Quantification of a sensitive soil carbon constituent

as affected by soil type, tillage system, and crop rotation

by

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A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Soil Science (Soil Microbiology and Biochemistry, Soil Management)

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2012

To my parents, Bill and Valerie Scott, for their support and guidance.

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LIST OF ABBREVIATIONS

R_r, potentially releasable reducing sugars

K_t, time required to release one-half of R_r

h, hours

min, minutes

C_{mic}, microbial biomass carbon

N_{mic}, microbial biomass nitrogen

C, carbon

N, nitrogen

CC, continuous corn

CS, corn-soybean rotation

MP, moldboard plow tillage system

CP, chisel-plow tillage system

NT, no-tillage system

SOC, soil organic carbon

ACKNOWLEDGEMENTS

First, I would like to express my thanks to my committee members - Dr. Moore, Dr. Castellano, and Dr. DiSpirito - for their thoughtful answers to many questions which have helped me in boundless ways. To my co-major professors, Dr. Al-Kaisi and Dr. Loynachan, I thank you each for the freedom, inspiration, and gentle cultivation of courage you have provided me with, and each for your help in the preparation of many documents. My immense gratitude is extended to Dr. Tabatabai for his mentorship, vision, and training; thank you for helping me understand "the language" both in and out of the laboratory. My appreciation also goes to the Agronomy Department and Research Training Fellowship Committee for providing me with the opportunity to forge together my passions for soil science and biochemistry. Thank you to all of the Iowa State Research Farm staff for their timely assistance. To my research team members: thank you for the countless hours of support and for also making me a part of the family. Thank you to my former teachers and mentors for being catalysts towards my purposed learning. I thank my friends for their unwavering loyalty and also for being advocates for my goals. To my family, I thank you all for your past and continued kind understanding. Most significantly, I thank my parents for their teamwork towards putting the utmost emphasis on education. It is my mother, Valerie, to whom I owe my creativity, and my father, Bill, to whom I owe my perseverance. Thank you for teaching me that hard work pays off.

ABSTRACT

Differences in tillage intensity and crop rotation management practices have been shown to lead to significant changes in the chemical, physical, and biological partitioning of soil carbon over various periods of time. Although many studies have focused on the roles of microbial biomass or specific enzymes in transformative processes within soils, the underlying net potential metabolic activity of a soil system remains to be addressed in terms of a quantitatively feasible constituent of the soil carbon pool. As carbohydrates are the primary source of carbon input into soil systems and one of the degradation products of carbohydrates is reducing sugars, analysis of a soil system's ability to either accumulate or release reducing sugars stands as a reasonable assessment of a soil's metabolic index of soil carbon. Therefore, the objectives of this research were to develop a method by which to assess the total potential release of reducing sugars from soils and then to use that method to assess the effects of soil type, tillage system, crop rotation, time, and physio-spatial distribution on that soil organic carbon pool.

The potential release of total reducing sugars from soil was quantified by incubation of surface soil (0-15 cm) in 60% methanol solution by volume at 30°C for time spans increasing in 2 h increments up to 24 h. The supernatant of eight distinct, uncultivated Iowa soils from under similar vegetation (grass areas predominated by *Bromus inermis* Leyss.) was colorimetrically analyzed by Somogyi-Nelson method. Release of reducing sugars from soils with respect to time fit a parabolic curve, and a double inverse transformation was done in order to calculate

a total releasable reducing sugar pool (R_r) and the time it would take to release one-half of that pool (K_t). The R_r values ranged from 39 to 152 mg kg^{-1} field-moist soils and from 11 to 98 mg kg^{-1} in air-dry soils. The K_t values ranged from 3.9 to 16 h in field-moist soils and from 1.6 to 12 h in air-dry soils.

Further findings of this research demonstrated that the decrease in rate of production of reducing sugars as the value of total reducing sugars released reached R_r was due to a decrease in the substrate pool from which these monosaccharides were nascent. After five days of incubation, concentrations of total reducing sugars released matched calculated R_r values. Therefore, incubation of field-moist surface soil (0-15 cm) for five days at 30°C in 60% methanol solution was used to estimate R_r and to assess the impacts of soil type at different locations, tillage system, and crop rotation on the total potential reducing sugar carbon pool.

Tillage, crop rotation, and location all significantly impacted the concentration of releasable reducing sugar in soils. On average, soil from the no-till system exhibited mean concentrations of 7.5 and 19.9 mg kg^{-1} soil more releasable reducing sugars than chisel-plow and moldboard plow tillage systems, respectively. The chisel-plow tillage system had concentrations that were, on average, 12.3 mg kg^{-1} soil greater than the moldboard plow tillage system. In general, releasable reducing sugar concentrations were 2.4 mg kg^{-1} soil greater in continuous corn than corn-soybean rotation. Although of different magnitude, the trends of these management effects were the same regardless of location.

The effects of a temporal variable on these releasable reducing sugar concentrations were significant; the impact of one single spring secondary tillage treatment was assessed and was found to be significant. Regardless of tillage management system, results show that releasable reducing sugar concentrations in soils had, on average, significantly decreased the following spring when compared to concentrations analyzed from samples that were collected the previous fall. Furthermore, when soil reducing sugar concentrations of spring baseline (prior to spring secondary tillage), were compared to concentrations after a single secondary tillage pass, concentrations averaged 18% lower in the moldboard plow tillage system, 6.9% lower in the chisel plow tillage system, and 9% greater in the no-till system (which was used as a control). Changes in reducing sugar concentrations during this six-day time period in the corn-soybean rotation, were as follows: decreases of 18.7% in reducing sugar concentration with moldboard plow tillage system, 8.3% with the chisel plow tillage system, and an increase of 11% with no-till were noted compared to decreases of 17.4%, 5.4%, and an increase of 6.9% for the same tillage treatments, respectively, in the continuous corn cropping system.

Analysis of the physio-spatial distribution of releasable reducing sugars in field-moist soil aggregates from no-till, chisel plow, and moldboard plow surface (0-7.5 cm) and subsurface (7.5-15 cm) soil samples demonstrated that soil aggregates of size fractions 1-2 and 2-4 mm held the greatest concentrations of releasable reducing sugars. A stratification effect was noted in the no-till system, where the average concentration of releasable reducing sugars from all aggregate fractions

was 63.9 mg kg^{-1} in the top 0-7.5 cm surface soil and 33.4 mg kg^{-1} in the 7.5-15 cm subsurface soil depth. Average concentrations were more homogenized in the other tillage systems with greater concentrations in subsurface soil (7.5-15 cm), and significantly greater concentrations in chisel plow subsurface soil depth than in the moldboard plow subsurface soil depth.

Greater potential reducing sugar values in no-till tillage systems lend support to the hypotheses of increased carbon sequestration and organic matter resilience associated with decreased disturbance of the soil. Overall, these findings provided evidence that the method developed for analysis of total releasable reducing sugars is a sensitive method for detecting and quantifying impacts of land-use, management practices, and crop rotations on soil carbon stocks, and should be useful in further study of mechanisms that regulate the transformative processes of soil carbon.

CHAPTER 1

GENERAL INTRODUCTION

Polyhydroxy aldehydes and ketones, or carbohydrates, are nature's way to store, modify, transport, and use the energy from the sun that is captured during the photosynthesis process. Fundamentally, carbon in soil must come from sources such as cellulose, murein, starch, chitin, pectin, and hemicellulose, each with variable rates of decomposition and degradation-reaction specific mechanisms. The energy stored in carbohydrates is used by non-photosynthesizing organisms in the processes known as glycolysis and respiration, or literally the breakdown of glucose and release of CO_2. It is widely agreed on that cellulose is the most abundant carbohydrate on earth, as it is found in all plants as the major structural component in the cell wall. Xylanes, polyphenols, chitin, and peptidomureins (linear polymers of alternating N-acetyl-glycosamine and N-acetyl-muramic acid which make up bacterial cell walls) are also all structural polysaccharides, while the amylose and amylopectin in starch are used as units of energy storage.

Regardless of the original form, however, each type of carbohydrate is broken down sequentially and cooperatively by a broad family of enzymes known as glycoside hydrolases (EC 3.2.1.-) to produce oligosaccharides, and to further produce monosaccharides. In the case of microbial degradation of cellulose, endo- and exo-targeted cellulase enzymes act to yield cellobiose; cellobiase, or ß-glucosidase, acts to cleave the ß-1,4 linkage between the individual glucose units in cellobiose (Wood, 1985). Enzymatic hydrolysis rates are generally governed by parameters such as

pH, temperature, enzyme cooperativity or inhibition, and the biophysical properties of the substrate. Uniquely, soil enzymes may be associated with viable, living cells, or as abiontic enzymes associated with dead cells or cellular debris (Skujins, 1976; Burns, 1982). There is evidence that even after a microbial population has been significantly reduced by extreme environmental factors, the soil organism itself retains some of the metabolic properties of enzymes immobilized on soil colloids (McLaren, 1975; Tripathia et al., 2007).

The production of enzymes in any system is governed by thermodynamic checks and balances in order to maximize returns of C, energy, and limiting nutrients while minimizing expenditures of resources (Allison et al., 2011). ß-glucosidases are considered to be useful in studying soil organic matter degradation and cycling because they are regarded as the most abundant extracellular enzyme in soil (Busto and Perez-Mateos, 2000). The amount of carbon residue added to a soil system will be met with an appropriate microbial enzymatic response for degradation of that carbon source into metabolic energy substrate. In consideration of cellulose being the most abundant carbohydrate on earth and ß-glucosidase being the most abundant enzyme in soil, it is easy to hypothesize that the majority of the soil organism's (when the soil itself is viewed as a single, dynamic living system) respiration of CO_2 comes from the degradation of cellulose and metabolism of subsequent constitutive glucose units. Similarly, the metabolic substrates nascent from the breakdown of other aforementioned carbohydrates are also reducing

sugars, or any sugar that has the potential to form a free hemiacetal or hemiketal group in solution (Robyt, 1998).

Among various sources, the soil microbiological community is responsible for the function of soil enzyme syntheses (Dick et al., 2006). It has also been shown that enzyme activity in soil, especially of glucosidases, is sensitive to soil management practices (Knight and Dick, 2004). Further research provides evidence that the soil structure, as affected by tillage, has the potential to significantly alter the composition of the soil microbiological community and strongly influence the function of soil ecosystem processes (van der Heijden et al. 2008; Xiang et al., 2011). Quantities and ease of rate of decomposition of organic carbon sources limit the growth and activity of microbial communities (Friedel et al., 1996). As changes in soil management practices occur, changes in resource inputs also occur, helping to shape the soil microbiological community, the enzymes produced in response to those inputs, and ultimately if soil organic matter is either degraded or preserved. Thus, more research is needed in the area of degradation of carbohydrates in soils, as impacted by land use and management practices, and the direct impact of these processes and mechanisms on soil processes, namely in support of agricultural ecosystems as an atmospheric carbon sink (Lal et al., 2011).

Several methods have been proposed for extraction and determination of the monosaccharides that comprise soil organic matter (Stevenson, 1994; Martens and Frankenberger, 1990). These methods provide data about constituent sugars that comprise the total carbohydrate substrate pool within soils, but little information is

available about the rates of release or the potential size or pool of total reducing sugars in soils that may be able to be metabolized. Likewise, numerous methods for detection and quantification of free reducing sugars have been developed, among which the Somogyi-Nelson method is best suited for determination of free reducing sugars in soils (Deng and Tabatabai, 1994). For the long-term quantitative recovery of reducing sugars from incubated soils (over 24 h) and to overcome interference from turbidity of the soil supernatant with the Somogyi-Nelson method, a 60% (v/v) methanol reagent was used as a plasmolytic agent for the biotic control of microorganisms.

The use of a 60% methanol reagent for quantitative recovery of enzymatic products in soils is a direct application of current methods for the extraction of metabolites in research that focuses on improving industrial bioprocesses. The standard protocol described by Sellick et al. (2011) uses 60% methanol solution (v/v) to quench cellular metabolism. This solution is supplemented by 0.85% ammonium bicarbonate (w/v) to avoid metabolite leakage (caused from lysis, particularly in centrifugation steps). The use of ammonium bicarbonate was reported to be comparable to other salts such as sodium chloride for extraction of the relevant TCA cycle metabolic intermediaries other than malate. Cells are then cooled to -40°C before further metabolite extraction in 100% methanol, as it was reported that temperatures above 0°C were not cold enough to totally ablate enzymatic activity. Other workers (Faijes et al., 2007) support the use of methanol reagents for the quenching and extraction of metabolites from *Lactobacillus plantarum* in order to

avoid degradation of metabolites from exposure to extreme pH values or temperatures and low solubility of metabolites in solutions that contain chloroform.

Other studies on the comparative kinetics and characterization of the biochemical mechanisms of action of different ß-glucosidases showed that this group of enzymes is optimally active in 60% methanol, when contrasted with a 20-50% increase in amount of p-nitrophenyl liberated from substrate at 20-30% concentrations of methanol, ethanol, propan-2-ol, and butanol, or up to 50% decrease in activity at 90-100% concentrations of methanol, ethanol, and propan-2-ol (Parry et al., 2001; Nazir et al., 2009). Furthermore, the use of methanol as an organic solvent for pesticides instead of ethanol (to avoid enzyme protein denaturation) has been used to study the effects of different pesticides on the activity of free and colloid-bound enzymes; the pesticide/methanol combination was noted to decrease activity of urease when compared to the amplified activity (1.48 and 7.47 fold, respectively) of urease from soil and soil extract treated with methanol as a control (Gianfreda et al., 2003). Increased activity exhibited by urease treated with methanol solutions was shown to be from increased cell wall permeability and cellular lysis, putting substrate molecules in closer proximity to the catalytic mechanisms of the enzyme (Fenton, 1982).

The focus of this research is on the incubation of soils in 60% methanol solution as a trap for the products of carbohydrate degradation, or the substrates for microbial metabolism in soils. The main objectives of this research were to develop a method for the determination of total potential reducing sugars releasable from

soils and to use that method to investigate the impacts of soil management practices on that soil carbon fraction.

Thesis Organization

This thesis is organized into four chapters, which address all components of this research. Chapter one is a general introduction outlining the relevance of this research. The second chapter focuses on the development of the method, while the third chapter describes the application of this method in the study of the impacts of crop rotation, tillage system management practices, time, spatial variability, and physio-spatial distribution on the total potential reducing sugar fraction of the soil carbon pool. The fourth chapter, or general conclusion of the thesis, highlights the implications of this method as one of the more sensitive quantitative analytical methods available for assessment of the impacts of land-use change on what may be the most sensitive fraction of soil carbon pools.

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CHAPTER 2

QUANTIFICATION OF POTENTIAL REDUCING SUGAR CONCENTRSTIONS IN SOILS

Abstract

Native and added organic C in soils is degraded by a complex set of biochemical reactions, leading to production of reducing sugars (e.g., glucose). The glucose produced is metabolized by microorganisms as an energy source, releasing CO_2 into the atmosphere. We hypothesized that if we could find a reagent to stop microbiological metabolism of the degradation products of soil organic carbon, reducing sugars released from soil could be accumulated in solution and a kinetic model could be applied to predict the total amount of reducing sugars releasable pool from soil and the time it would take to release half of that total pool.

The objective of this work was to develop a method for the determination of the total potential release of reducing sugars from soil. Five g of soil from eight different soil series was incubated with 60% methanol solution at 30°C for times ranging from 2 to 24 h, and the amounts of reducing sugar produced were determined colorimetrically. The methanol reagent prevents microbial metabolism of the reducing sugar produced, but it does not inhibit the hydrolytic activity of enzymes such as ß-glucosidase. Findings show that accumulation of reducing sugar followed non-linear curves, which were converted to a linear model to calculate the potential release of reducing sugar (R_r) in each soil and the incubation time (K_t) required to accumulate 50% of R_r. The R_r values in the eight surface soils used

ranged from 39 to152 mg kg^{-1} in field-moist soils, and from 11 to 98 mg kg^{-1} in air-dried soils. The K_t values ranged from 3.9 to 16 h for the field-moist soils and from 1.6 to 12 h for the air-dried counterparts. Replicated analyses showed that the method is highly accurate and precise. To our knowledge, this is the first report on the use of 60% methanol for inhibiting reducing sugar metabolism by microorganisms for determination of the potential release of reducing sugar pools in soils. This method should aid our understanding of agricultural land management impacts on the mineralization or humification of the most sensitive, or labile, fraction of soil organic carbon.

Introduction

The complex chemistry and biochemistry of carbohydrates in soil organic matter is the subject of much study (Clapp et al., 2005). Among the various C chemical structures, the monosaccharides, oligosaccharides, and polysaccharides are the most studied, because of their chemical significance as building blocks of carbohydrates and their role in soil aggregate stability (Stevenson, 1994). The total living soil system is made up of webs of complex chemical and biochemical reactions, and as such, soils degrade and metabolize added organic C and release CO_2 to the atmosphere (Quastel, 1946). The degradation of organic C in soils follows known biochemical pathways, including (in the case of cellulose) enzyme-catalyzed reactions that lead to the production of disaccharides, which are enzymatically hydrolyzed by glycosidases (e.g, ß-glucosidase), producing monosaccharides or reducing sugars, such as glucose.

Soil carbohydrates are readily degradable components of SOM and the major energy sources for microorganisms (Cheshire, 1985). Different carbon sources such as cellulose, starch, chitin, pectin, and hemicellulose, with variable rates of decomposition and reaction-specific mechanisms, are hydrolyzed when added to soils. Generally, glycoside hydrolases (GH's, or EC 3.2.1.-) represent one of the largest groups of enzymes, divided into 110 separate families and further into 14 clans (Coutinho and Henrissat, 1999, see http://www.cazy.org/). The purpose of GH's is to hydrolyze or rearrange glycosidic bonds between two or more carbohydrates, or between a carbohydrate and a non-carbohydrate moiety. The GH3 family of glycoside hydrolases is of particular interest in soils, as it includes ß-glucosidases (EC 3.2.1.21), xylan 1,4-ß-xylosidases (EC 3.2.1.37), ß-n-acetylhexosaminidases (EC 3.2.1.52), glucan 1,3-ß glucosidases (EC 3.2.1.58), glucan 1,4-ß-glucosidases (EC 3,2,1,74), exo-1,3-1,4-glucanases (EC 3.2.1.-), and α-L-arabinofuranosidases (EC 3.2.1.55), all of which have a retaining mechanism of action. Although only 204 of over 4000 GH3's have been characterized from the kingdoms of Archaea, Bacteria, and Eukaryota, it stands that aspartic acid serves as a catalytic nucleophile and glutamic acid serves at a catalytic proton donor in the active site of the protein (Coutinho and Henrissat, 1999).

The complex variety of steriochemical conformation of carbohydrates in nature is only rivaled by enzymatic mechanisms to degrade those carbohydrates. The enzyme system for the conversion of cellulose (the most predominant carbohydrate in nature) to glucose is comprised of the action of three cellulases:

endo-1,4-ß-glucanase (EC 3.2.1.4) acts to cleave the ß-1,4 glycosidic linkages of cellulose, exo-1,4-ß-glucanase (EC 3.2.1.91) attacks the ends of cellulose to produce dimers of glucose linked by ß-1,4 glycosidic bonds (cellobiose), and ß-glucosidase (EC 3.2.1.21) further hydrolyses cellobiose into two constitutive molecules of glucose. Cellobiose is a strong inhibitor of both exo-1,4-ß-glucanase and endo-1,4-ß-glucanase, but the action of ß-glucosidase cooperatively reduces this inhibition, enabling the sequential degradation of crystalline cellulose to glucose (Saha et al., 1995). Likewise, the action of α–D–glucosidase on starch and microbial residues with α bond glucose units, or ß–D–xylosidaseses on xylan units in hemicellulose, leads to the release of reducing sugars. As soil organic constituents and as the final products of decomposition of a variety of organic matters added to soil, reducing sugars are among the major sources of metabolic substrate for the soil organism, when viewed as a whole.

A reducing sugar is any sugar that, in solution, has an aldehyde or a ketone group which allows the sugar to act as a reducing agent (Robyt, 1998). The quantitative measurement of reducing sugar pools in soil systems is important because they are the end products of many biological processes and enzymatic reactions of interest that occur in soils. Different carbon sources such as cellulose, starch, chitin, pectin, hemicellulose, murein, sucrose, and α,α-trehalose, all with variable rates of decomposition and reaction specific mechanisms, can be degraded when added to soils to ultimately release reducing sugars. Carbon compounds are also known to be present in other sources such as exudates from plants and other

biota in forms such as glycoproteins and glycolipids, cell membranes of microorganisms both living and dead, and intracellular constituents of living soil biota.

Several methods have been proposed for extraction and determination of the monosaccharides that are constituents of soil organic matter (Stevenson, 1994; Martens and Frankenberger, 1990), but little information is available about the rates of release or the potential size or pool of total reducing sugars in soils. Such information is essential for assessing the potential metabolic index of the soil system and how agricultural management practices influence both short-term and long-term soil carbon processes.

Current protocol in metabolomics research involves the use of 60% methanol solution to quench the metabolic processes within cells (Sellick et al., 2011). Review of the literature revealed that plant acid phosphatase functions in 40% methanol solution (Bieleski, 1964), and that proteins extracted from *Brucella abortus* remain active after extraction with 60% methanol and 0.15 M sodium chloride (Tabatabai et al., 1979). Inactivation of *Brucella abortus* was demonstrated by lack of growth on tryptose agar plates. One of the proteins extracted was identified as Cu-Zn superoxide dismutase (Beck et al., 1990). This enzyme retained activity after inactivation of the *Brucella* cells with 60% methanol as determined by the inhibition of cytochrome C reduction by xanthine-xanthine oxidase reaction (McCord and Fridovich, 1969). Food science research in the dairy industry also provides evidence that lactase remains active in methanol solutions, as insufficient

mutarotation to the α-hydrate (or inactive) form is observed (Nickerson and Lim, 1974). Past research into the treatment and proposed cure of *herpes simplex* and the chemistry involved in contact lens disinfecting solution are both based on the original use of solutions of methanol as a sporicidal agent (Pepper and Lieberman, 1962). Further, an effective method for the quenching and extraction of metabolites from *Lactobacillus plantarum* is based on the use of 60% methanol solution, avoiding the degradation of certain metabolites from exposure to extreme pH values or temperatures and low solubility of metabolites in solutions that contain chloroform (Faijes et al., 2007). Other studies showed that ß-glucosidases are active in 60% methanol (Parry et al., 2001; Nazir et al., 2009).

We have discovered that by determining the amounts of reducing sugar released over time in soil incubated with 60% methanol, a parabolic curve is produced, which can be linearized for calculating the total potential reducing sugar pools in soils. Therefore, the objectives of this work were to develop an analytical method for accurate and precise determination of reducing sugars such as glucose in soils, and to determine the time required for the release of 50% of the total reducing sugar pool for different soils.

Materials and Methods

Soils and their properties

Soils were collected from minimally disturbed areas in fencerows adjacent to cultivated fields from eight diverse and representative Iowa soils. Surface soil samples (0-15 cm) were collected in May 2011 under fencerow vegetation predominated at all sites by smooth brome (*Bromus inermis* Leyss.). The field-moist (gravimetric moisture) soil was sieved in-field to pass a 4-mm screen, excluding roots and vegetation. Soils were homogenized in the laboratory, and one-half of the sample was then sieved through a 2-mm screen and air-dried, while the remainder was stored in sealed black plastic bags at 4^{o} C. A portion of the air-dried soil was then ground to pass an 80-mesh (180-µm) sieve. In the soil properties reported (Table 2.1), pH was determined by a glass electrode (soil: water or 0.01 *M* $CaCl_2$ ratio = 1:2.5), total N by a dry combustion CHN analyzer (LECO Corp., St Joseph, MI), organic C by the Mebius (1960) method, particle size distribution by pipette analysis (Kilmer and Alexander, 1949). Organic C and N determinations used the <180-µm soil samples, while pH and particle size distribution were determined on the <2-mm mesh air-dried soil samples.

Microbial biomass C and N

The microbial biomass C (C_{mic}) and N (N_{mic}) in soils (Table 2.1) were determined on the field-moist samples within two weeks after sampling, by the chloroform fumigation-extraction method described by Vance et al. (1987) and the

chloroform fumigation-incubation method described by Horwath and Paul (1994), respectively. All results reported are averages of duplicates and are expressed on an oven-dry equivalent weight basis. Moisture content of the soil was determined from weight loss after drying at 105˚C for 48 h.

Chemicals and reagents

Methanol (60%, v: v): Add 60 mL of methanol (Fisher certified reagent, Fisher Scientific Co., Chicago, Ill.) to 40 mL of deionized water, and mix.

Reducing sugar standard stock solution: 1.0 g of D-glucose was dissolved in 800 mL of deionized water in a 1-L volumetric flask. The volume was adjusted to one liter with deionized water, and mixed thoroughly. One mL of this stock solution contains one mg of glucose. This solution was stored in a refrigerator, and allowed to come to room temperature (22˚C) before preparation of any standard working solution.

Reducing sugar standard working solution: For preparation of the calibration graph, 0, 0.5, 1, 2, 3, 4, and 5 mL of the 1 mg L^{-1} D-glucose standard stock solution were pipetted into each of seven 50-mL volumetric flasks and the volumes were adjusted so that the total volumes contain 60% methanol, and mixed thoroughly. Those solutions contain 0, 10, 20, 40, 60, 80, or 100 μg of D-glucose per 2 mL, respectively.

Somogyi reagents SI and SII and Nelson reagent were prepared to have the same composition as those described by Wood and Bhat (1988).

Procedure

Unless otherwise specified, 5.0 g of field-moist soil sample (on an oven-dried weight basis) was placed in a 50-mL plastic centrifuge tube, treated with 25 mL of 60% methanol, sealed with a rubber stopper, shaken by hand for one minute, and incubated in duplicates for 0, 2, 4, 6, 8, 12, 18, or 24 h at 30°C. After incubation, the tube was shaken on an end-to-end shaker for 15 min, un-stoppered, and centrifuged at 17,390 *g* for 15 min. Supernatant was decanted, and a 2.0-mL aliquot was analyzed for reducing sugars by the Somogyi-Nelson method as follows**:** 40 mL the Somogyi Reagent I (SI) and 10 mL Somogyi Reagent II (SII) were mixed to yield a 4:1 ratio SI: SII (Somogyi) solution. Then, 2 mL of this Somogyi solution was quantitatively transferred to a standard 18 x 150-mm laboratory test tube, along with 2 mL of soil supernatant or 2 mL of 60% methanol for control. The level of the liquid in the test tube was marked, and the test tube was immersed in a boiling water bath for exactly 15 min, which was partially covered to maintain the boiling temperature. The test tube was immediately transferred to a cold-water bath to cool (*ca*. 5 min). After the sample was cooled, a few drops of deionized water was added up to the mark to compensate for any liquid loss during heating, mixed, and 2.0 mL of the Nelson reagent was immediately added with caution, as a strong effervescence may occur. The tube was then mixed on a vortex mixer. A blue color

was developed with a maximum intensity after 30 min at room temperature, and then 4 mL deionized water was added and mixed thoroughly by inverting the tube. After 10 min, or until CO_2 effervescence ceased, the absorbance of the color was measured with a spectrophotometer at 520 nm. The color is stable for at least 24 h.

The concentration of the reducing sugars in the sample analyzed was calculated by reference to a calibration graph prepared by analyzing 2 mL of the standard working solution as described above for determination of the D-glucose equivalent concentration of reducing sugars in the sample. For calculation purposes, the moisture content of the field-moist sample analyzed should be added to the 25 mL 60% methanol initially added to the soil sample analyzed. All results reported are averages of duplicates, expressed on an oven-dry weight basis, moisture being determined from weight loss after drying at 105°C for 48 h.

Kinetic transformations and calculations

To calculate potential maximum value of potentially releasable reducing sugars potentially (R_r) in a given soil, we used the following equation:

$$1/R_c = 1/R_r + K_t/R_r \cdot 1/t \qquad \text{(Eq. 2.1)}$$

where R_c is the cumulative amount of reducing sugar (D-glucose equivalent) released at time (t) and K_t is a constant (K_t = the incubation time required to produce 50% of R_r). When the results are plotted $1/R_c$ vs. 1/t, the intercept is $1/R_r$, and the slope is equal to K_t/R_r; R_r is in units of mg reducing sugar (D-glucose

equivalent) per kilogram of soil, and K_t is in hours. The initial amount of reducing sugar in the soil sample before incubation was subtracted from that released before plotting the results.

Results and Discussion

Effect of 60% methanol solution on quantitative recovery of reducing sugar with the Somogyi-Nelson method

By incubating soil in 60% methanol solution, products such as glucose, which would otherwise either metabolically participate aerobically in the TCA cycle producing CO_2 or ferment anaerobically to produce CH_4 and CO_2, are accumulated in the 60% methanol solution. Likewise in soil, CO_2 is produced from aerobic utilization of reducing sugars, and CH_4 and CO_2 from anaerobic processes. Incubating field-moist soil in 60% methanol leads to inactivation of the microorganisms (Appendix A), decreased CO_2 production (Appendix B), and accumulation of reducing sugars (Fig. 2.1). The accumulated reducing sugars (Table 2.2) were quantified by the colorimetric method of Somogyi and Nelson as described by Wood and Bhat (1988), which was shown by Deng and Tabatabai (1994) to be most suitable for use in soils, as it is sensitive and has a reasonable range of detection for concentrations of reducing sugars found in soil extracts (Appendices C-H).

Accuracy and precision

Tests with several soils representing a range in organic C showed that the method developed has high accuracy and precision (Table 2.3). The recoveries of glucose added to soils (replications of 0, 20, 50, and 100 mg kg^{-1} soil) and incubated for 24 h with 60% methanol as previously described were quantitative (means ranged from 97 to 101%). The recovery values from soils incubated with deionized water instead of 60% methanol ranged from 6 to 19% (Raw data in Appendix I). Results showed that the reducing sugar pools in air-dried soils are much lower than those of the corresponding field-moist soils (Table 2.2). This could be due to partial enzyme protein denaturation during air-drying of field-moist soils at room temperature (22°C).

Effect of 60% methanol solution on culturable organisms

Triplicate 0.1 mL soil suspension from either 5.0 g equivalent of air-dried soil and 25.0 mL 60% methanol solution or 25.0 mL deionized water was inoculated into pour plates of 10% tryptic soy agar and incubated to assess the effect of 60% methanol on culturable microbial populations. Samples were plated immediately after the solutions were mixed, and after 24 h of incubation at 30˚C. Plates were then also incubated at 30˚C and observed after 24 h. Results showed that the 60% methanol solution decreased culturable microbial populations by an estimated 90% in comparison with samples plated immediately after being mixed (Appendix A). After soil solutions were incubated for 24 h, greater decrease of culturable microbial

population was counted after plating on 10% tryptic soy agar plates, with approximately 95% of the microbial growth inhibited (Appendix A); the remainders probably were inactive spore forms resistant to the treatment.

Effectiveness of methanol in microbial inactivation

Glucose is water soluble and can be easily extracted from soils with water, but it is not persistent in soils, as it easily metabolized and converted to CO_2 by the microbial populations (Frankenberger and Dick, 1982; Martens and Frankenberger, 1990; Frey et al., 1999). To inactivate the microorganisms, we used 60% methanol, as this reagent has been used for microbial inactivation by many workers in different fields (Bieleski, 1964; McCord and Fridovich, 1969; Nickerson and Lim, 1974; Faijes et al., 2007; Sellick et al., 2011), including pathogenic microorganisms (Tabatabai et al., 1979). To confirm this, we studied the recovery of glucose added to soils. The recovery ranged from 97 to 101% (Appendix C to H), with greatest recoveries from soils rich in organic C. These results indicate that after 24 h, 97 to 101% of any glucose added could be recovered, in addition to the native release of reducing sugars from the soils' metabolic activity (with 0 mg glucose per kg soil used as a control). Soils incubated in 60% methanol showed evidence that microbial metabolism of added and released reducing sugars had been stopped, when compared to soils incubated with water and a glucose addition (spike), where the recovery ranged from 6 to 19%.

Reducing sugar pool in soils

The results obtained for the accumulation of glucose in three surface soils incubated in 60% methanol at 30°C for times ranging from 2 to 24 h are shown in Fig. 2.1 (full lists of data in Appendix J). The results obtained with the other soils studied were similar. Those results further support the finding of inactivation of the microbial populations. Application of Eq. 2.1 to the results reported in Fig. 2.2 showed that the results obeyed the parameters of the equation. Similar graphs were developed from the application of the equation to the results obtained with the other five soils. The potential reducing sugar pool in soils ranged from 39 to152 mg kg^{-1} (average = 88 mg kg^{-1}) in the field-moist soils, and from 11 to 98 mg kg^{-1} (average = 36 mg kg^{-1}) in the air-dried soils. The times required to release 50% of the glucose equivalent pools ranged from 3.9 to 16 h in the field-moist soils (average = 9.8 h) and from 1.6 to 11.6 h (average = 5.3 h) in the air-dried soils.

Relationships of the reducing sugars pool to other biochemical properties

In this study, analysis showed that the reducing sugar concentrations were not well correlated (r = 0.24 and 0.67, respectively) with organic C or C_{mic} concentrations of soils, perhaps because the observed total potential releasable reducing sugar pools are such a small fraction of the larger organic C pool. Also, soils had pH values close to neutral (changes in pH during incubation varied only within the margin of error of the electrode used, or 0.5 pH unit) and all soils were collected from under minimally disturbed areas with predominantly the same

vegetation and biomass input, where differences among the R_r values could not be attributed to soil management factors. The R_r and K_t values varied among the soils, but all soil samples had reached values equivalent to R_r values after four or five days of incubation. We tested the possibility of estimating the total reducing sugar pools in soils without constructing Figs. 2.1 and 2.2 by incubating soils with 60% methanol mixture for 1, 2, 3, 4, or 5 days. Results showed that the values obtained after 4 days of incubation were similar, and those obtained after 5 days were nearly identical to the R_r values calculated by using Equation 2.1 (Figure 2.3).

Potential usefulness of the method

The described method is centered on a functional-potential premise, as defined by the subject of much research: greenhouse gas flux from soil. Rather than pin-pointing specific carbon groups present in a soil at a given time, the method allows quantification of a group of substrates that can easily be metabolized to CO_2 (Cheshire, 1985). Although conceptualized from β-glucosidase hydrolysis of cellobiose to glucose, this pool is defined strictly by function; it is non-specific in origin, independent of enzyme activity assay, free from overlap or transformations due to extraction method and represents processes regulating mechanisms in soil not only as complex, but also as biological and unique.

Benefits of this approach include that results are not specific to any one substrate or catalytic agent of a pool-regulating reaction. Rather, this approach and the analytical methods developed should provide results indicating the capacity of a

soil system to metabolize soil carbon, which is a multivariable function. These reasons are important because soils are living systems with webs of complex chemical and biochemical reactions, especially in terms of a “young” or “labile” soil carbon. Spohn and Giani (2011) showed that soil carbohydrates reach new equilibrium concentrations faster than total organic carbon, glomalin-related soil protein, and mean weight-diameter of water stable soil aggregates in response to changes in land usage, as carbohydrates are susceptible to faster reactions. It may therefore stand that a quantitative measure of the products of degradation of carbohydrates and structurally related soil carbon constituents would serve as an appropriate and feasible short-term indicator of potential long-term impacts of land management strategies on soil-carbon residence times.

Several groups of ß-glucosidases have been identified (Saha et al., 1995). Some show product inhibition (i.e., inhibited by reducing sugars) while others do not. Therefore, we tested the possible effect of glucose on β-glucosidase activity in soils, the most predominant glycosidase (Eivazi and Tabatabai, 1988). Results showed that the activity of this enzyme in soils was not affected by 0.02 mM glucose (a concentration greater than that observed during analysis of potentially releasable reducing sugars) when the incubation mixture contained 60% methanol. Further tests showed that the pH values of the incubated soils remained relatively constant throughout incubation (±0.5 pH unit) (Appendix K). Therefore, the decreases in rates of reducing sugars produced in the soils (Figure 2.1) are related to the

decrease in the substrate (i.e., disaccharide) concentration with incubation time (first order reaction).

The described method may have the potential to assist in the study and partitioning of root respiration from total CO_2 flux measured during in-field CO_2 monitoring experiments. This method may also facilitate research and contrast of the roles of intracellular and extracellular soil enzymes' roles in nutrient cycling processes: an area with little previous exploration. It provides support for and new ideas in the areas of rate-limiting regulatory reactions involved in the study of otherwise amorphous dissolved organic carbon and soil biological community functions (Kemmitt et al., 2008). This method may also be used in validation of regionally appropriate models that include impacts of conservation practices such as no-till and reduced tillage and cropping systems that take advantage of diverse rotations and perennial systems (Greenhouse Gas Working Group, 2010). The mechanisms that govern the size and ease of movement of this sensitive fraction of soil C pool are controlled by the same laws of thermodynamics and kinetics that make the application of this method viable. As such, knowledge of the reducing sugar pools in soils may help to refine prediction of global climate change scenarios, as well as to provide data to support agricultural ecosystems as a source or sink of atmospheric carbon.

Conclusions

The predominant source of soil metabolic substrate, or reducing sugars, can be easily quantified by incubating soil with 60% methanol by volume at 30°C for times ranging from 2 to 24 h and calculating the pool size or by incubating the soil in 60% methanol for 5 days at 30°C and analyzing the reducing sugars produced by colorimetric method. Results were highly reproducible, and the method was shown to have great precision and accuracy. The potential reducing sugar pool (R_r) in eight soils varying in texture ranged from 39 to152 mg kg^{-1} (average = 88 mg kg^{-1}) in field–moist soils, and from 11 to 98 mg kg^{-1} (average = 36 mg kg^{-1}) in air–dried soils. The times required to release 50% of the glucose equivalent pools (K_t) ranged from 3.9 to 16 h in field–moist soils (average = 9.8 h) and from 1.6 to 11.6 h (average = 5.3 h) in air–dried soils. The differences in size and ease of turn-over of metabolic substrate pool among soil types represent differences in enzymatic hydrolysis processes. The decreases in both R_r and K_t in soils which were air-dried supports the need for the meaningful analysis of the regulatory mechanisms of soil at field-like conditions. The incubation of field-moist soil in 60% methanol solution for five days at 30° C and analysis of soil extract by Somogyi-Nelson colorimetric method is an accurate method for the assessment of soil management impacts on the potential reducing sugar pool in soil.

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Table 2.1 Summary of selected properties of soils.

Soil		pH†							
Series	Subgroup	H_2O	$CaCl_2$	Org. C	Org. N	Clay	Sand	C_{mic}‡	N_{mic}‡
				----------g kg^{-1} ----------				------mg kg^{-1}------	
Clarinda	Vertic Argiaquolls	6.7	6.6	21.6	2.3	440	30	315	70
Clarion	Typic Hapludolls	6.9	6.6	22.1	2.6	240	400	169	40
Exira	Typic Hapludolls	7.0	6.6	24.0	2.9	360	10	425	95
Marshall	Typic Hapludolls	6.8	6.5	25.7	3.0	290	10	292	83
Linder	Aquic Hapludolls	7.1	6.7	29.0	3.2	180	570	302	85
Nicollet	Aquic Hapludolls	6.8	6.5	30.0	3.3	240	360	292	87
Webster	Typic Endoaquolls	7.3	7.0	33.8	3.4	320	260	539	98
Harps	Typic Calciaquolls	7.4	7.0	35.7	2.9	230	90	292	74

† Soil:water or 0.01 *M* $CaCl_2$ ratio 1:2.5.

‡ C_{mic} and N_{mic} are microbial biomass C and N, respectively.

Table 2.2 Potential cumulative reducing sugars (R_r) and the time required to produce 50% of R_r (K_t).

	Field moist†			Air dried†		
Soil	R_o	R_r	K_t	R_o	R_r	K_t
	-------mg kg^{-1}------		---hours---	---------mg kg^{-1}--------		---hours---
Clarinda	1.2	115	6.1	7.1	98.0	11.6
Clarion	2.6	39.1	6.9	3.8	12.6	3.0
Exira	5.2	74.1	3.9	7.6	51.5	11.1
Marshall	6.6	90.9	9.2	8.4	26.6	3.3
Linder	3.7	52.6	7.3	7.6	21.7	1.9
Nicollet	5.8	132	13.3	10.6	11.3	1.6
Webster	1.7	153	16.0	3.8	49.8	6.6
Harps	2.0	90.1	15.8	4.4	16.3	3.0
Avg.	3.6	88.0	9.8	6.7	36.0	5.3

† R_o is reducing concentration in soil; R_r is the potential reducing sugar pool in soil; K_t is the time required to release 50% of R_r.

Table 2.3 Precision of the method.

Soil	D-glucose equivalent (mg kg^{-1} soil in 24 h)		SD‡	CV(%)§
	Range	Mean†		
Clarion	33.6 - 35.9	34.6	0.90	2.6
Linder	36.8 - 39.6	37.8	0.76	2.0
Marshall	70.3 - 74.9	72.7	1.47	2.0
Clarinda	94.1 - 101.7	98.3	2.50	2.5
Harps	35.2 - 43.3	38.5	2.67	6.9

† Mean of six replicated extractions and analyses.

‡ SD, Standard deviation.

§ CV, Coefficient of variation.

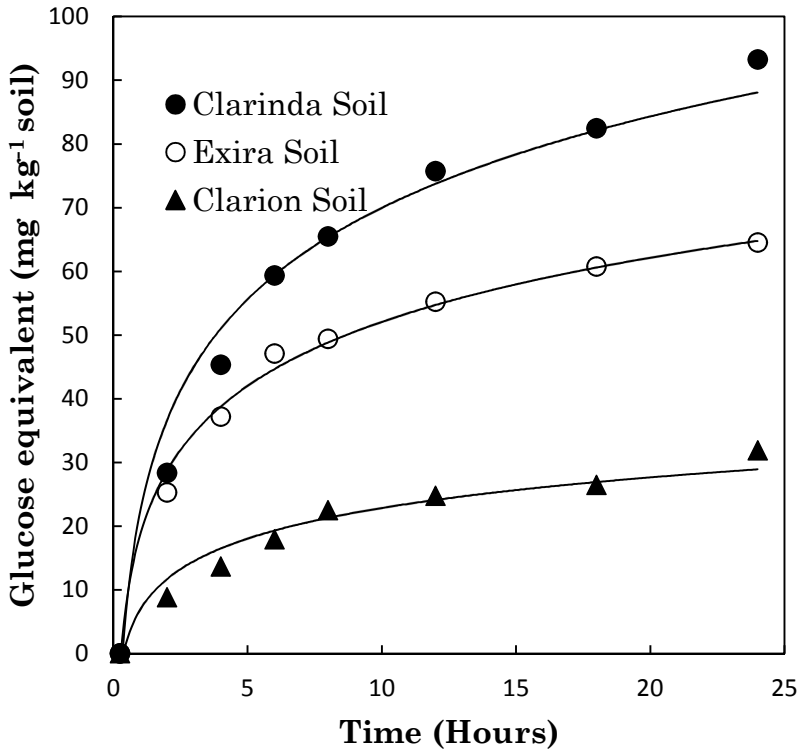

Figure 2.1 Production of reducing sugars from three Iowa soils over 24 hours. At all data points, the differences among duplicate values were smaller than the symbol used in the figure.

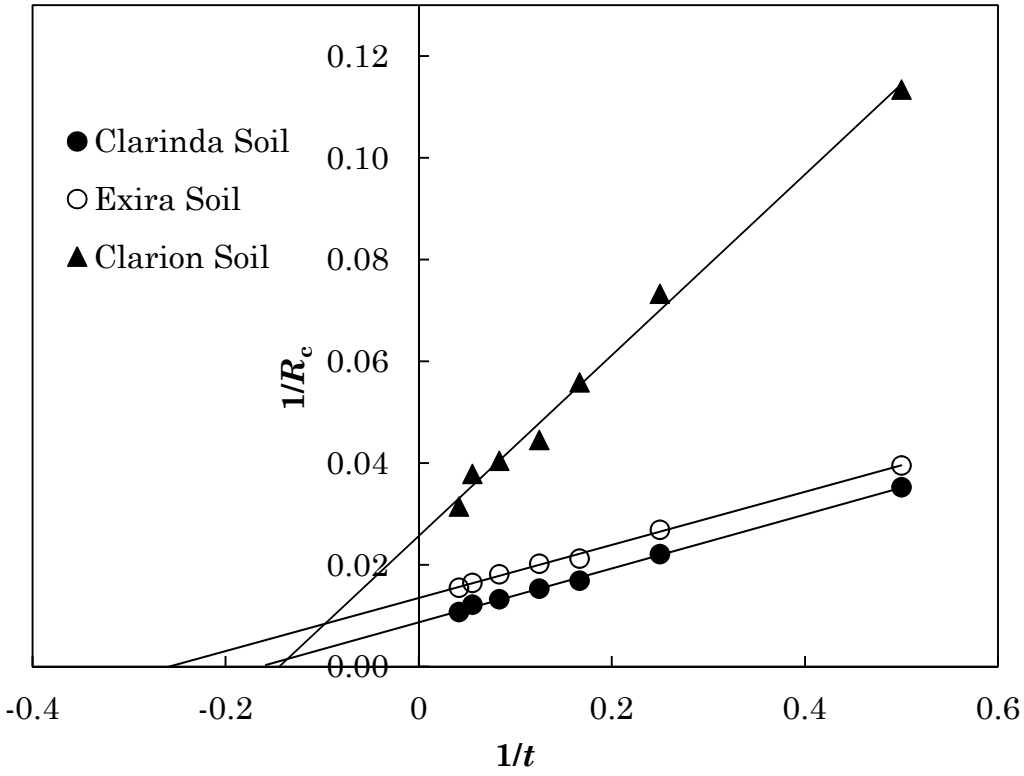

Figure 2.2 Transformation of observed production of reducing sugars over 24 hours for calculation of total potential reducing sugar released. At all data points, the differences among duplicate values were smaller than the symbol used in the figure.

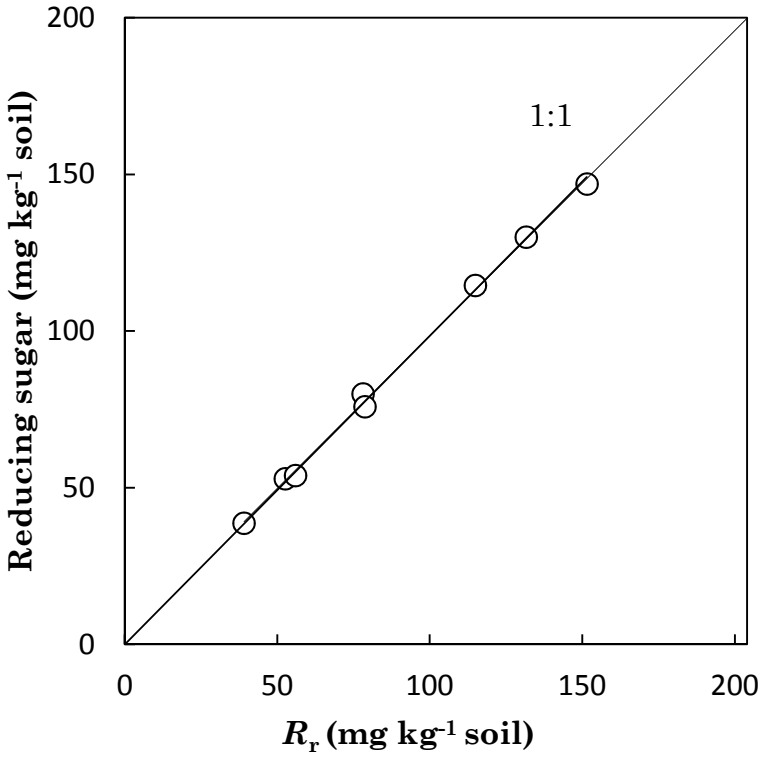

Figure 2.3 Relationship between the calculated amounts of potential reducing sugar pools in soils (R_r) and the values obtained after incubation for five days at 30°C. At all data points, the differences among duplicate values were smaller than the symbol used in the figure.

CHAPTER 3

TILLAGE SYSTEM AND CROP ROTATION SPACIOTEMPORAL EFFECTS ON A SENSITIVE SOIL CARBON FRACTION AND ASSOCIATED SOIL AGGREGATE FRACTION DISTRIBUTION

Abstract

Despite much focus on the effects of tillage and crop-rotation practices on soil carbon, there is still a need to assess the immediate or short-term impacts of soil management practices on sensitive constituents of soil carbon. The objectives of this study were to investigate the effects of tillage and crop rotation on the potential reducing sugar pool in soil as affected by tillage, time, soil depth, and soil aggregate size fractions. The experimental layout of the study was a complete randomized block design with three replications repeated at four locations on distinct and representative Iowa soils. Crop rotations were continuous corn (CC) and corn-soybean (CS), both managed under moldboard plow (MP), chisel plow (CP), and no-till (NT). Surface soil samples (0-15 cm) from all four locations were collected after harvest in fall 2011 and analyzed for pH, moisture, total carbon and nitrogen, and potential total releasable reducing sugars.

Soil moisture and potential total reducing sugars were analyzed in the spring of 2012, one week before a tillage treatment and again one day after spring secondary tillage treatment on soil samples collected again at the Ames location. Soil cores (0-15 cm) were collected from all three tillage systems within the corn-soybean rotation at Ames, and divided into 0-7.5 cm and 7.5-15 cm subsamples prior

to processing for dry sieving. Each subsample was dry sieved on a nest of sieves for the aggregate size fractions >8, 8-4, 4-2, 2-1, 1-0.5, and <0.5 mm. Each soil aggregate size fraction from each subsample depth and tillage system was then analyzed for soil moisture and potential reducing sugars by incubating 2.0 g of field-moist soil in 10.0 mL of 60% methanol solution for five days at 30°C, as recommended by previous research (chapter 2).

At all four locations, crop rotation and tillage had a significant effect on reducing sugar concentrations, with greatest differences noted in soils that were natively rich in organic matter. At each location, no-till (NT) systems had the greatest concentrations of reducing sugar, while moldboard plow (MP) had the lowest. There was a significant crop rotation and location interaction. Concentrations of reducing sugars decreased significantly over the winter for all tillage systems. Concentrations of reducing sugars also significantly decreased in the moldboard plow tillage system within a one-week period that included a single pass of secondary tillage in the spring when compared to concentrations of the previous week. Although not significant, concentrations of reducing sugars declined in soil under chisel plow tillage system during the same week of spring and after a single secondary tillage event. These decreases can be compared to concentrations that significantly increased in the no-till system in the spring within the same week.

Tillage also had significant effects on the concentrations of total potential reducing sugars of six different soil aggregate fractions within and between two

different depths, with greatest concentrations in the no-till system 0-7.5 cm subsample. On average, the 1-2 and 2-4 mm soil aggregate fractions held the greatest concentration of releasable reducing sugars. A stratification effect was noted in the no-till system between the averages for all size fractions in the 0-7.5 cm (surface) and 7.5-15 cm (sub-surface) soil samples, with significantly greater concentrations in surface soil. Total potential reducing sugar concentration is well correlated ($r = 0.764$) with total soil carbon pool. In less than ten years of continuous system management, crop rotation and tillage practices had significant effects on potential total reducing sugar concentrations in soils.

Introduction

Supply and degree of availability of fresh organic matter input drives soil organic matter dynamics by altering microbial community populations and biomass and thus the enzymatic activities of soil (Gude et al., 2012). A broad family of enzymes is responsible for the fast reactions, or degradation of glycosidic bonds in such a supply of fresh organic matter (Coutinho and Henrissat, 1999), providing the soil biological community ample opportunity to decompose any organic matter of natural origin (Lützow et al., 2006). Such opportunity, however, may be hindered by organic matter interactions with soil, or protection of carbon by the formation of and stabilization of macro-aggregates by root exudates, fungal mycorrhizal hyphae, and recycled microbial cell debris (Tisdall and Oades, 1982). Formation of stable soil aggregates that may secure carbon in soil is based on inputs of fresh organic matter, the degree to which that organic matter can be decomposed, the depth at which the

fresh organic matter input is introduced (i.e. growth of roots or incorporation of crop residue into soil subsurface), and the level of adsorption to soil particles (Jones and Donnelly, 2004). The length of time that this fresh carbon is protected in macro-aggregates, however, may be relatively short, as it is highly susceptible to decomposition by microorganisms and easily affected by tillage (Cambardella and Elliot, 1993). Management practices that reduce the levels of soil disturbance (such as no-till) create conditions which are favorable for greater carbon sequestration compared to conventional tillage (Paustian et al., 1997; Al-Kaisi et al., 2005). Work towards the documentation of the size of a more-labile, or more readily oxidizable, pool of the total soil organic carbon pool concludes that carbohydrate turn-over is highly sensitive to soil texture, climate (temperature and rainfall), and substrate availability. Hu et al., (1997) document that there were no significant differences in soil carbohydrate concentrations over the short-term (two years) between conventional tillage and no-till management practices.

Tillage and crop rotation have been found to not only alter the supply and availability of organic carbon, but to also alter microbial populations and biomass and subsequent enzyme activities (Xiang et al., 2011). The degree of disturbance exerted by a tillage practice has also been found to alter the type and amount of organic carbon distribution within soil-aggregate fractions as well as the actual distribution of soil aggregates themselves (Oades, 1984; Elliot, 1986; Paustian, et al., 1997). The mutualistic relationship between SOC and soil aggregates is based on SOC's role in formation of soil aggregates, but continues with the role of

aggregates in the physical protection or stabilization of SOC (Tisdall and Oades, 1982). In turn, the physical protection of SOC makes it less accessible to soil biological attack (metabolism) and more likely to become chemically or biochemically stabilized (Jacobs et al., 2010). Such chemical or biochemical stabilization of soil carbon in soil micro-aggregates within macro-aggregates is associated with older, or more decomposed organic matter, as humic compounds or polymers are left after decomposition by microorganisms (Tisdall and Oades, 1982).

Efforts towards understanding and quantifying the impacts of soil management on SOM quality provide evidence that discrete soil carbon pools such as soil carbohydrates reach new equilibrium concentrations faster than total organic carbon (TOC), glomalin-related soil protein (GRSP), and mean weight-diameter (MWD) of water stable soil aggregates in response to changes in land usage, as carbohydrates are susceptible to faster reactions (Spohn and Giani, 2011). However, there has been little assessment of a meaningful soil carbon pool that is highly sensitive (i.e. detects changes over short periods of time, or in under five years) and that reflects the impacts of different agroecosystems and management practices on relevant and quantifiable kinetic rates of turn-over within such a pool. Therefore, the major objectives of our study were to investigate the impacts of management practices, time, and space on a highly sensitive soil carbon constituent. Towards this purpose, potential total releasable reducing sugars (a product of SOM degradation by soil enzymes) from field-moist surface soil (0-15 cm) were trapped in a 60% methanol solution for five days at 30°C after metabolic

activity of the soil was quenched and quantitatively analyzed by Somogyi-Nelson method.

Materials and Methods

Site description, experimental design, and management

This study was conducted on soil samples collected from four locations in Iowa in the fall of 2011 at 1) the Marsden Research Farm near Ames, where the soils were Webster silty clay loams; 2) the Armstrong Research and Demonstration Farm near Lewis, where the soils were Marshall silty clay loams; 3) the Northeast Research and Demonstration Farm near Nashua, where the soils were Kenyon silt loams; and 4) the Northwest Research and Demonstration Farm near Sutherland, where the soils were predominantly Galva silty clay loams (Table 3.1).

All sites were established between 2002 and 2003 under corn-soybean (C-S) and corn-corn-soybean (C-C-S) rotation over moldboard plow (MP), chisel plow (CP), and no-till (NT) tillage systems and had previously been in C-S rotation under chisel plow tillage system. In 2006, the C-C-S plots were split and one half of each plot was changed to continuous corn (C-C) crop rotation. Average seasonal precipitation (April – October) in 2011 for the four locations was 574 mm, with a thirty-year average precipitation of 727 mm. The experimental design used in this study was a randomized complete block design with three replications. Plot sizes ranged from approximately 9 to 15-m wide and between 27 to 30-m long at each location.

On the moldboard plow plots, primary tillage consisted of fall moldboard plowing, which resulted in a soil disturbance to a depth of approximately 20 cm, followed by field cultivation (or disking) as the secondary tillage in the spring. On the chisel plow plots, primary tillage consisted of fall chisel plowing, which resulted in a soil disturbance of approximately 15 cm, also followed by field cultivation (or disking) as the secondary tillage in the spring. Under no-till, the only field operations performed were seed planting and N fertilizer application, where maximum disturbances up to 5 cm deep due to planting or due to injecting anhydrous ammonia. Actual N rates applied to all plots were of 45 kg ha^{-1} for corn after soybean and 190 kg ha^{-1} for corn following corn. For all treatments, weeds were controlled using pre-and post-emergence herbicides that are typically used in crop production for Iowa. Due to high soil tests for phosphorus (P) and potassium (K), no additional fertilizers were applied.

Soil samples (n = 72) were collected in October 2011 from all four locations. Eighteen samples were collected from each location, representing three replications of each tillage system (MP, CP, and NT) under both crop rotations (CS and CC). For each sample, field-moist surface soil (0-15 cm) was sieved in-field to pass through a 4-mm sieve, excluding plant roots and debris, into a black plastic garbage bag. Soils were homogenized in the laboratory, and a subsample was then sieved through a 2-mm screen and air dried, while the remainder was stored at 4˚C until analysis. The same procedure was repeated again in April 2012 for plots at the Ames site, and again one week later, one day after the spring secondary tillage treatment.

Soil cores were also collected the first week of April 2012 from two replications of each tillage system in the C-S crop rotation using a 7.6-cm diam. golf course hole-cutter to a soil depth of 15 cm. Soil cores were divided into two halves to represent 0-7.5 and 7.5-15 cm subsamples, and stored at 4˚C in sealed plastic bags. Subsamples were then gently broken apart by hand along planes of natural weakness into a nest of sieves sized 8, 4, 2, 1, and 0.5 mm and shaken up and down on a mechanical shaker (Sieve Tester, Model # SS15, Gilson Company, Inc.) for exactly two minutes.

Resulting field-moist soil aggregate fractions of sizes >8, 4-8, 2-4, 1-2, 0.5-1, and <0.5 mm were then weighed and promptly stored in reseal-able clear plastic bags at 4˚C until analysis. We chose to exclude the fractionation of the soil into any smaller soil aggregate size fractions due to the possibility of overwhelming soil particle size distribution disparities when compared to actual secondary organo-mineral complexes. Our choice was also supported by the potential differences in moisture contents of smaller size fractions, as moisture content of soils was shown in previous work (chapter 2) to have negative effects on concentrations of potential total releasable reducing sugars in soils. A subsample of each soil aggregate size fraction from each tillage system and depth was also air-dried for further analysis.

A portion of all of the air-dried soil samples was ground by mortar and pestle to pass through an 80-mesh (180-μm) sieve. Soil properties, including pH that was determined by a glass electrode (soil: water or 0.01 *M* $CaCl_2$ ratio = 1:2.5) using an AR15 pH meter (Accumet® Research, Fisher Scientific International Inc., and

organic carbon by a dry combustion CHN analyzer (LECO Corp., St Joseph, MI), are summarized in Table 3.1. Organic C determinations used the <180-µm soil samples, while pH was determined on the <2-mm mesh air-dried soil samples. All results reported are averages of duplicates, expressed on an oven-dry weight basis, moisture being determined from weight loss after drying at 105°C for 48 h.

Chemicals and reagents

Methanol (60%, v:v): add 60 mL of methanol (Fisher certified reagent, Fisher Scientific Co., Chicago, Ill.) to 40 mL of deionized water, and mix.

Reducing sugar standard stock solution: 1.0 g of D-glucose was dissolved in 800 mL of deionized water in a 1-L of volumetric flask, and the volume was adjusted to one liter with deionized water, mixed thoroughly. One mL of this stock solution contains one mg of glucose. This solution was stored in a refrigerator at 4°C and allowed to come to room temperature (22˚ C) before preparation of any standard working solution.

Reducing sugar standard working solution: for preparation of the calibration graph, 0, 0.5, 1, 2, 3, 4, and 5 mL of the 1 mg L^{-1} D-glucose standard stock solution were pipetted into each of seven 50-mL volumetric flasks and the volumes were adjusted so that the total volumes contain 60% methanol, and mixed thoroughly. Those solutions contain 0, 10, 20, 40, 60, 80, or 100 µg of D-glucose per 2 mL, respectively.

Somogyi reagents SI and SII and Nelson reagent were prepared to have the same composition as those described by Wood and Bhat (1988).

Potential total releasable reducing sugar procedure

For soil samples from the four different locations in the fall and spring samples in Ames, 5.0 g of field–moist soil sample (on an oven-dried weight basis) was placed in a 50-mL plastic centrifuge tube, treated with 25 mL of 60% methanol solution, sealed with a rubber stopper, shaken by hand for one minute, and incubated in duplicates for five days at 30°C. For soil samples from different field-moist soil aggregate size fractions, 2.0 g of field-moist soil was incubated with 10 mL of 60% methanol solution, and differences in amount of water added and oven-dried weight of the sample used were later adjusted after drying for 4 h at 105°C and calculating the gravimetric moisture content of the soils.

After incubation, the tube was shaken on an end-to-end shaker for 15 min, un-stoppered, and centrifuged at 17,390 *g* for 15 min. Supernatant was decanted, and a 2.0-mL aliquot was analyzed for reducing sugars by the Somogyi-Nelson method as follows**:** 40 mL the Somogyi Reagent I (SI) and 10 mL Somogyi Reagent II (SII) were mixed to yield a 4:1 ratio SI: SII (Somogyi) solution. Then, 2 mL of this Somogyi solution was quantitatively transferred to a standard 18 x 150-mm laboratory test tube, along with 2 mL of soil supernatant or 2 mL of 60% methanol for control. The level of the liquid in the test tube was marked, and the test tube was immersed in a boiling water bath for exactly 15 min, the boiling water bath

being partially covered to maintain the boiling temperature. The test tube was immediately transferred to a cold-water bath to cool (*ca.* 5 min).

After the sample was cooled, a few drops of deionized water was added up to the mark to compensate for any liquid loss during heating, mixed, and 2.0 mL of the Nelson reagent was immediately added with caution, as a strong effervescence may occur. The tube was then mixed on a vortex mixer. A blue color was developed with a maximum intensity after 30 min at room temperature, and then 4 mL deionized water was added and mixed thoroughly by inverting the tube. After 10 min, or until CO_2 effervescence ceased, the absorbance of the color was measured with a spectrophotometer adjusted to 520 nm. The color is stable for at least 24 h.

The concentration of the reducing sugars in the sample analyzed was calculated by reference to a calibration graph prepared by analyzing 2 mL of the standard working solution as described above for determination of the reducing sugars in the sample. The moisture content of the sample analyzed should be added to the 25 mL 60% methanol initially added to the soil sample analyzed. All results reported are averages of duplicated analysis from two replications, expressed on an oven-dry weight basis, moisture being determined from weight loss after drying at 105^{o}C for 48 h. All laboratory analysis raw data in duplicates are summarized in Appendices L and M.

Statistical Analysis

The results were analyzed using the SAS Statistical Software Package (SAS Institute Inc., 2005). The general linear model (GLM) procedure was used to perform the analysis of variance for potential total releasable reducing sugar concentration for the main effects of location, tillage system, and crop rotation. Soil samples from Lewis and Sutherland locations had a previous crop of soybeans in the corn-soybean rotation during sampling time, while Ames and Nashua location soil samples had corn as the previous crop in the corn-soybean rotation during sampling time. The average of two laboratory duplicates was used as an experimental unit for each of the 18 samples from the four different locations. Means were separated using a least significant difference (LSD) where treatment effects were significant at $P< 0.05$.

Proc GLM was also used to perform the analysis of variance for potential total releasable reducing sugar concentration distribution within soil aggregate size fractions, where, due to manageable workload, treatment comparisons were unavoidably based on pseudo-replicates. Means were separated using a least significant difference (LSD) where treatment effects were significant at $P< 0.05$. The Proc Mixed procedure with repeated measures for soil depth was also used to evaluate for differences between potential total releasable reducing sugar concentrations within aggregates. A compound symmetry covariance structure was used for repeated measures. ANOVA F-tests at $P< 0.05$ led to the same

interpretation of mean separations for treatment effects as when interpreted with least significant differences (LSD) at $P< 0.05$ generated by Proc GLM.

Results and Discussion

Organic Carbon Pool and Reducing Sugar

The relationship between soil organic carbon as an independent variable and potential total releasable reducing sugars as a dependent variable from all locations was linear with an R^2 value of 0.584 (Figure 3.1). These results suggest that although releasable reducing sugars are related to organic carbon, SOC as a source for R_r can only explain 58.4% of R_r liberation. These results suggest that other factors in addition to SOC pool influence the size of the R_r pool of soil carbon, which agrees with recent research that indicates that there may be more suitable analyses available for the quantification of land-use impacts on soil carbon changes (Culman et al., 2012).

The relationship between reducing sugar as a dependent variable and soil organic carbon as an independent variable were examined for each location (Figure 3.2). Results show that locations with greater stocks of native organic carbon are more susceptible to changes in potential total releasable reducing sugars from management impacts. The slope of the linear regression line was greater for locations where the average soil organic carbon value was greater (i.e. Nashua) when compared to the location with the lowest average soil organic carbon value (Lewis). These results support a body of work that suggests the more total organic

carbon is in a soil, the more sensitive or susceptible carbon pools at various stages of stability within the total soil carbon pool may be to cultivation or crop management (Hu et al., 1997; Blair and Crocker, 2000; Degryze et al., 2004).

Tillage Systems Effect on Soil Potential Total Reducing Sugar

The effect of tillage on total potential releasable reducing sugars in soil was significant, where reducing sugar concentration decreased with increasing tillage intensity. On average, R_r of soils under no-till, chisel-plow, and moldboard plow tillage systems were all significantly different at $P< 0.05$. When soils collected in the fall from each location are considered, on average, the no-till system soil exhibited mean concentrations of 7.5 and 19.9 mg kg^{-1} soil more releasable reducing sugar than chisel-plow and moldboard plow tillage systems, respectively. Chisel-plow tillage system had concentrations that were, on average, 12.3 mg kg^{-1} soil greater than moldboard plow tillage system (summary of raw data, Appendix L). These findings reveal the importance of conservation tillage systems in either building or helping to maintain the kind of SOM which may be easily degraded to metabolic substrate and subsequent greenhouse gases. Similar increased levels of soil carbon have been noted in conservation tillage systems when compared to conventional tillage systems (Lal et al., 1994; Jacobs et al., 2009).

When tillage systems were isolated and examined within crop rotations and locations, significant differences were also observed. Under continuous corn cropping system (Figure 3.3), the no-till system had the greatest concentrations of

releasable reducing sugars, regardless of the location, soil type, and soil organic carbon content, with intermediate concentrations in the chisel-plow tillage system, and the moldboard plow tillage system with the lowest concentrations. Concentrations within moldboard plow, chisel-plow, and no-till systems were each significantly different from each other at all locations except for Lewis site. This might be attributed to the relatively low native organic carbon found at this location.

Under corn-soybean crop rotation (Figure 3.4), the same significant trends were observed between all tillage systems at each location. However, concentrations of releasable reducing sugars under chisel-plow and no-till systems at Nashua showed that chisel plow was slightly greater than no-till, although differences were not significant. Another exception was again observed at Lewis site, where even though no-till system concentrations were greater than concentrations of soil under chisel-plow tillage system, differences between the two were not significant. Again, this might be attributable to the low native total carbon at Lewis location. This may also indicate that R_r pools are more resilient in CS crop rotation, as concentrations are maintained which are similar to NT.

In both the continuous corn and corn-soybean rotation cropping systems, the greater differences in releasable reducing sugars between tillage systems in soils with increased levels of native total carbon is similar to findings of Blair and Crocker (2000), Whitbread *et al.* (1998), and Blair and Daniel (1996) who report that

the impacts of soil management are more extreme in soils with greater native total carbon. They also report that changes attributable to soil management in labile soil carbon (oxidized with $KMnO_4$) were more pronounced than changes in total soil carbon. Similarly, after converting agricultural soils that have been traditionally intensely cultivated to no-till system, increases in total soil carbon are less pronounced in soils with lower total organic carbon (Fabrizzi et al., 2003). Mann (1986) reported that soils which are initially very low in carbon stand to gain slight amounts of carbon following cultivation, while most agricultural soils that are natively rich in organic carbon lose at least 20% upon initial cultivation.

In general, there were significant differences when means of each tillage system across both crop rotations at all locations were analyzed solely by tillage system (Figure 3.5). It appears that the magnitude of a tillage system's ability to store soil carbon instead of rapidly degrading it for release to the atmosphere is by site specific conditions and other intrinsic parameters like soil particle distribution, drainage, climate, or native organic carbon. Nevertheless, when each tillage system is used to compare the combined effects of location and crop rotation on a state-wide level, seldom is the case where means of the same tillage system are *not* significantly different between crop rotations at the same location. These results were expected, as quantities and ease of rate of decomposition of organic carbon sources limit the growth and activity of microbial communities (Friedel et al., 1996). The results of either increasing or reducing microbial access through residue placement (incorporation during tillage events) are highly significant in agricultural

soils, as greater proportions of SOM are present as carbohydrates (Hu et al., 1997). Moreover, the most sensitive measures of soil quality are known to be of carbohydrate nature: hot-water extractable and water-soluble carbon are two recently cited carbon fractions to be used to assess impacts of land use on soil carbon (Ghani et al., 2003).

As changes in soil management practices occur, changes in resource inputs such as fresh organic carbon also occur, influencing the soil microbiological community, the enzymes produced in response to those inputs, and ultimately if soil organic matter is either degraded or preserved. This supports recent conclusions about the driving factors behind soil organic matter dynamics, especially in terms of supply and availability of fresh organic carbon (Gude et al., 2012). In long-term studies, it seems that tillage intensity is the driving factor in reduction of total soil carbon when comparing soil management extremes, especially in surface soil (Chan et al., 2001).

Crop rotation effect on soil reducing sugar concentration

Mean separation for the effect of soil type (location) using least significant difference (LSD) at $P< 0.05$ showed significant differences in releasable reducing sugar pools between sites (Table 3.2). Average concentrations of Ames, Nashua, and Sutherland sites were significantly different from the average location mean of Lewis, regardless of tillage and crop rotation (Figure 3.4). In this study, effects of

the previous crop within corn-soybean cropping system, which was nested within location, was determined to not be significant.

In general, releasable reducing sugar concentrations were 2.4 mg kg^{-1} soil greater in continuous corn than corn-soybean crop rotation. These differences between the average concentrations of the two crop rotations were not significantly different. However, mean separation between locations for concentrations of releasable sugars in soils under either crop rotation revealed significant differences (Table 3.2).

In the continuous corn cropping system, Lewis site had the lowest concentrations, which were significantly different from Ames and Sutherland (Figure 3.4). The mean concentration of releasable reducing sugars from Nashua was significantly greater than all other locations under continuous corn. Studies from other long-term sites have also shown that cropping systems that have greater C inputs (such as the greater above and below ground biomass of a corn crop compared to a soybean crop) are more likely to have increased enzymatic soil activity (Dick, 1994). This potential increase in enzymatic activity, paired with increased C inputs, could help explain why soil from continuous corn cropping system, on average, had greater concentrations of releasable reducing sugars than did soil from the corn-soybean cropping system.

In the corn-soybean cropping system at all locations, mean separations of reducing sugar based on LSD's at $P< 0.05$ were significantly different between all

locations, regardless of tillage system (Figure 3.5). Lewis site's soil exhibited lowest concentrations of potentially releasable reducing sugar concentrations, followed by Nashua. Ames soil had greater concentrations than those at Nashua. However, mean concentration of releasable reducing sugars of soil under corn-soybean cropping rotation was greatest at Sutherland. Blair and Crocker (2000) report increases in total soil carbon and especially significant increases in labile soil carbon over the long-term (34 years) when a legume is used in cropping rotations when compared to mono-cropping systems without a legume species present. This increase in soil carbon concentrations may be attributable to increased nitrogen availability and subsequent growth (increased fresh carbon inputs) from the following year's crop (Karlen and Cambardella, 1996).

Comparisons between means of releasable reducing sugar concentrations of crop rotation treatments at each location regardless of tillage yielded significant results (Table 3.2). Average concentrations of releasable reducing sugars were greater in soils from under corn-soybean crop rotation at both Lewis and Sutherland locations, where the previous crop in the corn-soybean crop rotation at Lewis and Sutherland was soybean (Figure 3.6). However, at Ames and Nashua, where the previous crop in the corn-soybean crop rotation was corn, the reducing sugar concentrations were not significantly different. The experimental treatment effect of previous crop had to be nested within location for analysis of crop rotation effect and type of crop during soil sampling time for each location; as such the effect of previous crop was insignificant.

We believe, however, that such results may not be insignificant if not nested within location and crop rotation. Significant results may provide evidence that there may be an effect of one season's crop growth on releasable reducing sugars in soil. Such findings would lend support to evidence that concludes including a legume species in a crop rotation increases concentrations of discrete SOM pools such as the light fraction (LF) and intra-aggregate particulate organic matter (iPOM) (Zotarelli et al., 2007). Also, as this test for releasable reducing sugars represents a theoretical metabolic capacity of the soil system as a whole, the system may be driven by carbohydrate inputs and regulated by enzymatic activities of microbial communities, results could be directly related to differences in carbohydrate inputs from one year with one single different crop. Such findings could also be connected to evidence that the activities of ß-glucosidase, amidase, and L-asparaginase are significantly greater in soils where a legume species has been included in the crop rotation (Miller and Dick, 1995). Research also suggests that soil enzymes such as ß-glucosidase are more likely to retain abiontic activity through periods of extreme temperatures in soil that includes a legume species in the crop rotation (Miller and Dick, 1995).

Temporal Effect on Soil Reducing Sugar Distribution

Regardless of tillage management system, results show that releasable reducing sugar concentrations in soils had, on average, significantly decreased by the following spring when compared to baseline values analyzed from samples

collected the previous fall (Figure 3.7). Percentage decrease was greatest in the no-till system under corn-soybean rotation (Table 3.3). This may be linked to evidence that the inclusion of a legume crop in crop rotations that increases enzymes ability to withstand thermal stress, rendering abiontic humic-enzyme complexes more active after repeated freeze-thaw cycles (Miller and Dick, 1995). This decrease in potential reducing sugars in the no-till system might be attributed to potential leaching due to stable soil structure and increased infiltration rate leading to potential leaching of water-soluble compounds (such as disaccharides from which R_r is nascent) to subsurface depths (Beare et al., 1994). This large decrease in potentially releasable reducing sugars may also have some relationship to the increased amorphous dissolved organic carbon (DOC) concentrations noted after freeze-thaw cycles (Wang and Bettany, 1993), or from increased opportunities for intimate reactions hosted by soil surfaces that may signify a transition to a more recalcitrant pool of soil carbon (Oades, 1988).

The effect of a spring secondary tillage pass, or disking, six days after spring samples were collected, (with approximately 24 h additional time after disking to allow soil systems to equilibrate), significantly decreased concentrations of releasable reducing sugars in soil from both continuous corn and corn-soybean cropping systems under moldboard plow tillage system. The single secondary tillage pass also decreased concentrations in chisel-plow tillage systems, although differences were not significant. Once soil carbon is disturbed (i.e. from a tillage operation), it is then possible for the enzymatic activities of the soil organism to

degrade soil carbohydrates to reducing sugars such as glucose, and release CO_2 (Dick and Frankenberger, 1982). It is highly likely that such decreases in concentration of soil metabolic substrate are followed by increases in metabolic product, or CO_2, after a tillage event, and that cultural practices that invoke less disturbance would exhibit less significant decreases, as research done on the same soil type at the same location showed that soil CO_2 emissions were lower for less intensive tillage systems (19-41%) compared with CO_2 emissions from moldboard plow tillage system after a tillage event, with the most substantial differences directly after the soil was disturbed (Al-Kaisi and Yin, 2005).

Soil from the no-till system was also sampled six days after initial spring samples were collected. Concentrations of releasable reducing sugars had significantly increased (+11%) in the corn-soybean crop rotation in six days' time. Although concentrations also increased (+6.9%) in the continuous corn rotation, they were not significantly different when compared to the initial spring samples.

Soil aggregate fractions associated reducing sugar concentration

The effects of tillage, depth, and size of field-moist soil aggregate fractions were highly significant on concentrations of releasable reducing sugars within soil macro-aggregates of size classes < 0.5, 0.5-1, 1-2, 2-4, 4-8, and < 8 mm, as well as percentage distribution of releasable reducing sugars. Comparisons of reducing sugar concentrations for all soil aggregate size fractions at two depths of no-till tillage system were significantly greater than those from under chisel-plow tillage

system (summary of raw data, Appendix M). The average chisel-plow treatment effect was also significantly greater than the effect of moldboard plow tillage system on the concentration of releasable reducing sugars in soil. Differences in R_r within these soil aggregate size fractions can be attributed to the effects of differences of management practices on macro-aggregate bonding agents, which are highly transient due to their polysaccharide nature (Tisdall and Oades, 1982).

The same significant differences were observed among tillage systems within all aggregate size fractions within the 0-7.5 cm surface depth (Table 3.4). In the 7.5-15 cm subsurface depth, however, there was not a significant difference between no-till and moldboard plow tillage systems. These results indicate that the majority of the R_r-rich soil in NT systems is in the top 0-7.5 cm (3 inches) of minimally disturbed soil. The average concentration of releasable reducing sugars of all soil aggregate size fractions within the 7.5 cm depth for chisel-plow tillage system was significantly greater than that of all soil aggregate size fractions within the no-till and moldboard plow tillage systems. Regardless of tillage system, however, average concentration of releasable reducing sugars within all soil aggregate size fractions was significantly greater in the 0-7.5 cm depth when compared to the 7.5-15 cm depth. This may be attributed to the effects of tillage practices on incorporating crop residue into the plow layer, and stratification of biochemical activity near the soil's surface in minimally disturbed systems (such as no-till) where crop residues are not incorporated into the soil (Jacobs et al., 2010).

Figure 3.8 summarizes mean comparisons of releasable reducing sugar concentrations in field-moist aggregate fractions by depth in different tillage systems. Significant differences in releasable reducing sugar concentrations were observed among different aggregate size fractions within the same tillage system and depth. In the 0-7.5 cm depth, no-tillage reducing sugar concentration was significantly greater than that with chisel-plow or moldboard plow systems for the same soil aggregate fraction. The same significant differences were observed in the 7.5-15 cm depth for the same soil aggregates fractions of chisel-plow plots of all sizes except < 0.5 and 0.5-1mm. These results were expected in comparison to moldboard plow, as changes in the stabilization of organic matter in soils (or secured storage from microbial metabolism) may be attributed to change in spatial accessibility, biochemical recalcitrance, or organomineral association (von Lutzow et al., 2006). Moreover, these findings are supported by work which concluded that as cultivation is reduced, the byproducts of microbial activity have a chance to bind micro-aggregates into macro-aggregates with an enriched labile fraction of soil carbon (Cambardella and Elliot, 1994).

Mean comparisons of reducing sugar concentrations from each soil aggregate size fraction across all tillage systems and depths revealed that concentrations of releasable reducing sugars in the 1-2 and 2-4 mm range were significantly greater than concentrations in both the 0.5-1 and 4-8 mm range. Reducing sugar concentrations of soil aggregate fractions of 0.5-1 and 4-8 mm were significantly greater than those of the soil aggregate > 8 mm, which were in turn significantly

greater than concentrations measured in soil aggregates < 0.5 mm. Such low concentrations within the < 0.5 mm size fraction may be attributed to the increased percentage of fine sand inherent to the size-exclusion sieving processes, which was visibly noticeable during sample processing. This size fraction also had less gravimetric water content than did other fractions. Decreased concentrations of potential metabolic product may also be explained by research which indicates that 60-70% of the total activity of the soil enzymes of ß-glucosidase, amidase, and L-asparaginase (on a mass basis) is associated with macro-aggregates, particularly those larger than 1.0 mm (Miller and Dick, 1995). Differences in distributions in some of the smaller macro-aggregates supports work which shows that land use strongly impacts soil aggregation and the subsequent storage of soil carbon within those soil aggregates (Jastrow, 1996; Jastrow et al., 1996).

Additionally, the greater concentrations of potentially releasable reducing sugar in the 1-2 and 2-4 mm soil aggregate fractions support the hypothesis that the accumulation of C binding agents (such as polysaccharides from plant residue inputs) stabilizes micro-aggregates and smaller macro-aggregates within the large macro-aggregates. Such a mechanism provides a physical barrier and excludes this carbon from being used as an energy source for microbial decomposition (Six et al., 1999). Our findings show that the effect of physical stabilization of organic matter in macro-aggregates alone may not be a mechanism of labile carbon storage; micro-aggregates which have formed within macro-aggregates are probably the source of concentrations of releasable reducing sugars within those macro-aggregates. This

complements work which shows that longer storage of soil carbon likely occurs after micro-aggregate formation within macro-aggregates (Jacobs et. al., 2010).

In all soil aggregate fractions in the no-till system, concentrations of releasable reducing sugars were greater in the surface (0-7.5 cm) soil sample than in the subsurface (7.5-15 cm) soil sample, where the average concentration of releasable reducing sugars from all aggregate fractions was 63.9 mg kg^{-1} in the surface soil sample and 33.4 mg kg^{-1} in the subsurface soil sample. Such stratified character of properties is frequently attributed to soils under no-till management systems; the process of organic matter stratification may occur within three years after transition to no-till from conventional tillage management (McCarty et al., 1998). Average concentrations were more homogenized between surface and subsurface soils in moldboard plow and chisel-plow tillage systems. Greater concentrations in subsurface soil were observed in moldboard plow and chisel-plow tillage systems when compared to the no-till system, with significantly greater concentrations in chisel-plow subsurface soil. These findings are supported by work that shows that tillage practices decrease the occurrence and stability of macro-aggregates in soils compared to soils that are minimally disturbed (Jacobs et al., 2009), where amounts of releasable reducing sugars could be interpreted as a stabilizing factor, as soil carbohydrates are widely recognized for their roles in soil aggregate stability (Stevenson, 1994).

When the average organic carbon concentration of each soil aggregate size fraction from each tillage system and both surface and subsurface samples is considered (Table 3.5) in comparison to the average releasable reducing sugar concentration of those samples, results indicate that soils with greater concentrations of potentially releasable reducing sugars generally have greater concentrations of organic carbon. The percentage of the soil organic carbon that the releasable reducing sugar pool constitutes ranged from 0.06% in the <0.5 mm aggregate size in surface soil from under moldboard plow tillage to 0.3% in the 0.5-1 mm aggregate size in surface soil from under the no-till tillage system (unites were converted to g R_r kg^{-1} soil. Again, stratification in soil organic carbon concentrations and releasable reducing sugar concentrations was observed in the no-till system, whereas a homogenizing effect was noted in conventional tillage systems.

Overall, our findings on the impacts of tillage on releasable reducing sugars in soil aggregate size fractions support other theories about relationships between soil secondary physical structure and the biological regulation of biochemical stabilization of soil carbon. A study of a 110-year chronosequence of soils converted from pasture to row crops at different points in history found that water-stable aggregates, organic carbon, total soil carbohydrates (by modified acid hydrolysis method), and glomalin-related soil protein (GRSP, a fungal glycoprotein attributed with soil aggregate forming properties) reacted towards land use change at different speeds. In that study, soil carbohydrates reached a new equilibrium in the top 0-20 cm of soil after 14 (±6) years and mean-weight diameter (MWD) of water-stable soil

aggregates reaching equilibrium 33 (±2) years after change in land use (Spohn and Giani, 2011). In our work, both soil aggregate size fraction distributions (larger soil macro-aggregates) and increased concentrations of potentially releasable reducing sugars was evident in the surface soil when compared to the subsurface soil, especially in the no-till system. This is similar to the findings of McCarty and Meisinger (1997) which state that soil under long-term no-tillage is stratified towards the surface in composition and amount of soil organic matter.

Conclusions

This study indicates that although there is a relationship between releasable reducing sugars in soils and total organic carbon, the physical, biological, and biochemical impacts of tillage system and crop rotation management are highly significant on reducing sugar concentration. Differences between concentrations of releasable reducing sugars of different locations across Iowa indicate the effect of spatial variability and soil type on releasable reducing sugar pool in addition to the effects of soil management practices. Releasable reducing sugar concentrations in soils decreased over the winter, and were negatively affected by a single tillage treatment with appreciable decline even for a short period of time (days). These results indicate that this soil carbon pool component is a sensitive indicator to assess the effects of agricultural management practices such as tillage and crop rotation on soil carbon transformative and storage processes.

Furthermore, the impact of management practices on reducing sugar concentrations was also shown in the distribution of R_r in different soil aggregate fractions, where greatest concentrations of releasable reducing sugars were found in soil aggregate size fractions of 1-2 and 2-4 mm in the no-tillage system. As such, the mechanism behind increased CO_2 fluxes and decreased organic matter storage in intensely cultivated soils may be due to increased biological availability of releasable reducing sugars as the result of disrupted macro-aggregates and aggregate stabilizing carbohydrate compounds.

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Table 3.1 Summary of soil properties.

Location	Soil Series	Soil Classification	pH †		Organic C ‡
			H_2O ‡	$CaCl_2$ ‡	g kg^{-1}
Ames	Webster	Fine-silty, mixed, superactive, mesic Typic Hapludoll	5.0-5.6 (5.3)	4.6-5.2 (4.9)	18.0-32.6 (27.0)
Lewis	Marshall	Fine-silty, mixed, superactive, mesic Typic Hapludoll	5.8-6.4 (6.2)	5.3-6.2 (5.8)	15.7-24.3 (20.7)
Nashua	Kenyon	Fine-loamy, mixed, superactive, mesic Typic Hapludoll	5.2-5.9 (5.6)	4.5-5.3 (5.0)	24.9-32.1 (28.7)
Sutherland	Galva	Fine-silty, mixed, superactive, mesic Typic Hapludoll	5.3-5.9 (5.7)	4.6-5.4 (5.1)	14.9-36.2 (23.9)

† Soil:water or 0.01 *M* $CaCl_2$ ratio 1:2.5.

‡ Range of values reported is followed by the average for all plots at each location.

Table 3.2 Average potential total reducing sugar pool in soils with two crop rotations and four locations.

		Crop Rotation †	
	Average ‡	CC	CS
Location §	Potential total reducing sugar pool		
	----------mg kg^{-1} soil ¶----------		
Ames	41.2 B	43.3 Ba	39.1 Ca
Lewis	20.7 A	19.0 Aa	22.3 Aa
Nashua	39.4 B	49.0 Ca	29.7 Bb
Sutherland	44.2 B	39.0 Ba	49.4 Db

† CS is corn-soybean rotation and CC is continuous corn.

‡ Different uppercase letters in columns denote significant differences between averages of potential total reducing sugar pools of locations.

§ Different lower case letters in rows indicate significant differences in averages of potential total reducing sugar pools between crop rotations at each location.

¶ Comparisons were based on least significant difference (LSD) at $P < 0.05$.

Table 3.3 Temporal and secondary tillage effect on potential total reducing sugar pools of soils at Ames location.

Crop rotation†	Tillage system‡	Potential total reducing sugar §			Change in reducing sugar		
		Fall ¶	Spring	SPD	F–S	F–SPD	S– SPD
		----------mg kg^{-1} soil----------			----------percentage change----------		
CS #	MP ††	22.6 Ab*	21.9 Ab	17.8 Aa	-3.1	-21.2	-18.7
	CP	41.1 Bb*	36.3 Ba	33.3 Bb	-11.7	-19.0	-8.3
	NT	53.6 Cc	40.1 Ba*	44.5 Cb*	-24.6	-16.5	+11.0
CC ‡‡	MP	31.4 Ac*	20.7 Ab	17.1 Aa	-34.1	-45.5	-17.4
	CP	46.6 Bb*	36.9 Ba	34.9 Ba	-20.8	-25.1	-5.4
	NT	51.9 Cb	46.2 Ca*	49.4 Cab*	-11.0	-4.8	+6.9

† CS is corn-soybean rotation and CC is continuous corn.

‡ MP is moldboard plow tillage system, CP is chisel plow tillage system, and NT is no-till.

§ F is fall, S is spring, SPD is spring post disking. Change in reducing sugar estimated as a percentage of previous period for different periods of measurements.

¶ Different upper case letters within each column denote significant differences in potential total reducing sugars between different tillage systems for the same time period and the same crop rotation.

Different lower case letters within each row denote significant differences between time periods for the same tillage system and same crop rotation.

†† An asterisk indicates a significant difference between crop rotations for the same time period and the same tillage system.

‡‡ All comparisons were based on least significant differences (LSD) at $P<0.05$.

Table 3.4 Concentration of potential total reducing sugars in soil aggregate fractions under different tillage systems and from two depths.

Depth	Tillage System†	Total potential reducing sugar pool Aggregate size fraction (mm) §						
		<0.5	0.5-1	1-2	2-4	4-8	>8	Average¶
		----------mg kg^{-1} soil----------						
0-7.5 cm	MP ‡	11.4Aa	24.8Ba*	45.3Fa*	42.1Ea	38.8Da	31.2Ca*	32.2††
	CP	20.9Ab	43.2Bb	47.8Cb*	47.0Cb*	48.5Cb	45.4Cb*	42.1††*
	NT	68.0Dc*	82.6Ec*	71.3Cc*	57.7Bc*	54.3Bc*	49.7Ac*	63.9††*
Overall average								46.1‡‡
7.5-15cm	MP#	12.7Aa	28.2Ba*	38.1Cb*	42.5Db	38.5Cb	35.5Cb*	32.6
	CP	23.7Ab	41.1Bb	54.2Dc*	69.3Ec*	45.8Cc	40.9Bc*	45.8††*
	NT	30.8Ac*	46.5Cc*	34.5Ba*	30.5Aa*	28.1Aa*	30.1Aa*	33.4*
Overall average								37.3‡‡

† MP is moldboard plow tillage system, CP is chisel plow tillage system, and NT is no-till.

‡ Different upper case letters in each row denote significant differences in potential total reducing sugar concentration between different aggregate size fractions within the same tillage system and same depth.

§ Different lower case letters in each column denote significant differences in potential total reducing sugar concentrations between different tillage systems within the same aggregate size fraction and same depth.

¶ Asterisks denote significant differences in potential total reducing sugar concentration between different depths within the same tillage system and same aggregate size fraction, or between the averages of the same tillage system at different depths.

†† Indicates that potential total reducing sugar concentration averages for each tillage system across all aggregate size fractions were significantly different within the same depth.

‡‡ Indicates that potential total reducing sugar overall averages across all tillage systems and aggregate size fractions for two depths were significantly different.

Comparisons were based on least significant differences (LSD) at $P< 0.05$.

Table 3.5. Releasable reducing sugars as a percentage of total organic carbon.

		Tillage system†					
		MP ‡		CP		NT	
Depth --cm--	Aggregate size class -----mm-----	Org C --g kg^{-1}--	R_r § ----mg kg^{-1}----	Org C --g kg^{-1}--	R_r ----mg kg^{-1}----	Org C --g kg^{-1}--	R_r ----mg kg^{-1}----
0-7.5	<0.5	18.2	11.4 (0.06)	23.3	20.9 (0.09)	26.9	68.0 (0.25)
	0.5-1	18.4	24.8 (0.14)	24.3	43.2 (0.18)	27.3	82.6 (0.30)
	1-2	19.1	45.3 (0.24)	24.1	47.8 (0.20)	28.8	71.3 (0.25)
	2-4	19.3	42.1 (0.22)	23.3	47.0 (0.20)	28.6	57.7 (0.20)
	4-8	18.3	38.8 (0.21)	21.8	48.5 (0.22)	29.6	54.3 (0.18)
	>8	17.1	31.2 (0.18)	21.0	45.4 (0.22)	28.1	49.7 (0.18)
7.5-15	<0.5	18.8	12.7 (0.07)	23.1	23.7 (0.10)	24.0	30.8 (0.13)
	0.5-1	18.8	28.2 (0.15)	23.0	41.1 (0.18)	24.5	46.5 (0.19)
	1-2	18.7	38.1 (0.20)	23.7	54.2 (0.23)	24.4	34.5 (0.14)
	2-4	18.1	42.5 (0.24)	24.2	69.3 (0.29)	24.2	30.5 (0.13)
	4-8	17.7	38.5 (0.22)	22.8	45.8 (0.20)	23.6	28.1 (0.12)
	>8	17.4	35.5 (0.21)	21.1	40.9 (0.19)	23.2	30.1 (0.13)

† MP, CP, and NT are moldboard plow, chisel-plow, and no-till tillage systems, respectively.

‡ Org C is organic carbon in g kg^{-1} and R_r is the concentration of potential total releasable reducing sugars in each sample.

§ Numbers in parentheses represent R_r as a percentage of Org C, where R_r was converted to g kg^{-1}.

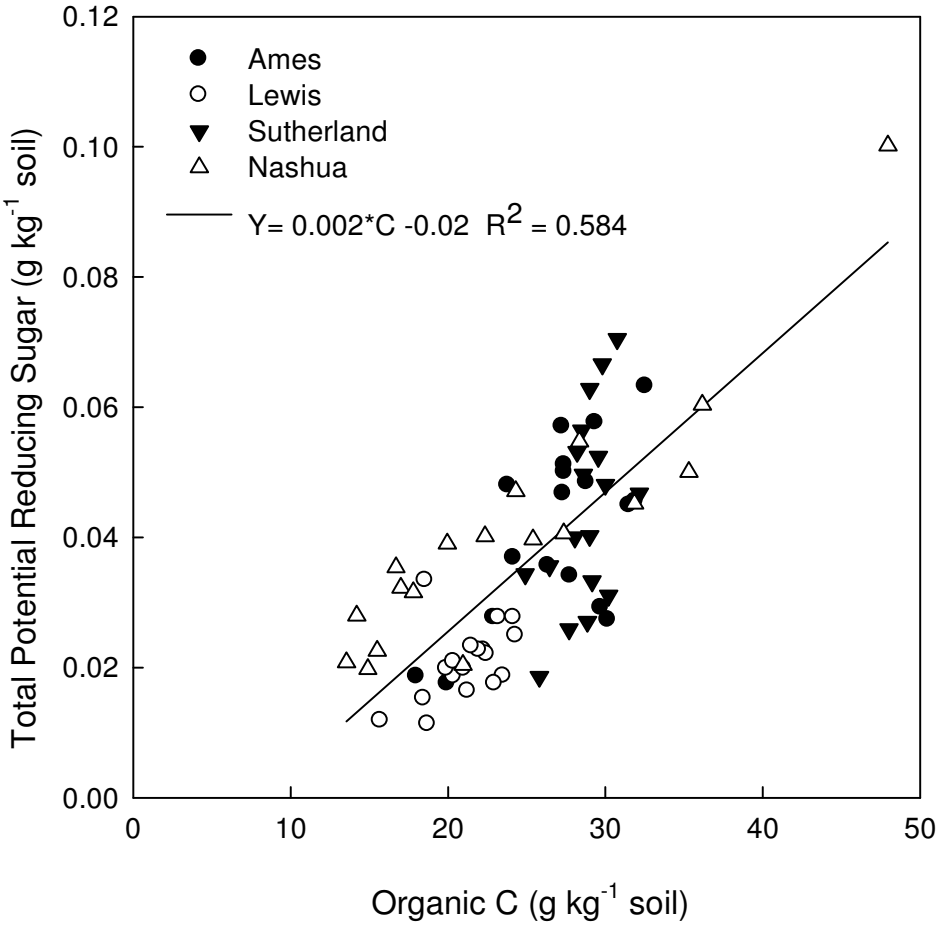

Figure 3.1 Relationship between potential reducing sugars in soil and total organic carbon.

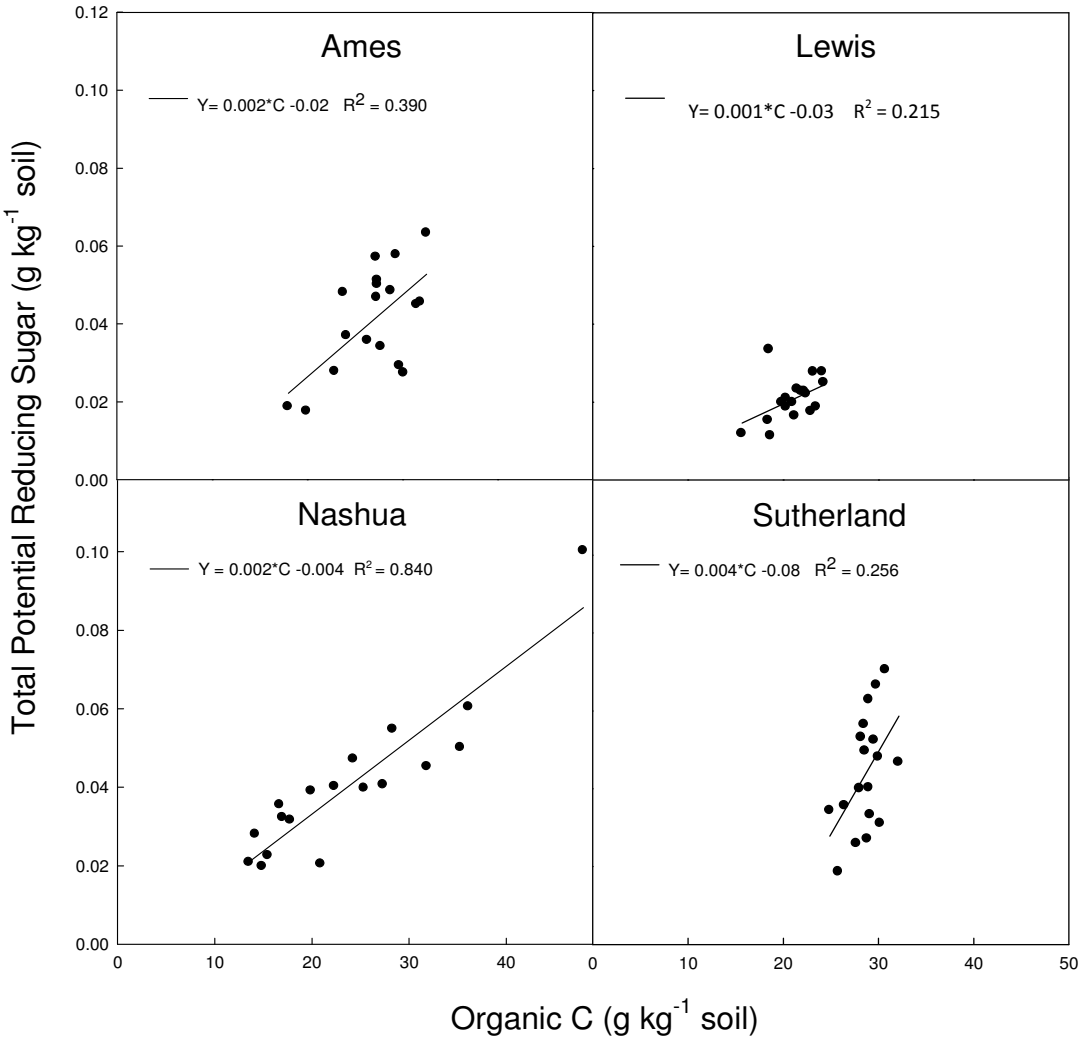

Figure 3.2 Relationship between potential reducing sugars in soil and total organic carbon at Ames, Lewis, Nashua, and Sutherland locations.

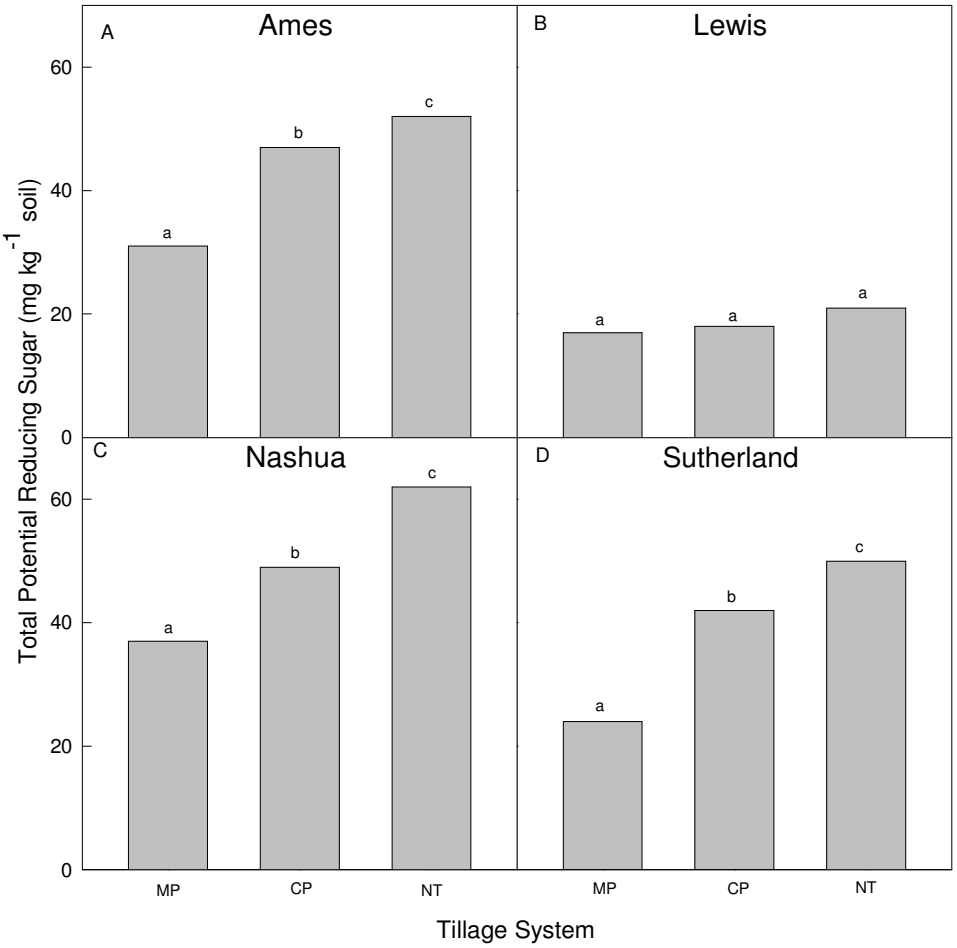

Figure 3.3 Effects of tillage within each location on potential total reducing sugars in soils under continuous corn. Different letters denote significant differences between tillage systems among each location based on least significant differences (LSD) at $P<0.05$.

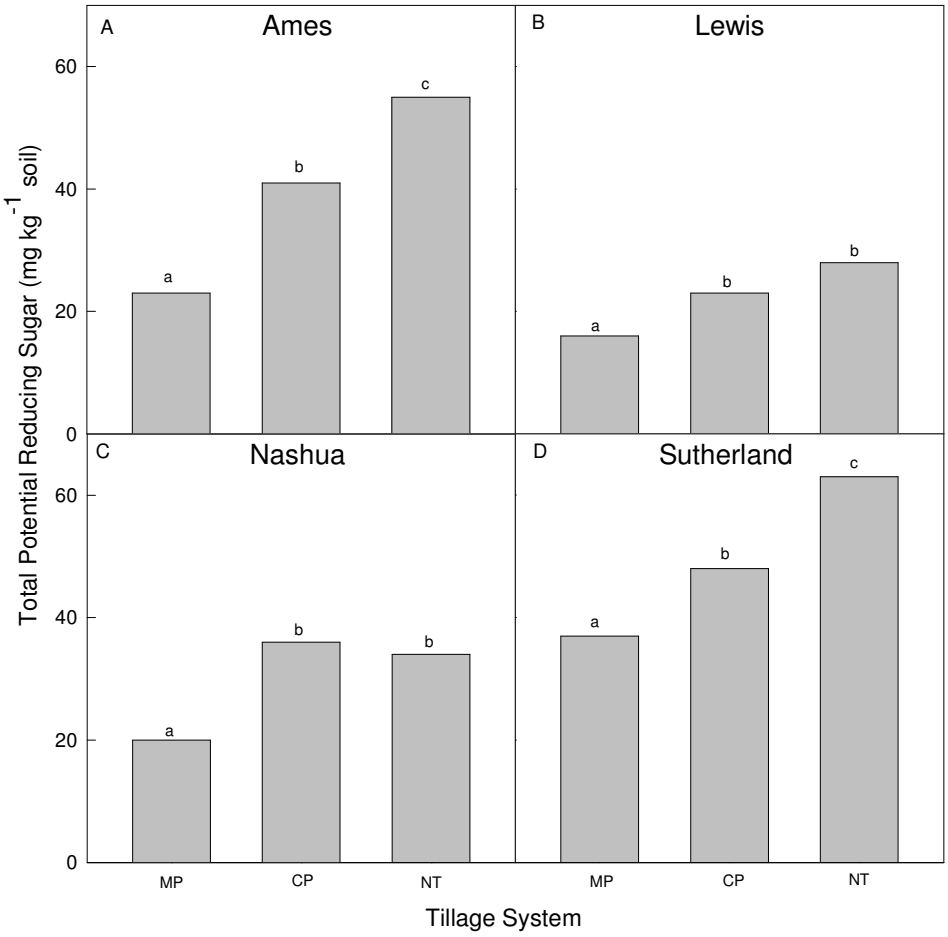

Figure 3.4 Effects of tillage within each location on potential total reducing sugars in soils under corn-soybean rotation.
Different letters denote significant differences among tillage systems within each location based on least significant differences (LSD) at P <0.05.

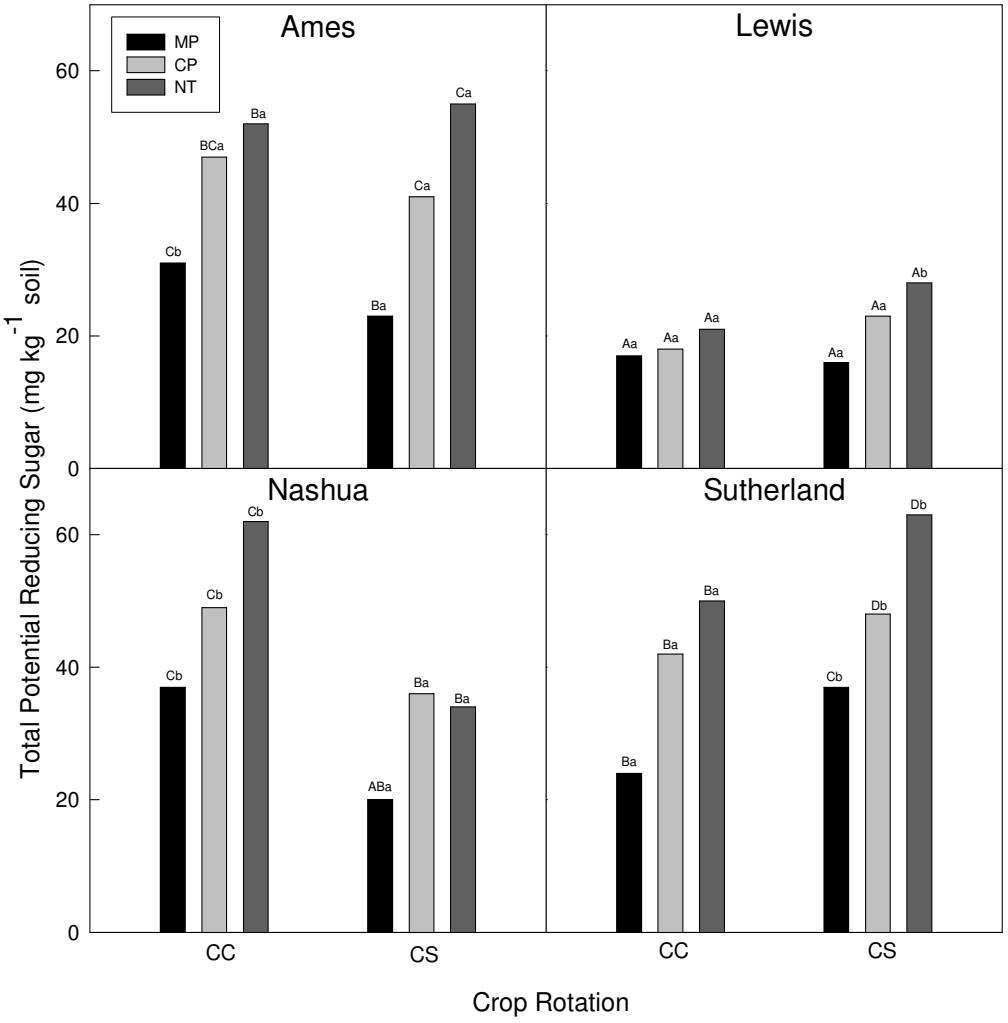

Figure 3.5 Effects of tillage and crop rotation within each location on potential total reducing sugars in soils.
Different upper case letters denote significant differences among locations within the same tillage system and crop rotation based on least significant differences (LSD) at $P < 0.05$.
Different lower case letters denote significant differences between crop rotations within the same location and same tillage system based on least significant differences (LSD) at $P < 0.05$.

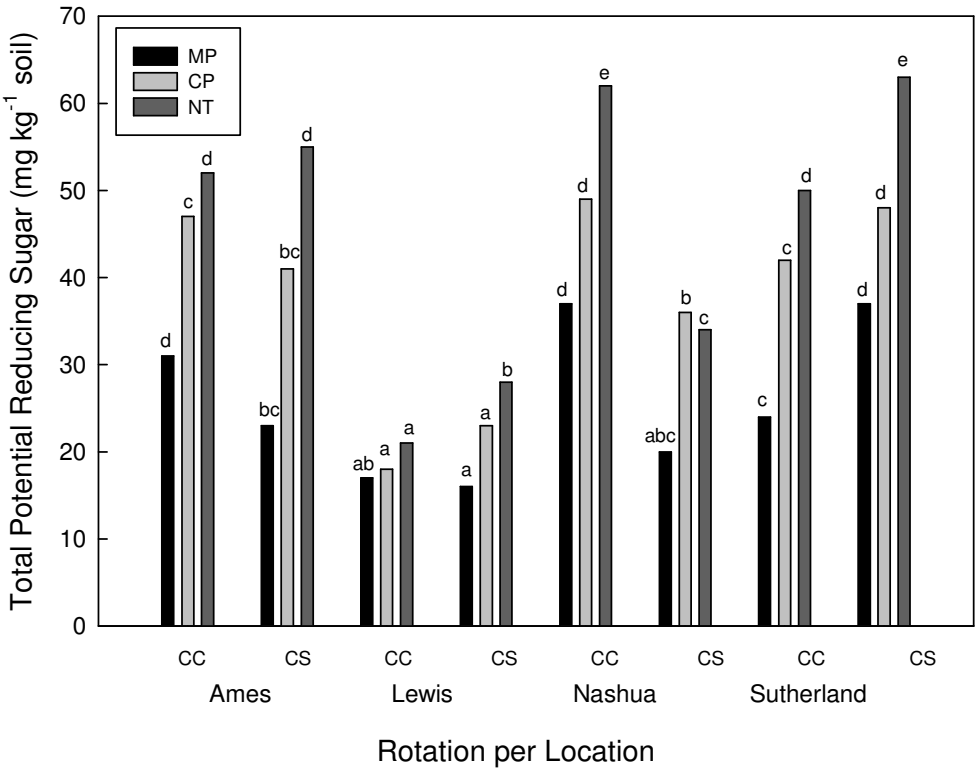

Figure 3.6 Potential total reducing sugars in soils at four locations, with two crop rotations (continuous corn and corn-soybean), and three tillage systems (moldboard plow, chisel plow, and no-till) at each location.
Treatments that have the same letters show no significant difference for the same tillage system across locations and crop rotations according to least significant differences (LSD) at $P < 0.05$.

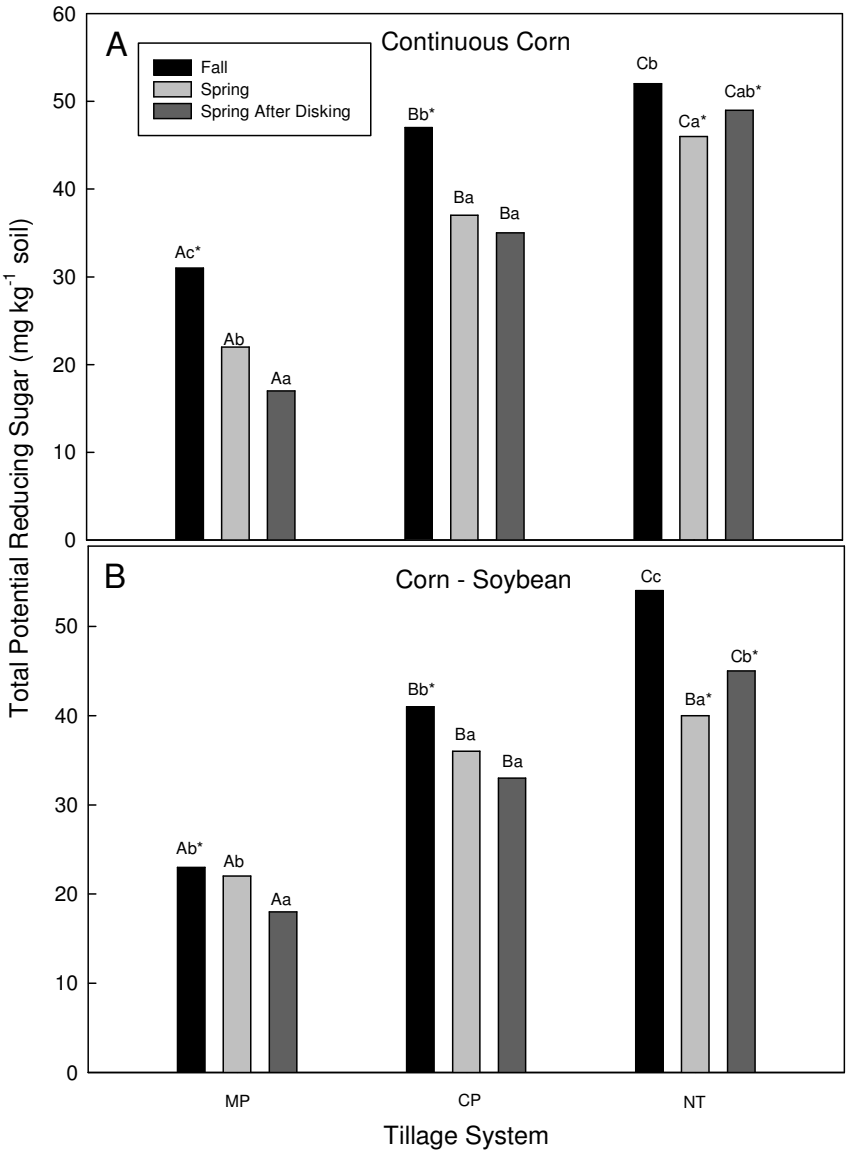

Figure 3.7 Short-term effects of tillage on potential total reducing sugar pools in soils as a function of time in continuous corn and corn-soybean cropping systems.
Different upper case letters denote significant differences in potential total reducing sugars in soils between tillage systems at the same time within each crop rotation.
Different lower case letters denote significant differences between times within the same tillage system and crop rotation.
An asterisk indicates a significant difference between crop rotations within the same time and tillage system. All comparisons were based on least significant differences (LSD) at $P < 0.05$.

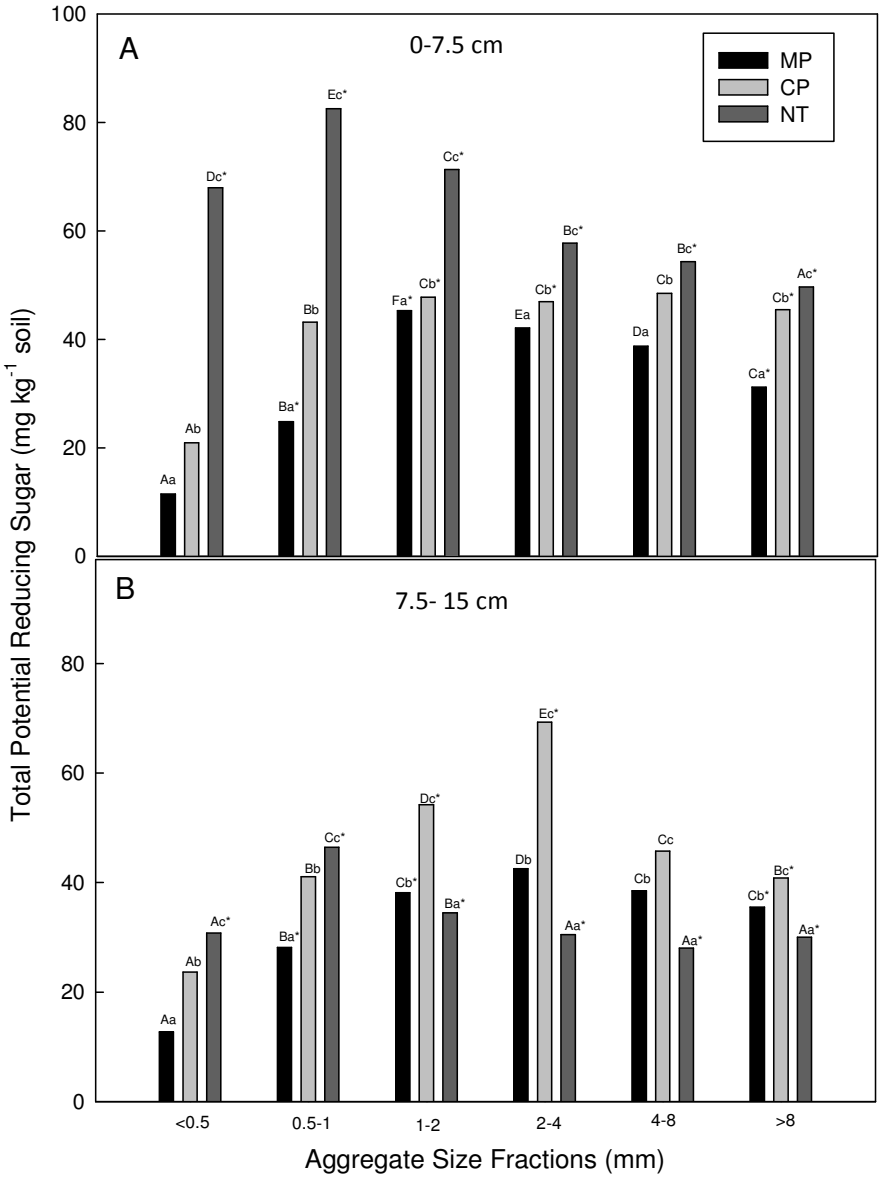

Figure 3.8 Impacts of tillage on potential total reducing sugars in field-moist aggregate fractions by depth.
Different upper case letters denote significant differences in potential total reducing sugar concentration of different aggregate size fractions for the same tillage system and depth.
Different lower case letters denote significant differences in potential total reducing sugars between different tillage systems and the same aggregates size fraction and depth.
Asterisks denote significant differences between depths within the same tillage system and same aggregate size fraction. Comparisons were based on least significant differences (LSD) at $P<0.05$.

CHAPTER FOUR

GENERAL CONCLUSIONS

The findings of this research show the importance of the development of new methodology for the relevant analysis and determination of a sensitive component of the soil carbon pool that we found to be significantly influenced by agricultural practices both in the short- and long-terms. To develop such an analytical method, the accumulation of reducing sugars was analyzed in a 60% methanol solution over time periods of 2 h increments (R_c) for a total of 24 h in order to kinetically predict the maximum (R_r), or potential total, releasable reducing sugars in soils. It was found that with eight different soils, this predicted kinetic maximum was reached within five days of incubation of soil at 30° C in 60% by volume methanol solution, with high precision and reproducibility with colorimetric analysis by Somogyi-Nelson method. The implications of this work may include the application of the method in determination of changes in this carbon pool constituent over short periods of time in order to assess land-use impacts on soil carbon transformative and storage processes. Furthermore, these finding should have significant application in managing and developing practices for minimizing such changes.

The application of this novel approach was examined with different management practices of tillage and crop rotations. Differences among tillage system, crop rotation, and locations were detectable in the releasable reducing sugar pool, or the soil carbon pool that is defined strictly by the metabolic capacity of a soil to either degrade (release as CO_2) or store soil carbohydrate carbon.

Detectable changes in soil releasable reducing sugars were noted with differences in intensity of tillage. Greatest concentrations were generally observed in no-till continuous corn systems at each location, except locations where the corn-soybean rotation had just had a soybean crop harvested (Lewis and Sutherland). At these locations, the no-till corn-soybean system had greater concentrations of releasable reducing sugars than did the no-till continuous corn system at the same location.

The R^2 value for the relationship between organic C and R_r was 0.584, indicating that although the two chemical properties are related, there are other driving factors which influence the size of the potential reducing sugar pool in soils. Locations with lower native organic C, however, were less likely to exhibit significant changes in R_r due to management practices. Locations with greater native organic C, however, were the most susceptible to decreased concentrations of releasable reducing sugars from more intense cultivation practices such as moldboard plow tillage, perhaps due to high availability of substrate source for reducing sugar production.

Changes in soil concentrations of releasable sugars were measurable and significant over a season's time, and also within one week's time that included a secondary tillage pass. Results from the analysis of differences in concentrations between different soil aggregate size fractions under different tillage systems at different depths indicated that tillage has a direct impact on how releasable reducing sugars are stored in soils. We found that there were significant differences

in concentrations of releasable reducing sugars among field-moist soil aggregates, with the highest concentrations found in the 1-2 and 2-4 mm size fractions. A stark gradient between average concentrations of aggregates from surface (0-7.5 cm) and subsurface (7.5-15 cm) soil was found in the no-till system, where the majority of potential releasable reducing sugars were stratified in surface soil. Contrastingly, the mixing effect of tillage practices in both the chisel-plow and moldboard plow tillage systems was exhibited.

This work lends insight into possible mechanisms of the storage of labile carbon and release of CO_2 from soils. As such, the total potential releasable reducing sugar pool in soils stands as a sensitive soil carbon pool, or measurement of labile soil carbon. Thus, the soil carbon pool that is regulated by enzymatic degradation of different forms of carbohydrate-carbon should be termed an 'ultra-labile' soil carbon pool. Applications of this work include the potential economic valuation of practices that lead to retention of greater concentrations of ultra-labile soil carbon. There is room for research into the measures to offset the potential carbon loss (CO_2 emissions) from soil and subsequent economic values; such relationships could lead to lower economic barriers in decision-making processes for land-managers.

APPENDICES

Appendix A: Microbial colony counts.

	Linder		Harps		Clarinda	
Time	60% MeOH †	DI H_20	60% MeOH	DI H_20	60% MeOH	DI H_20
0 h	11	96	7	142	22	193
	8	143	12	157	21	212
	15	111	26	152	15	160
24 h	3	206	11	269	6	333
	13	178	8	240	28	290
	14	225	17	272	12	265

† MeOH is methanol, DI H_2O is deionized water.

Appendix B. Gas chromatogram analysis of gas in headspace of incubation tube after five days.

	Linder		Harps		Clarinda		Control	
	CO_2	CH_4	CO_2	CH_4	CO_2	CH_4	CO_2	CH_4
H_2O	1489.4	1.8	1068.1	2.2	2177.5	2.0	869.1	2.2
MeOH	1196.1	2.8	760.5	2.6	1056.4	9.9	673.2	12.6

Concentrations of carbon dioxide (CO_2) and methane (CH_4) gas (ppm) in samples taken from headspace of capped centrifuge tube incubated 24 h with soil and water (H_2O) or soil and 60% methanol (MeOH), or controls of water or 60% methanol without soil.

Appendix C: Recovery of glucose from Linder soil (5.0 g) spiked with 20, 50, or 100 µg D-glucose g^{-1} soil and incubated in 25 mL DI water for 24 h at 30˚C.

Linder soil, deionized water

	0 µg		100 µg		250 µg		500 µg	
	abs†	µg/g	abs	µg/g	abs	µg/g	abs	µg/g
1a	0.018	6.01	0.022	7.62	0.025	8.66	0.022	7.62
1b	0.018	6.01	0.022	7.62	0.025	8.66	0.022	7.62
2a	0.022	7.34	0.02	6.93	0.018	6.24	0.023	7.97
2b	0.022	7.34	0.02	6.93	0.018	6.24	0.023	7.97
3a	0.024	8.01	0.02	6.93	0.024	8.32	0.026	9.01
3b	0.024	8.01	0.02	6.93	0.024	8.32	0.026	9.01
4a	0.021	7.01	0.02	6.93	0.022	7.62	0.029	10.05
4b	0.021	7.01	0.02	6.93	0.022	7.62	0.028	9.70
5a	0.018	6.01	0.022	7.62	0.019	6.58	0.028	9.70
5b	0.018	6.01	0.022	7.62	0.019	6.58	0.028	9.70
6a	0.024	8.01	0.02	6.93	0.02	6.93	0.026	9.01
6b	0.024	8.01	0.02	6.93	0.02	6.93	0.026	9.01
Average		7.06		7.16		7.39		8.86

† abs is spectrophotometric absorbance at 520 nm.

Appendix D: Recovery of glucose from Linder soil (5.0 g) spiked with 20, 50, or 100 µg D-glucose g^{-1} soil and incubated in 25 mL 60% methanol solution for 24 h at 30˚C.

Linder soil, 60% methanol

	0 µg		100 µg		250 µg		500 µg	
	abs†	µg/g	abs	µg/g	abs	µg/g	abs	µg/g
1a	0.067	37.91	0.1	58.756	0.149	87.55	0.23	135.14
1b	0.067	37.91	0.1	58.756	0.149	87.55	0.228	133.96
2a	0.068	38.48	0.093	54.643	0.144	84.61	0.23	135.14
2b	0.067	37.91	0.093	54.643	0.145	85.2	0.232	136.31
3a	0.066	37.34	0.096	56.406	0.143	84.02	0.235	138.08
3b	0.066	37.34	0.097	56.993	0.144	84.61	0.232	136.31
4a	0.068	38.48	0.093	54.643	0.144	84.61	0.219	128.68
4b	0.07	39.61	0.093	54.643	0.147	86.37	0.226	132.79
5a	0.066	37.34	0.095	55.818	0.149	87.55	0.235	138.08
5b	0.066	37.34	0.097	56.993	0.149	87.55	0.23	135.14
6a	0.066	37.34	0.095	55.818	0.147	86.37	0.228	133.96
6b	0.065	36.78	0.097	56.993	0.145	85.2	0.226	132.79
Average		37.82		56.259		85.93		134.7

† abs is spectrophotometric absorbance at 520 nm.

Appendix E: Recovery of glucose from Clarinda soil (5.0 g) spiked with 20, 50, or 100 µg D-glucose g^{-1} soil and incubated in 25 mL DI water for 24 h at 30˚C.

Clarinda soil, deionized water

	0 µg		100 µg		250 µg		500 µg	
	abs†	µg/g	abs	µg/g	abs	µg/g	abs	µg/g
1a	0.031	10.69	0.043	15.38	0.05	17.88	0.042	15.02
1b	0.035	12.07	0.04	14.31	0.051	18.24	0.042	15.02
2a	0.033	11.38	0.036	12.88	0.038	13.59	0.04	14.31
2b	0.033	11.38	0.032	11.45	0.04	14.31	0.04	14.31
3a	0.032	11.03	0.034	12.16	0.035	12.52	0.043	15.38
3b	0.032	11.03	0.034	12.16	0.034	12.16	0.044	15.74
4a	0.031	10.69	0.038	13.59	0.03	10.73	0.043	15.38
4b	0.032	11.03	0.038	13.59	0.03	10.73	0.043	15.38
5a	0.036	12.41	0.032	11.45	0.036	12.88	0.04	14.31
5b	0.034	11.72	0.033	11.80	0.036	12.88	0.04	14.31
6a	0.033	11.38	0.035	12.52	0.038	13.59	0.041	14.66
6b	0.033	11.38	0.036	12.88	0.038	13.59	0.041	14.66
Average		11.35		12.85		13.59		14.87

† abs is spectrophotometric absorbance at 520 nm.

Appendix F: Recovery of glucose from Clarinda soil (5.0 g) spiked with 20, 50, or 100 µg D-glucose g^{-1} soil and incubated in 25 mL 60% methanol solution for 24 h at 30˚C.

Clarinda soil, 60% methanol

	0 µg		100 µg		250 µg		500 µg	
	abs†	µg/g	abs	µg/g	abs	µg/g	abs	µg/g
1a	0.162	94.73	0.188	114.02	0.265	160.7	0.323	195.89
1b	0.168	98.23	0.182	110.38	0.233	141.3	0.332	201.35
2a	0.173	101.2	0.182	110.38	0.238	144.3	0.326	197.71
2b	0.174	101.7	0.185	112.2	0.233	141.3	0.322	195.28
3a	0.171	99.99	0.205	124.33	0.236	143.1	0.32	194.07
3b	0.169	98.82	0.202	122.51	0.236	143.1	0.326	197.71
4a	0.165	96.48	0.204	123.72	0.247	149.8	0.336	203.77
4b	0.161	94.14	0.198	120.08	0.25	151.6	0.312	189.22
5a	0.173	101.2	0.195	118.26	0.232	140.7	0.309	187.4
5b	0.169	98.82	0.202	122.51	0.251	152.2	0.3	181.94
6a	0.167	97.65	0.18	109.16	0.248	150.4	0.318	192.86
6b	0.165	96.48	0.187	113.41	0.209	126.8	0.324	196.5
Average		98.28		116.74		145.5		194.47

† abs is spectrophotometric absorbance at 520 nm.

Appendix G: Recovery of glucose from Harps soil (5.0 g) spiked with 20, 50, or 100 µg D-glucose g^{-1} soil and incubated in 25 mL DI water for 24 h at 30˚C.

Harps soil, deionized water

	0 µg		100 µg		250 µg		500 µg	
	abs†	µg/g	abs	µg/g	abs	µg/g	abs	µg/g
1a	0.023	7.82	0.023	8.12	0.014	4.94	0.02	7.06
1b	0.023	7.82	0.023	8.12	0.016	5.65	0.02	7.06
2a	0.02	6.80	0.015	5.29	0.022	7.77	0.018	6.35
2b	0.017	5.78	0.013	4.59	0.023	8.12	0.019	6.71
3a	0.016	5.44	0.022	7.77	0.02	7.06	0.014	4.94
3b	0.019	6.46	0.023	8.12	0.02	7.06	0.015	5.29
4a	0.026	8.84	0.02	7.06	0.019	6.71	0.022	7.77
4b	0.022	7.48	0.021	7.41	0.026	9.18	0.028	9.88
5a	0.023	7.82	0.016	5.65	0.022	7.77	0.035	12.35
5b	0.023	7.82	0.016	5.65	0.024	8.47	0.036	12.71
6a	0.018	6.12	0.023	8.12	0.018	6.35	0.024	8.47
6b	0.019	6.46	0.024	8.47	0.019	6.71	0.024	8.47
Average		7.06		7.03		7.15		8.09

† abs is spectrophotometric absorbance at 520 nm.

Appendix H: Recovery of glucose from Harps soil (5.0 g) spiked with 20, 50, or 100 µg D-glucose g^{-1} soil and incubated in 25 mL 60% methanol solution for 24 h at 30˚C.

Harps soil, 60% methanol

	0 µg		100 µg		250 µg		500 µg	
	abs†	µg/g	abs	µg/g	abs	µg/g	abs	µg/g
1a	0.063	36.34	0.084	50.278	0.145	86.79	0.242	144.85
1b	0.063	36.34	0.086	51.475	0.154	92.18	0.237	141.86
2a	0.064	36.92	0.107	64.045	0.162	96.97	0.251	150.24
2b	0.064	36.92	0.093	55.665	0.146	87.39	0.251	150.24
3a	0.061	35.19	0.089	53.271	0.14	83.8	0.238	142.46
3b	0.062	35.76	0.089	53.271	0.141	84.4	0.222	132.88
4a	0.068	39.22	0.083	49.68	0.148	88.59	0.249	149.04
4b	0.069	39.8	0.091	54.468	0.148	88.59	0.252	150.84
5a	0.074	42.68	0.099	59.257	0.157	93.97	0.234	140.06
5b	0.075	43.26	0.095	56.862	0.152	90.98	0.222	132.88
6a	0.069	39.8	0.103	61.651	0.15	89.78	0.238	142.46
6b	0.069	39.8	0.102	61.052	0.149	89.18	0.23	137.67
Average		38.5		55.915		89.38		142.95

† abs is spectrophotometric absorbance at 520 nm

Appendix I. Spectrophotometric absorbance of different reducing sugars at 520 nm.

Reducing sugar	Sample	20 µg	40 µg	60 µg
D-glucose (fw 180.16)	1a	0.120	0.216	0.316
	1b	0.120	0.216	0.316
D-galactose (fw 180.16)	2a	0.102	0.182	0.265
	2b	0.102	0.182	0.265
D-xylose (fw 150.13)	3a	0.109	0.210	0.305
	3b	0.109	0.210	0.305
D-mannose (fw 180.16)	4a	0.092	0.170	0.245
	4b	0.092	0.170	0.245
D-arabinose (fw 150.13)	5a	0.092	0.166	0.240
	5b	0.092	0.166	0.240
L-rhamnose (fw 164.16)	6a	0.070	0.122	0.180
	6b	0.070	0.122	0.180

Appendix J. Data of laboratory analysis of reducing sugar concentration (mg kg^{-1} soil) in duplicates of eight different soils at field-moist conditions in 60% methanol solution.

Time	Clarinda	Clarion	Exira	Marshall	Linder	Nicollet	Harps	Webster
---h---	----------mg kg^{-1} soil----------							
0	0.58	2.27	5.23	5.75	3.96	5.75	1.73	1.14
0	1.75	2.84	5.23	7.47	3.39	5.75	2.30	2.29
2	16.37	11.95	29.07	23.01	14.71	22.42	12.11	27.53
2	22.21	14.22	31.97	23.01	14.71	23.00	12.11	30.40
4	44.43	16.50	44.18	33.36	22.63	38.52	19.61	36.71
4	48.53	15.93	40.69	31.64	22.63	38.52	21.34	40.16
6	57.30	20.48	51.16	41.42	31.68	44.85	24.22	53.35
6	63.73	20.48	53.49	43.72	31.68	42.55	26.53	49.91
8	67.82	26.17	55.23	53.50	33.94	60.95	39.22	63.10
8	65.48	23.90	54.07	52.35	29.42	64.40	31.14	74.58
12	77.76	26.17	59.88	52.35	31.12	69.00	38.64	75.15
12	76.01	28.45	61.04	56.95	37.34	69.00	38.64	82.61
18	80.69	28.45	64.53	62.70	41.30	66.70	49.02	83.19
18	86.53	29.59	67.44	67.88	41.30	68.43	47.87	86.05
24	91.21	38.70	65.11	78.23	37.34	97.18	57.10	100.40
24	97.649	30.16	74.42	75.93	39.60	97.18	62.29	94.09

Appendix H (continued). Data of laboratory analysis of reducing sugar concentration (mg kg^{-1} soil) in duplicates of eight different soils at air-dry conditions in 60% methanol solution.

Time	Clarinda	Clarion	Exira	Marshall	Linder	Nicollet	Harps	Webster
---h---	----------mg kg^{-1} soil----------							
0	7.07	3.80	7.60	8.15	8.15	10.86	4.89	3.80
0	7.07	3.80	7.60	8.69	7.06	10.32	3.80	3.80
2	17.39	5.43	11.95	13.04	17.93	16.30	11.95	16.30
2	19.02	5.97	12.50	11.95	19.56	14.13	9.78	14.67
4	34.24	11.41	26.08	26.63	20.65	20.65	13.04	21.73
4	36.41	11.41	26.08	25.54	22.28	17.93	15.21	22.28
6	46.74	13.04	27.71	29.89	25.00	26.63	14.13	28.80
6	45.12	13.58	26.63	27.71	24.45	25.00	14.67	27.71
8	47.28	14.13	28.80	27.71	25.00	32.06	15.76	32.60
8	49.46	13.58	28.80	28.80	26.63	27.71	15.76	28.26
12	51.09	12.50	29.89	27.71	26.08	35.86	16.30	31.52
12	50.00	13.04	28.26	27.17	27.17	35.86	17.39	36.95
18	59.78	13.58	36.41	28.80	25.00	39.67	17.39	36.41
18	60.87	14.13	36.41	30.97	27.17	38.58	19.56	40.21
24	66.85	14.67	39.67	26.08	27.71	44.02	21.19	52.71
24	69.02	14.67	42.39	28.80	28.26	42.93	19.56	46.73

Appendix K. 5 Day pH of soils incubated in 60% methanol solution.

Sample	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
1a	5.65†	5.60	5.58	5.54	5.52	5.45
1b	5.68	5.62	5.56	5.48	5.40	5.62
2a	5.81	5.78	5.74	5.68	5.61	5.34
2b	5.80	5.62	5.53	5.62	5.48	5.35
3a	5.78	5.67	5.63	5.59	5.45	5.34
3b	5.76	5.54	5.50	5.46	5.44	5.36

† Margin of error of electrode used was ±0.5 pH unit.

Appendix L. Long-term tillage soils analyses data inventory.

							pH			
ID	**Site**	**ID #**	**Rotation**	**Tillage**	**Date**	**%H_2O**	**H_2O**	**$CaCl_2$**	**Total C**	**R.S.**
1	Ames	101	**C**/s	MP	9-23-11	16.39	5.23	4.96	1.8	17.5
2	Ames	103	**C**/s	CP	9-23-11	16.94	5.20	4.87	2.29	28.3
3	Ames	109	**C**/s	NT	9-23-11	18.84	5.31	4.91	2.74	49.5
4	Ames	303	**C**/s	MP	9-23-11	21.06	5.51	5.15	2.97	31.2
5	Ames	305	**C**/s	NT	9-23-11	19.40	5.63	5.20	3.255	63.2
6	Ames	307	**C**/s	CP	9-23-11	19.62	5.44	5.03	2.73	46.5
7	Ames	401	**C**/s	CP	9-23-11	19.70	5.51	5.05	2.88	48.5
8	Ames	403	**C**/s	NT	9-23-11	18.26	5.24	4.92	2.38	48.0
9	Ames	405	**C**/s	MP	9-23-11	16.97	5.10	4.62	1.995	19.2
10	Ames	502	**C**/c	MP	9-23-11	18.07	5.25	4.81	2.415	34.9
11	Ames	503	**C**/c	NT	9-23-11	18.41	5.12	4.70	2.74	50.3
12	Ames	504	**C**/c	CP	9-23-11	19.02	5.26	4.78	3.19	45.5
13	Ames	601	**C**/c	CP	9-23-11	19.90	5.53	5.08	2.725	57.3
14	Ames	602	**C**/c	NT	9-23-11	16.66	5.08	4.60	2.935	58.8
15	Ames	603	**C**/c	MP	9-23-11	19.58	5.03	4.61	3.015	24.2
16	Ames	701	**C**/c	MP	9-23-11	18.53	5.52	5.06	2.775	34.9
17	Ames	702	**C**/c	CP	9-23-11	17.26	5.04	4.68	2.635	36.8
18	Ames	705	**C**/c	NT	9-23-11	18.83	5.46	5.03	3.15	46.6
					avg	18.53	5.30	4.89	2.70	41.2
19	Lewis	102	c/**S**	CP	10-7-11	16.48	5.80	5.28	2.035	18.6
20	Lewis	103	c/**S**	NT	10-7-11	17.28	6.07	5.78	2.43	23.5
21	Lewis	105	c/**S**	MP	10-7-11	16.39	5.84	5.30	1.845	14.1
22	Lewis	302	c/**S**	CP	10-7-11	17.56	6.17	5.78	2.225	21.5
23	Lewis	304	c/**S**	MP	10-7-11	17.18	5.98	5.47	1.99	18.7
24	Lewis	305	c/**S**	NT	10-7-11	16.63	6.30	6.02	2.32	28.3
25	Lewis	401	c/**S**	MP	10-7-11	16.61	6.22	5.81	2.125	16.4
26	Lewis	402	c/**S**	NT	10-7-11	17.36	6.35	6.12	1.855	31.7
27	Lewis	403	c/**S**	CP	10-7-11	16.78	6.37	5.69	2.415	28.3
28	Lewis	902	c/**C**	CP	10-7-11	17.15	6.48	5.79	2.245	22.1
29	Lewis	903	c/**C**	NT	10-7-11	17.89	6.46	6.10	2.35	19.0
30	Lewis	905	c/**C**	MP	10-7-11	17.05	5.86	5.34	2.1	20.1
31	Lewis	1102	c/**C**	CP	10-7-11	17.09	6.48	6.14	2.295	19.8
32	Lewis	1104	c/**C**	MP	10-7-11	16.45	6.15	5.59	2.035	20.9
33	Lewis	1105	c/**C**	NT	10-7-11	17.81	6.48	6.14	2.195	22.1
34	Lewis	1201	c/**C**	MP	10-7-11	15.75	6.45	5.92	1.57	12.1
35	Lewis	1202	c/**C**	NT	10-7-11	17.99	6.47	6.28	2.15	22.7
36	Lewis	1203	c/**C**	CP	10-7-11	17.41	6.45	6.13	1.87	12.2
					avg	17.05	6.24	5.82	2.11	20.7

							pH			
ID	**Site**	**ID#**	**Rotation**	**Tillage**	**Date**	**% H_2O**	**H_2O**	**$CaCl_2$**	**Total C**	**R.S.**
37	Sutherland	201	**C**/c	MP	10-19-11	15.25	5.40	4.51	2.58	20.3
38	Sutherland	204	**C**/c	NT	10-19-11	15.99	5.80	5.07	2.98	65.2
39	Sutherland	205	**C**/c	CP	10-19-11	15.82	5.42	4.70	2.86	49.6
40	Sutherland	306	**C**/c	NT	10-19-11	15.42	5.63	4.92	2.955	52.4
41	Sutherland	309	**C**/c	CP	10-19-11	15.20	5.22	4.65	3.215	47.9
42	Sutherland	310	**C**/c	MP	10-19-11	14.78	5.27	4.72	2.77	24.2
43	Sutherland	402	**C**/c	MP	10-19-11	15.37	5.21	4.62	2.885	27.9
44	Sutherland	403	**C**/c	NT	10-19-11	15.61	5.42	4.75	2.915	33.8
45	Sutherland	404	**C**/c	CP	10-19-11	16.12	5.71	5.35	3.02	29.6
46	Sutherland	208	c/**S**	MP	10-19-11	15.10	5.80	5.06	2.49	32.4
47	Sutherland	209	c/**S**	CP	10-19-11	17.37	5.59	4.98	2.9	42.5
48	Sutherland	210	c/**S**	NT	10-19-11	17.07	5.87	5.21	2.9	64.5
49	Sutherland	312	c/**S**	MP	10-19-11	16.29	5.84	5.29	2.645	36.7
50	Sutherland	313	c/**S**	NT	10-19-11	18.65	5.82	5.26	3.075	72.5
51	Sutherland	315	c/**S**	CP	10-19-11	15.94	5.62	4.94	2.85	56.4
52	Sutherland	406	c/**S**	NT	10-19-11	16.82	5.76	5.15	2.82	53.4
53	Sutherland	409	c/**S**	MP	10-19-11	15.66	5.78	5.14	2.805	40.6
54	Sutherland	410	c/**S**	CP	10-19-11	17.00	5.72	5.18	3	45.5
					avg	16.08	5.60	4.97	2.87	44.2
55	Nashua	103	s/**C**	MP	10-21-11	16.29	5.62	4.96	1.49	19.8
56	Nashua	107	s/**C**	NT	10-21-11	17.03	5.37	4.65	1.7	31.7
57	Nashua	109	s/**C**	CP	10-21-11	16.92	5.57	5.06	1.995	39.0
58	Nashua	203	s/**C**	CP	10-21-11	14.75	5.55	4.97	1.67	36.0
59	Nashua	205	s/**C**	NT	10-21-11	12.92	5.90	5.38	1.42	28.3
60	Nashua	209	s/**C**	MP	10-21-11	14.18	5.78	5.16	1.355	18.5
61	Nashua	401	s/**C**	NT	10-21-11	16.81	5.49	4.93	2.235	40.7
62	Nashua	405	s/**C**	MP	10-21-11	15.87	5.63	5.02	1.55	20.6
63	Nashua	407	s/**C**	CP	10-21-11	15.57	5.92	5.35	1.78	33.0
64	Nashua	603	c/**C**	CP	10-21-11	20.89	5.56	4.97	3.185	43.5
65	Nashua	605	c/**C**	NT	10-21-11	19.53	5.24	4.61	2.835	52.1
66	Nashua	609	c/**C**	MP	10-21-11	17.41	5.90	5.29	2.095	20.7
67	Nashua	701	c/**C**	CP	10-21-11	18.04	5.89	5.25	2.43	42.3
68	Nashua	703	c/**C**	NT	10-21-11	17.57	5.67	5.13	2.54	39.4
69	Nashua	707	c/**C**	MP	10-21-11	20.66	5.86	5.30	2.735	39.2
70	Nashua	801	c/**C**	NT	10-21-11	24.69	5.87	5.36	4.795	93.8
71	Nashua	805	c/**C**	MP	10-21-11	22.74	5.96	5.45	3.53	50.1
72	Nashua	807	c/**C**	CP	10-21-11	22.39	5.91	5.38	3.615	59.8
					avg	18.01	5.71	5.12	2.39	39.35

Appendix M. Aggregate fraction reducing sugar data.

Plot	Depth(cm)	Size(mm)	ID	Fraction weight (g)	Obs a mg/kg	Obs b mg/kg
Ames 101 c/s moldboard plow	0-7.5	>8	1	399.2	26.8	26.8
	0-7.5	4-8	2	272.6	33.1	26.5
	0-7.5	2-4	3	126.9	35.8	34.5
	0-7.5	1-2	4	98.0	37.3	36.6
	0-7.5	.5-1	5	95.1	23.8	26.4
	0-7.5	<.5	6	75.8	7.9	13.4
	7.5-15	>8	7	489.1	29.1	31.8
	7.5-15	4-8	8	220.1	30.0	32.7
	7.5-15	2-4	9	70.4	38.4	31.1
	7.5-15	1-2	10	40.0	31.0	31.0
	7.5-15	.5-1	11	24.3	26.0	26.0
	7.5-15	<.5	12	7.6	7.7	13.0
Ames 103 c/s chisel-plow	0-7.5	>8	13	340.7	40.9	46.2
	0-7.5	4-8	14	171.7	49.0	54.3
	0-7.5	2-4	15	90.2	53.0	34.5
	0-7.5	1-2	16	71.3	40.5	38.6
	0-7.5	.5-1	17	95.6	29.6	33.5
	0-7.5	<.5	18	116.8	16.4	18.9
	7.5-15	>8	19	454.4	48.0	37.2
	7.5-15	4-8	20	177.7	42.2	45.0
	7.5-15	2-4	21	67.2	47.0	73.5
	7.5-15	1-2	22	39.1	45.9	53.6
	7.5-15	.5-1	23	29.7	37.8	31.6
	7.5-15	<.5	24	15.5	14.8	19.5

Plot	Depth(cm)	Size(mm)	ID	Fraction weight (g)	Obs a mg/kg	Obs b mg/kg
Ames 109 c/s no-till	0-7.5	>8	25	351.8	42.2	52.4
	0-7.5	4-8	26	223.4	47.4	52.1
	0-7.5	2-4	27	127.1	47.1	59.9
	0-7.5	1-2	28	109.7	66.0	67.3
	0-7.5	.5-1	29	96.5	76.2	76.9
	0-7.5	<.5	30	67.2	61.6	66.7
	7.5-15	>8	31	544.8	26.8	39.1
	7.5-15	4-8	32	230.5	19.3	32.4
	7.5-15	2-4	33	91.8	27.6	27.6
	7.5-15	1-2	34	49.9	25.3	29.4
	7.5-15	.5-1	35	14.5	56.7	22.4
	7.5-15	<.5	36	2.6	27.0	23.5
Ames 303 c/s moldboard plow	0-7.5	>8	37	455.2	36.2	34.8
	0-7.5	4-8	38	287.4	48.4	47.0
	0-7.5	2-4	39	140.9	47.1	51.0
	0-7.5	1-2	40	107.4	55.6	51.6
	0-7.5	.5-1	41	99.6	25.1	23.8
	0-7.5	<.5	42	78.9	12.8	11.6
	7.5-15	>8	43	561.0	39.9	41.3
	7.5-15	4-8	44	244.8	47.0	44.3
	7.5-15	2-4	45	104.3	51.0	49.6
	7.5-15	1-2	46	45.6	44.6	45.9
	7.5-15	.5-1	47	26.2	29.1	31.6
	7.5-15	<.5	48	8.2	14.8	15.4

Plot	Depth(cm)	Size(mm)	ID	Fraction weight (g)	Obs a mg/kg	Obs b mg/kg
Ames 305 c/s no-till	0-7.5	>8	49	515.8	52.4	51.7
	0-7.5	4-8	50	265.9	58.2	59.6
	0-7.5	2-4	51	137.2	60.6	63.3
	0-7.5	1-2	52	110.6	75.3	76.6
	0-7.5	.5-1	53	89.1	93.1	84.0
	0-7.5	<.5	54	42.8	69.2	74.3
	7.5-15	>8	55	628.8	26.8	27.5
	7.5-15	4-8	56	241.3	30.3	30.3
	7.5-15	2-4	57	105.9	33.1	33.8
	7.5-15	1-2	58	59.1	42.3	41.0
	7.5-15	.5-1	59	11.8	54.8	52.1
	7.5-15	<.5	60	3.6	35.8	37.0
Ames 307 c/s chisel-plow	0-7.5	>8	61	290.6	42.2	52.4
	0-7.5	4-8	62	168.3	47.4	43.3
	0-7.5	2-4	63	115.4	47.1	53.2
	0-7.5	1-2	64	87.4	59.3	52.6
	0-7.5	.5-1	65	75.6	51.5	58.0
	0-7.5	<.5	66	46.2	24.8	23.5
	7.5-15	>8	67	528.4	39.1	39.1
	7.5-15	4-8	68	227.1	47.6	48.3
	7.5-15	2-4	69	81.9	78.7	78.0
	7.5-15	1-2	70	45.1	60.7	56.7
	7.5-15	.5-1	71	8.2	46.8	48.2
	7.5-15	<.5	72	2.1	31.7	28.7