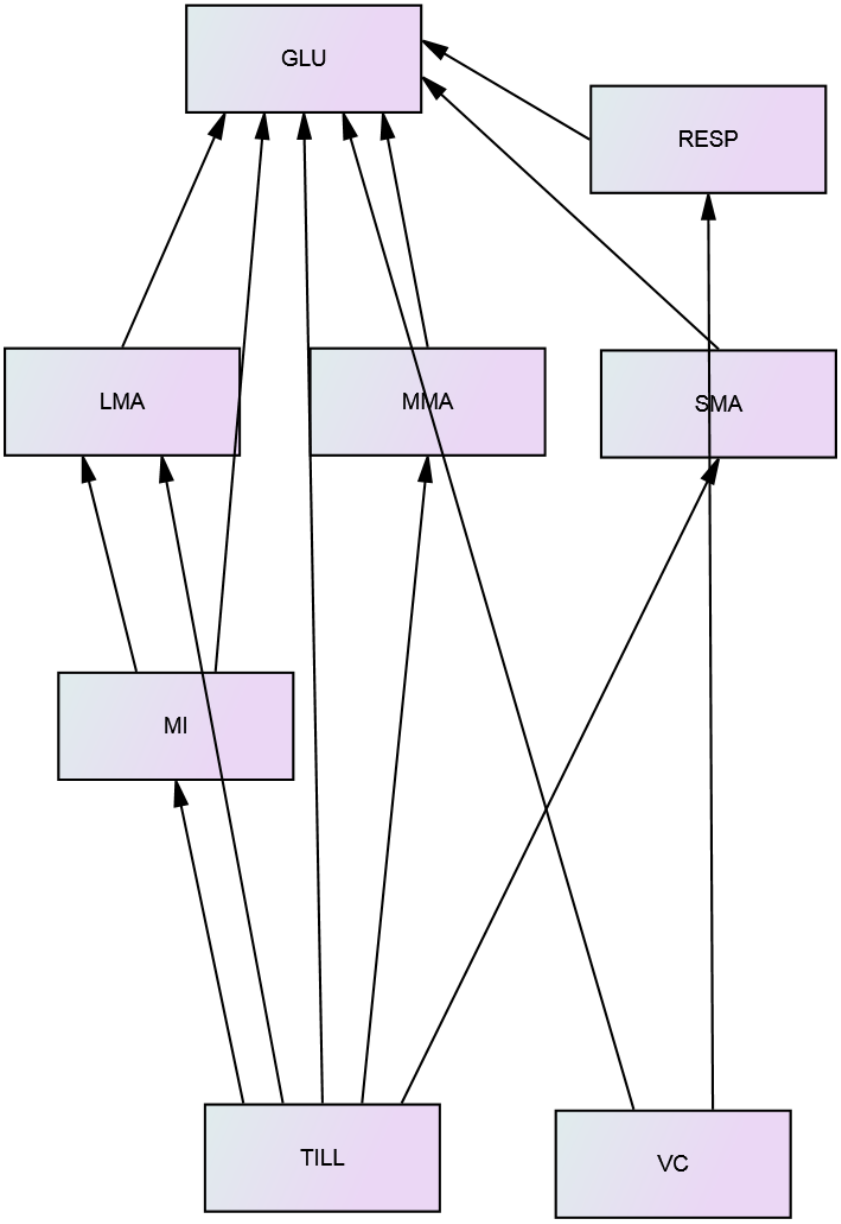

Figure 4.12 The *a priori* model for controls of glucosamine accumulation in bulk soil.

GLU: glucosamine; RESP: soil microbial respiration; LMA: large macroaggregate content; MMA: medium macroaggregate content; SMA: small aggregate content; MI: microaggregate content; VC: vetch cover crops; TILL: tillage. Boxes represent variables. Single headed arrows represent causal relationships.

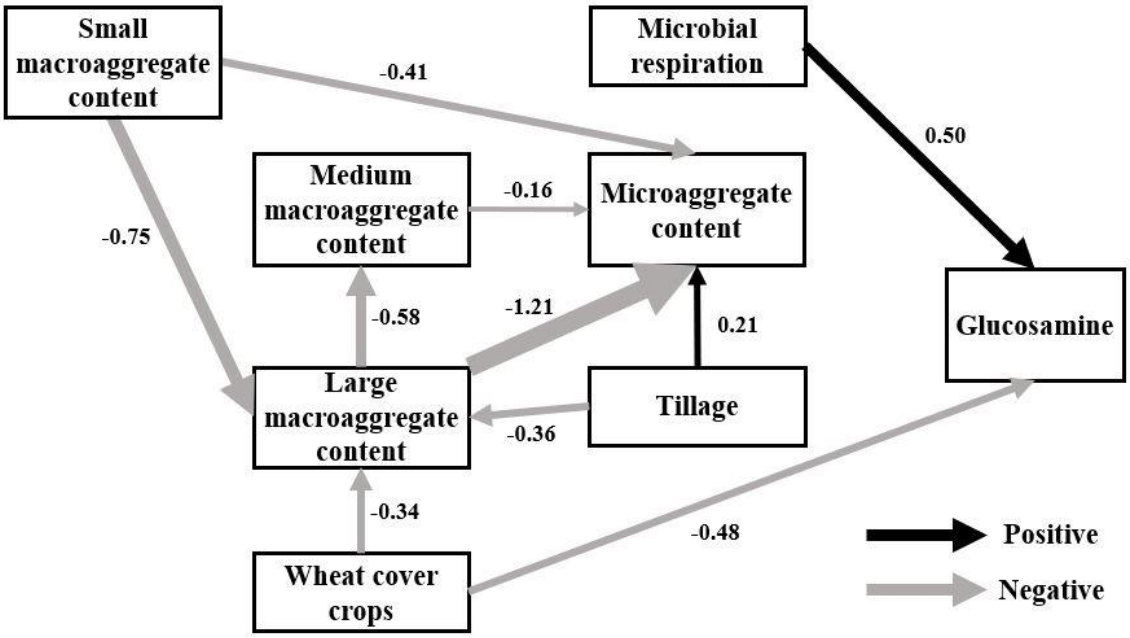

Figure 4.13 Structural equation model for controls of glucosamine accumulation in bulk soil.

Boxes indicate variables. A arrow represents a causal relationship ($P < 0.05$). Arrow direction indicates the direction of causation. Arrow width indicates effect size. A black arrow denotes positive relationship, and gray arrow negative relationship. Numbers beside arrows are standardized path coefficients. CMIN/DF = 0.902, $P = 0.576$, CFI = 1.000, RMSEA = 0.000, PCLOSE = 0.622, $R^2 = 0.48$. All variables were log transformed for normality.

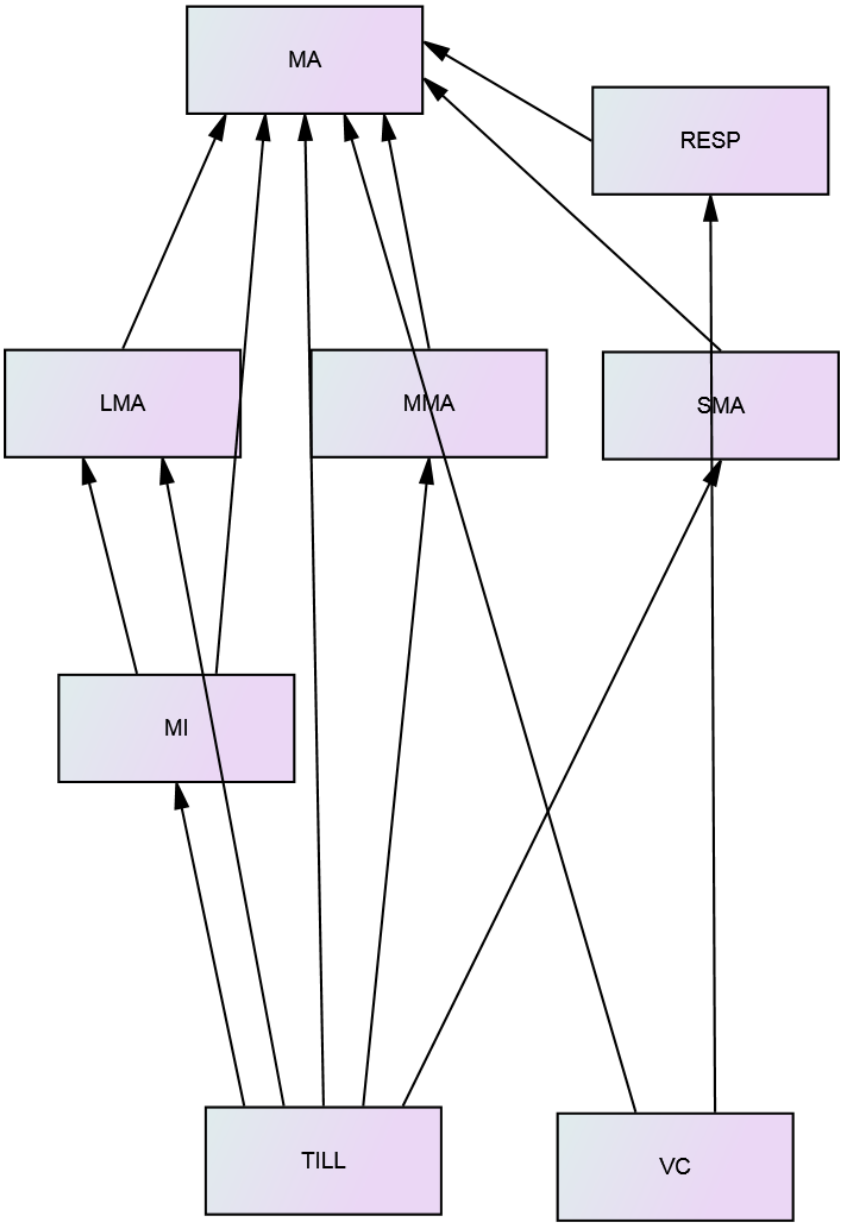

Figure 4.14 The *a priori* model for controls of muramic acid accumulation in bulk soil.

MA: muramic acid; RESP: soil microbial respiration; LMA: large macroaggregate content; MMA: medium macroaggregate content; SMA: small aggregate content; MI: microaggregate content; VC: vetch cover crops; TILL: tillage. Boxes represent variables. Single headed arrows represent causal relationships.

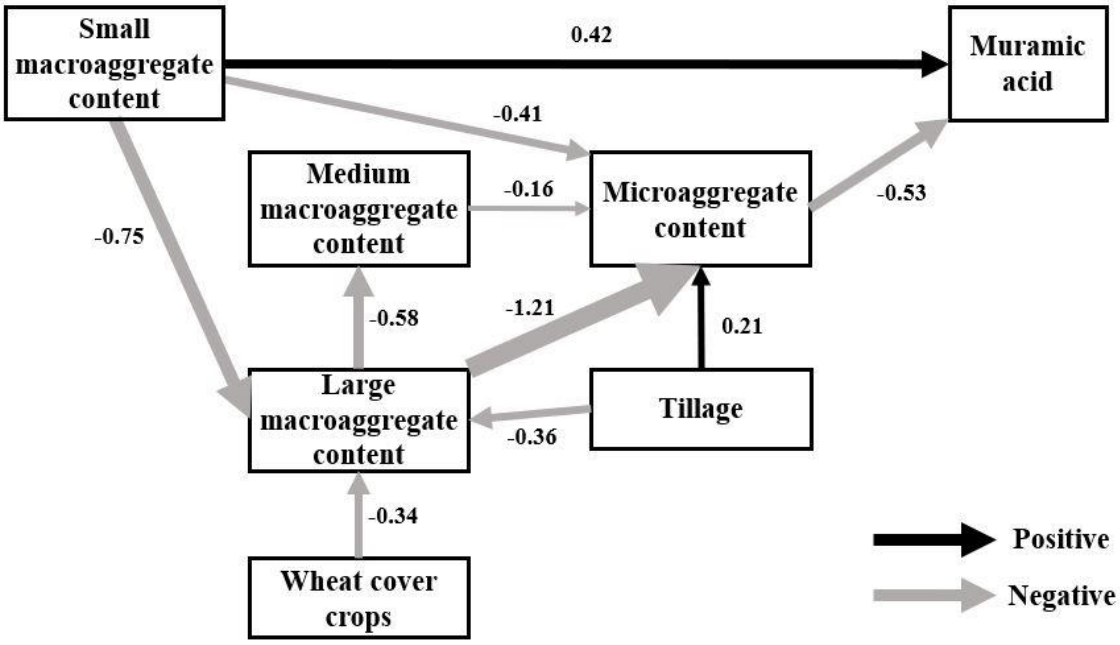

Figure 4.15 Structural equation model for controls of muramic acid accumulation in bulk soil.

Boxes indicate variables. A arrow represents a causal relationship ($P < 0.05$). Arrow direction indicates the direction of causation. Arrow width indicates effect size. A black arrow denotes positive relationship, and gray arrow negative relationship. Numbers beside arrows are standardized path coefficients. CMIN/DF = 0.351, $P = 0.974$, CFI = 1.000, RMSEA = 0.000, PCLOSE = 0.978, $R^2 = 0.27$. All variables were log transformed for normality.

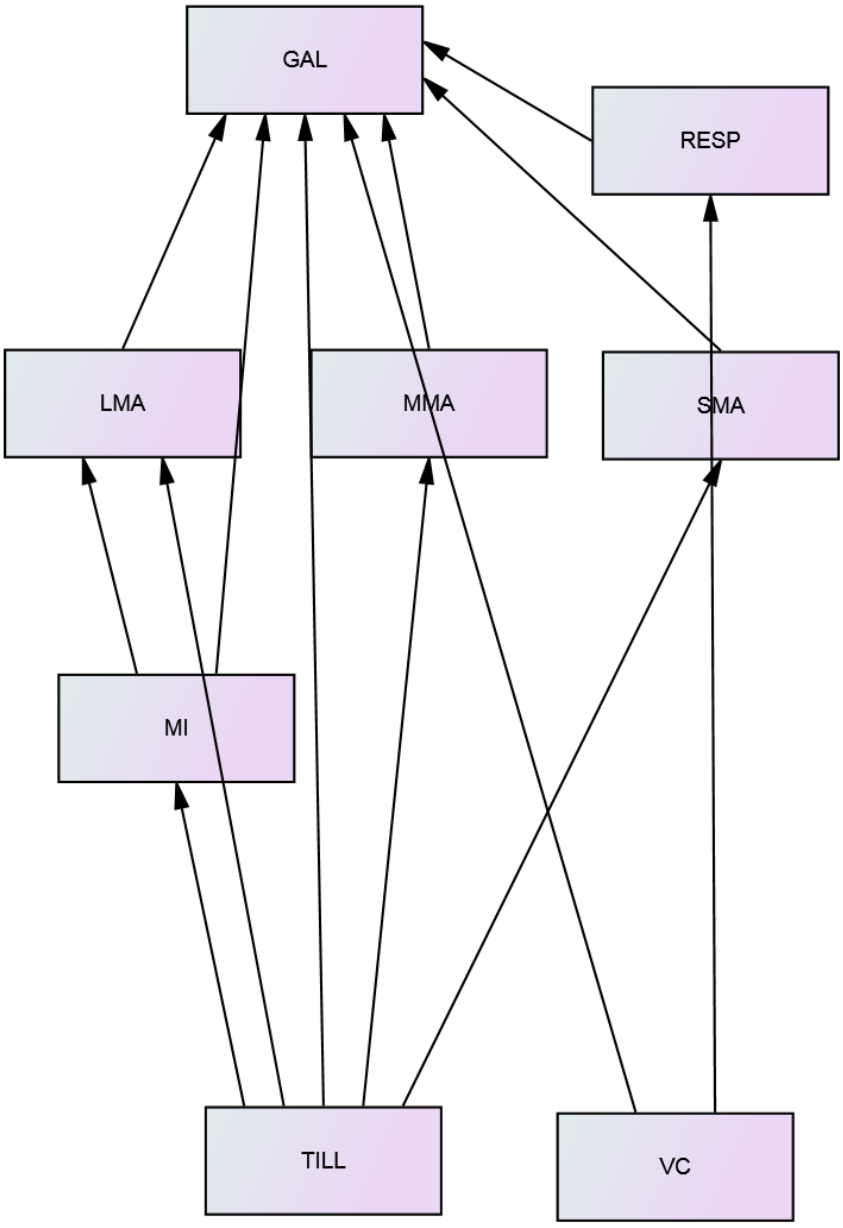

Figure 4.16 The *a priori* model for controls of galactosamine accumulation in bulk soil.

GAL: galactosamine; RESP: soil microbial respiration; LMA: large macroaggregate content; MMA: medium macroaggregate content; SMA: small aggregate content; MI: microaggregate content; VC: vetch cover crops; TILL: tillage. Boxes represent variables. Single headed arrows represent causal relationships.

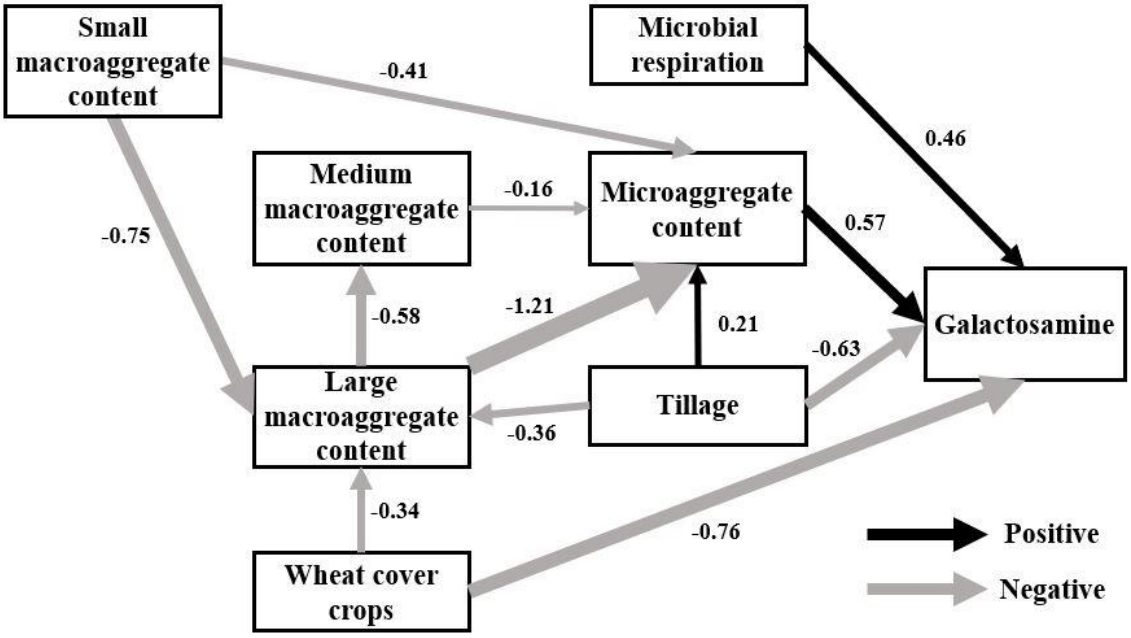

Figure 4.17 Structural equation model for controls of galactosamine accumulation in bulk soil.

Boxes indicate variables. A arrow represents a causal relationship ($P < 0.05$). Arrow direction indicates the direction of causation. Arrow width indicates effect size. A black arrow denotes positive relationship, and gray arrow negative relationship. Numbers beside arrows are standardized path coefficients. CMIN/DF = 0.918, $P = 0.577$, CFI = 1.000, RMSEA = 0.000, PCLOSE = 0.631, $R^2 = 0.74$. All variables were log transformed for normality.

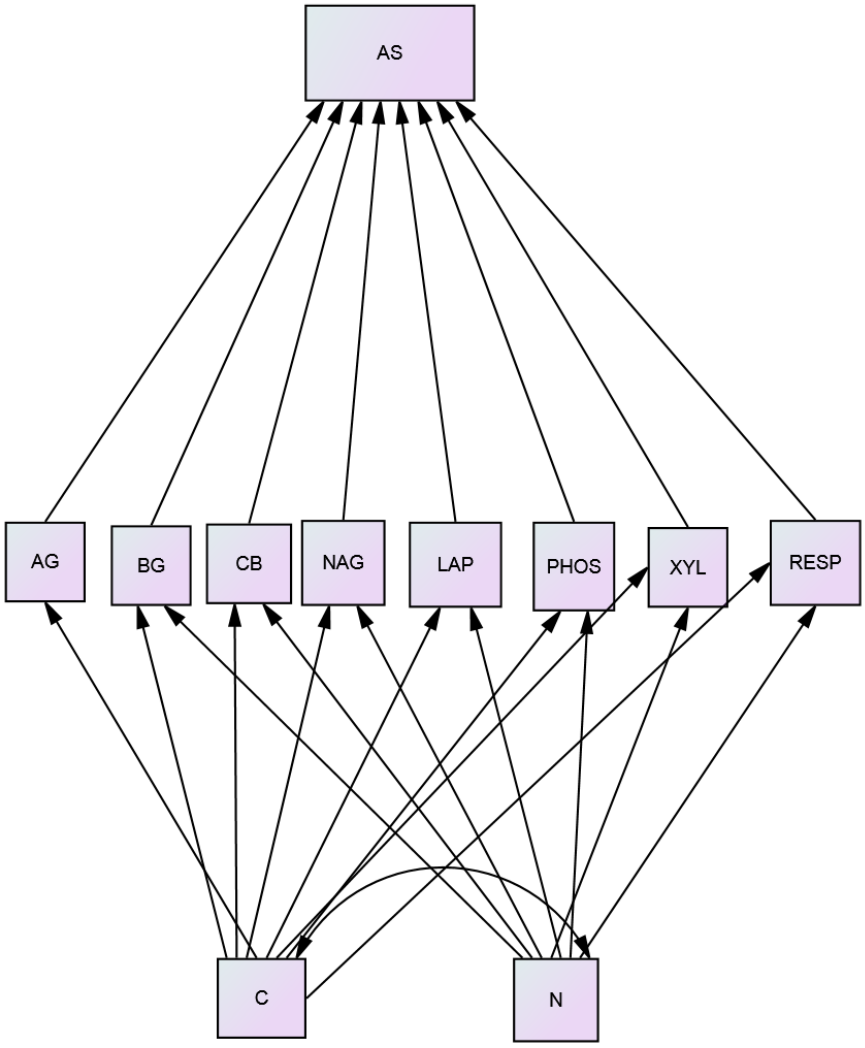

Figure 4.18 The *a priori* model for controls of total amino sugar accumalation in soil aggregate fractions.

AS: total amino sugars; AG: α-Glucosidase; BG: β-Glucosidase; CB: β-D-Cellubiosidase; NAG: N-actyl-β-D-Glucosaminidase; LAP: Leucine aminopeptidase; PHOS: Phosphatase; XYL: β-Xylosidase; RESP: soil microbial respiration; C: carbon; N: nitrogen. Boxes represent variables. Single headed arrows represent causal relationships. Double headed arrow represents correlation.

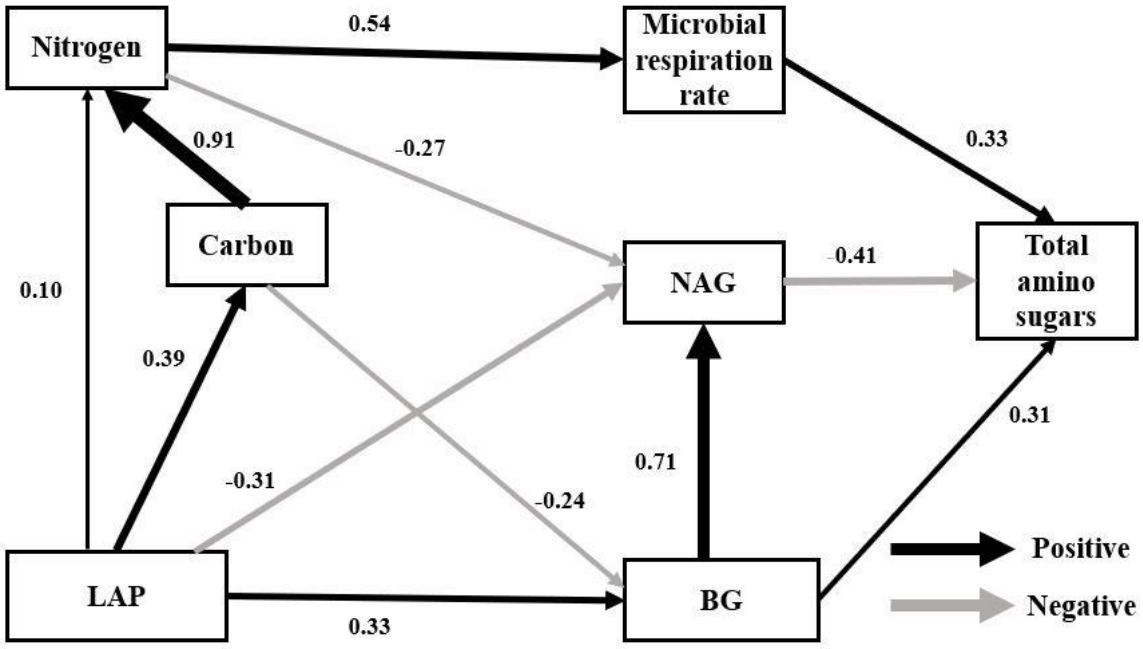

Figure 4.19 Structural equation model for controls of total amino sugar accumalation in soil aggregate fractions.

NAG: β-N-acetylglucosaminidase activity; LAP: leucine aminopeptidase activity; BG: β-Glucosidase activity. Boxes indicate variables. A arrow represents a causal relationship ($P < 0.05$). Arrow direction indicates the direction of causation. Arrow width indicates effect size. A black arrow denotes positive relationship, and gray arrow negative relationship. Numbers beside arrows are standardized path coefficients. CMIN/DF = 1.407, $P = 0.178$, CFI = 0.988, RMSEA = 0.076, PCLOSE = 0.293, $R^2 = 0.26$. All variables were log transformed for normality.

Table 4.107 Standardized total effects of microbial respiration, wheat cover crop, tillage, and soil aggregate composition on amino sugar accumulation in bulk soil and on soil aggregate composition.

Factors →	Total amino sugar	Glucosamine	Muramic acid	Galactosamine	> 2 mm	1-2 mm	0.25-1 mm	< 0.25 mm
Microbial respiration→	0.51	0.50	0.00	0.46	0.00	0.00	0.00	0.00
Wheat cover crop→	-0.48	-0.48	-0.20	-0.54	-0.34	0.20	0.00	0.38
Tillage→	-0.26	0.00	-0.33	-0.28	-0.36	0.21	0.00	0.62
> 2 mm→	-0.49	0.00	0.59	-0.64	-	-0.58	0.00	-1.12
1-2 mm→	-0.07	0.00	0.08	-0.09	0.00	-	0.00	-0.16
0.25-1 mm→	0.19	0.00	-0.23	0.24	-0.75	0.43	-	0.43
< 0.25 mm→	0.44	0.00	-0.53	0.57	0.00	0.00	0.00	-

> 2 mm: large macroaggregates; 1-2 mm: medium macroaggregates; 0.25-1 mm: small macroaggregates; < 0.25 mm: microaggregates. Arrows indicate directions of causal relationships. All effects are significant ($P < 0.05$), except small aggregates to muramic acid ($P = 0.07$).

Table 4.108 Characteristics of bulk soil samples used in the structural equation model.

Treatments	Total amino sugar (mg kg^{-1} bulk soil)	Glucosamine (mg kg^{-1} bulk soil)	Muramic Acid (mg kg^{-1} bulk soil)	Galactosamine (mg kg^{-1} bulk soil)	Large macroaggregate content* (%)	Medium macroaggregate content* (%)	Small macroaggregate content* (%)	Microaggregate content* (%)
NCNT	1302.57	906.18	39.51	356.87	0.57	0.13	0.17	0.13
NCNT	1715.48	1141.89	49.99	523.61	0.49	0.07	0.26	0.18
NCNT	1597.72	1089.51	54.28	453.93	0.76	0.06	0.08	0.10
NCCT	1014.26	700.18	34.78	279.29	0.56	0.11	0.14	0.19
NCCT	832.57	581.20	25.31	226.06	0.65	0.08	0.09	0.18
NCCT	1306.68	916.25	37.48	352.95	0.68	0.09	0.09	0.14
VCNT	1141.53	791.49	30.58	319.46	0.70	0.10	0.11	0.09
VCNT	1787.98	1202.77	44.55	540.65	0.54	0.12	0.14	0.19
VCNT	1211.52	869.52	39.37	302.63	0.75	0.06	0.08	0.11
VCCT	1340.63	949.10	60.01	331.52	0.60	0.13	0.13	0.15
VCCT	1375.40	966.61	61.59	347.20	0.54	0.12	0.14	0.19
VCCT	1439.51	1047.32	49.87	342.32	0.66	0.09	0.12	0.14
WCNT	963.95	711.71	37.14	215.10	0.68	0.09	0.10	0.13
WCNT	1168.40	823.18	38.84	306.38	0.75	0.07	0.07	0.10
WCNT	926.19	683.53	41.60	201.05	0.74	0.09	0.08	0.10
WCCT	806.40	584.57	25.80	196.03	0.50	0.11	0.08	0.32
WCCT	1136.26	812.66	29.10	294.50	0.46	0.11	0.15	0.28
WCCT	863.03	637.85	29.63	195.54	0.49	0.09	0.16	0.26

*Soil aggregate composition was measured by Wilson (2015). All variables were log transformed for normality before conducting structural equation modelling. NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage.

Table 4.109 Standardized total effects of microbial respiration, extracellular enzyme activity, and substrate availability on total amino sugar accumulation in soil aggregate fractions.

Factors →	Total amino sugars	NAG	Microbial respiration	BG	Nitrogen	Carbon
LAP →	0.26	-0.26	0.25	0.23	0.46	0.39
Carbon →	0.25	-0.41	0.49	-0.24	0.91	-
Nitrogen →	0.28	-0.27	0.54	0.00	-	0.00
BG →	0.02	0.71	0.00	-	0.00	0.00
Microbial respiration →	0.33	0.00	-	0.00	0.00	0.00
NAG →	-0.41	-	0.00	0.00	0.00	0.00

NAG: β-N-acetylglucosaminidase activity; LAP: leucine aminopeptidase activity; BG: β-Glucosidase activity. Arrows indicate directions of causal relationships. All effects are significant ($P < 0.05$).

Table 4.110 Characteristics of soil aggregate fraction samples used in the structural equation model.

Treatments	Aggregate size (mm)	Total amino sugar (mg kg^{-1} fraction soil)	BG* (nmol g^{-1} fraction soil h^{-1})	NAG* (nmol g^{-1} fraction soil h^{-1})	LAP* (nmol g^{-1} fraction soil h^{-1})	Respired C* (mg kg^{-1} fraction soil)	N (g kg^{-1} fraction soil)	C (g kg^{-1} fraction soil)
NCNT	> 2	985.86	99.07	49.51	546.40	348.46	0.71	5.93
NCNT	> 2	1037.81	119.43	38.35	1530.72	854.51	0.88	8.04
NCNT	> 2	1205.50	89.35	9.68	1145.23	686.86	1.15	11.21
NCCT	> 2	796.59	165.77	68.85	1483.86	482.00	0.88	8.72
NCCT	> 2	1062.57	94.84	15.11	931.46	456.88	0.83	8.08
NCCT	> 2	1061.23	119.85	42.12	1230.70	672.17	0.99	9.38
VCNT	> 2	1278.12	125.90	56.29	748.04	405.08	0.92	8.22
VCNT	> 2	1528.97	105.07	7.09	1366.15	690.26	1.16	11.01
VCNT	> 2	1603.39	112.88	13.37	1161.69	1031.71	1.52	14.99
VCCT	> 2	1189.72	144.85	53.54	1282.00	661.15	1.13	11.12
VCCT	> 2	1155.66	87.69	15.76	1274.01	707.26	1.11	10.76
VCCT	> 2	1531.86	100.86	32.50	1350.57	827.65	1.33	12.82
WCNT	> 2	1043.24	124.93	63.13	1085.33	469.13	0.84	8.25
WCNT	> 2	1203.45	96.48	15.71	1305.58	824.34	0.97	9.18
WCNT	> 2	1126.45	107.24	27.12	1222.65	1277.03	1.06	9.77
WCCT	> 2	635.35	168.60	69.31	1197.70	413.33	0.67	6.18
WCCT	> 2	932.20	52.62	16.92	1190.82	406.47	1.06	11.12
WCCT	> 2	682.22	78.46	33.56	507.38	619.25	1.06	14.33
NCNT	1-2	740.76	103.68	66.35	462.56	432.75	0.71	6.66
NCNT	1-2	910.25	125.00	38.07	1260.27	972.59	1.24	13.39
NCNT	1-2	1427.03	136.05	23.65	1391.48	692.99	1.22	11.81
NCCT	1-2	769.03	173.84	70.13	1567.92	564.15	0.90	9.46
NCCT	1-2	761.13	84.04	12.30	1021.19	492.25	0.98	11.39
NCCT	1-2	894.47	104.12	25.66	1291.85	715.92	1.04	10.17
VCNT	1-2	653.16	120.27	62.58	728.11	512.55	1.01	9.30
VCNT	1-2	765.15	91.68	11.57	1384.08	614.88	1.25	12.56
VCNT	1-2	1256.17	107.78	12.56	1236.17	348.46	1.87	19.41
VCCT	1-2	953.27	145.68	56.19	1460.26	854.51	1.57	19.01
VCCT	1-2	1081.51	82.81	14.48	1341.46	686.86	1.18	12.54
VCCT	1-2	873.87	116.53	28.40	1595.61	482.00	1.41	13.96
WCNT	1-2	810.60	126.62	82.43	1110.79	456.88	0.88	8.58
WCNT	1-2	1340.60	103.03	18.99	1412.79	672.17	1.01	10.22
WCNT	1-2	1153.75	114.97	35.58	1501.32	405.08	1.08	10.77
WCCT	1-2	663.06	147.02	68.67	1053.23	690.26	0.90	11.28
WCCT	1-2	497.35	61.02	20.39	1275.66	1031.71	1.02	10.44
WCCT	1-2	954.58	88.59	39.04	565.11	661.15	1.06	12.12
NCNT	0.25-1	487.13	84.31	49.34	502.29	707.26	0.63	6.34
NCNT	0.25-1	1319.24	96.99	16.43	1374.01	827.65	1.10	10.86
NCNT	0.25-1	1433.56	122.05	29.02	1492.46	469.13	1.29	12.72
NCCT	0.25-1	1050.69	150.84	60.03	1436.96	824.34	0.85	8.50
NCCT	0.25-1	738.56	80.95	14.74	1119.89	1277.03	0.87	9.67
NCCT	0.25-1	261.81	100.03	23.92	1478.04	413.33	1.16	13.42
VCNT	0.25-1	881.79	136.96	64.50	989.35	406.47	1.02	9.94
VCNT	0.25-1	1235.72	100.76	23.71	1265.59	619.25	1.24	13.52
VCNT	0.25-1	1305.71	96.95	12.59	1323.23	432.75	1.59	17.58

Table 4.110 continued

Treatments	Aggregate size (mm)	Total amino sugar (mg kg^{-1} fraction soil)	BG* (nmol g^{-1} fraction soil h^{-1})	NAG* (nmol g^{-1} fraction soil h^{-1})	LAP* (nmol g^{-1} fraction soil h^{-1})	Respired C* (mg kg^{-1} fraction soil)	N (g kg^{-1} fraction soil)	C (g kg^{-1} fraction soil)
VCCT	0.25-1	762.37	132.49	63.76	1087.31	1080.56	1.16	12.52
VCCT	0.25-1	1084.57	93.77	26.47	1357.16	915.46	1.08	10.85
VCCT	0.25-1	1630.76	117.31	23.66	1445.28	1024.76	1.40	16.85
WCNT	0.25-1	1096.38	141.23	93.03	1117.98	605.42	0.78	8.03
WCNT	0.25-1	2291.16	88.85	19.41	1421.13	814.25	1.02	11.92
WCNT	0.25-1	1034.43	87.11	23.66	1227.37	874.36	1.06	10.60
WCCT	0.25-1	997.53	118.18	62.83	1065.00	997.95	0.95	11.91
WCCT	0.25-1	1461.74	73.05	29.66	1143.98	434.92	1.08	12.64
WCCT	0.25-1	1096.67	98.78	38.58	562.98	555.79	0.97	10.60
NCNT	< 0.25	644.23	106.42	65.03	668.72	748.58	0.62	5.93
NCNT	< 0.25	942.88	92.02	19.07	1179.75	496.14	0.98	9.61
NCNT	< 0.25	1314.98	124.16	31.65	1509.85	799.30	1.18	11.35
NCCT	< 0.25	794.49	155.53	59.47	1361.42	1099.67	0.76	7.59
NCCT	< 0.25	803.74	77.41	10.90	1032.65	958.95	0.81	7.97
NCCT	< 0.25	985.16	111.21	22.12	1423.91	780.22	1.02	10.53
VCNT	< 0.25	1376.89	147.16	67.80	1116.48	979.85	1.04	10.65
VCNT	< 0.25	1435.24	138.71	28.75	1639.02	707.78	1.18	11.88
VCNT	< 0.25	1549.40	129.59	32.80	1496.23	999.70	1.57	16.53
VCCT	< 0.25	1229.63	171.23	59.26	1072.49	1656.02	1.07	11.32
VCCT	< 0.25	1258.07	106.56	21.38	1249.87	1122.68	1.05	10.71
VCCT	< 0.25	1326.65	130.81	34.98	1471.03	1028.58	1.30	13.02
WCNT	< 0.25	1008.51	156.18	94.20	1228.86	1197.31	0.81	8.34
WCNT	< 0.25	1211.11	98.02	20.73	1481.45	886.24	0.97	9.96
WCNT	< 0.25	864.54	111.66	43.84	1295.99	1170.36	1.14	11.65
WCCT	< 0.25	762.63	167.07	76.08	1373.08	956.90	0.66	7.02
WCCT	< 0.25	1149.38	78.48	27.49	1206.08	534.89	0.91	9.35
WCCT	< 0.25	987.45	115.55	49.96	719.25	847.11	0.85	8.89

*Enzyme activities and soil respiration were measured by Wilson (2015). All variables were log transformed for normality before conducting structural equation modelling. NAG: β-N-acetylglucosaminidase; LAP: leucine aminopeptidase; BG: β-1,4-Glucosidase; NCNT: no cover crop with no tillage; NCCT: no cover crop with conventional tillage; VCNT: vetch cover with no tillage; VCCT: vetch cover with conventional tillage; WCNT: wheat cover with no tillage; WCCT: wheat cover with conventional tillage; > 2 mm: large macroaggregates; 1-2 mm: medium macroaggregates; 0.25-1 mm: small macroaggregates; < 0.25 mm: microaggregates.

Chapter 5 Conclusions

My findings clarify the controls on turnover of newly added labile C and microbially derived organic matter, and the degree to which long-term agricultural management practices have affected them. This is the first time that the relative importance of different stabilization mechanisms of SOC have been quantified and the sequential order of biochemical reactions during microbial residue turnover have been identified, which could be a useful approach for understanding and modeling biogeochemical transformations of N and C in soil.

Physical, chemical, and biological transformation processes convert new C input to SOM. The accumulation of SOM is important for soil quality and agricultural productivity. Moisture pulse events and land use can affect how and where SOM accumulates in soils. The microcosm incubation experiment validated my hypothesis that drying-rewetting cycles deplete SOC and conservation agricultural management practices mitigate the depletion. Structural equation models support the hypothesis that chemical stabilization and biochemical recalcitrance rather than physical protection are responsible for long-term accumulation of new labile C input. The field experiment validates my hypothesis that chemical stabilization, biochemical recalcitrance, and physical protection jointly control the accumulation of amino sugars in soil under long-term conservation agricultural management practices. Structural equation models demonstrate that physical control, by aggregation, is mainly responsible for the stabilization of muramic acid in soil, while biochemical control for glucosamine. I also found that polypeptides are

hydrolyzed by microbial extracellular enzymes prior to amino saccharides when soil N is scarce.

My study shows that long-term conservation agricultural management practices had a beneficial effect on soil in terms of SOM sequestration, which is of importance for agronomy, food security, and biogeochemical transformations of C and N in soil. A mitigation effect of long-term conservation agricultural management practices on short-term drying-rewetting cycles in terms of SOC stabilization was observed. It implicates that future studies should further probe into how to maximize SOC accumulation by adjusting the quantity and quality of new C input.

Vita

Lidong Li was born in Benxi City, Liaoning Province, China on November 11, 1988 and graduated from high school in 2007. Later that year, she left home for college. She completed her Bachelor's degree in Environmental Science in the Northeast Agricultural University in 2011. Her Bachelor's research project was about bioremediation of atrazine polluted soil. As an undergraduate, she was also involved in a project using beer yeast to adsorb heavy metals in wastewater. This project won an award in a provincial competition. In the fall of 2011, Lidong was admitted by the University of Chinese Academy of Sciences to pursuit a Master's degree in Soil Science in the Institute of Applied Ecology. She was awarded the Master's degree in 2014. In August 2014, Lidong came to the US to pursue a Ph.D. in the University of Tennessee at Knoxville.